ASSOCIATION FOR RESEARCH IN OTOLARYNGOLOGY

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2022 ARO

ABSTRACT BOOK

FEBRUARY 5-9, 2022
VIRTUAL CONFERENCE

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Saturday, February 5, 2022

Presidential Symposium
7:00 a.m. – 11:00 p.m.

Mentoring and Training Scientists: What Works?
Chair: John Oghalai, *University of Southern California*

Virtual: Learning How to Learn: Powerful Mental Tools to Help You Master Tough Subjects
Barbara Oakley, *Oakland University*

Professor of Engineering (and former translator) Barbara Oakley is the instructor of Learning How to Learn (University of California-San Diego/McMaster – Coursera), one of the world's largest student massive open online course, with over three million students. In this presentation, she will show you useful information about how you and those you work with can more easily master new and difficult material—and establish an even richer learning culture for your students.
•   Learn why it's perfectly normal to not understand something difficult the first time its encountered. You'll see how knowledge of how the brain works can help improve test scores and protect against feelings of frustration and failure when something seems difficult to learn.
•   See what the patterns of expertise look like in the brain and learn how to build those patterns more quickly and with less frustration.
•   Discover simple tools to tackle procrastination. (You might be surprised to learn that even just thinking about something you don't like causes your brain to experience pain.)
•   Learn the surprising advantages of having a bad memory and sometimes being a slower learner.

Dr. Oakley's previous book, A Mind for Numbers, is a New York Times and international best-seller translated into over a dozen languages. Her most recent book is the critically acclaimed Uncommon Sense Teaching: Practical Insights in Brain Science to Help Students Learn.

Mentoring Graduate Students: Tales From the Trenches and New Evidence From Neuroplasticity Across the Lifespan
Daniel Levitin, *Minerva University*

Sciences training requires that graduate students succeed in making a paradigm shift in the way they view knowledge; they must transition from being consumers of knowledge to creators of knowledge; and from assuming published findings are true to questioning their truth. At the same time, they need to change a fundamental aspect of their self-image, from thinking they know everything (that's how they got into grad school in the first place) to realizing that they—and we as scientists—actually know very little. In parallel, neuroplastic changes in the brain can support or hinder these attitudinal shifts as a function of age and individual differences. Particularly after the age of 50, it is critical that we continue learning, and exercising our brains by taking on new challenges and learning new things.

Funds Flow To Support The Education Mission: A Dean's Perspective
Joseph Kerschner, *Medical College of Wisconsin*

Academic Health Systems (AHS) have a tripartite mission of clinical care, research and education. An AHS is generally defined as a clinical delivery system with a medical school with the medical school having the responsibility for the research and education missions. The research and education missions are often considered together as the academic mission. The clinical missions for AHS are generally net margin positive. The academic missions are generally net margin negative. Therefore, most AHS require cross-subsidization of their academic missions from their clinical missions. There are numerous structures for AHS from fully integrated to more loosely affiliated. Each structure has its own particular aspects related funds flow in supporting the academic missions of the AHS. There is no single "best" model, however, greater integration between the clinical health care delivery system and the medical school has been the trend for the past several decades. Regardless of an AHS's structure, there is a natural tension around the conversation related to, "how much clinical cross-

subsidization should occur to support the academic missions of the AHS?" This presentation will discuss the different models of AHS, the different points of emphasis for funds flow to support the academic missions and specifically emphasize the education mission as it relates to learners with a career interest as a clinician-scientist.

Mentoring the Budding Physician Scientist
Lloyd Minor, *Stanford University School of Medicine*

For decades, physician scientists have transformed biomedical science and the practice of medicine through their discoveries, including in otolaryngology, where breakthroughs in research gave rise to advances in the diagnosis and treatment of disorders in the scope of our specialty. Yet today, the number of physician scientists is in decline – a trend that must be reversed if we are to realize the full potential of biomedicine's next chapter.

Mentorship is one of our most powerful tools in developing the next generation of physician scientists. The challenging nature of the profession, which requires intense focus in both laboratory research and patient care, can be daunting. This challenge is compounded by the increasingly complex and competitive landscape for research funding. On all of these fronts, mentors can play an important role in the careers of budding physician scientists, helping them to pursue their passions while navigating obstacles that are inherent to the field.

In this presentation, Lloyd Minor, MD, Dean of the Stanford University School of Medicine, will discuss the importance of mentorship in his journey as a physician scientist, and share strategies for cultivating the careers of physician scientists in otolaryngology and beyond. The presentation will consider key themes including:
• 	Broadening the pipeline of young physician scientists
• 	Ways to prepare mentees for failure and adversity
• 	The importance of diversity, equity, and inclusion to the future of the field

Recruiting and Mentoring Diverse Trainees
Chris Manning, *University of Southern California*

In this talk, we will discuss building pipeline relationships with institutions of higher learning that are educating the kinds of diverse researchers whose viewpoints and backgrounds can enhance the excellence of your profession, targeting your position advertisements to subcommittees of professional organizations of diverse identity groups, and ensuring that your hiring rubrics evaluate the contribution that all potential colleagues can make to enhancing the diversity of your institution. In addition to recruitment strategies will we also discuss efforts to enhance retention of incoming diverse scientists.

Intentional Mentoring
Chair: John Oghalai, *University of Southern California*
Co-Chair: Linda von Hoene, *University of California, Berkeley*
Co-Chair: Sabrina Soracco, *University of California, Berkeley*

Panel:
Christopher Shera, *Keck School of Medicine, University of Southern California*
Anthony Ricci, *Stanford University*
Karina Cramer, *UC Irvine*
Paul Fuchs, *Johns Hopkins University of Medicine*
Ruth Litovsky, *University of Wisconsin*
Monita Chatterjee, *Boys Town National Research Hospital*

11:30 a.m. - 1:30 p.m.
Symposium #1

Consideration of Cross-Sensory Influences on Audition
Chair: Kelly Harris, *Medical University of South Carolina*
Co-Chair: James Dias, *Medical University of South Carolina*

Speech understanding in ecological environments benefits from the integration of information across multiple sensory inputs. The overarching theme of this symposium concerns the behavioral and neural mechanisms involved in cross-sensory integration and auditory processing. The research presented at the ARO meeting traditionally focuses on unimodal aspects of auditory processing, yet cross-sensory contributions to auditory processing are well-established. If selected, this symposium will bridge this gap and highlight how cross-sensory factors can contribute to auditory processing and speech recognition. We have assembled a diverse, 5-member panel that includes several leaders in the field of multisensory perception, two of whom are new to ARO, who will provide an in-depth and comprehensive overview of cross sensory factors relevant to auditory processing, aging, and neuroplasticity. Of 5 panel members, 3 are women, 1 is an early-career investigator, and 1 is underrepresented in science. Dr. Dias (15 min) will introduce the session with an overview of cross-sensory influences on auditory processing and present novel data finding older listeners exhibit improved auditory-visual integration to preserve audiovisual speech perception. Focusing on the importance of multisensory temporal integration, Dr. Lalor (30 min) models EEG responses to describe how temporal visual cues aid auditory perception. Our ability to incorporate cross-sensory information is dependent on the coherence across time and space. Dr. van Wassenhove (30min) will present her neural and behavioral research examining how comodulation of these cues contributes to the synthesis of incoming multisensory speech. The final two speakers will examine neuroplasticity associated with unimodal auditory deprivation. Dr. Sharma (30 min) will present her research focused on how hearing loss results in cross-modal recruitment of auditory areas during visual and somatosensory tasks and the effects of intervention. Dr. Harris (15 min) will present new evidence suggesting that a sub-total loss of auditory nerve function can contribute to cortical reorganization and lead to increased dependence on cross-sensory input.

Audition and the Multisensory Brain
James Dias, *Medical University of South Carolina*

A recent "multisensory revolution" has highlighted the broad extent to which vision can modulate audition. Converging evidence suggests that the brain uses input from across the senses to support and enhance perception of sounds heard in complex listening environments. This symposium will present recent work from leaders in the field of multisensory perception concerning how the brain processes auditory-visual information, how auditory-visual integration can enhance speech perception, and how hearing loss can result in greater reliance on visual input. We will also present our own recent finding that older adults with hearing loss rely more on multisensory sources of information.

Modeling the Electrophysiology of Natural Audiovisual Speech Processing
Edmund Lalor, *University of Rochester*

Seeing a speaker's face benefits speech comprehension, especially in challenging listening conditions. This perceptual benefit is thought to stem from the neural integration of visual and auditory speech at multiple stages of processing, whereby movement of a speaker's face provides temporal cues to early auditory cortex, and articulatory information from the speaker's mouth can aid recognizing specific linguistic units (phonemes, syllables). In this talk I will describe attempts to explore this multisensory integration framework – and how it is affected by attention – by modeling EEG responses to natural audiovisual speech in terms of different acoustic, visual, and linguistic features.

Temporal Comodulation in Multisensory Causal Inference
Virginie van Wassenhove, *Cognitive Neuroimaging Unit, CEA DRF/Joliot, INSERM, CNRS, Université Paris-Saclay*

Perception relies on inferences about the causal structure of the world provided by multiple sensory inputs. In ecological settings, multisensory events that cohere in time and space benefit inferential processes, e.g. hearing and seeing a speaker enhances speech comprehension. Using psychophysics and functional connectivity characterization of human brain activity measured with magnetoencephalography (MEG), I will discuss how temporal comodulation may contribute to the analysis and synthesis of incoming speech (and non-speech) signals.

Cross-Modal Neuroplasticity in Hearing Loss
Anu Sharma, *The University of Colorado Boulder*

A basic tenet of neuroplasticity is that the brain will re-organize following sensory deprivation. Our high-density EEG experiments suggest that cross-modal plasticity, one form of cortical re-organization, occurs in hearing loss. We find evidence of cross-modal recruitment of higher-order auditory cortical areas by visual and somatosensory modalities in deaf children with cochlear implants, and in older adults with mild-moderate hearing loss. Cross-modal plasticity is associated with difficulties in speech-in-noise and cognitive processing in untreated hearing loss. New findings suggest that cross-modal recruitment in hearing loss may be reversed by timely and well-fitted intervention with hearing aids and/or cochlear implants.

Compensatory Multi-Sensory Integration With Auditory Nerve Dyssynchrony
Kelly Harris, *Medical University of South Carolina*

Peripheral deficits in sensory processing result in widespread changes in the cortex. Unimodally, sub-total loss contributes to reorganization within the cortex of the affected modality. Auditory nerve dysfunction contributes to difficulties understanding auditory speech. However, it is uncertain the extent to which unimodal sub-total losses may impact multi-sensory integration. These associations are of ecological importance, as audiovisual speech is often more accurate than unimodal speech. We examined the neural mechanisms and structure contributing to audiovisual speech. Results discussed add to the growing literature that sensory loss may enhance not only unimodal processing in preserved senses but also integration across senses.

11:30 a.m. - 1:30 p.m.
Podium Session #2 – Anatomy and Physiology of the Inner Ear
Moderators: Jonathan Bird, Ph.D. & Gregory Frolenkov, Ph.D.

1. Molecular and Cellular Manifestations of Biological Aging of Cochlear Inner and Outer Hair Cells
Category: Hair Cells: Anatomy and Physiology
Huizhan Liu[1], Kimberlee Giffen[2], Yi Li[3], Zhi-Zhou He*[4]
[1]*Creighton University School of Medicine,* [2]*Stanford University School of Medicine,* [3]*Beijing Tongren Hospital,* [4]*Creighton University*

Background: Age-related hearing loss (ARHL) negatively impacts quality of life in the elderly population. Loss of the mechanosensitive hair cells (HCs) in the cochlea is the prevalent cause of ARHL. The molecular and cellular mechanisms of HC degeneration remain poorly understood. The goal of our study is to depict molecular and cellular changes associated with the biological aging of inner and outer HCs (IHCs and OHCs) for a better understanding of the mechanism of HC aging.
Methods: We utilized RNA-seq to analyze transcriptomes of 1,000 IHCs, 1,000 OHCs, and 1,000 stria vascularis cells individually collected from cochleae of 9 and 26 month-old CBA/J mice to identify age-related transcriptional changes. The cellular pathophysiology of aging HCs was examined using advanced imaging and electrophysiology techniques. Specifically, we examined ultrastructural and cytological changes in HC stereocilia and soma, as well as changes in mechanical activity and property of OHCs.
Results: RNA-seq transcriptomic analysis of IHCs and OHCs isolated from young and aged mice revealed differential expression of genes related to DNA damage/repair, transcription, autophagy, and oxidative stress. Furthermore, genes essential for HC function such as Lhfpl5, Tmc1, Tmie, Cib2, Kcnq4, and Slc26a5 were downregulated with age. Advanced imaging showed degeneration of HC stereocilia and soma, and voltage-clamp recording indicated a reduction in outer HC motility and axial stiffness.
Conclusions: This is the first comprehensive and in-depth study to examine molecular and cellular mechanisms associated with the biological aging of IHCs and OHCs. HCs share most common hallmarks of aging as seen in other cell types. At the molecular level, HC degeneration is driven by dysregulation of genes related to transcription, DNA damage/repair, autophagy, and oxidative stress as well as a shift in the balance from pro-survival and anti-apoptotic pathways toward pro-apoptotic and pro-inflammatory pathways. At the cellular level, HC degeneration is manifested by loss of stereocilia, shrinkage of HC soma and reduction in OHC mechanical properties. Such decline in mechanotransduction and OHC electromotility accelerates HC death and drives ARHL before HC loss. Our study provides novel molecular and cytological profiles of aging HCs and identifies Foxo3, Jund, and Cbx3 as biomarker and potential targets for therapeutic intervention for ARHL.

2. Regulation of Membrane Homeostasis by TMC1 Mechanoelectrical Transduction Channels is Essential for Hearing

Category: Hair Cells: Anatomy and Physiology

Angela Ballesteros*[1], Kenton J. Swartz[1]
[1]*NINDS*

Background: The mechanoelectrical transduction (MET) channel complex of auditory hair cells converts sound into electrical signals, allowing us to hear. After decades of research, the transmembrane-like channel 1 and 2 (TMC1 and TMC2) have been recently identified as pore-forming subunits of the MET channels, but the molecular peculiarity that differentiates these two proteins and makes TMC1 essential for hearing remains elusive. Interestingly, while TMC1 or TMC2 are sufficient for MET, expression of TMC2 is insufficient for maintaining normal hearing in mice lacking TMC1, suggesting that TMC1 must have additional functions required for hearing. Moreover, although some TMC1 deafness-causing mutations have been studied extensively, the deafness phenotype of these mutations does not appear to relate to the functional properties of the MET channel. Thus, the molecular mechanisms of TMC1-related deafness remain enigmatic.

Methods: Inspired by the evolutionary and structural relation between the TMC proteins and the TMEM16 lipid scramblases, we investigated the potential role of TMC proteins in the regulation of hair cell membrane homeostasis using super-resolution confocal microscopy and organ of Corti explants from 13 different transgenic mouse lines. mT/mG reporter mice expressing a membrane-targeted tomato fluorescent protein in a wild type or TMC1/TMC2 knockout background were used to visualize and study the hair cell membrane.

Results: Here we report the first physiological difference between TMC1 and TMC2 in murine auditory hair cells. We show that TMC1, but not TMC2, is essential for a regulatory mechanism activated by a decrease in intracellular calcium that triggers membrane remodeling and lipid and protein mislocalization at the hair cell mechanosensory organelle. We demonstrate that pharmacological inhibition of MET channels, breakage of the tip links, or buffering of intracellular calcium lead to pronounced phosphatidylserine externalization, membrane blebbing and ectosome release at the hair cell sensory organelle, culminating in the loss of TMC1 protein. Importantly, our findings show that TMC1 dominant (M412K and D569N) and recessive (D528N) deafness-causing mutations (DFNA36 and DFNB7/B11) alter phosphatidylserine externalization by different mechanisms, and that the constitutive externalization of phosphatidylserine correlates with the deafness phenotype in TMC1 mice carrying the mutation in heterozygosity or homozygosity.

Conclusions: Our work reveals a novel role for TMC1 in regulating hair cell membrane homeostasis that is essential for hearing and has consequences in hair cell development, repair, and death.

3. Solving the Multiple Roles of GNAI Proteins in the Polarized Morphogenesis of Sensory Hair Cells

Category:Hair Cells: Anatomy and Physiology

Amandine Jarysta*[1], Abby Tadenev[1], Matthew Day[1], Benjamin Low[1], Michael Wiles[1], Basile Tarchini[1]
[1]*The Jackson Laboratory*

Background: Studies over the last decade have shown that inhibitory G alpha (GNAI) proteins are critical for sensory hair cell (HC) development and hearing. Limited in vitro evidence suggest that GNAI may be involved in the off-center shift of the basal body that breaks cytoskeleton symmetry at the surface of prospective HCs. GNAI also appear to signal downstream of the GPCR GPR156 to reverse HC orientation downstream of the transcription factor EMX2. Finally, GNAI have been best characterized as interacting with the GPSM2 protein scaffold to regulate stereocilia elongation and row identity during hair bundle morphogenesis. Importantly, insight into these surprisingly diverse GNAI functions remains fragmentary. This work was indeed largely based on the use of Pertussis toxin, a bacterial protein that globally downregulates functionally redundant GNAO, GNAI1, GNAI2 and GNAI3, and may also cause unrelated defects. We thus embarked on a comprehensive study to definitively determine the role(s) of individual GNAI proteins.

Methods: We obtained or generated single Gnai1(ko), Gnai2(ko), Gnai3(ko) and Gnao(ko) constitutive mouse mutants, and derived viable double mutants: Gnai1(ko); Gnai2(ko) and Gnai1(ko); Gnai3(ko). Because combined loss of Gnai2; Gnai3 is embryonic lethal, we generated a Gnai3Flox allele and conditional FoxG1-Cre; Gnai2(ko); Gnai3(Flox) mutants. We used scanning electron microscopy to quantify hair bundle dimensions in adults, auditory brainstem response to record hearing, and confocal microscopy to quantify protein signals, and measure HC eccentricity and orientation during development.

Results: We find that only GNAI3 is essential for the organization and elongation of stereocilia, as well as for auditory function. As reported previously (Beer-Hammer et al., 2018), we confirm that GNAI2 can mitigate the

loss of GNAI3 during hair bundle morphogenesis, and we now provide a detailed molecular explanation. Interestingly, functional rescue by GNAI2 is incomplete because GNAI2 does not uniformly occupy the subcellular compartments where GNAI3 is missing: the HC surface (bare zone) and stereocilia tips. Strikingly, our FoxG1-Cre; Gnai2ko; Gnai3Flox mutants recapitulate for the first time two distinct types of defects so far only observed with Pertussis toxin: 1) a delay or failure of the basal body to shift off-center at early HC developmental stages, and 2) a graded inversion of OHC orientation similar to the one we observed in Gpr156 mutants. As these latter defects show variable expressivity, GNAO and GNAI1 might be involved in HC symmetry breaking (1) and orientation (2), but they play at best a marginal role in hair bundle morphogenesis.

Conclusions: To date, GNAI1 and GNAO function remained untested, and Beer-Hammer et al. did not involve GNAI proteins in HC symmetry breaking or orientation. Here we directly assign distinct and partially redundant roles to all individual GNAI proteins, and validate all Pertussis-based GNAI roles as physiologically relevant.

4. A Novel Isoform of Myosin 15 Traffics EPS8 in Adult Hair Cell Stereocilia
Category: Hair Cells: Anatomy and Physiology
Ghazaleh Behnammanesh*[1], Jonathan Bird[1]
[1]*University of Florida*

Background: On the apical surface of hair cells, actin-based stereocilia transduce fluid movements into electrical signals that are transmitted to the brain. Stereocilia assemble into rows of precisely graded heights and the preservation of this architecture is essential for mechanoelectric transduction (MET) and hearing. Mutations in MYO15A, encoding the molecular motor myosin 15, disrupt the hair bundle and cause human hereditary hearing loss, DFNB3. Alternative splicing creates multiple MYO15 isoforms with different functions in the hair bundle. A long isoform (MYO15-1, aka MYO15-L) postnatally maintains the size of shorter row stereocilia with active MET. A short isoform (MYO15-2, aka MYO15-S) controls stereocilia growth during developmental trafficking of the elongation complex (EC), WHRN, EPS8, GNAI3, GPSM2, in addition to potentially stimulating actin polymerization directly. We recently identified a new isoform (MYO15-3) with an alternative transcription start site encoding a novel 50-residue N-terminal domain. The function of MYO15-3 is unknown. Here, we investigate the expression of MYO15-3 in cochlear hair cells and examine if it can traffic the EC, like MYO15-2.
Methods: We measured the expression of Myo15 mRNA by qPCR through key stages of hair bundle development and maturation. Total RNA was extracted from P0 – 60 mouse cochleae and relative expression for Myo15 transcripts was normalized to Tbp and Hprt. HeLa cells were transfected with either EGFP-MYO15-2, EGFP-MYO15-3, and EGFP-MYO10 plasmid DNA, fixed, labeled with anti-EPS8 antibody and imaged using a confocal microscope. Mouse cochleae from a CRISPR engineered deletion (Myo15Δ3) were fixed and labeled with anti-EPS8 antibody and fluorescent phalloidin, before imaging on a confocal microscope.
Results: Using qPCR, we found that Myo15-3 expression was upregulated during postnatal development in C57B6/J cochleae, increasing significantly by P60. To test our hypothesis that MYO15-3 can traffic the EC in vitro, we examined the colocalization of EGFP-MYO15-3 and EPS8 in HeLa cell filopodia. The relative intensity of endogenous EPS8 labeling at filopodia tips versus the cell cortex was significantly increased in cells expressing EGFP-MYO15-3 or EGFP-MYO15-2. EGFP-MYO10 did not enhance EPS8 accumulation at filopodia tips (negative control). We further tested our hypothesis in vivo and found that EPS8 antibody labeling was absent from the stereocilia tips of mutant Myo15(Δ3/Δ3) hair cells compared with control Myo15(+/Δ3) samples at P30.
Conclusions: Myo15-3 is expressed in postnatal and adult hair cells, in a similar profile to the longer Myo15-1 isoform that maintains stereocilia architecture. The stereocilia tip localization of EPS8 is dependent upon MYO15-3 in adult hair cells, consistent with these proteins interacting in vitro. Our results suggest that MYO15-3 traffics EPS8 in adult stereocilia, and that these proteins may be involved in maintaining stereocilia architecture.

5. Epigenetic Silencing of Hair Cell Gene Regulatory Network by DNA Methylation in Postnatal Maturing Supporting Cells
Category: Genetics A: Genomics and Gene Regulation
John Nguyen*[1], Juan Llamas[2], Talon Trecek[3], Litao Tao[3], Neil Segil[4]
[1]*USC,* [2]*USC Keck School of Medicine,* [3]*University of Southern California,* [4]*Keck School of Medicine, University of Southern California*

Background: The main cause of hearing loss is damage of cochlear sensory hair cells. In non-mammalian vertebrates, hair cells can be replaced by regeneration that involves the transdifferentiation of existing supporting cells. In mice, a latent ability to transdifferentiate persists at birth, but is lost during the first postnatal week. The

ability to transdifferentiate relies on the expression of a hair cell gene regulatory network (GRN), led by the master-gene regulator of hair cell differentiation Atoh1. Atoh1 and the hair cell GRN is normally repressed in supporting cells through epigenetic mechanisms controlled by the Notch signaling pathway (Tao et al., 2021). In perinatal mice, this repressed epigenetic state of the hair cell GRN in supporting cells can be reversed in the service of transdifferentiation by blocking Notch signaling. As the mouse organ of Corti matures during the first postnatal week, the ability to transdifferentiate is lost. We hypothesize that this is the result of changes in the epigenetic status of the hair cell GRN during maturation. Examples of this changing epigenetic status during postnatal maturation include loss of H3K4me1 enhancer licensing, and increases in the repressive histone modification H3K27me3 specifically within the hair cell enhancers of supporting cells (Tao et al., 2021). Here we report on additional changes in the epigenetic status of supporting cells during postnatal maturation, specifically on the changing patterns of DNA methylation within the hair cell gene GRN.

Methods: We used mice with a NuTRAP transgene to label nuclei in a cell type-specific manner, allowing FACS-purification of labeled nuclei for downstream epigenetic profiling from whole cochlea. Atoh1-creER mice were bred to NuTRAP mice to label hair cell nuclei, while Lfng-creER mice were used to label supporting cell nuclei. Whole genome bisulfite sequencing was used to profile DNA methylation at CpG dinucleotides; CUT and Tag was used to profile the histone modifications H3K4me1, H3K4me3, and H3K27me3; and chromatin accessibility was assayed by analysis of H3K4me2 (CUTAC).

Results: The promoters of many developmentally regulated genes harbor an increased frequency of CpG dinucleotides in what are known as CpG islands (CGIs). DNA methylation of CpG islands is generally associated with silencing of genes. We show that there is a developmentally regulated increase in DNA methylation at CpG islands in the hair cell GRN of supporting cells, suggesting that these genes are undergoing a more permanent form of silencing. Many of the sites that gain DNA methylation are direct targets of Atoh1.

Conclusions: The hair cell GRN in cochlear supporting cells undergo multiple forms of epigenetic remodeling during postnatal maturation, including DNA methylation. These changes are likely responsible for silencing the developmentally regulated hair cell GRN, whose reactivation is needed for regeneration.

6. p27Kip1 a Validated Cellular Regeneration Target in the Cochlea of Mature Mammals
Category: Inner Ear: Drug Delivery
Jonathan Kil*[1]
[1]*Sound Pharmaceuticals, Inc.*

Background: In adult mammals, absence of a cochlear progenitor cell capable of cellular regeneration is thought to be a primary reason why sensorineural hearing loss is often permanent and progressive. P27 is a cyclin-dependent kinase inhibitor, that acts to inhibit cell proliferation by binding and inactivating cyclin/cyclin-dependent kinases, predominantly cyclinE/cdk2. During cochlear embryogenesis, p27 expression begins as cell division stops in the organ of Corti, and before supporting-cells and hair-cells have begun to differentiate. P27 is not highly expressed in hair-cells but is highly expressed in the nuclei of developing and adult supporting-cells. Furthermore, cyclinE/cdk2 is highly expressed in the cytoplasm of developing and adult supporting-cells. Germline deletion of p27Kip1 in mice allows supporting-cells to continue to proliferate through hearing onset, resulting in extra rows of auditory supporting-cells and hair-cells. These supernumerary hair-cells display critical molecular functions although the adult p27 knockout mouse is hearing impaired when compared to wildtype and heterozygous littermates. In addition, conditional knockout of p27Kip1 induces renewed supporting-cell proliferation resulting in some hair-cell regeneration in the adult cochlea.

Methods: To stimulate proliferative regeneration in mature cochleae following ototoxic and acoustic insults, three different p27Kip1 gene knockdown methods: antisense oligonucleotides (AONs), short hairpin RNAs (shRNA), and short inhibitory RNAs (siRNA) were administered by intracochlear injection to adult guinea pigs. BrdU (a proliferation marker) was provided systemically through the recovery period. Supporting-cell proliferation was determined by BrdU immunocytochemistry and hair-cell regeneration was determined by immunocytochemistry involving hair-cell specific markers: myosin-VI and -VIIa. Whole mount sections of the cochlea and serial cross-sections were analyzed for proliferative regeneration (BrdU/myosin-VI and -VIIa) under light and fluorescence microscopy. ABRs were performed before and after cochlear injury, after intracochlear injection, and at the end of study.

Results: p27Kip1 AONS, shRNA and siRNA can effectively decrease p27 mRNA and protein expression in vitro using quantitative PCR and semi-quantitative western blot analysis. P27Kip1 shRNA and siRNA were able to induce significant cellular proliferation and regeneration in vivo following aminoglycoside induced or noise induced hearing loss. Within 4 to 8 weeks after a single 5 microL injection of an optimized p27 siRNA (SPI-

5557), improvements in ABR thresholds were observed. After 8 to 12 weeks, no significant inflammation was observed in the siRNA injected cochlea, and all animals appeared to exhibit normal activity.

Conclusions: These findings support p27Kip1 as a validated regeneration target in the adult mammalian cochlea. p27 inhibition may allow the endogenous activity of cyclinE/cdk2 to stimulate effective supporting-cell proliferation. The progeny of these new cell divisions may be more amenable to other endogenous or exogenous cell fate-determining molecules that could facilitate effective hair-cell regeneration. p27Kip1 plays a significant role in maintaining cellular quiescence and terminal differentiation in the mature organ of Corti.

7. Regeneration of Type I and Type II Hair Cells by Drug Treatment of Mature Mouse Utricle
Category: Inner Ear: Drug Delivery

Hanae Lahlou*[1], Kohsuke Tani[2], Albert S. B. Edge[3]
[1]*Eaton-Peabody Laboratory-Massachusetts Eye and Ear Infirmary/Harvard Medical School,* [2]*Eaton-Peabody Laboratory, Massachusetts Eye and Ear, Boston, USA,* [3]*Massachusetts Eye and Ear Infirmary/Harvard Medical School*

Background: The vestibular sensory organs of the inner ear contain highly specialized mechanoreceptive hair cells essential for balance. Vestibular hair cells regenerate spontaneously in response to damage; however the extent of regeneration declines from a high point in the first postnatal weeks through adulthood. In this study, we sought to develop a pharmacological treatment to enhance vestibular hair cell regeneration in damaged adult utricle.

Methods: We used adult mice that express the diphtheria toxin receptor in hair cells (Pou4f3-DTR) for targeted ablation of hair cells. After in vivo administration of diphtheria toxin, damaged adult utricles were treated in vitro with a combination of a glycogen synthase kinase inhibitor and a histone deacetylase inhibitor, a combination previously shown to stimulate hair cell differentiation in the cochlea.

Results: We observed a significant increase in cells expressing MYO7A, specific vestibular hair cell marker, relative to the spontaneous regeneration observed in utricles with damage only (28% vs 8% of hair cells in a normal utricle of the same age). The fate mapping, using lineage tracing of supporting cells, demonstrated that the newly regenerated hair cells after drug treatment displayed both type I (MYO7A+SOX-) and type II (MYO7A+/SOX+) hair cell features. The same drug combination injected into damaged adult mouse ear via the semicircular canal resulted in regeneration of 58% of the normal number of hair cells after 8 weeks as compared to 32% in the damaged, non-drug-treated ear.

Conclusions: Ultimately, this work provides further knowledge of the molecular mechanisms required for vestibular hair cell differentiation and may pave the way toward the development of new therapeutic strategies to produce hair cells to restore vestibular function.

8. Slc26a4-Insufficiency Mouse Model: Correlating Hearing Instability With Stria Vascularis Dysfunction
Category: Inner Ear: Membranes and Fluids

Rafal Olszewski[1], Shoujun Gu[1], Michael Hoa*[1]
[1]*Auditory Development and Restoration Program, National Institute on Deafness and Other Communication Disorders, National Institutes of Health*

Background: Hearing instability disorders in humans, including Meniere's disease, autoimmune inner ear disease, sudden sensorineural hearing loss, and enlarged vestibular aqueduct syndrome, remain poorly understood with few, if any, effective treatments. Observations of endolymphatic hydrops in patients with these diseases suggests the possibility of dysfunctional cochlear ionic homeostasis and one of the regions in the cochlea responsible for this function is the stria vascularis (SV) and lateral wall of the cochlea. Mechanisms underlying hearing instability are not well defined and few mouse models exist with which to model these diseases. Fortunately, at least one mouse model, the Slc26a4-insufficiency mouse model, exists where hearing fluctuation occurs without surgical manipulation. Missense mutations in SLC26A4 have been implicated in Meniere's disease. Studying hearing instability in this mouse model represents an opportunity to study the mechanisms underlying this phenomenon. In this study, we seek correlate hearing instability with changes in stria vascularis gene and protein expression, as well as function.

Methods: The Slc26a4-insufficient mouse model (DE17.5 mice) as published by Ito and colleagues (Ito et al, 2014) was used in this study. DE17.5 mice which have a Slc26a4 null background (experimental group) and heterozygous (control group) animals underwent ABR starting at age p30, once weekly, for 4 consecutive weeks. Endocochlear potential was measured one day after last ABR test. Inner ear tissue has been collected immediately

after EP test termination. Histological studies (immunofluorescence (IF) and RNAscope in situ hybridization) for SV cell type-specific protein and gene expression were performed on PFA-fixed, frozen cochlear tissue.
Results: ABR monitoring over 4 weeks distinguished ears with fluctuating from non-fluctuating hearing among tested animals. SV cell type-specific protein and gene expression was compared and quantified between DE17.5 ears with and without hearing fluctuation as well as to a control group of ears from animals heterozygous for Slc26a4 mutation.
Conclusions: We characterize expression of SV cell type-specific in the Slc26a4-insufficiency mouse model in the setting of hearing fluctuation. We seek to correlate changes in SV cell type-specific expression with hearing fluctuation.

11:30 a.m. - 1:30 p.m.
Podium Session #3 – Vestibular Function and Aging: Basic Research
Moderators: Benjamin T. Crane, M.D., Ph.D. & Anna Lysakowski, Ph.D.

1. Temporal Binding Window for Visual-Vestibular Heading Integration
Category: Vestibular: Basic Research and Clinical
Raul Rodriguez[1], Benjamin Crane*[2]
[1]*University of California, Berkley*, [2]*University of Rochester*

Background: Natural environments include complex multisensory stimuli that must be integrated into a unified perception. Multiple factors determine whether and how these sensory signals are integrated, with the temporal relationship being one potentially important factor. The temporal binding window for visual-auditory stimuli, the range during which cues are perceived to be simultaneous, is ±100 ms. For auditory and vestibular stimuli, the temporal binding window has been reported to be within a 200–300 ms window. Heading direction is perceived based on visual and inertial cues, but the temporal window over which these can be integrated is unknown.
Methods: Seven healthy human subjects (3 female, mean age 39 ± 18 years) experienced 2 s of translation along a heading of 0°, ±35°, ±70°, ±105°, or ±140°. A 6-degree-of-freedom (6-DOF) motion platform (Moog, East Aurora, NY model 6DOF2000E) was used to deliver the inertial motion stimuli. These inertial headings were paired with 2 s duration visual headings that were presented at relative offsets of 0°, ±30°, ±60°, ±90°, or ±120. A 55" color LCD screen with a 1920 x 1080 pixel resolution delivered the visual component. The screen was mounted to the motion platform and set 50 cm from the subject, filling 117° horizontal field-of-view. The subjects wore a helmet coupled to the motion platform while they sat in an automotive-style racing seat. The visual stimuli were presented at 17 temporal delays ranging from -500 ms (visual lead) to 2,000 ms (visual delay) relative to the inertial stimulus. After each stimulus, subjects reported the direction of the inertial stimulus using a dial.
Results: The bias of the inertial heading towards the visual heading was robust at ±250 ms when examined across subjects during this period: 8.0 ± 0.5° with a 30° offset, 12.2 ± 0.5° with a 60° offset, 11.7 ± 0.6° with a 90° offset, and 9.8 ± 0.7° with a 120° offset (mean bias towards visual ± SE). The amount of bias varied between subjects. However, the mean bias was consistently diminished with temporal misalignments of ±500 ms, and there was no longer any visual influence on the inertial heading when the visual stimulus was delayed by 1,000 ms or more. Although the amount of bias varied between subjects the effect of delay was similar.
Conclusions: Visual headings influence inertial heading perception when timing differences are within 250 ms. This represents a larger temporal binding window than is seen with visual-auditory stimuli.

2. Electrophysiological Correlates of Amplitude Modulation Depth Detection and Discrimination
Category: Aging
Sam Watson*[1], Jonatan Märcher-Rørsted[1], Anders Søe[1], Jens Hjortkjær[1]
[1]*Technical University of Denmark (DTU)*

Background: Both aging and hearing loss impacts amplitude modulation (AM) depth detection and discrimination thresholds measured behaviourally, but objective correlates of the underlying neural AM processing are missing. This study was designed to probe AM depth processing at both the neural and behavioural level using the same novel stimulus, with simultaneous measures of cortical and sub-cortical processing.
Methods: We used a 128 Hz amplitude modulated broad-band noise stimulus that alternated between two levels of AM depth at an alternation rate of 2 Hz. The stimulus was first used to elicit cortical (2 Hz) and sub-cortical (128 Hz) envelope-following responses (EFRs) recorded with electroencephalography (EEG). Next, the same

depth-alternating stimulus was used to measure behavioral AM detection and discrimination thresholds psychophysically. Both EFRs and behavioral thresholds were measured with either unmodulated or 40 % modulated reference stimuli, and varying target depths. Eight young (18-28 years) and ten elderly (55-70 years) listeners with normal hearing sensitivity thresholds participated in the study.

Results: From the EEG data, it was found that increasing AM depth relative to the reference increased the EFRs at both 2 Hz and 128 Hz in both listener groups. However, small depth differences failed to yield significant EFRs in many subjects. For fully modulated stimuli alternating with an unmodulated reference, EFR magnitudes at 2 Hz and at 128 Hz were correlated. Yet, only EFRs at 2 Hz, but not at 128 Hz, were significantly correlated with behavioural AM detection thresholds. Thus, subjects with lower psychophysical modulation discrimination thresholds showed stronger cortical responses to AM depth differences. Elderly subjects had significantly reduced 128 Hz EFRs for fully modulated stimuli compared to the young group. A significant interaction between age and the rate of the EFR (2 Hz vs 128 Hz) was found, possibly indicating a cortical gain mechanism that compensates for worse sub-cortical AM coding in the aged at high modulation frequencies.

Conclusions: We propose that the presented paradigm is useful for probing the origins of the age-related changes in AM processing observed in behaviour.

3. Inference of Epithelial and Mesenchymal Cell Communication in the Postnatal Mouse Utricle Using Single Cell RNAseq

Category: Vestibular: Basic Research and Clinical

Abel David*[1], Yasmin Eltawil[1], Sarah Easow[1], Stefan Heller[2], Alan Cheng[3], Taha Jan[1]
[1]*University of California – San Francisco,* [2]*Stanford University School of Medicine,* [3]*Stanford University*

Background: The utricle is a vestibular sensory organ that continues to expand postnatally. Prior studies have focused on the development of the sensory epithelium and transitional epithelial cells. We hypothesize that there is interaction between the mesenchymal and epithelial compartments during the postnatal period that may drive proliferation and homeostasis. Identifying these cell populations and inferring their signaling pathways can provide insights into utricle development and structural organization.

Methods: We utilized recently published single cell RNAseq (scRNAseq) data of the utricular sensory epithelium (Jan et al., 2021) from P4 and P6 utricles. Non-sensory cells were collected at these same time points following enzymatic digestion and mechanical peeling of the sensory epithelium. Following flow cytometry for single cell isolation, the Smartseq2 platform was used to generate scRNAseq data. We performed alignment, quality control, dimension reduction, and differential gene expression analyses. Using CellChat, we quantitatively inferred and analyzed intercellular communication networks.

Results: A total of 1,155 cells were sequenced with 955 cells passing quality control metrics. There were 329 non-sensory cells with a median read count of 610,636 with 2,655 genes per cell; 626 sensory epithelial cells with a median read count of 569,002 with 3,306 genes per cell. The 955 cells were clustered and projected onto a 2-dimensional UMAP space. We were able to annotate 12 unique cell populations including: mesenchymal cells, type I and II hair cells, transitional epithelial cells, supporting cells, glia, roof cells, pericytes, Schwann cells, endothelial cells, macrophages, and melanocytes. Within the mesenchymal cell cluster, we found 3 unique cell sub-clusters. CellChat analysis identified mesenchymal cells as the dominant sender of signaling pathways with statistically significant pathways including WNT, pleiotrophin (PTN), and midkine (MK). Sender cells, receiver cells, signaling influencers, and mediators were identified for each of the detected interactions. Significantly detected ligand-receptor combinations for each cell type is determined.

Conclusions: We identified 12 cell populations with unique gene expression markers from the sensory and non-sensory cells of the postnatal mouse utricle. Within the mesenchymal cells, there were 3 computationally distinct cell sub-populations. CellChat was able to infer dominant cell signaling in the WNT, PTN, and MK pathways. This dataset serves as a resource for druggable targets and for identifying previously unexplored cell-cell interactions.

4. Changes in Electric Potential and Potassium in the Synaptic Cleft Mediate Fast and Slow Nonquantal Transmission at the Vestibular Hair-Cell-Calyx

Category: Vestibular: Basic Research and Clinical

Aravind Chenrayan Govindaraju*[1], Imran Quraishi[2], Anna Lysakowski[3], Ruth Anne Eatock[4], Robert Raphael[1]
[1]*Rice University,* [2]*Yale University School of Medicine,* [3]*University of Illinois at Chicago,* [4]*University of Chicago*

Background: In the vestibular inner ear, type I hair cells, which detect head motion, sit within and transmit to cup-shaped terminals (calyces) of afferent neurons. These neurons guide motor reflexes that maintain gaze, balance, and our sense of orientation. In addition to glutamate release from vesicles (quantal transmission), ions flow through the basolateral hair cell (pre-synaptic) membrane into the synaptic cleft and through the inner calyx (post-synaptic) membrane (nonquantal (NQ) transmission). The enclosed vestibular hair cell-calyx (VHCC) synapse cannot be accessed by recording electrodes without disrupting its structure and function. As a result, ion concentrations [Ion] and electric potential (ϕ) within the synaptic cleft (SC) cannot be measured and pre-/post-synaptic membrane voltages cannot be directly obtained. This has limited our understanding of driving forces underlying NQ transmission and how they contribute to responses recorded from vestibular calyces. We have developed a computational biophysical model of the synapse to overcome this limitation.

Methods: To simulate transmission between hair cell and afferent neuron, our VHCC model uses Hodgkin-Huxley-style ion currents based on whole-cell recordings, continuity equations to describe changes in electric potential within the hair cell, cleft, afferent calyx and fiber, and electro-diffusion equations for cleft K^+ and Na^+. Step or sinusoidal hair bundle deflections or voltage step protocols were used as input. Membrane currents are calculated as a function of varying $[K^+]\_SC$, $[Na^+]\_SC$ and ϕ_SC which alter driving forces across the presynaptic and postsynaptic membranes. The model allows $[K^+]\_SC$ or ϕ_SC to be held constant as required, and permits the separation of these two processes which cannot be achieved experimentally.

Results: Model outputs include capacitive and resistive membrane currents, spatio-temporal changes in $[K^+]\_SC$, $[Na^+]\_SC$, and ϕ_SC and the electric potential within the afferent neuron. Other results include currents through the low-voltage-activated potassium conductance (gK,L) of the hair cell and Kv7 and HCN channels on the post-synaptic calyx membrane in response to hair bundle deflection and voltage protocols.

Conclusions: The VHCC model captures putative roles of channels and transporters during NQ transmission and explores their interaction in an in-silico representation of the vestibular hair cell-calyx synapse. gK,L and Kv7 appear to be the primary mediators of NQ transmission during physiological operation. Simulations demonstrate that changes in electric potential and potassium in the synaptic cleft are necessary to explain the fast and slow NQ calyx responses that have been observed. Short-latency calyx responses to hair bundle deflections (Songer and Eatock 2013) and fast NQ currents recorded in paired hair cell and calyx voltage clamp experiments (Contini et al. J Physiol 2020) both provide evidence for changes in electric potential within the synaptic cleft.

5. Conditional Deletion of POU4F3 From Type II Hair Cells Leads to Hair Cell Loss in Murine Horizontal Cristae
Category: Vestibular: Basic Research and Clinical

Kendra Stansak*[1], Caroline Nall[1], Tianwen Chen[1], Wu Zhou[1], Hong Zhu[1], Brandon Cox[2], Bradley Walters[1]
[1]*University of Mississippi Medical Center,* [2]*Southern Illinois University School of Medicine*

Background: The vestibular sensory epithelia of the inner ear give rise to our senses of balance, motion, and proprioception. Age-related vestibular dysfunction represents a major human health issue as it affects a large proportion of the population and is the leading cause of fatal falls among aged individuals. Despite the prevalence of vestibular dysfunction and its impact on mortality and morbidity, the causes of age-related vestibular dysfunction are currently unknown. Pou4f3 is a transcription factor that is required for the development and proper innervation of cochlear inner ear hair cells, though significantly less is known about its role in vestibular development and function

Methods: To better characterize the distribution of Pou4f3 in the adult vestibular epithelia, we quantified POU4F3 immunoreactive nuclei in whole-mounted horizontal cristae from adult mice at 2, 8, and 14 months of age. To test whether Pou4f3 is necessary for vestibular hair cell survival and function, we conditionally deleted Pou4f3 from ~50% of type II hair cells in male and female mice at two time points (aged 2 weeks or 8 weeks) using an Atoh1-CreER:Pou4f3loxP/loxP mouse model. Vestibuloocular reflexes were recorded 6 weeks after tamoxifen induction and hair cell survival was quantified using MYO7A and SOX2 immunolabeling. The hair cell bundles were also assessed via phalloidin labeling.

Results: Quantification of POU4F3-;MYO7A+ hair cells across age suggests that a moderate number of cristae hair cells downregulate POU4F3 with age. Conditional deletion of Pou4f3 from type II hair cells at two weeks or at 8 weeks of age led to decreased gain and increased phase in rotational VOR assessments. Pou4f3 conditional deletion also resulted in loss of phalloidin+ hair cell bundles and MYO7A+ cells in the central and peripheral zones of the horizontal cristae, when compared to wildtype littermates. Conditional deletion of Pou4f3 at 8 weeks

of age did not significantly affect the survival of type I hair cells (SOX2-; MYO7A+) but did reveal a significant decrease in type II hair cells (SOX2+; MYO7A+) consistent with the expression pattern of the Atoh1-CreER. **Conclusions:** The data suggest that Pou4f3 plays a role in vestibular hair cell survival in adolescent and adult animals, and that loss of Pou4f3 can lead to loss of hair cells in the horizontal cristae. Future studies will endeavor to quantify hair cells in the other vestibular end-organs. These results and future directions will help us better understand the causes of vestibular dysfunction that may be mediated by Pou4f3 loss, such as aging or DFNA15 mutations, and may lead to targeted treatments for preventing or reversing vestibular deficits during the adult mammalian lifespan.

6. Chronic Inflammation Occurs in Both the Cochlea and the Cochlear Nucleus During Age-Related Hearing Loss
Category: Aging
Ben Seicol*[1], Shengyin Lin[1], Ruili Xie[1]
[1]*The Ohio State University*

Background: Age-related hearing loss (ARHL) is an increasingly common age-related pathology characterized by structural damages in the auditory pathway including the cochlea and the cochlear nucleus (CN). Cochlear macrophages and CN microglia provide host defense and tissue surveillance functions as the primary resident innate immune cell within their respective niches. Both cochlear macrophages and microglia are long-lived cell populations that respond to environmental insults to promote and resolve inflammation. Failure to resolve inflammation leads to chronic inflammation that can enhance age-related pathology. While it is known that cellular and tissue damage following acoustic insults are worsened by acute inflammation, the impact of chronic inflammation during ARHL remains unclear. We hypothesized that chronic accumulation and activation of cochlear macrophages and CN microglia occurs during ARHL in mice corresponding with loss of function. **Methods:** To test this hypothesis, we investigated chronic inflammation in the cochlea and CN using 6 young, 9 middle-aged, and 11 aged CBA/CaJ mice. All mice were tested for auditory brainstem response (ABR) to assess hearing status. Temporal bones for whole-mount cochlea preparation and cryopreserved parasagittal brainstem slices containing the CN were collected and immunohistochemistry was used to label tissue with markers targeting ionized calcium binding adaptor molecule 1 (Iba1) and CD68—a marker of phagocytic activity. Stained tissue was imaged using confocal microscopy and analyzed by quantitative image processing to measure the accumulation and activation of both immune cell populations across the lifespan. **Results:** We found consistent ABR threshold increases during aging characteristic of late-onset ARHL in CBA/CaJ mice. We also observed progressive increases in the area covered by Iba1-labeled macrophages in the osseous spiral lamina (OSL) that correlated with increased ABR threshold during aging. Notably, we found significant accumulation and activation of cochlear macrophages in middle-aged mice, which have relatively normal ABR threshold and prior to overt ARHL. CD68-labeled area increased during aging in both the OSL and CN indicating activation. C1q deposition significantly increased during ARHL in the CN consistent with elevated neuroinflammation. **Conclusions:** Our study showed that chronic inflammation occurs in both the cochlea and the CN during aging, even in middle age prior to overt ARHL. These findings suggest that chronic inflammation may precede significant tissue damages of the auditory system and contribute to the development of ARHL.

7. Effect of Aldosterone on Connexin 30 and 43 Expression and Hearing in CBA/CaJ Mice
Category: Aging
Jennifer Pineros*[1], Xiaoxia Zhu[1], Bo Ding[1], Robert Frisina[1]
[1]*Medical Engineering Dept., Global Ctr. Hearing and Speech Res., University of South Florida*

Background: Connexin proteins (Cx) are essential for intercellular communication via gap junctions. Cx gene mutations have been linked to human syndromic and nonsyndromic deafness. Our previous work showed that Cx30 and Cx43 downregulate with age in the stria vascularis (SV) and revealed that hydrogen peroxide (H_2O_2) and aldosterone modulated the expression of Cx gene expression in SV-k1 and HEI-OC1 cochlear cell lines. In the present study, we analyzed changes in Cx30 and Cx43 expression levels in aged mice following aldosterone treatment as a potential therapy option for ARHL. **Methods:** Two groups of CBA/CaJ mice aged 15-18 months at baseline were used: control (n=3) and treated (n=3). The treated animals were given 1.67µg of aldosterone per day via subcutaneous, extended-release pellets over a period of four months. Cochleae from the mice were collected and prepared for immunohistochemistry

experiments. The cochleae were analyzed utilizing confocal laser microscopy and Nikon NIS-Elements Analysis software. Auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE) were recorded to measure hearing changes. In vitro cell treatment studies using SV-k1 and HEI-OC1 cell lines are treated with different compounds– H2O2 and aldosterone. Western blots were used to measure Cx30 and Cx43 protein expression levels.

Results: Dose-dependent treatment with H2O2 produced a decrease in protein expression for Cx30 in HEI-OC1 cells and increased following aldosterone treatment. Currently, Western Blot experiments repeating the treatments for SV-k1 cells is underway and experiments for both cell-lines will be repeated to measure Cx43 protein expression. Confocal images confirmed the presence and distribution of both Cx proteins in the cochlea as well. Moreover, mean signal-intensity comparisons between the control and aldosterone-treated cochlear samples confirmed an upregulation for both Cx30 and Cx43 following aldosterone treatment. ABR wideband noise thresholds stayed at the same level for the treated animals, while control animal's thresholds increased following the pattern for ARHL. Future experiments will be performed to study the biological mechanisms underlying these changes.

Conclusions: Our study indicates an upregulation of both Cx proteins in HEI-OC1 cells following aldosterone treatment but downregulated with application of H2O2. The in vitro cell treatments appear to be a good option to investigate changes of Cx expression levels. Western blot experiments for Cx30 and Cx43 using H¬2O2 and aldosterone to treat SV-k1 cells are ongoing. Confocal results validate these findings by exhibiting an upregulation of both, Cx30 and Cx43, in aged CBA/CaJ mice treated with aldosterone compared to aged control mice. The long-term goal is to determine the mechanisms affecting the cochlear connexin family gap junction proteins during ARHL and the feasibility of aldosterone as part of a clinical treatment.

8. Age-Related Hearing Loss is Exacerbated by Alzheimer´s Disease Amyloid Pathology: Evidence From APPNL and APPNL-F "Knock-In" Mice

Category: Aging

Jose Juiz*[1], Veronica Fuentes-Santamaría[2], Juan C Alvarado[2], Takashi Saito[3], Hiroki Sasaguri[4], Takaomi C Saido[4], Thomas Lenarz[5]

[1]*Department of Otolaryngology, NIFE-VIANNA, Cluster of Excellence Hearing4All, Hannover Medical School, Hannover, Germany/IDINE-School of Medicine in Albacete, UCLM, Albacete, Spain,* [2]*IDINE-School of Medicine, UCLM, Albacete, Spain,* [3]*Laboratory of Proteolytic Neuroscience, RIKEN Center for Brain Science, Saitama, Japan. Department of Cognitive Science, Institute of Brain Science, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan,* [4]*Laboratory of Proteolytic Neuroscience, RIKEN Center for Brain Science, Saitama, Japan.,* [5]*Department of Otolaryngology, Hannover Medical School, NIFE-VIANNA, Cluster of Excellence Hearing4All (DFG), Hannover, Germany*

Background: Age-related hearing loss (ARHL) is a major modifiable risk factor for Alzheimer´s disease (AD). However, mechanistic links between ARHL and AD are unknown. Focus is currently put on the induction of AD pathology by altered auditory input, whereas the converse, i.e., the impact of AD pathological traits on ARHL, is receiving less attention. This is relevant, because a pathophysiological "two-way road" represents a vicious circle, with consequences for therapeutic strategies.

"Knock-in" (KI) mouse strains APPNL and APPNL-F contain in their genome "humanized" amyloid precursor protein (APP) constructs with mutations which induce, by proteolytic cleavage, increased levels and different proportions of beta-amyloid fragments, without confounding APP overexpression. These KI mice are built on the genetic background of the C57/BL6J mouse. Therefore, the early onset ARHL, starting at six months, which characterizes this strain, in combination with the amyloid pathology induced by the APP KI constitutes a privileged model to unravel links between ARHL and AD.

Methods: Homozygous APPNL and APPNL-F mice aged 4, 8 and 15 months were used, along with age-matched wild type (WT) C57/BL-6J as controls. ABR recordings were carried out, followed by immunocytochemistry in the cochlea for detection of pro-inflammatory cytokines IL-1 beta and TNF alpha, microglial reactivity with Iba-1 and localization of Na+/K+ ATPase.

Results: WT C57/BL6J mice click thresholds of 20.6+11,3 dB (SPL) at 4 months, 34.2+14,6 dB at 8 months and 72.9+7,6 dB at 15 months. In APP NL mice, click thresholds were comparatively higher at all tested ages: 33.8+8,5 dB at 4 months, 72.1+11 dB at 8 months and 78,6+9 dB at 15 months. Threshold elevations were more conspicuous for frequencies above 24 kHz in WT mice and above 16 kHz in APPNL mice, starting at 8 months. Comparable threshold elevations at high frequencies were also recorded in APPNL-F mice. At 15 months,

thresholds were increased across the entire frequency range (4-32 kHz), with no significant differences among WT and KI mice. Therefore, early onset increases in auditory thresholds characteristic of the C57/BL6-J mouse seem to be exacerbated by abnormal processing of APP, linked to AD. In correlation with this, cochlear immunolabeling for the inflammatory markers IL-1 beta and TNF alpha was more intense in APPNL mice when compared with WT C57/BL6-J, particularly in the stria vascularis and spiral ligament. Up-regulation of inflammatory markers is also seen in increased labeling for the microglial/macrophage marker Iba-1 in cells in the spiral ligament and stria vascularis of APPNL mice relative to WT C57/BL6-J. In addition, immunolabeling for the Na+/K+ ATPase is comparatively less intense in the stria vascularis, at least in APPNL mice, suggesting larger functional deficit.

Conclusions: These findings suggest that ARHL may be exacerbated by amyloid pathology related to AD.
Support: DFG-Cluster of Excellence EXC 1077 "Hearing4All 2.0" and JCCM SBPLY/17/180501/000544

2:00 p.m. – 4:00 p.m.
Workshop – Diversity and Minority Affairs

Diversity and Minority Affairs Workshop
Chair: Avril Genene Holt, *Wayne State University School of Medicine*
Co-Chair: Tejbeer Kaur, *Creighton University*
Co-Chair: Jeffrey Cheng, *Mass. Eye and Ear Infirmary/Harvard Medical School*

The Association for Research in Otolaryngology (ARO) is an inclusive community of more than 2200 scientists and technical experts putting science to work for better understanding of hearing and balance disorders and treatments. Our ARO community embraces racial, gender, and ethnic diversity of scientists and is committed to stamping out inequity in all forms ensuring that every member has an equal opportunity to contribute to and reap benefits from research, collaboration, and leadership. To sustain ARO's mission, the Diversity and Minority Affairs Committee is motivated and excited to continue building upon the theme of last year's well received Diversity and Minority Affairs workshop and newly implemented coffee hour conversations. After the broad overview of diversity equity themes presented at the last MidWinter Meeting the workshop will provide an opportunity to engage in a focused in-depth conversation on topics such as microaggression and dynamics of power. Participants will work together in small group to employ skills introduced during discussion in the larger session.   To introduce the topics, facilitate discussion, and guide small group activities among attending ARO members, we will again invite Dr. Deborah Willis from the University of Michigan. Dr. Willis is a Diversity, Equity and Inclusion (DEI) certified senior program leader and manager for professional and academic development. This one-hour workshop will include a 30 – 40-minute platform presentation by Dr. Willis followed by small group (4 – 6 attendees) activities pertinent to the topic. Dr. Willis' goal is for ARO members to move continually from awareness to action. The objective of this workshop is to educate ARO members about the concepts of the topics and ultimately learn to identify and exercise strategies to address barriers to diversifying the scientific workforce such as power, privilege, oppression, and microaggression within our ARO scientific community and workplaces more broadly.

Deborah Willis, *University of Michigan*
Avril Genene Holt, *Wayne State University School of Medicine*
Tejbeer Kaur, *Creighton University*

This workshop is designed to continue our year-round coffee hour conversations on social accountability, unconscious bias in scientific research, dynamics of power, privilege and oppressions and their impacts on individuals and communities, and many more topics within the mission of ARO Diversity and Minority Affairs Committee. Dr. Deborah Willis, a senior program lead of DEI certificate program from University of Michigan, was our last year round-table workshop facilitatory and we would like to invite her back to lead an interactive discussion on two specific topics this year: dynamics of power and microaggression. Dr. Willis, an African American female will speak and facilitate the discussion and activities. Dr. Holt (African American, female) will chair the workshop with Drs. Kaur (South Asian American female) and Cheng (Asian American male) as Co-Chairs.

Sunday, February 6, 2022

7:00 a.m. – 9:00 a.m.
Symposium #4

Noise Induced Hearing Loss – an 'Omic Path to Treatment
Chair: Ronna Hertzano, *University of Maryland School of Medicine*
Co-Chair: Barbara Canlon, *Karolinska Institute*

Noise induced hearing loss (NIHL) underlies much of the pathophysiology of age-related hearing loss, nevertheless, to date – there are no therapeutics to prevent or counter the molecular and cellular changes induced by noise over-exposure. Omic analyses – whether in bulk or single cell, transcipromic, proteomic or metabolomic – have become the workhorse to identify molecular changes in development, aging, health and disease. Here we first begin with a tour-de-force of current tools to map cell states and understand molecular changes at the single cell level using novel cutting-edge biological methods. We then put the focus on the cellular and physiological outcomes of a variety of calibrated noise exposures, to put the search for therapeutics in context. The four talks that follow, provide elegant examples of omic analyses of the inner ear following noise exposures, that all resulted in identification of different candidate drugs to prevent or treat NIHL. These four talks will focus on novel and unpublished data. The goal of the symposium are to familiarize the audience with cellular and physiological changes following noise exposures and provide real-world examples where researchers have used 'omic analyses for drug discovery and testing. The symposium should ignite the imagination of the listeners and provide the necessary tools to consider asking similar questions in analogous lines of research.

From Single Cell 'omic to Therapeutics – Lessons from the Brain
Sten Linnarsson

The mammalian brain emerges from the neural tube through a complex developmental process involving spatiotemporal interactions and intrinsic maturation programs. To understand this process, we have combined dense spatial and temporal sampling of first trimester human brain using single-cell RNA-seq, with spatial molecular maps at key timepoints. In addition, we have sampled the adult human brain across 100 regions in three donors, revealing a tremendous cellular diversity. Comparative analysis with mouse and non-human primates revealed conserved and species-specific aspects of brain cellular diversity. We are now leveraging these atlases of the normal brain to map and discover molecular targets in human glioblastoma.

From Tts to Pts Noise – A Review of Changes to the Inner Ear Structure and Function
Keiko Hirose, *Washington University School of Medicine*

Research on noise induced hearing loss (NIHL) has progressed over many decades. Early work in this field included microscopic examination of the mammalian cochlea after prolonged and loud exposures to sound, noting structural changes resulting from varying levels of noise. With the advent of controlled noise dosage and more rigorous calibration, patterns of histologic change could be replicated and systematically quantified. This presentation will review the physiologic and histologic changes that have been identified in NIHL, with the goal to provide background for the following talks that use a proteomic, metabolomic or genomic approach to the study of hearing loss.

Drug Repurposing by Transcriptomic Analysis Identifies Otoprotective Therapeutics for Noise-Induced Hearing Loss
Jian Zuo, *Creighton University School of Medicine*

We performed functional enrichment and pathway analyses using cochlear transcriptomes from acoustic overstimulation mouse models. L1000 CDS2 search engine tool was used to probe library of integrated network-based cellular signatures (LINCS) small molecule expression profile dataset. We identified 16 novel compounds that either mimic or reverse the gene signature associated with mouse models of NIHL. Afatinib, an epidermal growth factor receptor (EGFR) inhibitor and an FDA-approved drug for human lung cancer treatment, shows

protection against NIHL in both zebrafish and mice through EGFR. Thus, transcriptomic in silico screens can be used for repurposing FDA-approved drugs as NIHL otoprotectants.

A Cell Type-Specific Analysis of the Inner Ear Response to PTS-Inducing Noise Reveals FDA-Approved Drugs as Candidate Therapeutics
Ronna Hertzano, *University of Maryland School of Medicine*

Identification of treatments for noise induced hearing loss (NIHL) is important – both for prevention of noise trauma, as well as for identification of treatments to prevent age related hearing loss. Here we took a cell type-specific approach to identify the molecular changes induced in the various cell types of the inner ear following noise exposure. These results were then intersected with drug-central to identify candidate FDA-approved therapeutics to oppose to molecular changes induced within the first 24 hours after PTS inducing noise. We present here our results as well as functional studies to validate identified candidate therapies.

Cochlear Proteostasis in Noise Induced Hearing Disorders
Jeffrey Savas, *Northwestern University Feinberg School of Medicine*

Excessive sound causes noise-induced hearing loss through complex mechanisms. We investigated how noise insults affect the cochlea with proteomics and found that it causes proteotoxicity. We identified hundreds of proteins that accumulate, including cytoskeletal proteins, and several nodes of the proteostasis network. Global cochlear protein ubiquitylation levels build up after exposure to noise and transcripts encoding proteasome subunits are acutely elevated. Notably, we have recently discovered that activating the heat shock response with a small molecule can minimize the effects of loud noise on the cochlea. Thus ensuring cochlear proteome fidelity during noise exposure represents an opportunity for therapeutic intervention.

7:00 a.m. – 9:00 a.m.
Podium Session #5 – Aging Effects on Auditory Neural Encoding and Perception
Moderators: Ruili Xie, Ph.D. & Inyong Choi, Ph.D.

1. Cortical Compensation for Afferent Loss: Associations With GABA and Speech-In-Noise Recognition
Category: Aging
Kelly Harris*[1], James Dias[1], Jeffrey Rumschlag[1], Carolyn McClaskey[1]
[1]*Medical University of South Carolina*

Background: Age-related deficits in auditory nerve (AN) function reduce afferent input to the auditory cortex. The extent to which the auditory cortex adapts to this loss of afferent input, and the mechanisms that contribute to these responses are not well understood. Evidence from animal models suggest that a loss of afferent input results in reduced or absent AN responses. In contrast, cortical responses are relatively preserved, in a phenomenon known as 'central gain'. A loss in central inhibition, specifically the neurotransmitter GABA, is thought to underlie central gain mechanisms. While central gain may contribute to enhanced cortical responses, animal evidence suggests that reduced inhibition contributes to deficits in complex auditory processing. Building upon our previous work showing robust deficits in AN function with advancing age, we examined the extent to which central gain is present in older
adults, is associated with changes in the inhibitory neurotransmitter GABA and contributes to individual differences in speech-in-noise recognition.
Methods: Participants included 28 younger and 60 older adults. AN compound action potentials (CAP-N1) and cortical P1-N1 responses were elicited by a 100 dB pSPL click. CAP-N1 amplitude was used as the metric of AN afferent input and was compared to the cortical P1-N1 response amplitude. Associations between CAP-N1 and P1-N1 amplitudes were modeled in younger adults and the resulting coefficients were applied to older adults to estimate the predicted cortical P1-N1 response amplitudes based on the observed CAP-N1 values. Observed P1-N1 values greater than those predicted served as an indicator of central gain in older adults. Proton Magnetic resonance spectroscopy was collected in a subset of participants to estimate cortical levels of GABA (GABA+). To test hypotheses that central gain and decreased GABA+ affect speech recognition in noise, we examined the

extent to which central gain and GABA+ predicted individual differences in speech-in-noise recognition (QuickSIN).

Results: Older adults exhibited significantly reduced AN responses and significantly larger P1-N1 response amplitudes than younger adults. In older adults, cortical responses that were larger than predicted were associated with poorer speech-in-noise performance. Additionally, in those older adults that exhibited central gain, decreased GABA+ was associated with larger cortical responses. Finally, across younger and older adults, decreased GABA+ was associated with poorer speech-in-noise performance.

Conclusions: Our results are consistent with animal models of central gain and suggest that individual differences in AN afferent input may contribute to changes in cortical encoding, inhibitory neurotransmission, and speech-in-noise difficulties in some older adults. A significant advancement in our understanding of the changes that occur throughout the auditory system in response to the gradual loss of afferent input with age is needed and may provide potential therapeutic targets for intervention.

2. Age Effects on Temporal Processing and Auditory Figure-Ground Segregation, and Relationship to Speech Perception in Noise
Category: Aging
Varsha M Athreya*[1], Ravinderjit Singh[1], Hari Bharadwaj[1]
[1]*Purdue University*

Background: Speech perception in noise (SPIN) is highly variable amongst individuals with normal audiometric sensitivity, and can worsen with age. Behavioral studies suggest that temporal processing degrades with age. Physiological data from animal models suggest aging is accompanied by both cochlear hearing loss (e.g., cochlear synaptopathy, loss of hair-cells), and by downregulation of inhibitory neurotransmission in the central auditory systems. While both peripheral loss and reduced central inhibition can contribute to temporal processing deficits, auditory figure-ground segregation may also be impacted by the changes in the central auditory system. This is because inhibition-mediated temporal-coherence processing is thought to be important for scene segregation. Here, we sought to understand the contribution of these two factors to speech perception in noise as a function of age.

Methods: We measure SPIN scores using two tasks with two speech-in-noise tasks with a different balance of energetic and informational masking, and with differing cognitive demands. Temporal processing is quantified by measuring behavioral gap detection thresholds (GDT) for gaps in 4 kHz tones embedded with an octave-band of noise centered at 4 kHz. A neural measure of temporal processing is also obtained using electroencephalographic (EEG) responses to gaps in tones. Figure-ground segregation is measured behaviorally using a co-modulation masking release (CMR) paradigm with a stimulus consisting of three-bands of noise with the middle band centered at 4 kHz, and the spacing between bands being 50% wider than cochlear tuning bandwidths.
A neural correlate of auditory figure-ground segregation is also obtained using a novel stimulus where the comodulation statistics of a set of 20 tones can be parametrically manipulated without changing the modulation statistics of individual tones. The frequency spacing between the tones was 50% wider than established cochlear tuning bandwidths, such that peripheral interactions between the tones are minimal, and sensitivity to the co-modulation statistics would have to arise primarily from temporal coherence processing in the central auditory system. Data collection is ongoing using the full battery of measures from subjects with near-normal audiometric sensitivity (4-frequency pure-tone thresholds average of <25 dBHL) and a wide age range (18-70 years).

Results: Preliminary results suggest that robust neural measures can be obtained both in response to the gap stimulus, and in response to temporal-coherence manipulations using the 20-tone figure-ground stimulus. CMR values fall in the 6-10 dB range, and GDTs in the 3-8 ms range.

Conclusions: By comparing the different behavioral and neural temporal processing and figure-ground segregation measures to SPIN scores, we aim to gain insight into the contributions of the two factors to age-related changes in auditory processing.

3. Assessment of Functional and Cognitive Declines in Alzheimer's Disease by a New Method of Recording Auditory Evoked Cortical Potentials in Mice
Category: Aging
Ling Mei[1], Liman Liu[1], Kaitian Chen[1], Hong-Bo Zhao*[1]
[1]*University of Kentucky Medical Center*

Background: Alzheimer's disease (AD) is a common neural degeneration disease characterized with progressive memory loss and cognitive decline. Early detection of AD is critical for intervention of AD development and progression. However, assessment of early AD-associated functional and cognitive changes is still a big challenge, usually hard to distinguish from normal aging effect. In particular, it lacks reliable, objective biomarkers to assess cognitive decline at the early stage. Auditory evoked cortical potential (AECP) is an event-related potential reflecting not only neural activation in the auditory cortex but also cognitive activity in the brain. In this study, we intend to record AECP in AD mice to assess AD generation and dementia development. However, unlike AECP recorded from humans, AECP in animals was usually recorded by implanted electrodes in previous studies; the recorded AECP also usually lacked later cognitive waveforms. In this study, a new ARCP recording method in mice with subdermal electrodes was established. AECP changes with normal aging and its changes in AD mice were assessed by a new AECP recording.

Methods: APP/PS1 AD mice (Stock No: 004462, mixed C57BL/6;C3H genetic background) were crossed with CBA/CaJ mice at least 4 generations to diminish C57BL/6 genetic background. Wild-type (WT) littermates were used as control. AECP was recorded with the same subdermal needle electrodes and setting as ABR recording. The cortical potentials were evoked by clicks (85 dB SPL) in alternative polarity with a stimulating rate of 1/s.

Results: AECP in mice usually appeared as three positive peaks, i.e., P1, P2, and P3, and three corresponding negative peaks N1, N2, and N3, similar to AECP recorded from humans. In normal aging CBA mice, the early sensory peaks P1, N1, and P2 were reduced as age increased, whereas the later cognitive peaks N2, P3, and N3 were increased or had no changes with aging. Moreover, the latency of the P1 peak was increased as age increased, although the latencies of later peaks had a significant reduction with aging. In AD mice, peak P1 was significantly reduced in comparison with WT littermates at young ages, proceeding AD phenotype presentation. In particular, the later cognitive peak P3 was diminished after 3 months old, different from the normal aging effect. However, the latencies of AECP peaks in AD mice generally had no significant delay or changes with aging. Finally, consistent with AECP changes, the accumulation of amyloid precursor protein (APP) at the AC was visible in AD mice as early as 2 months old.

Conclusions: In this study, we established a new AECP recording method in mice, which can be used to assess early brain-functional and cognitive changes in AD.

4. Central Compensation Underlies Over-Representation of Speech Signals in the Aging Auditory Cortex
Category: Aging

Samira Anderson*[1], Janani Perera[1], Stefanie Kuchinsky[2], Jonathan Simon[1]
[1]*University of Maryland,* [2]*Walter Reed National Military Medical Center*

Background: Investigations of neural auditory processing have demonstrated over-representation of the cortical envelope in older compared to younger listeners. Several explanations have been posited, including an inefficient use of cognitive resources, reduced neural inhibition, and redundant local processing. Animal models have demonstrated central compensation for disrupted afferent input, but the role of compensation in age-related enhancement of cortical responses is not well understood. The aim of this study was to determine the extent to which subcortical compensation predicts cortical envelope enhancement.

Methods: We recruited 19 listeners with audiometrically normal hearing to participate in the study, including 8 young normal hearing (YNH) and 11 older normal hearing (ONH) listeners. Audiometric thresholds were measured from 125 to 14,000 Hz. Subcortical measures included auditory brainstem responses (ABRs) to 100-μs broadband click stimuli and auditory steady-state responses (ASSRs) to 100-Hz and 400-Hz bandpass-filtered click trains. Central compensation was calculated from the Wave V/Wave I (brainstem to auditory nerve) amplitude ratio and from the 100 Hz/400 Hz (high brainstem to low brainstem) phase locking ratio. Magnetoencephalography (MEG) was used to evaluate encoding of the speech envelope. One-minute audiobook passages were presented diotically to the listeners in a single-competing talker condition at a 0 dB signal-to-noise ratio. The listeners were instructed to attend to either the male or female speaker while ignoring the other speaker. A step-wise regression model was conducted that included MEG reconstruction accuracy as the dependent variable, and Wave V/I ratio, ASSR ratio, and pure-tone average (PTA; 500, 1000, 2000, and 4000 Hz) as the independent variables.

Results: The older listeners had lower pure-tone thresholds, higher Wave V/I amplitude ratios, and higher ASSR phase locking ratios than the younger listeners, demonstrating compensation for reduced auditory input. However, reconstruction accuracy did not differ between the groups in this data sample. Reconstruction accuracy positively correlated with Wave V/I ratio and ASSR ratio but not with the PTA. The step-wise regression model revealed

that the ASSR ratio significantly predicted variance in the reconstruction accuracy (40%), but no other variables contributed.

Conclusions: These results provide support for the hypothesis that age-related over-representation in the cortical envelope results from compensation for reduced afferent input. Although older listeners typically having higher cortical response amplitudes and reconstruction values, they often report having difficulty understanding speech in noisy environments. Therefore, central compensation may be occurring at the expense of effective inhibitory mechanisms, leading to a reduction in neural precision and decreased speech-in-noise performance. This work was supported by the National Institute of Aging (P01 AG055365).

5. How is Preparatory Spatial Attention Affected by Age and Hearing Loss?
Category: Aging
Emma Holmes*[1], Timothy Griffiths[2]
[1]*UCL,* [2]*Newcastle University; UCL*

Background: We often face the challenge of understanding speech when competing speech is present. Listeners with normal hearing can deploy preparatory spatial attention to improve intelligibility in 19patialized settings, but children who have hearing loss from a young age deploy preparatory spatial attention to a lesser extent than do children with normal hearing. It is currently unclear whether age-related hearing loss has similar detrimental effects on spatial attention, or whether older adults' prior experience with sounds preserves (or, perhaps, increases reliance on) preparatory spatial attention despite hearing loss. Here, we investigated how age and audiometric thresholds relate to preparatory spatial attention.
Methods: We recruited two groups of participants, age 18-35 years and 60-80 years. We measured their audiometric thresholds and tested their ability to understand a target phrase in the presence of two competing phrases, which were spoken by different talkers and presented from different locations. The target talker was cued visually by an arrow (left or right), which was presented 100 or 2000 ms before the talkers started speaking—thus providing a short or longer interval for participants to prepare spatial attention. We measured intelligibility in both conditions at SNRs between -18 and +18 dB.
Results: Preliminary results suggest that intelligibility for both groups is better in the 2000 than 100 ms condition. Further statistical analyses will be conducted when the full sample is recruited, and will be reported at the meeting.
Conclusions: Preliminary results imply that both older and younger adults may benefit from preparatory spatial attention. The final analyses will compare the magnitude of benefit between the groups and examine how it relates to audiometric thresholds, thresholds for discriminating spatial location, and WAIS scores.

6. Investigating the Role of Epigenetic Mechanisms in Age-Related Hearing Loss
Category: Aging
Marie Roche*[1], Denise Yan[1], Pei-Ciao Tang[1], Feng Gong[1], Xue Liu[1]
[1]*University of Miami School of Medicine*

Background: Presbycusis, also known as age-related hearing loss (ARHL), is the most frequent disability affecting elderly adults worldwide. Presbycusis is typified by a bilateral, progressive, sensorineural hearing loss that is pronounced in high frequency. The molecular development of Presbycusis entails both extrinsic and intrinsic factors. Genetic predisposition to hearing loss is responsible for a substantial proportion of the variations between individuals. Additionally, it is hypothesized that Presbycusis could result from unclarified epigenetic susceptibility. Nevertheless, there is a shortage of information on the exact contribution of aberrant epigenetic regulations to Presbycusis. This study aims at examining whether DNA methylation mediated silencing could be a risk factor contributing to Presbycusis.
Methods: This project is a hospital-based cohort study comprising 134 DNA samples; obtained from 55 ARHL subjects and 79 controls. The inclusion criteria for subjects with presbycusis consisted of age greater than 40 years and greater than 30 dB HL hearing loss (bone conduction pure tone average [PTA] of frequencies 500, 1000, 2000, and 4000 Hz). Hearing measurements were used to determine the audioprofiles. The control samples consisted of a Caucasian diversity panel of unrelated people with normal hearing, aged 40-81 years old. DNA was extracted from patients, clinical, audiometric patterns, DNA testing, and methylation pattern screening were undertaken. Specifically, quantitative interrogation of methylation sites across the genome at single nucleotide has been performed using the Illumina Methylation EPIC array analysis.
Results: Our preliminary analysis showed that the audiometric patterns that were more frequent in the cohort study are "High frequency Steeply Slopping" or HFSS (33%)," High-frequency Gently Slopping" or HFGS (31%)

and "FLAT" (27%) while the other patterns were less prevalent. No statistical significance was found in terms of gender, age, ear side, and PTA values among the audiometric types. The Illumina Infinium® Methylation EPIC Beadchip has been used to identify regions with aberrant levels of methylation across genomes from 16 patients and data analysis is ongoing. A PCR-based bisulfite DNA methylation detection assay has been established as a validation method to confirm methylation levels at specific gene locus in Presbycusis patients.

Conclusions: Aberrant DNA methylation and its impact on gene expression have been implicated in many biological processes. By interrogating methylation status across the genome at single-nucleotide resolution, our study will help establish the association between audiometric patterns and methylation status in deafness-associated genes, Importantly, our study will identify novel epimutations that lead to hearing loss.

7. Longitudinal Analysis of Auditory Dysfunction in a Mouse Model of Alzheimer's Disease
Category: Aging

Daxiang Na*[1], Patricia White[2]
[1]University of Rochester Medical Center, [2]University of Rochester School of Medicine

Background: The Alzheimer's Disease (AD) is a neurodegenerative disease without any cure. All current therapies require accurate diagnosis and staging of AD to ensure proper strategy. The increasing prevalence of the disease has caused a great burden to the health care system worldwide. Thus, it has become an urgency to find efficient approaches for AD diagnosis and progression. The comorbidity between Alzheimer's disease and hearing loss has been shown to be significant. Beyond hearing loss, AD patients also showed auditory processing disorders. However, it remains unclear if auditory dysfunction and Alzheimer's disease progression are correlated.

Methods: In the present study, we performed longitudinal analysis of auditory dysfunctions in AD using 5xFAD, a transgenic AD mouse model. Those animals were bred in CBA/B6 hybrid background to compensate the hearing loss deficits in C57/B6 strain. The auditory features were monitored by Auditory Brainstem Response (ABR) test and Distortion Product Otoacoustic Emission (DPOAE) test.

Results: Consistent with previous studies, the AD animals showed accelerated age-related hearing loss. The ABR threshold increase showed up at lower frequencies first, indicating that the underlying mechanism might be distinct from canonical age-related hearing loss. In addition to the increased hearing threshold, ABR waveform abnormalities were also observed in the AD animals. Longitudinal analysis revealed that ABR waveform abnormalities develop along with the progression of AD. Those abnormalities suggest auditory neuropathy and auditory processing deficits in AD animals.

Conclusions: With those results, we conclude that auditory dysfunction is a possible outcome of AD, and the ABR abnormalities could serve as a biomarker for AD progression. Overall, this study suggests auditory measurement, a low-cost, non-invasive test, as a possible approach for AD diagnosis and the estimation of disease stage.

8. The Effects of Lifetime Noise Exposure and Age on Hearing Ability: An Online Study
Category: Aging

Adnan Shehabi*[1], Garreth Prendergast[1], Hannah Guest[1], Christopher Plack[1]
[1]University of Manchester

Background: Animal research shows that both aging and excessive noise exposure can damage the synapses connecting the inner hair cells with the auditory nerve. Additionally, aging has been associated with deficits in central neural processing. Since humans typically get exposed to a wide range of occupational and recreational noise throughout their lifespan, the effects of noise exposure may be more apparent for older adults. The current study aims to assess the effects of lifetime noise exposure and aging on (i) self-reported hearing ability, (ii) the presence of chronic tinnitus, and (iii) speech-perception-in-noise (SpiN) hearing thresholds.

Methods: Two hundred and eighty-seven adults with no past diagnosis of hearing and memory impairments were recruited online. Participants were divided into two groups: 211 "young" (females: 145, age range: 18-35, mean age: 24.5) and 76 "older" (females: 49, age range: 50-70, mean age: 58). Subjects completed a set of online instruments including an otologic health and demographic questionnaire, the AD8 dementia screening tool, the online forward and backward digit span test, a noise exposure questionnaire that evaluates lifetime occupational, recreational, and firearm noise exposure, the Khalfa hyperacusis questionnaire, the speech-spatial and hearing qualities (SSQ12) questionnaire, the tinnitus handicap inventory (THI), an online digits-in-noise (DIN) test, and an online coordinate response measure (CRM) speech test. Multiple linear regressions were employed to test the study aims (i) and (iii), while logistic regression was used to evaluate aim (ii). The covariates of the sex of

participants and their cognitive function, as reflected by the forward and backward digit span test score, were accounted for in all the statistical models. Exploratory analyses were performed to determine the effects of lifetime noise exposure and age on CRM thresholds, hyperacusis scores, and THI scores.

Results: Preliminary analysis showed that neither lifetime noise exposure nor age was a significant predictor of (i) the self-reported hearing ability as reflected by the SSQ12 scores, (ii) the presence of chronic tinnitus, or (iii) the SpiN hearing thresholds as shown by the DIN test. Exploratory analyses showed that neither lifetime noise exposure nor age significantly predicted performance on the CRM test, the hyperacusis scores, or the THI scores.

Conclusions: The present preliminary data, derived using online instruments, provides no evidence that lifetime noise exposure or age affects self-reported hearing ability, the risk of chronic tinnitus, or SpiN hearing thresholds in adults with no past diagnosis of hearing impairment.

7:00 a.m. – 9:00 a.m.
Podium Session #6 – Organ of Corti Micromechanics
Moderators: Hamid Motallebzadeh, Ph.D. & Anna Vavakou, M.Sc.

1. A Revised WKB Approach to the 3-D Viscous Cochlear Model
Category: Inner Ear: Cochlear Mechanics
Renata Sisto*[1], Daniele Belardinelli[1], Arturo Moleti[2]
[1]*INAIL Research,* [2]*Physics Dept., University of Roma Tor Vergata*

Background: Although 1-D transmission line cochlear models have been successful in modeling the BM response, the traveling wave propagation, and the otoacoustic emission generation, they do not fully account for the 2-,3-D hydrodynamics. The coupling to the fluid not only ensures the propagation of a slow cochlear traveling wave, but it also provides, through the fluid-focusing phenomenon, a relevant contribution to the cochlear gain. This phenomenon yields amplification of the pressure in the peak region, within a fluid layer of the order of the TW wavelength. Hydrodynamics also yields enhanced viscous losses near the BM in the peak region, where steep vertical velocity gradients arise. Viscous damping, being proportional to the real part of the wave vector amplitude, ensures the stability of the BM response, counteracting both pressure focusing and active amplification. Although a WKB approach to the solution of 3-D cochlear models was already proposed long ago (summarized in Yang et al., 2016), using the acoustic potential formalism, normalization factors and boundary conditions are still an unsolved issue. Imposing the no-slip condition at the membrane-fluid interface implies annihilation of the vertical gradient of the vertical fluid velocity, so the main contribution to the viscous dissipation vanishes. In this paper, a different normalization is proposed.

Methods: The scalar and vector potential normalization was chosen in order to get equality between the BM velocity and the fluid vertical velocity in the peak region (adhesion condition). The equation for the pressure averaged over the cross section was used to get a relation between the longitudinal component of the wave vector and the BM admittance (Shera et al., 2005). The fluid vertical velocity and the pressure were compared to the BM velocity and to the pressure at the BM-fluid interface computed in a 2-D WKB model (Sisto et al., 2021), including focusing and viscosity effects.

Results: A satisfactory agreement was obtained between the pressure calculated in a 3-D model starting from the field potential components and that calculated in the 2-D WKB model. The adhesion condition was satisfactorily fulfilled particularly in the peak region (short-wave) . The WKB solution of the 3-D model permitted the computation of the fluid velocity and its gradients, in particular, of the vertical velocity gradient, which is proportional to the main viscous force acting at the BM-fluid interface. This term was calculated and compared to the OHC active force.

Conclusions: The scalar and vector potential approach permits calculating the fluid velocity and pressure, keeping into account both focusing and viscosity effects. Relaxing the no-slip condition is necessary to get a correct estimate of the dissipation force due to the BM-fluid viscous coupling, and of the OHC force counteracting it. Analytical models can be useful for interpreting the results of more complex models.

2. Higher Order Vibration Mode of the Basilar Membrane
Category: Inner Ear: Cochlear Mechanics
Wei-Ching Lin[1], Jonathan Becker[1], Jong-Hoon Nam*[1]
[1]*University of Rochester*

Background: The basilar membrane is the mechanical foundation of cochlear mechano-transduction. The stiffness gradient of the basilar membrane along the length of the cochlea underlies the cochlear tonotopy. The basilar membrane interacts with the cochlear fluids to form the traveling waves. A few studies by Nigel Cooper and his colleagues showed that the basilar membrane deforms like a pair of chase doors, justifying the assumption of first-mode vibration in cochlear studies. Recent measurements using OCT (optical coherence tomography) began to provide more data regarding the vibration profiles both in the longitudinal and in the radial directions. From high resolution OCT measurements, we examined whether the basilar membrane could vibrate at a higher mode.

Methods: The cochleae were isolated from young gerbil (15-30 day old). After being reduced to a single turn, the excised cochlea, between 50 and 80-percentile location from the basal end, was placed in a micro-chamber. Mechanical and electrical stimulations were applied to the tissue at different frequencies to evoke the passive and active vibrations. Resulting vibrations were measured using an OCT system (Jabeen et al., 2020). The optical plane was aligned with the basilar membrane. At a radial section, the vibrations were measured by running 40-60 M-scans across the OoC span. In some preparations, the OoC subjected to different levels of hydrostatic pressure were scanned from which static deformation patterns were obtained.

Results: Consistent with previous observations, the basilar membrane deformed as if a beam with clamped-ends has a hinge in the middle. The hinge point where the peak occurs was near the root of the first-row Deiters cell. Our measurements provided further details regarding basilar membrane vibration patterns. The radial deforming pattern of the basilar membrane became narrower as the stimulating frequency increased. The full width at the half-maximum amplitude (FWHM) decreased by 20-30 percent at the best-responding frequency as compared to the case of static deformation. As the stimulating frequency further increased, a clear sign of higher mode vibration appeared—zero-displacement nodes other than the medial and lateral ends. The basilar membrane vibration pattern due to electrical stimulations was distinguished from the passive vibration patterns. Outer hair cell motility consistently created higher order vibration patterns regardless of stimulating frequencies.

Conclusions: Our observations suggest that outer hair cell motility promotes the mode shift from the primary to a higher mode of the basilar membrane vibration. This may explain how the BF of sensitive cochlea is higher than that of insensitive cochlea.

3. Using Volumetric Optical Coherence Tomography to Achieve Spatially Resolved Organ of Corti Vibration Measurements

Category: Inner Ear: Cochlear Mechanics
Brian Frost*[1], Clark Strimbu[2], Elizabeth Olson[1]
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Background: Optical coherence tomography (OCT) allows for the measurement of vibrations of structures within the Organ of Corti complex (OCC). While structures within the cochlea move in three dimensions (the anatomical longitudinal, radial and transverse directions), OCT measurements only record a one-dimensional projection of this motion onto the optical axis of the device. The optical axis is not usually perpendicular to the basilar membrane (BM), and structures within a single OCT measurement lie at different longitudinal cross-sections of the cochlea. These features complicate the interpretation of OCT data, especially because the direction of the optical axis with respect to the anatomical orientation of the OCC is not known a priori.

Methods: We have developed a program that uses the volumetric imaging capabilities of OCT to relate the optical coordinate axes to the anatomical coordinates of the cochlea. By making a first-order approximation of the BM as a plane, we can determine a) the components of the measurement axis in anatomical coordinates, and b) the longitudinal distance between measured locations. The experimenter can use this program to determine the scan locations required to measure points specified in anatomical coordinates, facilitating interpretation of OCT vibration data.

Results: In vivo measurements made through the round window in gerbil often have significant components in all three anatomical directions, and structures within a single measurement can be far enough apart longitudinally that the travelling wave phase varies significantly between them. For example, we often find that measured outer hair cells (OHCs) may lie up to 60 micrometers apical from the measured BM location. Using the program described above, the experimenter can take two measurements so that the BM in the second measurement lies in the same cross-section as the OHC in the first measurement. This allows for a more meaningful comparison of the motion between the two structures. Using this method, we have measured the motions of the BM, OHCs and Hensen's cells within the same cross-section as one another, using a measurement axis containing significant longitudinal

and transverse components. We have found that, in this direction, OHC motion leads BM motion across frequency, with the lead being largest at low frequencies and shrinking close to the best frequency. This is in concordance with OHCs moving in elliptical patterns suggested by fluid dynamics, as forwarded in Cooper et al, 2018.

Conclusions: The value of one-dimensional motion measurements is enhanced by analysis that orients that direction in anatomical space.

4. Reticular Lamina and Outer Hair Cell Gain Continue to Increase Above the Best Frequency in the Gerbil High-Frequency Hook-Region
Category: Inner Ear: Cochlear Mechanics

Nam Hyun Cho*[1], Sunil Puria[2]
[1]*Harvard Medical School,* [2]*Harvard Medical School, Mass. Eye and Ear Infirmary*

Background: The basilar membrane (BM) and reticular lamina (RL) of the organ of Corti (OoC) are coupled through three rows of a Y-shaped arrangement of outer hair cells (OHCs), Deiter's Cells (DCs), and Phalangeal Processes. Previous measurements reported motion of the BM, DC and OHC (Cooper et al., 2018), BM and RL (He et al, 2018), and BM and OHC (Fallah et al., 2019), made through the round window membrane for 20-30 kHz best frequencies (BFs). Here we report measurements of BM, OHC, and RL motion in the same animal for the ~45 kHz BF region.

Methods: Cross-sectional imaging and vibrometry measurements were made using a Spectral-Domain Optical Coherence Tomography system with a 900-nm center wavelength and up to 240-kHz line-scan camera rate (GAN620C1, Thorlabs, Germany). The axial and lateral resolutions (in water) were ~1.45 µm and ~4 µm, respectively. Stimuli were sequences of pure-tones (2-63 kHz) equalized for constant levels from 30 to 95 dB SPL in the ear canal. All reported in vivo measurements are for animals (N=5) that passed a "≥" 10 dB SPL DPOAE threshold criterion (50 dB SPL primary tones). We report displacements greater than 3 dB over the ~0.2 nm noise floor, for multiple points on the BM, near OHC-DC junctions, and at the RL.

Results: Consistent with previous observations, BM motion had the largest displacement near the arcuate-pectinate junction (BM-APJ) and the phase showed a travelling wave. Motion gain was calculated relative to the BM-APJ displacement at 90 dB SPL (BM-APJ-90). For sub-BF frequencies (2-30 kHz), BM-APJ and RL gains near the third OHC row were similar while the OHC-DC-junction gain was higher by up to 15 dB. The RL phase tended to lead BM phase while the OHC-DC-junction phase lagged the BM by close to ½-cycle.

As frequency increased from 30 kHz to BF, RL gain increased, was level dependent and showed compression. The BM gain was less than RL gain and had less compression. From 30 kHz to BF, the OHC-DC-junction gain decreased and approached the gain of the BM. The RL phase decreased. The OHC-DC-junction phase also decreased and surprisingly changed sign near BF.

Above BF (43-50 kHz), the RL gain continued to increase reaching as high as 45 dB for the 50 dB SPL input, and then about 1/6-oct beyond the broad peak decreased rapidly. The BM and OHC-DC-junction gains also increased but with lower gains than RL.

Conclusions: Some of our results are consistent with previous measurements below BF while others differ. A new observation is that above BF, RL and OHC-DC-junction gains increased while OHC-DC-junction phase changed sign. Reasons might include the higher BF and the more transverse angle approach.

5. Vibrations of the Apical Organ of Corti Complex at Distortion Product Frequencies
Category: Inner Ear: Cochlear Mechanics

Sebastiaan Meenderink[1], Wei Dong*[2]
[1]*Department of Veteran Affairs,* [2]*VA Loma Linda Healthcare System*

Background: Distortion products (DPs) are generated in the cochlea by the outer hair cell (OHC) based nonlinearity. DPs that can propagate back to the ear canal where they are measured as an otoacoustic emission (DPOAE). It is believed that DPs contributing to a DPOAE primarily originate from the traveling wave peak region of the stimulus tones, although this generation region may extend further basally for higher-intensity stimulus tones. Observations from OHC or the reticular laminar in the high-frequency basal region of the cochlea however demonstrate the existence of a broad nonlinear region that generates DPs. What limits the extent over which OHC-generated DPs couple to the basilar membrane (BM) and how they travel back to the ear canal to form a DPOAE is still not well understood. Moreover, the low-frequency apical region responds to sound differently than the base, which underlies the different characteristics of both low-frequency auditory nerve

responses and DPOAEs. Understanding the generation of low-frequency DPOAEs is critical in the improvement of precisely detecting sensory damages in the clinic.

Methods: Two-tone induced DPs were recorded from the apically located organ of Corti complex [OCC includes organ of Corti, the BM, and the tectorial membrane I] of alive gerbil cochleae using optical coherence tomography (OCT) together with ear canal DPOAEs. The OCT measurement beam was almost parallel to the shearing motion between the reticular laminar and the TM, which results in an ~40o angle re. the BM at these apical recording locations. The care and use of animals were approved by the Institutional Animal Care and Use Committee (IACUC) of the VA Loma Linda Healthcare System.

Results: With equal-intensity stimulus at a frequency ratio of f2/f1=1.25, DPs are robust within the OCC. Motions within the OCC at DP frequencies are directly compared with the simultaneous motions at the stimulus frequencies. The tuning of DPs correlates directly to the tuning properties of the stimulus at different OCC locations: they are broadly tuned in OHC and lateral support cell (i.e., Hensen's) regions and more sharply tuned on the BM. Different tuning and phase responses suggest that locally measured DPs include both local and nonlocal components. The latter can be a forward or a reversely traveling component depending on the f2 re. the local best frequency (BF).

Conclusions: Like responses at stimulus frequencies, coupling of motions between OHC and BM is stronger at the peak region and weaker at low frequencies. Their phase relationship plays a key role in the motion coupling. In addition, physical properties of OCC subcomponents appear to play an important role in shaping the responses at both the stimulus and DP frequencies, i.e., tuned responses are observed at stiffer locations.

6. Impedance Matching Explains the Relationship Between Outer Hair Cell Length, Conductance and Characteristic Frequency in the Cochlea
Category: Inner Ear: Cochlear Mechanics
Monika Buczak[1], Tamara Bidone[1], Richard Rabbitt*[1]
[1]University of Utah

Background: Outer hair cells (OHCs) length, basolateral conductance and capacitance vary systematically with characteristic frequency (CF) along the length of the cochlea, but the precise factors driving tonotopic variations remain unclear. The relationship between length and CF is universal across diverse mammalian species, with 65µm long OHCs located near 1kHz CF, 35µm long OHCs located near 10kHz CF, and 14µm long OHCs located near 100kHz CF. OHC conductance increases roughly linearly 25 fold with CF, from 1kHz to 100kHz, while the passive capacitance decreases nearly in proportion to membrane area. The increasing conductance combines with decreasing capacitance to set the passive RC corner (RCF) along the tonotopic map below CF (RCF~0.35*CF). Here, we examine the hypothesis that OHC length and RCF are set along the tonotopic map to optimize OHC power output to the viscous cochlear load through impedance matching, and that action of the medial olivo-cochlear (MOC) efferent system modulates the match and therefore power efficiency of OHCs.

Methods: OHC electro-mechanical power conversion was analyzed as a function of OHC properties, frequency and cochlear load using a relatively simple model in silico. OHCs were assumed to be powered by the endolymphatic potential driving current into the cell via the mechanically gated transduction channels. Electro-mechanical power conversion by the prestin-motor complex was modeled using nonlinear piezoelectricity, and the cochlear load was modeled using a simple spring-mass-damper system.

Results: OHC power output is predicted to be tuned to match CF in the cochlea, with maximum power delivery occurring when the impedance of OHCs near CF combine to match to the local impedance of the cochlear partition. Impedance matching requires the OHC length, compliance and RCF to be related to the stiffness of the cochlear load and CF in a specific way. The optimum OHC length and conductance predicted by impedance matching compares favorably to experimental data across the entire frequency bandwidth of mammalian hearing in multiple species. Results further predict that the increased OHC membrane conductance caused by activation of MOC efferent neurons reduces OHC power output by reducing the receptor potential and also by disrupting the impedance match.

Conclusions: Results support the hypothesis that outer hair cell length and conductance are optimized along the tonotopic map to maximize power delivered to the cochlear amplifier. Results further support the hypotheses that the MOC system controls OHC power output primarily through action on impedance matching and secondarily through the reduction in receptor potential. Results suggest that tonic MOC activity tunes impedance of OHCs to match the local cochlear load, and transient MOC activity disrupts impedance matching to reduce power output beyond what would occur from the reduction in the receptor potential alone.

7. Cochlear Supporting Cells Require GAS2 for Cytoskeletal Architecture and Hearing
Category: Inner Ear: Cochlear Mechanics

Tingfang Chen*[1], Alex M. Rohacek[2], Matthew Caporizzo[3], Amir Nankali[4], Jeroen Smits[5], Jaap Oostrik[5], Erdi Kücük[6], Christian Gilissen[6], Jiddeke M. van de Kamp[7], Ronald J.E. Pennings[5], Staci M. Rakowiecki[2], Kevin K. Ohlemiller[8], Klaus Kaestner[2], John S. Oghalai[4], Hannie Kremer[9], Benjamin L. Prosser[3], Douglas J. Epstein[2]
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Background: The organ of Corti is a specialized sensory epithelium unique to mammals that lines the length of the cochlear duct and is responsible for sound reception. It comprises a single row of inner hair cells (IHCs), three rows of outer hair cells (OHCs)and a variety of interspersed supporting cells that sit atop the basilar membrane. In comparison with hair cells, relatively little is known about the role that supporting cells play in the transmission of mechanical vibrations across the cochlear partition. Computational models predict that the unique geometry and cytoskeletal composition of inner ear supporting cells provides a structural framework for the exchange of forces between the basilar membrane and the apical surface of the organ of Corti. Nevertheless, experimental evidence in support of these models, especially from in vivo studies, is limited. It also remains unclear whether mutations in genes that selectively perturb supporting cell mechanical properties would cause hearing loss, and if so, by what means.

Methods: Statistical analysis was performed using GraphPad Prism 8 software (Graphpad Software Inc., San Diego, CA, USA). Normality was assessed using Shapiro-Wilk and Kolmogorov-Smirnov tests. Relevant information for each experiment including sample size (n≥3), statistical tests and p-values are described in the figure legends. In all cases $P<0.05$ is considered statistically significant.

Results: In this study, we identified the Gas2 gene in a screen for Sonic hedgehog dependent regulators of cochlear development. We demonstrate that inner ear supporting cells provide a structural framework for transmitting sound energy through the cochlear partition. Humans and mice with mutations in GAS2, encoding a cytoskeletal regulatory protein, exhibit hearing loss due to disorganization and destabilization of microtubule bundles in pillar and Deiters' cells, two types of inner ear supporting cells with unique cytoskeletal specializations. Failure to maintain microtubule bundle integrity reduced supporting cell stiffness, which in turn altered cochlear micromechanics in Gas2 mutants. Vibratory responses to sound were measured in cochleae from live mice, revealing defects in the propagation and amplification of the traveling wave in Gas2 mutants.
loss.

Conclusions: We propose that the microtubule cross-linking function of Gas2 affords support cells with mechanical properties needed to transmit sound energy through the cochlea.

8. Three-Dimensional Cochlear Micromechanics Measured in Vivo
Category: Inner Ear: Cochlear Mechanics

Derek Liu*[1], James Dewey[1], Wihan Kim[1], Sangmin Kim[2], Kumara Ratnayake[1], Brian Applegate[1], John Oghalai[1]
[1]*Caruso Department of Otolaryngol–gy - Head and Neck Surgery, USC*, [2]*Department of Bioengineering, Texas A&M University*

Background: Cochlear amplification, driven by outer hair cell (OHC) electromotility, is maximized when OHCs are stimulated at the correct phase relative to the traveling wave. However, recent work using 1D-OCT systems has revealed the presence of complex, three-dimensional vibrational modes within the organ of Corti (OoC). We developed a 3D-OCT system to precisely reconstruct the 3D vibration patterns within the OoC.

Methods: The 3D-OCT system consists of three optical axes positioned symmetrically around a dissecting microscope and utilizes a depth-encoding scheme to combine three images into a single A-line. The three volumetric images are separated and registered to produce a single, combined volume with independent measurements obtained from each optical axis at each point. 3D vectors of motion in Cartesian coordinates were reconstructed for each point in the combined volume.

To accommodate the angled positioning of three optical axes, our standard surgical exposure of the cochlea in CBA/CaJ mice was extended to include resection of a portion of the tympanic annulus, resulting in a 10-15 dB SPL conductive hearing loss. The degree of hearing loss was quantified and added to the desired sound pressure input to produce equivalent responses. Vibrations throughout the OoC were measured in response to tones at the CF (9 kHz) or below the CF (5 kHz). Vibration vector magnitudes were compared at the basilar membrane (BM), outer hair cell/Deiters' cell (OHC/DC) junction, and reticular lamina (RL) in three mice.

Results: Previously described patterns of vibration in the transverse, radial, and longitudinal directions were found to be replicated in these measurements. However, the 3D reconstruction permits the integration of these findings, together with a more detailed characterization of vibrational modes within the OoC. As a first pass interpretation, we found that the 3D vibration vector magnitude at the BM was smaller than the OHC/DC and the RL by a factor of 2-3 at the CF using a 40 dB SPL stimulus. However, using an 80 dB SPL stimulus, the 3D vibrational vector magnitude of the BM was similar to that of the OHC/DC and the RL. Post-mortem, the BM magnitude both at the CF and below the CF was greater than both the OHC/DC and the RL by a factor of 2.

Conclusions: Using our 3D-OCT system, we measured 3D vibration vector magnitudes of different OoC structures that were similar to those obtained with previous 1D OCT measurements. These three-dimensional vectors can be further decomposed into transverse, radial, and longitudinal components.

9:30 a–m. - 11:30 a.m.
Symposium #7

Individual Listening Strategies: From Physiological to Perceptual Responses in Experiments and Simulations
Chair: Alejandro Osses Vecchi, *École Normale Supérieure, PSL University, CNRS*

In this session we shed light into individual listening strategies that can be revealed using empirical data or methods that combine experimental responses with simulations. The common factor in these methods is how the variability in the obtained responses is interpreted. This variability can stem from internal aspects of the auditory processing or from external intrinsic variations in the (noisy) stimuli that are being listened to, resulting in cues that can be differently coded and weighted at a more central auditory level. In this session we cover methods used in animal physiology (auditory nerve recordings) as well as in human objective (EEG recordings) and behavioral tasks (speech discrimination), where simulations of the peripheral auditory processing or the use of advanced statistical methods allow a quantitative analysis of the time-frequency cues that may lead to such variability. The studies covered in this session have diverse goals, ranging from the improvement in the fundamental understanding of auditory processes, hearing diagnosis, and cochlear implant fitting using speech-in-noise sounds or artificial stimuli. However, what we want to emphasize in this session, is that the close loop between experimental data –the baseline– and simulation data –that set the quantitative expectations based on previous scientific advances– is beneficial to generate and test hypotheses, and that this knowledge not only improves our understanding of auditory processes but can be directly used for improving listener-targeted technologies (e.g., cochlear-implant fitting). For the previous reasons, this session targets an audience from experimental physiology (AN recordings, EEG), audiology (speech perception, hearing assessment), psychoacoustics (speech perception), and researchers of all these areas who are also interested in auditory modeling.

Auditory Classification Images: A Psychophysical Paradigm to Explore Listening Strategies in Phoneme Perception
Léo Varnet, *Laboratoire des Systèmes Perceptifs (ENS Paris/CNRS)*

The identification of the exact acoustic cues that listeners use when categorizing phonemes still constitutes an open question for research on speech perception. In this talk, we present a psychoacoustic reverse-correlation approach allowing direct investigation of individual listening strategies in phoneme perception. The Auditory Classification Image technique relies on a penalized Generalized Linear Model to link categorization errors in a speech-in-noise comprehension task with the corresponding distribution of the noise, on a trial-by-trial basis. We will also discuss recent improvements of the method designed to reduce the duration of the experiment.

Auditory Nerve Fiber Discrimination and Representation of Naturally-Spoken Vowels in Noise

Amarins Heeringa, *Carl Von Ossietzky University*

To understand how vowels are encoded by auditory nerve (AN) fibers, previous research suggested a number of representation schemes that extract the vowel's formant frequencies from spiking patterns of AN fibers. Here, we will show the application of these representation schemes for AN-fiber responses, recorded in the Mongolian gerbil, to naturally-spoken vowels presented in a speech-shaped background noise. Behavioral experiments in the same species revealed which of these vowels are easy and which are difficult to discriminate from each other. A spike-timing based discrimination metric agreed well with perceptual performance, while mean discharge rate was a poor predictor. Consistent with that, only spike timing-based, but not rate-based, representation schemes revealed peaks at the formant frequencies, which are paramount for perceptual vowel identification and discrimination. Making use of these perceptual discrimination data, this study showed that difficulties with naturally-spoken vowel discrimination in noisy listening conditions originate peripherally and can be studied in the spike timing patterns of single AN fibers.

Variability in Auditory Nerve Responses in Animals: How to Extrapolate to Individual Human CI Users?
Dyan Ramekers, *University Medical Center Utrecht*

The electrically evoked compound action potential (eCAP) is a direct measure of the responsiveness of the auditory nerve to electrical stimulation. It offers a unique opportunity to study the auditory nerve's electrophysiological behavior in individual cochlear implant (CI) users over time. In guinea pigs several eCAP measures correlate well with neuronal survival, but in human studies eCAP measures often lack predictive value, at least partly because of substantial variability among CI users. Here we evaluate the variability in eCAP measures and auditory-nerve histology in guinea pigs, in order to better understand variability among CI users.

The Impact of Internal Noise on Amplitude Modulation Detection for Children and Young Adults: Empirical and Simulation Data
Laurianne Cabrera, *Universite de Pa–is - CNRS*

The consistency of auditory judgments in a forced-choice amplitude-modulation (AM) detection task was assessed for children and adults using a constant-stimuli procedure and a double-pass paradigm. Sinusoidal AM was applied to narrowband-noise carriers and presented at detection threshold. Percent correct detection and percent agreement between the two passes were measured and used to assess internal noise thanks to a modulation-filterbank model using a template-matching decision strategy. At the group level, internal noise was higher in children than adults. At the individual level, performance variability was not only explained by lower internal noise with age but possibly by sub-optimal templates.

Re-Interpreting Experimental Results With Computer Model Simulations: The Case of a Peripheral Compression Study
Gerard Encina-Llamas, *Technical University of Denmark*

Usually we have an interesting hypothesis about the function of the auditory system that we aim at prove or reject with experimental data. In humans, experimental data is generally indirect and their interpretation is subjected to a number of assumptions. Computer simulations can assist in the interpretation of the results and avoid erroneous conclusions. We present here a case where we investigated the use of envelope following responses (EFR) to estimate peripheral compression. Computer model simulations indicated that the observed experimental effect was not related to changes in cochlear compression, despite to what the experimental data suggested.

Power Law Adaptation in the Electrically Stimulated Auditory Nerve
Margriet van Gendt, *Leiden University Medical Center, the Netherlands*

Computer models may help speed up the evaluation of new cochlear implant sound-coding strategies. For this, temporal detail and effects of longer stimulation, such as adaptation, have to be implemented. Here, various forms of adaptation were implemented in a model; a single decaying exponent, multiple exponents and a power law. Measured neural responses to electrical stimulation with both short and long duration pulse trains were compared in terms of spike rate and vector strength (VS) with outcomes of these models. Overall, power law adaptation most closely mimics neural adaptation of the electrically stimulated auditory nerve.

Trial-by-Trial Analysis of Phoneme-in-Noise Perception using a Model of Monaural Auditory Processing
Alejandro Osses Vecchi*, *École Normale Supérieure, PSL University, CNRS*

In this presentation we provide insights into the discrimination of vowel-consonant-vowel (VCV) words embedded in white noise and speech-shaped noise by adopting an auditory model that uses a modulation filter bank front-end and a speech back-end decision module. Our analysis is focused on the discrimination cues available to the model and their interaction with specific processing stages in the model, evaluating whether these cues might be further used to simulate listener-dependent performance. For that purpose we rely on a reverse-correlation approach by comparing the noise representations that lead to the choice of one or other consonant.

9:30 a–m. - 11:30 a.m.
Podium Session #8 – Inner Ear Development
Artur Indzhykulian, M.D., Ph.D. & Ksenia Gnedeva, Ph.D.

1. CCER2: A Novel Gene Upregulated in the Early Stages of Mammalian Sensory Hair Cell Differentiation
Category: Development: Cellular/Systems

Emilia Luca*[1], Joanna F. Mulvaney[1], Gianluca Sampieri[1], Daniel Nouri Nejad[1], Alain Dabdoub[2]
[1]*Biological Sciences, Sunnybrook Research Institute,* [2]*Biological Sciences, Sunnybrook Research Institute, Toronto, Canada. Department of Otolaryngology - Head and Neck Surgery, University of Toronto, Toronto, Canada. Department of Laboratory Medicine and Pathobiology, University of Toronto*

Background: Hair cells (HCs) are specialized sensory cells that detect sounds and movements in the auditory and vestibular systems, respectively. It has been demonstrated that Atoh1, a helix loop helix transcription factor, is necessary and sufficient for HC formation and differentiation. To discover the genes that are downstream of Atoh1 and involved in HCs development we characterized the transcriptome of Atoh1-induced ectopic HC at the early stage of mammalian cochlear development.
Methods: We electroporated embryonic (E) day 13.5 mouse cochlear explants with an Atoh1 GFP reporter construct, or with an empty GFP vector as a control. At this stage of development, overexpression of Atoh1 results in a ~100% conversion of electroporated cells into HCs. To identify the immediate genes regulated by Atoh1 overexpression, we used fluorescence-activated cell sorting (FACS) and sorted the cells overexpressing GFP 24 hrs after electroporation. RNA was extracted from both Atoh1 GFP and control cells, and bulk RNA-sequencing (RNA-seq) was performed.
Results: We found more than 800 differentially expressed genes (~700 upregulated and ~100 downregulated), and our bioinformatic analysis detected several known hair cell genes (e.g. Dll1, Gfi1, Jag2) in the Atoh1 expressing cells. Furthermore, we identified Ccer2 (coiled-coil glutamate-rich protein 2), a novel gene that was significantly upregulated (6-fold change). CCER2 is an uncharacterized protein; there is no published information about its structure, localization, or function. We confirmed the expression of CCER2 in endogenous cochlear and vestibular HCs. We investigated its spatiotemporal expression during mouse cochlear and vestibular development and found that in the cochlea, CCER2 has a developmental base-to-apex gradient and is transiently expressed starting at E13 up to postnatal day 6, following the spatiotemporal expression of Atoh1. In the utricle and saccule, the protein is expressed embryonically and throughout adult stages. We investigated the function of Ccer2, in hearing and balance, by generating Ccer2 mutant mice (FVB/NJ background) using CRISPR/Cas9 technology, and performed ABR and DPOAE, as well as rotarod balance tests.
Conclusions: In conclusion, our transcriptomic analysis is the first RNA-seq study that characterized Atoh1 downstream targets activated in the early stages of HC differentiation, which led to the discovery of CCER2, a novel and specific protein marker for inner ear sensory hair cells. Furthermore, it is one of the earliest markers expressed during HCs development. The characterization of CCER2 will provide insights into both Atoh1 and other signalling pathway(s) where it is involved, advancing our understanding of inner ear development.

2. Gene Expression Signatures of Extracellular Matrix and Integrins During Human Otic Sensory Differentiation From Pluripotent Stem Cells
Category: Development: Cellular/Systems
Azel Zine*[1], Lejo Johnson Chacko[2], Claudia Steinacher[2], Hanae Lahlou[3], Yassine Messat[4], Said Assou[5], Consolato Sergi[6], Anneliese Schrott-Fischer[2]

[1]*Bioengineering and Nanoscience Laboratory, University of Montpellier, [2]Medical University Innsbruck, [3]Mass Eye and Ear Infirmary, [4]University of Montpellier/ Laboratory of Nanoschioence Bioengineering, [5]IRMB, Univ Montpellier, INSERM, CHU Montpellier, University of Montpellier, France, [6]Children's Hospital of Eastern Ontario, University of Ottawa

Background: Two approaches have been subjected to restore inner ear HCs that do not regenerate, i.e., gene and stem cell-based cell therapies. The stem cell approach requires the robust production and characterization of human otic sensory progenitor cells.
To gain new insights into early human otic neurosensory lineage, we analyzed transcriptomic data from otic sensory cells differentiated from induced pluripotent stem cells (hiPSCs) by our previously described method (Lahlou et al., 2018).
Methods: We analyzed transcriptomic data from otic sensory cells differentiated from human induced pluripotent stem cells (hiPSCs) by a previously described method to gain new insights into the early human otic neurosensory lineage.
Results: These analyses identified and ranked genes known to be part of the otic sensory lineage program (SIX1, EYA1, GATA3), in addition to a number of novel genes encoding extracellular matrix (ECM) (COL3A1, COL5A2, FN) and integrin (ITG) receptors (ITGAV, ITGA4, ITGA) for ECM molecules. The results were confirmed by quantitative PCR analysis of a comprehensive panel of genes differentially expressed during the time course of hiPSC differentiation in vitro. Results were validated by immunochemistry for select otic and ECM/ITG gene markers in the human fetal inner ear.
Our screen shows ECM and ITG gene expression changes coincident with hiPSC differentiation towards human otic neurosensory cells.
Conclusions: In summary, we report a critical role of ECM-ITG interactions with otic neurosensory lineage genes in early neurosensory development and cell fate determination in the human fetal inner ear.

3. Norrie Disease Protein is Essential for Cochlear Hair Cell Maturation
Category: Development: Cellular/Systems
Yushi Hayashi*[1], Hao Chiang[1], ChunJie Tian[1], Artur A. Indzhykulian[1], Albert S. B. Edge[1]
[1]Harvard Medical School, Department of Otolaryngology

Background: Norrie disease is an X-linked, recessive, inherited disease that can be caused by mutations in the NDP gene. Major manifestations of the disease are bilateral blindness with a prominent intraocular mass and avascularity of the retina, intellectual disability, and progressive sensorineural hearing loss beginning in adolescence.
Ndp binds to Fzd4, of which signal leads to stabilization and nuclear translation of beta-catenin. Loss of Ndp/Fzd4 signaling in endothelial cells causes defective retinal vascular growth during development (Ye et al., 2009) and enlarged vessels in the stria vascularis with loss of hair cells (HCs) (Rehm et al., 2002; Xu et al., 2004).
To better understand the pathophysiology of the disease, we sought to learn whether the HC deterioration could be a direct result of the defect in the Ndp signaling.
Methods: We analyzed expression site of Ndp and Fzd4 mRNA in the cochlea by immunohistochemistry and RNA in situ hybridization, respectively. We investigated Ndp KO mice with physiological (ABR/DPOAE), histological (immunohistochemistry/SEM), and gene expression analysis (qRT-PCR/RNA-sequencing). We also prepared Ndp KO (X-Y);Sox2-CreER;Z/Norrin;tdTomato (Tm) mice where Sox2-positive supporting cells (SCs) are forced to express Ndp and Tm in the Ndp KO cochlea and Ndp KO (X-Y);Atoh1-Cre;beta-catenin flox(Exon3) mice where beta-catenin is stabilized in Atoh1-positive HCs in the Ndp KO cochlea, respectively, to elucidate downstream molecules of the Ndp/Fzd4 signaling.
Results: Ndp was expressed in SCs and the greater epithelial ridge (GER)/inner sulcus (ISu) and Fzd4 mRNA was strongly expressed in HCs, indicating that HCs receive Ndp secreted from SCs and the GER/ISu.
Ndp KO mice exhibited Myo7a and/or Pou4f3-negative HCs during postnatal stage, which was progressive during their maturation, leading to some HC death. To support this phenotype, transcripts of Myo7a, Pou4f3, and Gfi1 were down-regulated in sorted HCs of Ndp KO mice. As a consequence of Myo7a-negative HCs, ABR/DPOAE thresholds were elevated during maturation of Ndp KO mice. RNA-sequencing from sorted HCs of Ndp KO mice unveiled Setd7 and Nr4a3 as downstream molecules of the Ndp signaling pathway.
Both Ndp KO (X-Y);Sox2-CreER;Z/Norrin;Tm and Ndp KO (X-Y);Atoh1-Cre;beta-catenin flox(Exon3) mice attenuated elevation in ABR/DPOAE thresholds, Myo7a-negative HCs, and HC death as compared with Ndp KO mice, demonstrating that HC deterioration in Ndp KO mice was primarily due to lack of Ndp secreted from SCs

rather than secondary to vascular deficiency and beta-catenin regulates Myo7a expression in the downstream of Ndp/Fzd4.

Conclusions: ・Ndp secreted from SCs directly affects HCs that strongly express Fzd4.
・Ndp KO mice show Myo7a-negative HCs and HC death, indicative of failure in HC maturation and survival presumably caused by down-regulation of Pou4f3 and Gfi1.
・The Ndp/Fzd4 signaling pathway controls a network of transcriptional regulators such as Setd7 and Nr4a3.
・Our transgenic mouse models also show the possibility to clinically treat patients of Norrie disease.

4. PRDM16 is Required for the Development of Kölliker's Organ and Its Derivatives in Mouse Cochlea
Category: Development: Cellular/Systems

Michael Ebeid*[1], Kathy Barnas[1], Amal Yaghmour[1], Gabriele Noreikaite[1], Bryan Bjork[1], Hongji Zhang[1]
[1]*Midwestern University*

Background: The PR domain containing 16 (PRDM16) is a key transcription regulator in the development of different tissues including craniofacial, adipose, neural, and hematopoietic tissues. Our lab identified PRDM16 expression in the epithelial cells of the Kölliker's organ (KO) starting around E13.5 and maintained till the disappearance of the KO around P10. KO undergoes remodeling during development to give rise to the cells of the inner sulcus region and is thought to be involved in tectorial membrane formation as well as generating intrinsic spontaneous activity. This work aims at understanding the role of PRDM16 in cochlear development utilizing loss-of-function approach.
Methods: A transgenic mouse model (Prdm16cGT/cGT) carrying a gene trap allele for Prdm16 was used to characterize the impact of Prdm16 deletion on cochlear development. Whole-mount/section immunostaining, Hematoxylin and Eosin staining, EdU proliferation assay, as well as scanning electron microscopy were used to characterize the phenotype of Prdm16-null cochlea at different time points. Bulk RNA sequencing of cochlear duct cells at E14.5 was used to identify differentially expressed genes (DEGs) in Prdm16-null versus littermate control cochleae. Quantitative real time PCR and mRNA Fluorescence in-situ hybridization (FISH) were used to validate RNA sequencing data. Since Prdm16-null mice die at birth, we utilized Fgf20Cre to conditionally delete Prdm16 from the cochlear duct as early as E13.5. Analysis of Prdm16 conditional mutant (cKO) cochleae was done to understand postnatal development of cochlea lacking PRDM16.
Results: Prdm16-null cochlea at P0 exhibited hypoplastic KO, shortened cochlear duct (60% compared to control), increased density of hair cells and supporting cells in the apical turn as well as multiple isolated islands of ectopic hair cells within the KO domain. Ectopic HCs were Sox2+ and Myo6+ with immature stereocilia bundles, and consistent innervation from the spiral ganglion neurons. Proliferation of KO epithelial cells was deficient in the apical turn of E14.5 and E16.5 Prdm16-null cochlea versus controls. Upregulated genes at E14.5 in Prdm16-null cochlea versus controls included Fgf20, as well as several Notch pathway genes (Lfng, Hes1 and Jag1). mRNA FISH and immunostaining confirmed expanded Lfng, Jag1 and Fgf20 expression domains to include the KO in Prdm16-null cochlea. Analysis of Prdm16 cKO at P0 showed same phenotype as Prdm16-null cochlea. Postnatal development of Prdm16 cKO cochleae shows hypoplastic spiral limbus, lack of inner sulcus and detached tectorial membrane. ABR recordings of Prdm16 cKO are in progress.
Conclusions: PRDM16 is required for KO development and its derivatives in mouse cochlea. It regulates KO cell proliferation and represses Notch signaling activity in KO during development. Individuals with 1p36 deletion syndrome suffer sensorineural hearing loss that is not yet attributed to a certain gene. Prdm16 is located in the area of deletion and is a potential candidate.

5. Step-Wise Characterization of Inner Ear Cell Types Derived From Pluripotent Stem Cells in 3D Organoids
Category: Development: Cellular/Systems

Daniela Doda[1], Simon Sennhauser[2], Marta Roccio*[1]
[1]*University Hospital Zurich,* [2]*University of Zurich*

Background: Inner ear hair cells and auditory neurons are essential for sound detection. Their damage or loss is irreversible in humans and is a major cause of permanent hearing deficit. A major bottleneck for the development and clinical translation of novel therapies for sensorineural hearing loss (SNHL) is represented by the lack of suitable cellular assays, based on human sensory cells, for the validation of compounds or gene therapies in pre-clinical phase.

Differentiation of human pluripotent stem cells (PSC) in vitro to inner ear cell types opens new opportunities to gain insight into inner ear development, the pathophysiology of SNHL and for the validation of therapeutic strategies. The reproducibility and robustness of this methodology need to be further optimised to fully exploit its' potential.

Methods: Human iPSCs are coaxed to differentiate towards the otic lineage by providing morphogens and growth factors to recapitulate key development steps occurring in vivo. Specifically, by guiding the consecutive specification of non-neural ectoderm, placodal ectoderm and subsequently otic placode tissue. This is achieved by transient activation of BMP and FGF signaling, followed by Wnt activation. In combination with 3D culture methods, this leads to the formation of otic vesicle-like structure that develop hair cell bearing sensory epithelia, non-sensory epithelia, as well as otic-like neurons and glia. We are making use of knock-in fluorescent reporter lines to mark otic (PAX-2-GFP) and prosensory domain progenitors (SOX2-GFP), in combination with immunostaining-characterization, to optimize the stages of the differentiation protocol.

Results: hIPSC aggregates, incubated with a TFGb inhibitor to suppress mesendoderm differentiation, respond to BMP (BMP4) stimulation in a dose dependent manner. Low concentrations (<1ng/ml) lead to neural crest differentiation, intermediate BMP doses (1.5-2.5 ng/ml) induce placode differentiation and high doses (3-10ng/ml) result in epidermis induction. At day 8 of in vitro differentiation, the derived placodal tissues is characterized by the co-expression of ECAD/NCAD/SIX1/AP2/PAX8. Some neural crest (NC) cells (CD271+/SOX10+) co-develop with placodal cells at this stage.

Otic vesicle structure expressing PAX2/PAX8/ECAD/FBXO2/SOX10 further develop into non-sensory and sensory epithelia, the latter comprising hair cells, supporting cells and innervating neurons by day 60-70 of in vitro culture. TUBB3+/BRN3A+/ISL1+ neurons are identified in the culture at the periphery of the aggregates and a subset of these innervates the sensory epithelial patches. The developmental origin of these sensory neurons is currently under investigation.

Conclusions: Optimization of the initial starting conditions and growth factors delivery will set the frame to reliably differentiate inner ear cell populations à la carte. Robust and reliable differentiation methods will enable, in a second step, testing novel therapeutics to counteract SNHL.

6. Single-Cell Atlas of the Developing Human Inner Ear Highlights Heterogeneity of the Cochlear and Vestibular Epithelia

Category: Development: Cellular/Systems

Amar Sheth*[1], Richard Perez[2], Sonia Scaria[2], Shengyang Kevin YU[2], Stacey Frumm[2], Smita Krishnaswamy[1], Aaron Tward[2]
[1]*Yale School of Medicine,* [2]*UCSF*

Background: Hearing loss (HL) and vestibular dysfunction (VD) are major causes of morbidity in the world, causing disability in over 50% of the aging population over 75 [1, 2]. Furthermore, congenital hearing loss is one of the most prevalent chronic conditions in children with environmental and genetic etiologies. Despite the growing burden of hearing loss, our molecular understanding of the inner ear physiology is lacking [3].

In an era of access to high-resolution single cell sequencing [4] and new avenues for gene therapy, there is a growing impetus to study the inner ear. While our group has recently uncovered the developmental transcriptomic signature of the murine inner ear, to-date, the literature is yet to identify similar developmental trajectories in the developing human inner ear[5]. Through our research, we mapped the trajectories of early cell lineages and identified distinct cell types in the developing inner ear.

Methods: All single-cell RNA sequencing was done using 10X genomics digital droplet technology. Afterward, we performed quality filtering on each data following best practices to remove dying cells, doublets, and cells with low gene expression. Lastly, we log-normalized, combined, and regressed out batch-correction. Our analysis comprised of three steps:

I)   Lineage Trajectory Analysis

First, we created a high-dimensional graph of the processed data. Then, we identified the parameters to embed the data in 2D and 3D visual representations using the PHATE algorithm [6].

II)   Cell Type Identification

To identify both broader cell type clusters and sub-clusters, we used Louvain clustering recursively. We set the parameters to continue clustering sequentially into smaller from larger clusters [5].

III) Gene co-expression networks

We used a stochastic kinetic modeling method to create gene networks [7].

Results: We sequenced the inner ear at six distinct time-points: 15-weeks, 17-weeks, 18-weeks, 23-weeks, 24-weeks, and adult. Our processed data comprised of 140,007 transcriptomes.

Through clustering, we identified epithelial, endothelial, immune, mesenchymal, and neural crest lineages. In the cochlear epithelia, we identify roof cells, interdental cells, Reissner membrane, sick cells, outer sulcus, hair cells, stria vascularis, and supporting cells. In the vestibular epithelia, we isolate keratinizing epithelium, semicircular canal, sick cells, interdental cells, hair cells, dark cells, and supporting cells.

PHATE embedding and RNA velocity analysis produced an unbiased map of developmental trajectories followed by each distinct lineage. Lastly, we created genetic modules for each lineage using stochastic modeling to uncover how genes interacted.

Conclusions: Our results yield an improved understanding of the genetic regulation underlying normal human inner ear cellular development. Next, we aim to conduct meta-analyses identifying the modules in which HL and VD-associated genes lie. Our findings and subsequent analyses will serve as a roadmap for directed differentiation approaches, reprogramming and novel gene-based therapies.

7. Identification of Molecular Mechanisms That Confer Tonotopic Identity During Cochlear Duct Extension
Category: Development: Cellular/Systems

Shuze Wang[1], Saikat Chakraborty[1], Yujuan Fu[2], Mary Lee[1], Scott Jones[1], Joerg Waldhaus*[1]
[1]*University of Michigan,* [2]*University of Washington*

Background: The organ of Corti is tonotopically organized; low frequencies are detected in the apex, while high frequencies are detected in the base. Tonotopic function is established in part through graded gene expression requiring the cochlear duct cells to acquire spatial identities along the organ's longitudinal axis. Manipulations of SHH-signaling were shown to interfere with tonotopic gene expression, however, additional molecular mechanisms conferring spatial identity in mammals remain to be determined. Considering the current knowledge, we hypothesize two models. 1) Strict control over the temporal aspect of gene expression during organ of Corti development could directly translate to spatial information, as observed in determination of the anterior-posterior axis in vertebrate hindbrain development. 2) Alternatively, morphogen gradients spanning the apex to base axis could account for graded gene expression which dynamically adjusts to the length of the cochlear duct.
Methods: To test both hypotheses, we analyzed whole transcriptome profiles of 1711 individual cells isolated from the E12.5 and E14.5 developing cochlea duct.
Results: Each cell's relative position within the developing cochlea duct was reconstructed in 3D-space analyzing the transcriptome data using a PCA-based approach. After projecting the cells onto a cylinder surface, gradually expressed genes (GEGs) exhibiting linear gradients along the apex-to-base axis for both time points were identified. Using the GEGs as features, a similarity matrix was calculated comparing tonotopically organized pairs of meta-cells along the cochlea duct. The results indicated that E12.5 meta-cells aligned with the tonotopically corresponding E14.5 meta-cells within the cochlea. Since the cochlear duct extends through cell division at the basal end, this finding indicates the presence of dynamically regulated gene expression gradients, supporting the morphogen model. Based on the prediction that morphogen gradients contribute to tonotopic identity, we performed signaling pathway analysis for the E12.5 and E14.5 time points, respectively. First, enrichment scores for GO-term pathways were calculated at single-cell resolution. Next, pairwise comparison between the apex and base compartments were performed to determine the differentially active signaling pathways. Among other pathways, retinoic acid signaling was identified to be asymmetrically active between cochlear apex and base. Analyzing individual components of the retinoic acid pathway revealed that a retinoic acid signal emanates from the roof compartment as indicated by differential expression of genes involved in retinoic acid synthesis like Rdh10 and Aldh1a3. Expression of retinoic acid receptors was found in the cochlea floor and known retinoic acid target genes including Cyp26b1 and Dhrs3 were identified as GEGs of the cochlear floor as well.
Conclusions: In this work, we used single-cell whole transcriptome sequencing to reconstruct the developing cochlear duct in 3D-space. In combining bioinformatics and experimental approaches, we identified the morphogen retinoic acid to potentially confer spatial information during cochlear duct extension.

8. Single-Cell Analysis Reveals Cochlear and Vestibular Developmental Trajectories in Organoid-Derived Sensory Cells
Category: Development: Cellular/Systems

Joerg Waldhaus*[1], Linghua Jiang[1], Liqian Liu[1], Jie Liu[1], Robert Duncan[1]
[1]*University of Michigan*

Background: Inner ear organoids are novel model systems for studying development and disease, but the degree to which they mimic the complexity of the normal inner ear remains uncertain. To date, organoid sensory cells appear to default toward a vestibular fate, but these judgements have been based on a relatively small number of morphological and physiological markers. In this study, we sought to examine the complexity of inner ear organoids using single-cell RNA sequencing.

Methods: Murine Atoh1/nGFP embryonic stem cells were used to generate inner ear organoids following standard protocols. Whole spheroids were collected after 20 days of culture and dissociated for flow sorting and downstream analysis. Reporter-positive cells were divided into GFP-high and GFP-low samples and libraries were generated on the 10X Genomics platform. The analysis pipeline included Seurat for clustering, Velocity to examine developmental trajectories, and AUCell to calculate and compare auditory and vestibular enrichment scores.

Results: Spheroids produced Pax2- and Six1-positive otic vesicles by culture day 12 and cystic organoids with MyoVIIa-positive hair cells and Sox2-positive supporting cells by day 20. In total, 6,339 single cells were captured and analyzed after stringent quality control. Overall, 13 clusters were identified and known marker genes used to annotate the clusters, revealing three hair cell clusters based on shared MyoVIIa expression and two supporting cell clusters based on Gjb2 expression. Analysis of developmental markers suggested that hair cell clusters represented distinct maturation stages. Differential expression of Atoh1 and Tmc1 outlined young versus more mature hair cells in two of the clusters, while the third cluster was annotated as a transient population owing to the detection of both markers. Based on trajectory analysis using an RNA velocity algorithm, two independent trajectories were predicted giving rise to two distinct types of hair cells in vitro. Joint analysis of the in vitro generated hair cells with postnatal day (P) 1 utricle and P2 organ of Corti revealed that the two trajectories predicted for in vitro generated hair cells segregated with the asymmetric distribution of auditory and vestibular lineages. Auditory versus vestibular hair cell enrichment scores were calculated and projected onto the in vitro data confirming differentiation of both cell types in inner ear organoids.

Conclusions: Single-cell analysis reveals a complex ensemble of GFP-positive, Atoh1-expressing cell types in day 20 organoid cultures, including at least two classes of sensory hair cells that span several development stages with both auditory and vestibular fates. Notably, our approach required dissociation of a pooled sample of spheroids and thus eradicated information regarding aggregate-specific and vesicle-specific differentiation. Further study is required to determine whether organ-specific hair cells are intermingled within an organoid or segregated between individual vesicles or spheroids and to identify the signaling events that specify these fates.

9:30 a.m. - 11:30 a.m.
Podium Session #9 – Spatial Hearing and Development
Moderators: Ruth Y. Litovsky, Ph.D. & Melissa Polonenko, Ph.D.

1. Spatial Hearing Skills of Young Children With Prelingual Single-Sided Deafness and a Cochlear Implant
Category: Auditory Prostheses

Tine Arras*[1], Birgit Philips[2], Christian Desloovere[3], Jan Wouters[1], Astrid van Wieringen[1]

[1]*ExpORL, Dept. Neurosciences, KU Leuven, University of Leuven, Leuven, Belgium,* [2]*Cochlear Technology Center Belgium,* [3]*University Hospitals Leuven, Dept. Otorhinolaryngology, Head and Neck Surgery, Leuven, Belgium*

Background: Children with single-sided deafness (SSD) have no access to binaural hearing, which affects their ability to localize sounds and understand speech in noisy environments. Additionally, the children are at risk for speech-language delays and the aural preference syndrome. A cochlear implant (CI) can restore bilateral hearing and may improve spatial hearing skills. The aim of the current study was to investigate hearing outcomes in children with SSD with a CI compared to their peers with untreated SSD.

Methods: We assessed speech perception in noise and sound localization in three groups: children with SSD with a CI (SSD+CI group), children with SSD without a CI (SSD group), and children with bilateral normal hearing (NH group). For the SSD+CI group, we collected additional data on CI use (data logging) and speech understanding with the CI only.

Results: Data were collected from 48 children (13 SSD+CI, 9 SSD, 26 NH) aged 4-8 years. The children in the SSD+CI group received their implant before the age of 2.5 years.

On average, the children wore their device for 8.6 ± 2.6 hours per day (range: 2.9 to 12.2 hours). Nine children were regular users (> 8 hours/day), while the four others used their device for less than 7 hours per day. All children but one were able to understand speech when sound was presented directly to the CI. This one child was a limited user who recently stopped using the device, due to a lack of perceived benefit (despite measurable improvements in spatial hearing).

Speech perception in noise: the speech reception threshold (SRT) was significantly different between groups when speech was presented on the deaf/CI side with noise on the NH side ($p < 0.001$). Children with NH outperformed both SSD groups ($p < 0.001$). Children with SSD and a CI outperformed their non-implanted peers when using the CI ($p < 0.001$) and when the CI was switched off ($p = 0.04$). In all groups and conditions, SRTs improved with age ($p < 0.001$).

Sound localization: the mean absolute error (MAE) differed significantly across groups ($p < 0.001$) and improved with age ($p < 0.001$). Children with NH outperformed both SSD groups ($p < 0.001$). Children with untreated SSD performed around chance level (44°). In the group of children with SSD and a CI, some were able to localize sounds significantly better than chance (MAE < 34°). As a group, these children outperformed their non-implanted peers when using their CI ($p < 0.001$), but not when the device was switched off.

Conclusions: Early cochlear implantation improves spatial hearing outcomes in children with SSD. Most children learn to understand speech using only their CI. These results confirm that a CI is a viable treatment option for children with SSD.

2. Adjustment of the Interaural Stimulation Timing Leads to Improved Sound Localization in Bimodal Listeners

Category: Binaural Hearing and Sound Localization

Stefan Zirn*[1], Julian Angermeier[2], Werner Hemmert[3]

[1]*University of Applied Sciences Offenburg, *[2]*Faculty of Electrical Engineering, Medical Engineering and Computer Sciences, University of Applied Sciences Offenburg, *[3]*Technical University of Munich*

Background: In bimodal cochlear implant (CI) / hearing aid (HA) users a constant interaural time delay in the order of several milliseconds occurs due to differences in signal processing of the devices. For MED-EL CI systems in combination with different HA types, we have quantified the respective device delay mismatch (Zirn et al. 2015).

Methods: In the current study, we investigate the effect of the device delay mismatch in actual bimodal listeners on sound localization accuracy. To deal with the device delay mismatch we delayed the CI stimulation according to the measured HA processing delay and two other values. To determine potential effects of procedural learning, we applied an A-B-B-A testing paradigm.

Results: With all delay values highly significant improvements of the rms error were observed compared to the test without the delay (14.7° improvement in average). Also the signed bias of sound localization improved significantly from 25.2° to 10.5° averaged across listeners.

Conclusions: The results reveal that bimodal listeners benefit from a reduction of the device delay mismatch between CI and HA. With this form of temporal adjustment of modalities, spatial re-centering seems possible.

3. Effects of Large Interaural Delays on Cue Dependent Spatial Release From Masking: Experimental Results and Modelling

Category: Binaural Hearing and Sound Localization

Julian Angermeier*[1], Stefan Zirn[1], Werner Hemmert[2]

[1]*Peter Osypka Institute of Medical Engineering, Faculty of Electrical Engineering, Medical Engineering and Computer Sciences, University of Applied Sciences Offenburg, *[2]*Bio-Inspired Information Processing, Munich Institute of Biomedical Engineering, Technical University of Munich*

Background: In asymmetric treatment of hearing loss, different processing latencies in each ear can lead to a change of the reference interaural time differences (ITD) (i.e., the ITD at 0° azimuth) of several milliseconds (Zirn et al., 2015). Considering the biggest naturally occurring ITDs being around 700 µs, this change in reference ITD leads to drastic changes in interaural timing. When trying to understand factors that determine spatial release from masking (SRM) in subjects using hearing aids (HA) or cochlear implants (CI), asymmetric processing latencies of these devices and the following change in reference ITD are often overlooked. This study aims to investigate the effects of a rising reference ITD on SRM with respect to the binaural cues available for spatial separation of sound sources.

Methods: SRM was measured in ten normal hearing subjects with a reference ITD of 0, 1.75, 3.5, 5.25 and 7 ms by measuring speech reception thresholds (SRT) for spatially collocated speech and noise (S0N0) and spatially separated speech and noise (S0N90) using the Oldenburg Sentence Test (OlSa). The available cues for spatial separation of target and masker were manipulated to measure the effect of reference ITD on SRM with only interaural level differences (ILD), interaural time differences (ITD) or both cues available. A blind equalization-cancellation (EC) model by Hauth et al. (2020) was applied to model SRTs for the same conditions that were measured experimentally.

Results: With a rising reference ITD, a significant decrease in SRM was measured with ITD and ILD combined, as well as with only ITD as the available cues. When only ILD are present, a rising reference ITD does not lead to a significant change in SRM. The applied model could accurately simulate SRM for the different cues available and matched the behavior for a rising reference ITD seen in the experimental results.

Conclusions: Asymmetric processing latencies in the treatment of hearing loss can significantly impact SRM, depending on the available cues that users utilize for spatial separation of sound sources. This effect as so far not been described in the literature. These outcomes can be reliably simulated by an equalization-cancellation model.

4. Differences Between Fetal and Preterm Infant Language and Auditory Environments
Category: Development: Human Subjects
Brian Monson*[1], Sophie Ambrose[2], Derrick Rollo[3]
[1]*University of Illinois at Urbana-Champaign*, [2]*Boys Town National Research Hospital*, [3]*Carle Foundation Hospital, Urbana, IL*

Background: Human auditory function begins during fetal development, as early as 23 weeks' gestation. Thus extrauterine language and auditory exposures during gestation affect neurobehavioral outcomes for full-term newborns. However, the typical prenatal auditory experience has not been well characterized. Such characterization is important for comparison with abnormal auditory experience during this stage of development, such as that which occurs with premature birth. In this study, we compared (extrauterine) auditory exposures for fetuses and preterm infants during the final months prior to term.

Methods: We analyzed 24,000 hours of auditory exposure data obtained during the final three months of prenatal/preterm brain development for typically developing fetuses and very preterm (VPT; born ≤ 32 weeks' gestation) infants, using multiple 24-hr audio recordings per week for each participant. Extrauterine exposures for fetuses were captured by having pregnant women (n = 27) wear LENA recorders near the abdomen twice weekly throughout the third trimester of pregnancy. The same devices were used to capture auditory exposures for VPT infants (n = 24) by placing the recorders with the infants in their hospital beds three times per week during their stay in the neonatal intensive care unit (NICU).

Results: Fetuses received 2.6 ±0.6 hr/day of exposure to adult near language, nearly five times greater than the 0.53 ±0.2 hr/day for VPT infants. A similar magnitude of difference was reflected in adult word count estimates, as fetuses were exposed to 36,679 ±8,873 words/day and VPT infants were exposed to 7,108 ±3,091 words/day. There was a main effect of talker gender, with greater exposure to female speech than male speech, and a significant interaction between talker gender and group. Fetuses had less exposure to TV/electronic sounds (1.3±0.6 vs. 5.1±2.5 hr/day) and airborne noise (2.9±2.8 vs. 4.4±2.1 hr/day) than VPT infants. Additionally, whereas fetuses never experience silence, owing to the presence of mother's heartbeat and other biological noise in utero, VPT infants spent 4.7 ±3.9 hr/day in silence. Finally, language and total extrauterine sound exposure for fetuses showed an expected marked circadian pattern, with low exposure during nighttime hours, whereas VPT infants showed significantly less change across the 24-hr cycle.

Conclusions: Our findings provide the first characterization of auditory exposures for typically developing fetuses. Although it has been presumed that language exposure for NICU infants is low, typical language exposure for the equivalent stage of brain maturation has been unknown. Our data indicate VPT infants incur a deficit of hundreds of hours of language exposure over the course of the prenatal/preterm period. Given the previously demonstrated effects of prenatal language exposure on neurobehavioral outcomes, this magnitude of deficit is alarming. Our data provide meaningful targets for interventions designed to optimize auditory exposures in NICU settings.

5. Sensitive Indicators of Childhood Listening Difficulties: Overview of 4 Year Longitudinal Study
Category: Development: Human Subjects
David Moore*[1], Lisa Hunter[2], Listening Lab[1]
[1]*Cincinnati Children's Hospital*, [2]*Cincinnati Children's Hospital*

Background: Scientific study of the role played by hearing in childhood learning disorders has a long history. Today, hearing is usually excluded as a root cause of language, speech, attention, intellectual, and autism disorders because most children with these disorders have "normal hearing". However, understanding of what constitutes normal hearing is changing rapidly. For example, people of all ages may have difficulty hearing speech in challenging environments despite normal pure tone audiograms. Here, we test the general hypothesis that sensitized measures of childhood hearing and listening difficulties (LiD) predict a wide range of disorders.
Methods: We examined ear, brainstem and cortical physiology, and auditory and cognitive performance in children (n=132; 6-13y.o. at enrollment) with 'normal' sensitivity (0.25-8kHz) across 3 assessments (Waves, W) over 4 years. Based on caregiver report (ECLiPS), individual children were assigned a Total Listening Score (TLS) and, by design, divided into age-matched typically developing (TD) and LiD groups.
Results: For W1, TLS scores correlated strongly (r=0.8) with a benchmark Children's Communication Checklist. Extended high frequency hearing was slightly less sensitive in the LiD group (p<0.05). Auditory brainstem responses and middle ear muscle reflexes were enhanced in the LiD group (p<0.05). Speech reception thresholds in distracting sentences (LiSN-S) were overall poorer in LiD group, but spatial hearing did not differ between groups. Fluid and crystallized reasoning was impaired in LiD group (p<0.001). Resting state connectivity (rsfMRI) was reduced between speech-related cortical nodes in LiD group, but did not differ across groups between non-speech, sound nodes. Across all kinds of sound processing and in both groups, connectivity was stronger in the older than in the younger children. Among known speech processing pathways, connectivity between the thalamus and cortex was also weaker in the LiD group. However, the left cortex was more highly connected for speech processing in both groups. All behavioral indices of hearing continued to show these patterns of differences across W2 and W3.
Conclusions: Distinct patterns of differences between groups were found from the periphery to the cortex. Overall, results support the hypothesis that LiD in children with sensitive hearing predict a range of other documented developmental problems. Novel physiological findings were speech-specific impairments in neural pathways, including widespread cortico-cortical and sub-cortical connections. Surprisingly, we found enhanced activation of brainstem circuitry in children with LiD, suggesting possible disinhibition of efferent pathways. The widespread and longitudinally persistent nature of listening problems, and their intimate connection with speech processing, suggests that auditory perceptual mechanisms contribute to normal development and maintenance of speech and language, and possibly to cortical circuits underlying other developmental disorders. We are currently analyzing auditory data for age-matched children with high functioning autism using a similar data acquisition and processing pipeline to the one described here.

6. Development of Attentive Tracking of Sound Sources
Category: Development: Human Subjects

Axelle Calcus*[1], Elena Benocci[1]
[1]*Université Libre de Bruxelles*

Background: From lively playgrounds to busy classrooms, children communication usually happens in noisy settings. Perceiving speech in noisy is a complex task that requires an adequate combination of sensory perception and cognitive processing. In spite of their functionally mature auditory system, school-age children's perception of speech in noise remains poorer than adults'. The main aim of this study was to better understand the mechanisms underlying this protracted auditory development. In particular, we focused on auditory selective attention and its relationship with speech perception in noise throughout development.
Methods: Participants were included in one of three groups based on their age: 8-11 years (n = 31); 12-15 years (n = 38); 16-19 years (n = 26). Children were presented a selective attention task (Woods and McDermott, 2015) as well as several speech perception tasks (in quiet and in noise). Testing took place online.
Results: Results of the selective attention task revealed a significant developmental effect: the youngest children were consistently poorer than both groups of older children. Although all three age groups performed similarly at perceiving speech in quiet, the youngest group was significantly poorer than both groups of older children in noisy conditions. Interestingly, across all children, there was a significant relationship between stream segregation and speech perception in noise.
Conclusions: This is in line with previous studies showing that auditory scene analysis relies on selective auditory attention, an ability that develops until late childhood.

7. Eye-Movement and Hand-Pointing Can Both Reveal the Cue Saliency Rule in Multisensory Spatial Localization
Category: Binaural Hearing and Sound Localization

Colton Clayton*[1], Yi Zhou[1]
[1]*Arizona State University*

Background: In the real-world, environmental objects are often both seen and heard. Visual information provides a frame of reference to guide action. Visual stimuli (Pick et al., 1969) and the visual environment itself (Platt and Warren, 1972) can both influence the accuracy and variability of sound source localization, but in different ways. Our previous work showed that uncertainty in auditory-alone localization led to increased visual bias in auditory-visual localization for both normal-hearing and hearing-impaired individuals (Montagne and Zhou, 2016; Venskytis et al. 2019). In these studies, responses were registered through button pushing on a graphic user interface (GUI) – this method requires rescaling a perceived sound direction to the button layout, which is not a natural sensorimotor response in multisensory tasks. To address this concern, the present study measured eye saccades, a natural orienting behavior, in response to auditory and visual stimuli similar to those presented in our previous work. The experiments were designed to evaluate both the effects of visual stimuli and visual environment on sound source localization.

Methods: Experiments used level- and timing-based stereophony to render brief 15-ms noise bursts in a horizontal range of ±30°. Our lab previously demonstrated that uncertainty in auditory-alone responses and visual bias are greater in response to timing-based manipulations than to level-based manipulations (Zhou et al. 2018). Two visual environmental texture effects were evaluated: (1) No visual references and (2) Seven square (1 in 2) visual references placed 5° apart and spanning ±15°. Listeners were tasked with localizing a sound source by making a saccadic eye movement toward the perceived sound source location. We measured the accuracy of responses, compared responses between timing- and level-based stereo conditions, and evaluated the changes in response when a sound stimulus was accompanied by an LED light presented at +/- 8°. We also compared response accuracy between the two visual environments. We tested twelve normal-hearing (NH) listeners in the no reference environment and ten NH listeners in the reference environment; six of these completed the study in both environments.

Results: Results from saccade responses confirmed our previous findings based on button-pushing responses. That is, visual bias is significantly correlated with the uncertainty in auditory-alone responses. However, the between and within-subject variability were both significantly reduced for the eye-localization condition compared to the hand-localization condition. The visual environment also impacted the performance, with the no-reference visual environment resulting in increased response variability relative to the reference environment.

Conclusions: Simultaneously presented visual stimuli and the texture of the visual environment both have a significant influence on eye saccade responses to auditory targets, influencing both the accuracy and the variability of responses, especially for less salient auditory stimuli.

8. WITHDRAWN

12:00 p.m. - 2:00 p.m.
Symposium #10

Neural Basis of Auditory Decision-Making
Chair: Justin Yao, *New York University*
Co-Chair: Yale Cohen, *University of Pennsylvania School of Medicine*

Auditory decision-making involves the brain's interpretation of information within and across discrete stimuli to detect, discriminate, or identify their source or content. Consequently, it is a principal outcome of the neural computations underlying auditory-object perception. Despite a great deal of scientific energy, we still do not have a fundamental understanding of the underlying principles and neurocomputational processes that subserve auditory decision-making. Recent evidence from studies that simultaneously measure neural activity from many individual neurons during task performance emphasize a critical role for local computations during sound-driven decision-making (e.g., Runyan et al., 2017; Francis et al., 2018). However, in addition to knowing where these computations occur, it is vital to know when they occur (e.g., Tsunada et al., 2019; Napoli, Camalier et al., 2021). Our goals for this symposium is to highlight and synthesize studies of auditory perceptual decision-making across

multiple spatial and temporal scales. Thus, we will examine the neurocomputational processes of auditory perceptual decision-making at multiple levels (local and across brain regions), in a variety of organisms (rodents, nonhuman primates, and humans), and across sensory contexts (auditory and audiovisual cues). We have assembled a diverse array of speakers (some of whom have never given an oral presentation at ARO) with the aim of engaging a broad audience, including those interested in sensory-driven decision-making, auditory perception, auditory encoding, computational theory, and psychophysics.

Correlates of Auditory Decision Making in Prefrontal, Auditory, and Basal Lateral Amygdala Cortical Areas
Bruno Averbeck, *National Institutes of Health, NIMH*

We developed a spatial selective detection paradigm for monkeys and recorded activity in primary auditory cortex (AC), dorsolateral prefrontal cortex (dlPFC) and the amygdala (BLA). We found that AC encoded cues and targets before dlPFC and BLA. AC also encoded choices before dlPFC and around the time of BLA. BLA showed robust activity only during choices. Similarities between AC and dlPFC were abolished during passive sensory stimulation, suggesting that sensory encoding in dlPFC is contextually gated. Thus, AC neural activity represents sensory and decision information before dlPFC and BLA does not appear to be robustly involved in selective spatial processing.

Neural Coding of Task-Related Information in Auditory Cortex During Pure-Tone Frequency Discrimination
Nikolas Francis, *University of Maryland*

Functionally connected neuronal networks in auditory cortex encode behavioral choice during the performance of a pure-tone detection task. However, naturalistic auditory tasks often include non-target sounds and delayed behavioral responses that engage auditory short-term memory. To investigate how these additional complexities of task performance affect neural coding of auditory decision-making, we imaged neuronal activity in auditory cortex of mice performing pure-tone frequency discrimination tasks, both with and without a short-term memory component. Here, we will discuss how populations of functionally connected neurons encode auditory task-related information about sound and behavioral choice during task performance.

Temporal and Spatial Scales of Population Codes in Auditory and Parietal Cortex
Caroline Runyan, *Univ. of Pittsburgh*

The cortex represents information across widely varying timescales. For instance, auditory cortex (AC) encodes stimuli that fluctuate over milliseconds, whereas in posterior parietal cortex (PPC), behavioral choices can require the maintenance of information over seconds. I will discuss our recent findings, that the temporal properties of population codes differ fundamentally between sensory and association cortices, as well as ongoing work in the lab to compare the function of inhibitory microcircuits and their contributions to the population activity dynamics in AC and PPC.

Excitatory and Inhibitory Subnetworks Are Equally Selective During Audiovisual Decision-Making
Farzaneh Najafi, *Allen Institute for Brain Science*

Inhibitory neurons, which play a critical role in decision-making models, are often simplified as a single pool of non-selective neurons lacking connection specificity. The selectivity of excitatory and inhibitory neurons within decision circuits, hence, the validity of decision-making models is unknown. We simultaneously measured excitatory and inhibitory neurons in the posterior parietal cortex of mice judging audiovisual stimuli. Surprisingly, both cell types were equally selective for the animal's choice and exhibited similar changes in selectivity during learning. These observations, combined with modeling, argue for selective subnetworks of excitatory and inhibitory neurons that are shaped by experience to support decision-making.

Delineating Between Multisensory Binding and Integration to Better Understand Perceptual Decision-Making Processes
Adrian KC Lee, *University of Washington*

Our sensory world is inherently multisensory. There are many ways to leverage cross-modal information and combining information across sensory modalities can be particularly beneficial in low signal-to-noise environments. Integration and binding are two concepts related to how information is combined in sensory domains. The two concepts are relatively well delineated in audition, yet, they are used interchangeably in the multisensory literature. In this talk, we will explore how sorting out these concepts may potentially help us to better understand multisensory decision-making processes.

Flexibility of Timescales of Auditory Evidence Evaluation for Decision Making

Tim Hanks, *University of California, Davis*

Auditory signals provide critical information for guiding behavior. Auditory processing often relies on the evaluation of information across time, with the optimal timescale depending on the situation. Therefore, determining how auditory information from multiple timescales is used for these purposes is critical for understanding hearing. Our central goal is to understand the neural and computational basis for how the brain evaluates information across multiple timescales to guide behavior. I will discuss our work towards this goal that connects experimental approaches using shared paradigms in rodents and humans with methods that include psychophysics, high density neural recordings, optogenetics, and computational modeling.

Flexibility in Auditory Decision-Making

Joshua Gold, *University of Pennsylvania*

Decisions about the presence, identity, or source location of auditory stimuli require the brain to flexibly balance the relative contributions of expectations and evidence. I will first describe computational and behavioral work showing how this balance should be, and is, controlled to facilitate our ability to make flexible and effective decisions under a broad range of conditions. I will then describe ongoing work examining neural substrates of flexible decision-making, focusing on multiple auditory and prefrontal brain regions that combine learned expectations with incoming sensory information to form categorical judgments that guide behavior.

12:00 p.m. - 2:00 p.m.
Podium Session #11 – Inner Ear Therapeutics
Moderators: Wade Chien, M.D. & Gwen Géléoc Ph.D.

1. DFNA9 Dominant Coch p.A449T in a New Humanized Mouse Model: CRISPR-Cas9 Mediated Targeting of the Pathogenic Variant for Gene Therapy

Category: Genetics A: Genomics and Gene Regulation

Hila Romi[1], Nahid Robertson[2], Sarath Vijayakumar[3], Channabasavaiah Gurumurthy[4], Benjamin Kleinstiver[5], Richard Sherwood[6], Maryna Ivanchenko[7], Kevin Booth[7], David Corey[7], Cynthia Morton*[8]
[1]*Brigham and Womens Hospital, Harvard Medical School, Boston,* [2]*Brigham and Women's Hospital,* [3]*Creighton University School of Medicine,* [4]*University of Nebraska Medical Center,* [5]*Massachusetts General Hospital, Harvard Medical School,* [6]*Brigham and Women's Hospital, Harvard Medical School,* [7]*Harvard Medical School,* [8]*Brigham and Women's Hospital, Harvard Medical School, University of Manchester*

Background: Dominant gain-of-function mutations in COCH cause DFNA9, an adult-onset progressive sensorineural hearing loss and vestibular disorder. Individuals with the p.A449T variant (in the vWFA domain) display an early-onset hearing loss, typically in the first to second decade of life, compared with individuals with mutations in the LCCL domain. Specific disruption of the p.A449T dominant mutation using CRISPR-Cas9 could keep the normal allele intact and might rescue hearing and balance.
Methods: We first generated a fibroblast cell line using a skin biopsy from an individual with the COCH p.A449T variant. We then wanted to determine if disruption of the pathogenic variant could rescue hearing in vivo. Using EasiCRISPR, we created a humanized mouse model in CBA/J with the COCH p.A449T mutation and flanking human sequence to match the gRNAs targeting the human mutation. This is the first mouse model of a Coch mutation in the vWFA domain and it allows testing rescue at earlier time points.
Results: We transfected cells with a plasmid carrying SaCas9-KKH and a guide RNA targeting A449T, and assessed editing with next-gen sequencing. The Cas9 successfully created indel disruption only in the variant

allele, with an editing efficiency of 43% and with no disruption of the normal allele. Auditory brainstem response (ABR) as well as distortion product otoacoustic emission (DPOAE) analyses of 9-month-old mice showed significantly elevated thresholds for both CochA449T/+ (heterozygous) and CochA449T/A449T (homozygous) mice, as compared to their Coch+/+ (wild-type) littermates, at all tested frequencies. There was no significant difference between the heterozygotes and homozygotes, reflecting the dominant nature of the mutation. Vestibular sensory evoked potentials (VsEP) were measured at 8-10 months of age and thresholds were significantly elevated for heterozygous and homozygous mice compared to the wild-type littermate controls. Two of the homozygous mutants had absent responses. Coch p.A449T mice showed severe swimming abnormalities: both heterozygous and homozygous mutants exhibited underwater tumbling, with homozygous mutants performing much worse than heterozygous animals. Mice at younger ages are currently being evaluated. We now have an appropriate animal model. The next step is to perform round window micro-injection of an AAV encoding CRISPR-Cas9 and guide RNA, and to assess phenotype rescue.

Conclusions: We have shown successful targeting of a COCH pathogenic variant using a CRISPR-Cas9 mediated approach in vitro and are poised to test rescue in a novel mouse model in vivo. If successful, this strategy is expected to inform gene therapy in other autosomal dominant HL disorders, the majority of which are due to missense pathogenic variants rather than truncation or loss of gene function. Notably, these AD disorders are often characterized by late-onset presentation, providing a window of opportunity for therapeutic intervention.

2. High-Efficiency Editing of CRISPR/Cas9 in Hair Cells Improved Hearing in a Dominant Deafness Mouse Model
Category: Genetics A: Genomics and Gene Regulation

Chong Cui*[1], Daqi Wang[1], Bowei Huang[1], Jun Lv[1], Yuxin Chen[1], Fang Wang[1], Genglin Li[1], Huawei Li[1], Yilai Shu[1]
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Background: Pathogenic mutations in KCNQ4, a voltage-gated potassium channel protein essential for auditory function, cause non-syndromic autosomal dominant deafness-2 (DFNA2), but there are no effective clinical drugs to treat KCNQ4-induced deafness. CRISPR/Cas9 technology has shown great potential in the prevention and treatment of deafness, but there is no means to accurately detect its editing efficiency in hair cells. In this study, we propose to explore precise gene therapy protocols to improve the hearing function of Kcnq4G229D/+ mice that mimics KCNQ4 mutation (p.G228D) in deaf patients; furthermore, we intend to establish a method that can accurately detect the editing efficiency of CRISPR/Cas9 in hair cells.

Methods: The sgRNA was designed for the Kcnq4G229D/+ mouse model, and plasmids expressing SaCas9-KKH and sgRNA were transfected into skin fibroblasts of neonatal mice in vitro, and the editing efficiency was detected by next-generation sequencing (NGS). A novel adeno-associated virus variant, AAV-PHP.eB, packaged with SaCas9-KKH-gRNA agent was microinjected into the inner ear of mice and auditory function and sensory cell characteristics were assessed by ABR, DPOAE and immunohistochemistry. Atoh1-GFP/Kcnq4G229D/+ mice with GFP specifically labeled hair cells were injected with the treatment agent, and GFP-positive cells in the inner ear were sorted and then the editing efficiency was analyzed by NGS.

Results: Four sgRNAs were designed for the Kcnq4G229D site and screened in vitro, and efficient and specific sgRNA3 was obtained. After injecting AAV-SaCas9-KKH-gRNA3 into the inner ear of Atoh1-GFP/Kcnq4G229D/+ mice, the editing efficiency was found to be up to 54.2% (mean 34.1%) for the sorted GFP-positive hair cells, which accurately reflected the editing of hair cells by the therapeutic agent. The ABR showed a reduction in hearing thresholds in the injected ear compared to the un-injected ear at 8 weeks and 12 weeks post-injection, and hearing improvement lasted up to 6 months in some mice; lower DPOAE thresholds, shorter ABR I-wave latencies, higher I-wave amplitudes and increased surviving hair cells were also observed in the treated ear compared to the untreated ear.

Conclusions: It is the first study to accurately assess the editing efficiency of the CRISPR/Cas9 system in cochlear hair cells and demonstrate that mutated genes can be efficiently and specifically knocked out with the CRISPR/Cas9 technology, which provides a strong support for the application of CRISPR/Cas9 in gene therapy for hearing loss. The hearing of Kcnq4G229D/+ mice can be effectively and safely improved by AAV-KCNQ4-SaCas9-gRNA therapeutic agents, which provide a new strategy for the treatment of deafness caused by KCNQ4 mutations, and further provide theoretical and scientific evidence for gene editing technology to treat hereditary deafness.

3. Selection of Viral Capsids and Promoters Impacts Rescue Efficacy of TMPRSS3-Deficient Cochlea

Category: Inner Ear: Drug Delivery
Ksenia Aaron*[1], Katja Pekrun[1], Ina Lee[1], Yasmin Eltawil[1], Patrick Atkinson[1], Sara Billings[1], Yuan-Siao Chen[2], Rick Nelson[2], Mark Kay[1], Alan Cheng[1]
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Background: Transmembrane protease/serine3 (TMPRSS3) is expressed in multiple sensory and non-sensory cell types in the cochlea and is critical for hearing. Thus, hearing loss may result from degeneration of multiple cell types, making this an ideal model to study the application of gene therapy on a multicellular level. Exogenous gene transfer has been shown to rescue numerous mouse models of hearing loss. However, whether the level of gene expression affects cytotoxicity and overall efficacy within the inner ear is unknown. Here, we examine the effects of using different recombinant adeno-associated virus (rAAV)s and promoters to deliver Tmprss3.
Methods: Tmprss3tm1Lex, heterozygous, and wildtype littermates were used. Temporal bones were harvested at postnatal day (P)1-120. Auditory brainstem responses (ABR) were evaluated at P21-120. Capsids KP1 and DJ were used to package expression cassettes into AAV particles. We expressed tdTomato and Tmprss3 driven by the CAG or EF1□ promoter. Recombinant AAVs were used to transduce 293T/17 and HeLa cells and cytotoxicity was assessed. rAAVs were delivered in vivo via a posterior semicircular canalostomy to P1 mice. Cochleae were stained for Myo7a, Sox2, and Tuj1 and labeled cells quantified. In situ was performed using RNAscope protocols.
Results: Tmprss3 mRNA is expressed in both hair cell and supporting cell subtypes at P5. Tmprss3tm1Lex mice display rapid and severe hair cell degeneration at P14, no detectable ABR at P21, and delayed loss of spiral ganglion neurons at P120. When examined at P21, rAAV-KP1-CAG-tdTomato transduces hair cells and supporting cells with high efficiency >88.2%, whereas rAAV-DJ-CAG-tdTomato transduces 26.1-65.6% of hair cells and supporting cells. rAAV-KP1-CAG-Tmprss3 caused severe cytotoxicity in vitro and in vivo in wildtype cochleae and failed to prevent hair cell degeneration or auditory dysfunction in Tmprss3tm1Lex cochlea. Reducing titers or using rAAV-DJ1 diminished cytotoxicity in vitro and in wildtype cochlea in vivo, but failed to rescue cell loss or dysfunction of mutant cochlea. Finally, the use of rAAV-KP1-EF1α-Tmprss3 abolished cytotoxicity in vitro and in vivo, and partially prevented hair cell degeneration, auditory threshold shifts, and delayed loss of spiral ganglion neurons in Tmprss3tm1Lex mutant mice.
Conclusions: Tmprss3 deficiency causes hair cell degeneration, hearing loss, and progressive loss of spiral ganglion neuron. AAV-KP1 capsid has high transduction of hair cells and supporting cells. Transgene expression of Tmprss3 under CAG promoter is cytotoxic in vitro and in vivo and a relatively weaker EF1α promoter is able to drive expression of Tmprss3, while preventing hair cell degeneration. Our study highlights the potential for exogenous transgene toxicity and serves as a proof-of-concept that the use of optimal promoters to calibrate exogenous gene expression can optimize gene therapy in the inner ear.

4. Tailoring Regulatory Elements in Gene Therapies for Hearing Loss

Category: Inner Ear: Drug Delivery
Danielle R. Lenz*[1], Hao Chiang[1], Yukako Asai[1], Shimon Francis[1], Enping Qu[1], Priya Bansode[1], Katherine D. Gribble[1], Richard Churchill[1], Francesc Puig-Basagoiti[1], Yuanzhao Darcy[1], Bifeng Pan[1], Junaid Syed[1], Pascal Schamber[1], Eva Andres-Mateos[1], Michelle D. Valero[1], Emmanuel J. Simons[1], Gregory S. Robinson[1], Robert Ng[1]
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Background: In the development of AAV gene therapy, we aim to generate a safe and efficacious therapeutic vector driving transgene expression in cells where a gene is normally expressed and avoiding ectopic expression in non-target cells. We and others have previously demonstrated that despite broader ectopic delivery, AAVAnc80-hOTOF, under the control of a ubiquitous promoter, leads to significant preferential expression of hOTOF in the inner hair cells (IHC), where otoferlin is normally expressed. This spatially restricted expression of ubiquitous promoter-driven transgene has been observed for other genes, such as VGLUT3.
It was recently demonstrated that delivery of AAVAnc80-hGJB2 under the control of a ubiquitous promoter resulted in robust expression in supporting cells. However, hair cell degeneration was observed, suggesting the potential need for spatial regulation for GJB2 gene therapy. In recognition of this transgene-dependent need, we have undertaken two parallel approaches: (1) extensive characterization of AAVAnc80-hOTOF, which uses a ubiquitous promoter, in target and non-target cells; and (2) a targeted fit-for-purpose approach for other transgenes to generate cell-selective regulatory sequences for applications where this is warranted.
Methods: To explore expression of AAVAnc80-hOTOF, we have administered AAVAnc80-hOTOF.FLAG intracochlearly to mice and NHPs and compared transgene expression to native OTOF expression, using immunohistochemical analysis. For expression of transgenes other than OTOF, some of which may benefit from

spatial regulation of expression, we have initiated a targeted approach to identify cell-selective promoters and regulatory elements for various cochlear cell types. Using RNA-Sequencing data and available NGS datasets, we identified groups of genes that are normally expressed in the cells of interest. We then utilized an integrated in silico approach to identify regulatory sequences of these gene groups and constructed promoter sequences that can be evaluated using Anc80 vectors.

Results: The CAG ubiquitous promoter used to drive expression of hOTOF.FLAG clearly enabled robust expression of OTOF in IHCs, while no OTOF expression was detected in non-target cells. For the selective promoters evaluated, for conditions other than OTOF-mediated hearing loss that may benefit from spatial regulation of expression, consistent mRNA and protein transgene expression was detected for all regulatory sequences in vitro, and evaluation in cochlear explants further enabled the identification of regulatory sequences that promoted selective gene expression in different cell types.

Conclusions: Ubiquitous promoters have been demonstrated to provide gene expression necessary for effective gene therapy, with cell selectivity achieved endogenously and without the need for additional regulatory elements. However, for some applications, cell-selective promoters may be preferred. We identified regulatory elements that drive selective gene expression in cells of the cochlea. Future in vivo evaluation will guide the selection of regulatory sequences for selective, physiologically relevant expression, for applications in which spatial regulation of expression is preferred.

5. Demonstration of Secreted Protein Expression Levels Following Intracochlear Delivery of AK-antiVEGF (AAVAnc80-AntiVEGF Vector) Across Multiple Doses in Non-Human Primates
Category: Inner Ear: Drug Delivery

John Connelly[1], Francesc Puig-Basagoiti[1], Brian Lin[1], Ann E. Hickox[1], Timothy Boyd[1], Ignacio Navas Enamorado[1], Shimon Francis[1], Ivy K. Hughes[1], Christopher A. Shera[2], Eric D. Horowitz[1], Kathleen Lennon[1], Jean Phillips[1], Jenna Soper[1], Michelle D. Valero*[1], Jennifer A. Wellman[1], Gregory S. Robinson[1], Emmanuel J. Simons[1], William F. Sewell[3], Michael J. McKenna[1], Eva Andres-Mateos[1]
[1]*Akouos, Inc., Boston, MA,* [2]*Consultant to Akouos, Inc.,* [3]*Co-founder and consultant to Akouos, Inc.*

Background: AK-antiVEGF (AAVAnc80-antiVEGF) is a gene therapy candidate in preclinical development. AK-antiVEGF is designed for the potential treatment of patients with vestibular schwannomas (VS), or benign tumors that originate from the cells surrounding the vestibulocochlear nerve within the internal auditory canal. Common symptoms associated with VS include hearing loss, tinnitus, and dizziness; as tumors continue to grow, they can compress the brainstem, representing a concern for more serious morbidity and, in very rare cases, mortality. Current interventional options are invasive alternatives such as surgical resection and/or radiation therapy, which can cause significant morbidity (e.g., facial paralysis and hearing loss). An alternative treatment approach using systemic Avastin® (bevacizumab) has been shown to decrease VS tumor size and improve hearing in neurofibromatosis type 2 (NF2) patients; however, long-term systemic administration of vascular endothelial growth factor (VEGF) inhibitors can be associated with significant safety concerns. Local delivery of AK-antiVEGF to the inner ear is intended to transduce cochlear and vestibular cells, resulting in secreted anti-VEGF protein into perilymph, an inner ear fluid that is in diffusional continuity with the interstitial and perineural spaces of the vestibulocochlear nerve where VS tumors are located. The limited systemic exposure of this approach has the potential to minimize adverse effects. Previously, we evaluated tolerability and exposure levels of anti-VEGF after administration of two different doses of AK-antiVEGF via bilateral intracochlear administration in non-human primates over 2-month and 6-month follow-up periods. This prior study showed tolerability and biologically active levels of anti-VEGF in the perilymph samples collected. Here we evaluated expression of anti-VEGF protein in systemic fluids and tissue at increasing doses of AK-antiVEGF.

Methods: We administered four different intracochlear doses of AK-antiVEGF bilaterally, and we evaluated the resulting anti-VEGF protein expression in perilymph and other fluids and tissue after a 2-month follow-up period. We also performed computational modeling of anti-VEGF protein diffusion as a function of distance from the inner ear to the location in the internal auditory canal where VS tumors typically originate.

Results: We show anti-VEGF protein detection at biologically active levels in perilymph fluid at all dose levels. The modeling predicts that anti-VEGF protein concentrations measured in perilymph, once diffused to the tumor site, are expected to remain within a predicted therapeutically relevant range at all dose levels evaluated, supporting the feasibility of the intended clinical route of administration for AK-antiVEGF.

Conclusions: Expression of anti-VEGF protein following intracochlear administration of AK-antiVEGF in NHP is observed across all dose levels evaluated. These anti-VEGF protein expression data in a translationally relevant

animal model, taken together with the computational modeling, provide support for the future clinical development of AK-antiVEGF for the treatment of VS.

6. Dual-AAV PCDH15 Gene Therapy for Usher Syndrome Type 1F Deafness and Blindness
Category: Inner Ear: Drug Delivery

Maryna Ivanchenko*[1], Eric Mulhall[1], Daniel Hathaway[1], Mantian Wang[2], Alex Klein[1], Yaqiao Li[1], Xudong Wu[1], Cole Peters[1], Kevin Booth[1], Bence Gyorgy[2], David Corey[1]
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Background: Usher syndrome type 1F is caused by mutations in the PCDH15 gene, which encodes the tip-link protein PCDH15. It is a recessively inherited syndrome characterized by profound congenital deafness and absence of vestibular function, and by progressive blindness beginning in the second decade. Gene addition therapy could be an attractive treatment; however, the PCDH15 coding sequence of ~5.8 kb is too large to fit into a single AAV capsid. We used a dual-AAV strategy to circumvent the size limitation and to treat the deafness in an Usher 1F mouse model.

Methods: We engineered two vectors that each encode part of the full-length protein. In a cell, AAV genomes can recombine to create a full-length coding sequence. We first assessed function in vitro. We treated HEK cells with dual AAVs and assessed recombination and translation using RT-PCR and immunofluorescence microscopy. To evaluate function in cochlea in vivo, Pcdh15 conditional knockout mice were injected with dual AAVs at P1 through the round window membrane. Hearing tests and histological analyses were performed at P30. To assess the potential of dual-AAV-mediated gene expression in human retina in vitro, we transduced retinal organoids from human iPSCs with dual AAVs and five weeks later evaluated HA-tagged PCDH15 expression and localization with immunofluorescence and immunogold SEM imaging.

Results: In HEK cells in vitro, full length PCDH15 was successfully produced using dual-AAV delivery. Proper recombination and splicing were confirmed with RT-PCR and Sanger sequencing. With an anti-PCDH15 antibody, strong labeling at HEK cell membranes was observed.

We found that Pcdh15 conditional knockout mice, injected at P1 through the round window membrane with dual AAVs encoding HA-tagged PCDH15, displayed HA immunoreactivity at the tips of stereocilia, four weeks after injection. They also showed robust rescue of hair bundle morphology observed by both actin labeling and SEM, and rescue of mechanotransduction assessed with FM1-43 loading. While uninjected Pcdh15 conditional knockout mice were deaf and had degenerated hair bundles, mice treated with dual AAVs encoding PCDH15 demonstrated good hearing rescue at low and middle frequencies.

We also characterized the ultrastructure of photoreceptors in retinal organoids. SEM showed that a majority of photoreceptors developed inner segments, while some formed outer segments and connecting cilia. We observed nascent calycal processes at the apical ends of inner segments. Retinal organoids from human iPSCs transduced with dual AAVs encoding HA-tagged PCDH15 showed antibody labeling of the HA-tag in photoreceptors, which was localized on the surface of the inner segments and at the inner/outer-segment junction where calyceal processes develop.

Conclusions: Dual-AAV delivery of PCDH15 mostly restores hearing in a mouse model of Usher 1F, and mediates expression and normal localization of PCDH15 in human photoreceptors in vitro. It holds promise for treatment of Usher 1F.

7. Development of an AAV-Based Gene Therapy for Children With Congenital Hearing Loss Due to Otoferlin Deficiency (DB-OTO)
Category: Inner Ear: Drug Delivery

Adam Palermo[1], Xichun Zhang[1], Arun Senapati[1], Joseph Goodliffe[1], Orion Keifer[1], Tera Quigley[1], Pete Weber[1], Eduardo Corrales[2], Kathy S. So[1], Sarah Cancelarich[3], Danielle Velez[3], Tyler Gibson[1], Lars Becker[1], Ning Pan[1], Leah Sabin[3], Meghan Drummond[3], Jonathon Whitton*[1]
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Background: Otoferlin is a calcium sensor protein expressed in the inner hair cells and is important for proper synaptic transmission between inner hair cells and the afferent fibers of the spiral ganglion. Biallelic loss of function mutations in the OTOF gene lead to congenital severe-to-profound auditory neuropathy in both humans and in mice. These mutations are believed to be causal in 2-3% of individuals born with hearing loss. Infants with biallelic OTOF mutations are currently managed with assistive devices, but several groups are developing AAV-based gene therapies to address this population.

Methods: We have developed DB-OTO, an Adeno-Associated Virus (AAV)-based gene transfer therapy for rescue of hearing in Otoferlin deficiency. DB-OTO expresses a corrected OTOF cDNA from a hair cell-specific Myo15 promoter and is delivered using an AAV1-based capsid. Because human OTOF cDNA exceeds the packaging capacity of a standard AAV, DB-OTO uses a dual AAV system to reconstitute the full-length OTOF coding sequence.

Results: We have previously shown that DB-OTO can rescue hearing function in OTOFQ828X/Q828X mutant mice as measured by ABR, that it can be successfully delivered to the primate ear via RW infusion with vestibular fenestration, and that using a cell-specific promoter is key to its function. Here, in preparation for the initiation of clinical studies, we further characterized the dose translatability of DB-OTO between mice and non-human primates. To better characterize the dose-response of DB-OTO, we dosed mice over a >10X dose range and evaluated their hearing recovery over several months. We found that at higher doses, tone-burst response in previously deaf OTOFQ828X/Q828X mice extends to the apex of the cochlea, whereas with low doses, the best recovery is seen around 22 kHz. We also treated OTOFwt/wt mice to characterize the tolerability of DB-OTO over the same dose range. Using RT-PCR, we showed that DB-OTO expression increases continuously for the first several weeks after dosing in mice, consistent with functional recovery. In parallel, we evaluated the expression timing in non-human primates using the same assay. There, the expression appeared to plateau in a comparable timeframe following dosing. Using this assay, DB-OTO expression levels at plateau in mice and primates were compared and used to confirm prior assumptions about dose translation based on expression from surrogate vectors. Together, these data support and inform our plans for clinical translation of DB-OTO.

Conclusions: DB-OTO is a promising emerging therapeutic for genetic hearing loss and has the potential to provide the first clinical proof-of-concept for gene therapy in the inner ear.

8. Supraparticle-Mediated Neurotrophin 3 Delivery Preserves Drug Bioactivity and Promotes Its Retention in the Guinea Pig Cochlea
Category: Inner Ear: Drug Delivery

Niliksha Gunewardene*[1], Patrick Lam[1], Yutian Ma[2], Yingjie Hu[2], Frank Caruso[2], Sherryl Wagstaff[1], Rachael Richardson[1], Andrew Wise[1]
[1]*Bionics Institute,* [2]*The University of Melbourne; Bionics Institute*

Background: Neurotrophins have long been established as a potential treatment for hearing loss, however its delivery into the inner ear at therapeutic levels over a sustained period has remained a challenge restricting clinical translation. We explored the potential of supraparticles for neurotrophin-3 (Ntf3) delivery into the inner ear. Supraparticles are compact spheroid structures composed of smaller colloidal particles that that provide a platform for long-term controlled release of therapeutics. We previously reported that supraparticle-mediated Ntf3 delivery significantly enhances spiral ganglion neuron (SGN) survival in the guinea pig cochlea. However, for clinical translation a thorough characterisation of the bioactivity and pharmacokinetics and of the supraparticle-released Ntf3 is required.

Methods: Protein integrity and bioactivity of supraparticle-eluted Ntf3 was evaluated at multiple timepoints in vitro using western blots, spiral ganglion neuron cultures and a NFAT beta-lactamase reporter cell line expressing human Trk-C, the Ntf3 receptor. Unpackaged Ntf3 was used as the control. Drug pharmacokinetics and biodistribution was evaluated by labelling Ntf3 with a radioactive tracer (iodine 125: 125I). The loading and elution profiles of the supraparticles were evaluated by obtaining gamma measurements from the supraparticles in vitro. Guinea pigs were subsequently implanted with 125INtf3 loaded supraparticles via the round window and intracochlear delivery routes. Gamma counts and autoradiography analyses of cochleae were performed to assess drug retention and biodistribution in the cochlea.

Results: Gamma measurements taken from 125Ntf3 loaded supraparticles revealed high drug loading (an average of 5.3 μg of Ntf3 loaded per supraparticle) and elution capacities in vitro (67% cumulative release over one month). Drug bioactivity testing using a Trk-C cell line assay showed maintained drug bioactivity of supraparticle-eluted Ntf3 after 1 month. Consistently, supraparticle-eluted Ntf3 improved SGN survival and neurite extension compared to the controls in vitro. Whole cochlea gamma measurements from supraparticle-implanted guinea pig cochleae harvested at various time points revealed detection of 125INtf3 in the cochlea after one month. Autoradiography analysis of cochlear micro-sections revealed widespread 25INtf3 distribution after intracochlear supraparticle delivery, but more restricted distribution with the round window delivery approach.

Conclusions: Supraparticles preserve drug bioactivity and support sustained, long-term release of neurotrophins in the inner ear. The methods applied here, and our findings can provide valuable information useful for the development of therapeutic treatments for hearing impairment.

12:00 p.m. - 2:00 p.m.
Podium Session #12 – Cochlear Implant Advances
Moderators: Julie Arenberg, Ph.D. & Karen Gordon, Ph.D.

1. BlurCAP: Investigating Changes in Current Spread With Focused and Blurred Stimulation in Cochlear Implant Users Using the Panoramic ECAP Method
Category: Auditory Prostheses

Charlotte Garcia*[1], Charlotte Morse-Fortier[2], Francois Guerit[1], Tobias Goehring[1], Robert P. Carlyon[1], Julie G. Arenberg[2]
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Background: The Panoramic ECAP (PECAP) Method uses Electrically-Evoked Compound Action-Potentials (ECAPs) to estimate the variation in current spread and neural health along the length of the electrode array in individual cochlear implant (CI) users. While PECAP's neural health estimate has previously been shown to correlate with focused thresholds and to identify localised areas of simulated reduced neural responsiveness (Garcia et al 2021, https://doi.org/10.1007/s10162-021-00795-2), the current-spread estimate has not been independently validated. In some CI devices it is possible to focus electrical stimulation by returning part of the delivered current to the adjacent electrodes (partial tripolar stimulation), as well as to blur it by stimulating multiple electrodes instead of one (Goehring et al 2020, https://doi.org/10.1007/s10162-020-00758-z). We applied these manipulations to evaluate the ability of PECAP to detect differences in current spread at the neurons.
Methods: ECAPs were recorded using the forward-masking artefact-cancellation technique from Advanced Bionics CI users in monopolar mode for all electrodes activated in the participant's map. Focused (partial tripolar, pTP) thresholds were also obtained for these electrodes. Two test electrodes were then selected that showed high and low focused thresholds. ECAPs were then obtained for every combination of masker and probe electrode in monopolar mode, and, for the test electrodes only, in pTP mode and with blurred stimulation in which 3 or 5 electrodes were stimulated simultaneously in phase. Stimuli for all presentation modes (monopolar, pTP, or blurred) was always at the same loudness level, as determined using a scaling procedure. Data were then submitted to PECAP, and the current spread estimate for the test electrodes was compared between the monopolar condition and the focused and blurred conditions. The magnitude of the effect of current manipulation was then compared across subjects between the electrodes that showed low focused thresholds compared to high focused thresholds.
Results: PECAP analysis applied to preliminary data from one pilot participant showed an increase in current spread at both test electrodes for both blurring conditions when compared to monopolar stimulation. Importantly, it did not reveal a change in estimated neural health at the test-electrode position, consistent with PECAP's ability to discriminate between changes in current spread and neural health. However, it did not show a difference in the estimated current spread for the pTP condition when compared to the monopolar stimulation for either electrode.
Conclusions: Complete results will provide evidence on the ability of the PECAP algorithm to discriminate between changes in current spread and in neural health. The data will also provide an objective measure of the extent to which differences in current spread are reflected in ECAP masking patterns, and test the hypothesis that focused stimulation is more effective at reducing the neural spread of excitation in electrodes with lower focused thresholds.

2. Deep Neural Networks for Decoding EEG Responses to the Speech Envelope
Category: Auditory Prostheses

Michael Thornton*[1], Tobias Reichenbach[2], Danilo Mandic[1]
[1]*Imperial College London,* [2]*Friedrich-Alexander-University Erlangen-Nürnberg*

Background: Humans with normal hearing are remarkably good at selectively attending to specific sounds in complex auditory scenes. It is now widely accepted that human selective attention to speech can be decoded from a listener's EEG recordings, and it is anticipated that advances in this field could translate into a neuro-steered hearing device that is capable of restoring auditory selectivity in patients with sensorineural hearing loss. However, such a practical application requires auditory attention decoding to work under different real-world

listening conditions, as well as with a high accuracy. Here we sought to investigate how the use of deep neural networks can help to address these challenges.

Methods: We focussed on reconstructing the envelope of the attended speech stream from a listener's EEG. We thereby compared the traditional method of linear filtering against the use of two distinct deep neural networks. The listening conditions in our dataset included clean speech in native and foreign languages; speech in background babble noise; and two competing speakers. We used an additional clean-speech dataset to pretrain listener-independent decoders.

Results: We found that the use of listener-specific deep neural networks considerably improved the clean-speech reconstruction accuracy, in comparison to traditional individualised linear methods. Even when listener-independent methods were used, the deep neural networks offered a significantly improved performance in comparison to the listener-independent linear method. We also found that listener-independent algorithms trained on clean speech generalised well to new listeners in different listening conditions, with the pre-trained DNNs achieving a significantly higher performance across all listening conditions except for the case of clean speech in babble noise (for which there was no significant difference between the reconstruction scores of the linear filters and the DNNs.)

Conclusions: Our results show that linear filters and DNNs can be trained to reconstruct the envelope of clean speech from EEG recordings and applied effectively when the listening conditions are noisy and/or the level of speech comprehension is low. Importantly, the deep neural networks were capable of generalising between listeners and listening conditions, even though such techniques are known to suffer from overfitting issues. Consequently, deep learning algorithms for auditory attention decoding offer prospects for real-world neuro-steered hearing devices.

3. Dexamethasone-Eluting Cochlear Implants Reduce Intracochlear Foreign Body Response Following Surgery

Category: Auditory Prostheses

Muhammad Rahman*[1], Brian Mostaert[2], Bryce Hunger[3], Peter Eckard[4], Cristina Garcia[3], Rong Zhuo Hua[3], Douglas Bennion[5], Jonathon Kirk[6], Keiko Hirose[7], Marlan Hansen[8]

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Background: In cases of sensorineural hearing loss, non-functional sensory cells can be bypassed by a cochlear implant (CI) to restore hearing which can dramatically improve the quality of lives of patients. The foreign body response (FBR) after implantation can result in fibrosis and reactive soft tissue that can negatively affect the outcome of CI. These reactions have been associated with increased electrode impedances, decreased battery life, further loss of acoustic hearing after initial hearing preservation, or in rare cases, device failure. This study aims at investigating the effect of dexamethasone, a well-established, potent anti-inflammatory glucocorticoid, on the intracochlear FBR after cochlear implantation in a murine model of CI.

Methods: 10-12-week-old CX3CR1+/GFP Thy1+/YFP mice on C57Bl6 background with normal hearing, were implanted with a 3-channel cochlear implant (dexamethasone-eluting or control implant) in the left ear via a round window insertion. The right ear served as the unoperated control ear. Implant functionality was tested with serial impedance and neural response telemetry (NRT) measurements. Programming for electrical stimulation was based on NRT. Starting from postoperative day 7, electrodes were stimulated for 5 hrs/day, 5 days/week up to 28 days with stimulation threshold 30 CL below NRT threshold and comfort level determined with behavioral response. Cochleae harvested at 10, 28 or 56 days post-operatively, fixed with 4% PFA, cryopreserved, were sectioned at30 μm parallel to the mid-modiolar plane, were labeled with antibody against α-Smooth Muscle Actin (α-SMA) to label myofibroblasts to quantify the fibrotic response. The outlines of scala tympani, Rosenthal canal, modiolus and lateral wall for each turn were traced manually to measure the volume of each and to quantify nuclei, and the density of CX3CR1+ macrophages, and spiral ganglion neurons (SGNs). The volume of α-SMA-positive fibrotic area was measured and the ratio of the volume of α-SMA+ area/volume of scala tympani calculated.

Results: At all times points, cochlear implantation caused infiltration of cells and CX3CR1+ macrophages into the cochlea. The inflammatory response is initially generalized and gradually becomes predominantly localized to the scala tympani of the basal region of the cochlea by 56 days after implantation. Fibrosis is seen in the scala tympani throughout the time investigated. Compared to cochleae implanted with standard arrays, cochleae implanted with

dexamethasone-eluting arrays showed significant reduction in the inflammatory response by reduced macrophage density and cellularity in scala tympani, Rosenthal's canal, the lateral wall and modiolus and reduced fibrosis in scala typmani.

Conclusions: Conclusion: Specimens implanted with dexamethasone-eluting electrodes displayed a reduce the intracochlear inflammatory response up to 9 weeks postoperatively, suggesting that anti-inflammatory reagents could be useful in preservation of the normal cochlear environment. This may further support long term preservation of cochlear anatomy and help to improve preservation of low frequency acoustic hearing after implant.

4. Neural Health Measures Are Correlated With Place Pitch Sensitivity of Cochlear Implant Users
Category: Auditory Prostheses
Niyazi Arslan*[1], Xin Luo[1]
[1]*Arizona State University*

Background: Most cochlear implant (CI) users show great progress in speech recognition after implantation, but there are also patients who receive limited benefits from the device. An important factor that may contribute to the variable CI outcomes is the health of spiral ganglion neurons (SGNs). Different neural health measures have been shown to be correlated with the SGN density in animal studies. However, in human CI users, these measures were not correlated with each other and may thus reflect different aspects of neural health. These measures also were not correlated with speech recognition performance. This study investigated whether neural health measures have stronger correlations with local pitch sensitivity than with speech recognition performance, due to less involvement of top-down processing in temporal/place pitch discrimination than in speech recognition.
Methods: Eight adults using the Advanced Bionics CI devices were tested with a total of 12 implanted ears. On five different electrodes from the base to the apex of each ear, polarity effect (PE), multipulse integration (MPI), and interphase gap (IPG) effect on the amplitude growth function (AGF) of electrically evoked compound action potential (ECAP) were measured to reflect different aspects of neural health, while thresholds of amplitude modulation frequency discrimination (AMFD) and virtual channel discrimination (VCD) were measured to indicate temporal and place pitch sensitivity. AzBio sentence recognition in noise was also measured using the clinical CI processor of each ear.
Results: There was a significant effect of electrode on AMFD and VCD. Based on post-hoc analyses, only VCD thresholds were significantly better on an apical electrode pair 4-5 than on a basal electrode pair 12-13. Different participants showed different patterns of PE, MPI, and IPG offset effect on ECAP AGF across electrodes, leading to no significant effect of electrode on these neural health measures. Across electrodes and ears, MPI was significantly correlated with PE and IPG offset effect on ECAP AGF. VCD was significantly correlated with AMFD. Importantly, VCD was also significantly correlated with IPG offset effect on ECAP AGF. For the mean values averaged across electrodes, only PE and IPG offset effect on ECAP AGF were significantly correlated across ears. AzBio sentence recognition scores were not correlated with neural health measures or temporal/place pitch discrimination thresholds across ears.
Conclusions: Neural health measures at different stimulation sites of individual CI users may better predict local pitch sensitivity than speech recognition performance. Specifically, the central axon demyelination reflected by IPG offset effect on ECAP AGF may play an important role in place pitch perception with CIs. Considering the importance of place pitch perception to speech recognition, IPG offset effect on ECAP AGF may be used as an objective measure to help customize the clinical programming of individual electrodes and CI users.

5. Predicting Spectral Selectivity of Optical Cochlear Implants in the Human Cochlea
Category: Auditory Prostheses
Lakshay Khurana*[1], Daniel Keppeler[1], Lukasz Jablonski[1], Tobias Moser[1]
[1]*Institute for Auditory Neuroscience, University Medical Center Göttingen*

Background: Cochlear implants are often used to treat sensorineural hearing loss by direct stimulation of tonotopically organized spiral ganglion neurons (SGNs). An electrical cochlear implant (eCI) converts the sound into electrical pulses, which are delivered to an electrode array in the cochlea of the inner ear. Although eCI users show satisfactory speech intelligibility in quiet environments, the electrical hearing is far from natural hearing. Increasing the number of frequency channels could provide more sound information but the wide spread of electrical current in the cochlea limits the number of independent channels. One solution to overcome this limitation is to use optogenetic stimulation, as light can be conveniently confined in space. An optical cochlear

implant (oCI) would limit the spread of neural activation and, hence, improve spectral coding by enabling a greater number of independent stimulation channels. The aim of this project is to predict the spread of light, hence the spectral selectivity, in a human cochlea, and therefore, to assist the development of clinical oCIs.

Methods: An optical engineering software, TracePro, was used to study the spread of light emitted from optical cochlear implants in silico. A human cochlea model was reconstructed from μCT scan data from the SICAS Medical Image Repository. Light sources were added and the model was simulated with different spectral and angular properties of the emitters to generate irradiance profiles along the cochlea length.

Results: The spectral spread, approximated from the full width at half-maximum of irradiance profiles in Rosenthal's canal, housing the SGN somata, was found to be lower in the middle and basal regions as compared to the apical region. The maximum irradiance increased and the spectral spread decreased when the emission profile was changed from Lambertian to Gaussian. When compared to electrical stimulation of the cochlea, the spectral spread was found to be lower for optical stimulation. The source-to-SGNs distance, formation of scar tissue, and orientation of the sources were found to impact optical stimulation of the cochlea.

Conclusions: The spread of SGN activation by oCIs is predicted to be lower than that of eCIs. Therefore, optical stimulation can potentially improve spectral selectivity of the cochlear implants.

6. Towards Behavioral Evaluation of a Multichannel Optogenetic Cochlear Implant System
Category: Auditory Prostheses

Bettina Wolf*[1], Lukasz Jablonski[2], Tamas Harczos[2], Alexander Dieter[2], Gerhard Hoch[2], Christian Dullin[3], Patrick Ruther[4], Tobias Moser[2]

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Background: Optogenetic cochlear implants (oCI) promise to overcome the issue of large current spread of electrical cochlear implants (eCI). Therefore, there are major efforts towards clinical translation of oCIs. However, the expected enhanced frequency resolution of an oCI has not been shown on the behavioral level yet. In order to evaluate the perceived selectivity of acoustic, electrical, or optical stimulation of the cochlea we established wireless multichannel e/oCI systems and used them in freely moving rats performing an auditory-cued avoidance task.

Methods: The ShuttleBox, a negative reinforcement paradigm encouraging avoidance behavior, was used to train Wistar rats (n=20) on click detection or pure tone discrimination tasks. Subsequently, animals were deafened via kanamycin injection and implanted either with a multichannel eCI (MED-EL electrode array with up to 5 electrodes) or a multichannel oCI (up to 10 LEDs). The position of the implant was confirmed via in vivo CT scan. Animals for oCI implantation had been injected with adeno-associated viruses carrying the channelrhodopsin-2 variant CatCh under the synapsin promoter at the age of 6–7 days. Functional expression of opsins was verified by recording optically evoked auditory brainstem responses (oABRs) prior to oCI implantation. Using the head-worn wireless CI system, animals were first subjected to a detection task via wireless triggered communication from outside the ShuttleBox inducing electrical or optical stimulation of the cochlea. Secondly, the CI system was used to directly process sounds and transform them into electrical/ optical stimuli. To control that rats responded to CI stimulation instead of sound, deafness was confirmed via ABR and behavior sessions with switched off CI systems.

Results: Functional expression of CatCh was confirmed in 87.5% of virus injected rats. Rats carried the CI system without any obvious burden. All animals pre-trained for detection also showed an avoidance behavior when cued by stimulation of the CI system (eCI, n=6; oCI, n=4). This was also true when acoustic clicks were transformed to stimuli using the CI system, instead of an external trigger. As expected, performance dropped to guessing level, when the CI system was switched off in control sessions. Using all available stimulation contacts mean behavioral thresholds were ~70 µA for eCI and ~0.31–3.1 mA LED current per channel for oCI. For both implant types behavioral responses could be elicited using single stimulation source. So far, for discrimination experiments an acoustic mean frequency differentiation limen of 0.08 Weber fraction was identified.

Conclusions: We established CI systems handling multichannel e/oCIs enabling their comparison in freely moving rats We demonstrated that the single LED-driven optogenetic stimulation evoked perception and is strong enough to elicit behavior in deaf animals. From here we aim to demonstrate higher spatial selectivity of optogenetic stimulation vs. electrical stimulation on the behavior level using the established setup.

7. Probing the Spectral Resolution of Red Fiber Based Optical Stimulation of Spiral Ganglion Neurons From Inferior Colliculus Recordings

Category: Auditory Prostheses

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Background: The development of the optical cochlear implants (oCI) strives to overcome the issue of large current spread and limited dynamic range of electrical cochlea implants (eCI). To improve both temporal and spatial precision as well as dynamic range and to avoid phototoxicity, we worked with f-Chrimson, which combines fast kinetics and red shifted light absorption. Here, we investigated the effect of different beam profiles on the inferior colliculus activity elicited by f-Chrimson mediated optogenetic stimulation of the auditory nerve.
Methods: The cochlea of Mongolian gerbils was injected with adenoassociated viruses (AAV2/6-hSyn-f-Chrimson-EYFP) seven days after birth inducing f-Chrimson expression in spiral ganglion neurons (SGNs). 1 to 2 months following the injection, cochleostomies were performed at the basal, medial and apical cochlea turn under isoflurane anesthesia. Laser-coupled optical fibers (594nm) with 200μm and 50 μm diameter and numerical apertures of 0.39 and 0.22 (NA) were placed at the cochleostomy and directed orthogonally toward the modiolus. Optically evoked auditory brainstem responses (oABR) were collected to confirm functional f-Chrimson expression. This was followed by recordings of multiunit activity (MUA) in response to optogenetic SGN stimulation using a 32-channel electrode array (Neuronexus, A1x32-6mm-50-177-A32) inserted in the contralateral IC. After mapping the IC tonotopy using pure tones to confirm placement of neural probe, MUA was collected using light pulses of varying intensities, durations and stimulation frequencies.
Results: Preliminary analysis showed peristimulus time histograms with ton at 4.25ms and toff at 12.5ms. Responses followed stimulation frequencies of up to 200 Hz. The spread of excitation tended to be lower with the 50μm diameter optical fiber (NA of 0.22) than with the 200μm fiber (NA of 0.39).
Conclusions: f-Chrimson appears to be a promising candidate for future clinical oCI as it offers good spectral selecitivity and temporal fidelity of bionic sound coding while use of red light reduces the risk of phototoxicity.

8. Effects of Different Current Levels on Type I Spiral Ganglion Neuron Density Along the Cochlea

Category: Auditory Prostheses

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Background: A wider electrical dynamic range, achieved through increasing the most comfortable levels (M-level), can be important for the outcomes of cochlear implant recipients, since this is associated with better speech perception scores (Robinson et al., 2012). However, this could lead to current levels which exceeded safe limits for spiral ganglion neuron (SGN) health. The present study aimed to determine the SGN density along the cochlea upon chronic intra-cochlear stimulation with different fixed current levels.
Methods: Four or five electrode contacts of a HiFocus1j electrode array (Advanced Bionics HiRes 90k®) were inserted into the first turn of the left cochlea in 10 normal hearing adult guinea pigs. After 4 weeks, an Auria© sound processor was fitted (HiRes strategy) with a pulse wide of 41.3 μs and a pulse rate of 1512 pulses per second per channel. All unused electrode contacts were set to zero. The M-levels were randomly assigned to form three groups with: a low current stimulation intensity ("LSI"; mean M-level of appr. 100 CU), a middle – ("MSI"; with a mean M-level of appr. 150 CU), or a high current stimulation intensity ("HSI"; with a mean M-level of appr. 235 CU). T-Levels were set at 10 % of M-levels. All animals were acoustically stimulated (16 h per day) with a radio play presented at 65 dB SPL. After 90 days of electrical stimulation, animals were perfused and their modioli were cut und stained histologically with hematoxylin-eosin staining. The corresponding areas of the Rosenthal´s canals were imaged and the number of SGN were counted.
Results: The mean SGN density was calculated for three regions: basal, medial and apical. The basal SGN density was significantly lower in the "HSI"-group in comparison to the "LSI"- and "MSI"-groups (p = 0.001 and p = 0.013). The medial area showed significantly lower SGN density for the "LSI"-group in comparison to the "MSI"-

and "HSI" groups (p = 0.000 for both comparisons) and significantly higher SGN density for the "HSI"-group in comparison to the "MSI"-group (p = 0.008). The apical SGN density of the "HSI"-group was significantly higher in comparison to the "LSI" – and "MSI"-groups (p = 0.000 and p = 0.002).

Conclusions: These results show that chronic high current stimulation leads to a significant SGN loss close to the electrode contacts (basal). However, the same stimulation had a protective effect for SGN´s in the medial and apical regions, where the current would be limited to lower levels. A low stimulation current does not affect SGN density in the basal area but was less protective than a higher stimulation current for the apical region. These data suggest the existence of an optimal current range for SGN protection and preservation.

This study was supported by Advanced Bionics GmbH

Monday, February 7, 2022

7:00 a.m. - 9:00 a.m.
Podium Session #13 – Hearing Loss: the Road From Genetics to Therapies
Moderators: Kevin Booth, Ph.D. & Aziz El Amraoui, Ph.D.

1. Confirmation of True Pathogenic Variant of OTOA, Overcoming Errors Caused by Pseudogene Contamination: Based on Long-Read Sequencing and Knock-In Mouse Study
Category: Genetics B: General

Bong Jik Kim*[1], Ju Ang Kim[2], Chung Lee[3], Jin Hee Han[4], Min Young Kim[4], Sungjin Park[5], Byung Yoon Choi[4]
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Background: OTOA encodes Otoancorin, which is required for the development of the tectorial membrane in the inner ear. Alterations in the gene cause non-syndromic sensorineural hearing loss (SNHL)(DFNB22). Previous study revealed that disrupted glycosylphosphatidylinositol (GPI) anchorage of Otoancorin played an important role in the pathogenesis of DFNB22. OTOAP1 shows very high sequence homology with OTOA, which raises pseudogene contamination issue with conventional short-read sequencing (SRS). To address the issue that potential candidate variant might reside in pseudogene (OTOAP1) not in OTOA, thus not exerting pathogenic potential in reality. We performed the long-read sequencing (LRS) in one hearing-impaired proband harboring on p.Glu787* of OTOA sequenced by SRS and further generated the Knock-in (KI) mouse of p.Glu787* to directly prove its pathogenicity.

Methods: A 10-month-old boy visited the clinic presenting with bilateral moderate SNHL. Routine SRS resulted in two potential candidate variants of OTOA: p.Gln589Argfs*55 (known pathogenic variant) and p.Glu787*. p.Glu787* is expected to disrupt the GPI anchorage, which should be pathogenic according to the previous report if it is real. However, too high minor allele frequency (0.23) in Korean population was highly against being considered as pathogenic. DNA samples of proband and parents were collected and SRS and LRS were performed to identify the causative variants. To confirm whether p.Glu787* resides in OTOAP1 or not, data from SRS and LRS were compared using IGV, BLAST and other bioinformatics tools. KI mouse (OtoaE787*E787*) was generated using CRISPR/Cas9 editing. ABR was performed to assess the hearing and H and E, IHC and electron microscopy (EM) were done to observe the immunohistology in the cochlea.

Results: LRS and bioinformatics analysis confirmed the presence of p.Glu787* in the OTOAP1. ABR at P28 showed severe hearing loss (around 80 dB SPL) and histologic study revealed detached tectorial membrane in KI mouse of p.Glu787*.

Conclusions: LRS revealed the pseudogene contamination that made genetic diagnosis difficult. KI mouse study confirmed the pathogenic potential of p.Glu787* if it is present in the true Otoa. Attention should be taken to interpret the NGS data in case of genes with possible pseudogene contamination.

2. Population-Scale Analysis of Common and Rare Genetic Variation Associated With Hearing Loss in Adults
Category: Genetics B: General

Kavita Praveen*[1], Lee Dobbyn[2], Lauren Gurski[2], Ariane Ayer[2], Jeffrey Staples[2], Shawn Mishra[3], Yu Bai[3], GHS-REGN DiscovEHR Collaboration[4], Decibel-REGN Collaboration[5], Olle Melander[6], Marcus Jones[2], Jonathan

Marchini[2], Suganthi Balasubramanian[3], Brian Zambrowicz[3], Meghan Drummond[3], Aris Baras[2], Goncalo Abecasis[2], Manuel Ferreira[2], Eli Stahl[2], Giovanni Coppola[2], Alexandra Kaufman[2], Arden Moscati[2], Christian Benner[2], Esteban Chen[2], Siying Chen[2], Alexander Popov[2], Janell Smith[2], Regeneron Genetics Center (banner author)[2]
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Background: The loss of hearing can have a debilitating impact on quality of life, requiring major adjustments to day-to-day activities. Significant co-morbidities are also associated with hearing loss including social isolation, depression, cognitive impairment, and dementia, which further deteriorate quality of life. These challenges, combined with a lack of therapeutics to stop or slow hearing loss progression, have contributed to its status as a growing global health issue. Novel therapies based on genetic evidence, therefore, will be crucial in addressing this unmet need.

Methods: We performed a genome-wide association meta-analysis of hearing loss with 125,749 cases and 469,497 controls across five cohorts, including UK Biobank (UKB), Geisinger DiscovEHR (GHS), the Malmö Diet and Cancer Study (MALMO), Mount Sinai's BioMe Personalized Medicine Cohort (SINAI), and FinnGen. We also generated exome sequence data and performed combined GWAS and exome-wide association study (ExWAS) on a subset of 108,415 cases and 329,581 controls across GHS, MALMO, SINAI, and UKB. Phenotypes were derived from ICD-10 diagnosis codes in GHS, MALMO, SINAI and FinnGen, and combined self-report and ICD-10 codes in UKB.

Results: We identified 53 loci affecting hearing loss risk, 18 of which are novel, including coding variants in COL9A3 and TMPRSS3. Through exome-sequencing, we identified an association with rare predicted loss-of-function variants in a gene that has been uncharacterized in hearing loss, KLHDC7B (odds ratio [OR] = 2.14, P = 5.2×10^{-30}), and with coding variants in two genes previously implicated in animal models of hearing loss (SYNJ2, OR = 1.31, P = 1.3×10^{-14}; FSCN2, OR = 1.24, P = 4.1×10^{-15}). We also observed single-variant and gene-burden associations with 11 genes known to cause Mendelian forms of hearing loss, including an increased risk in heterozygous carriers of mutations in the autosomal recessive hearing loss genes GJB2 (Gly12fs; OR = 1.21, P = 4.2×10^{-11}) and SLC26A5 (gene burden; OR = 1.96, P = 2.8×10^{-17}).

Conclusions: Our results show that Mendelian hearing loss genes contribute to the burden of hearing loss in the adult population, and suggest a shared etiology between common and rare forms of hearing loss. This work illustrates the potential of large-scale exome sequencing to elucidate the genetic architecture of common traits in which risk is modulated by both common and rare variation.

3. Rabbit Models of USH3A Have Progressive Hearing Loss
Category: Genetics B: General

Dongshan Yang*[1], Diane Prieskorn[2], Jun Song[1], Lisa Beyer[2], David Dolan[2], Jie Xu[1], Jifeng Zhang[1], Yehoash Raphael[2], Y Eugene Chen[1]
[1]*Center for Advanced Models for Translational Sciences and Therapeutics, University of Michigan Medical School,* [2]*University of Michigan, Kresge Hearing Research Institute*

Background: Usher syndrome type 3A (USH3A) is characterized by progressive loss of hearing and vision due to recessive pathogenic variants in the clarin-1 (CLRN1) gene. CLRN1 Y176X nonsense and N48K missense variants are the most common variants identified in individuals with USH3A. Affected individuals hear at birth but lose that ability over time. Animal models are needed to understand the function of CLRN1, the pathophysiology associated with USH3A, and to develop therapies. Mouse knockout mutants generated to mimic nonsense (Clrn1 KO/KO; referred to as "KO") or missense (Clrn1 N48K/N48K) variants in humans develop profound hearing loss by postnatal day 21 to day 25, which limits their usefulness for developing novel therapeutics. To address this problem, conditional knock out and transgenic models in the KO background or wildtype background were generated, which further complicated the study.

Methods: To address the need for USH3A models, we developed a rabbit model system using CRISPR/Cas9 mediated rabbit genome editing. We designed a guide RNA (gRNA) targeting the rabbit CLRN1 gene close to the N48 coding sequence. To produce the CLRN1 N48K mutation, a single strand DNA donor harboring the N48K mutation (c. 144T>G) flanked by 50 nucleotides homologous arms on both sides were co-injected with the gRNA and Cas9 protein into the rabbit pronuclear stage embryos.

Results: As a result, we successfully generated 3 founder rabbits carrying the CLRN1 c.144T>G mutation. In addition, alleles with insertion/deletion (indels) mutations were also present in the founder animals. By breeding a pair of the founder rabbits, both KI and KO (the frameshift indels causing premature stop codon) rabbits were generated. The hearing function of rabbits at 3 and 14 months of age was tested using auditory brainstem response (ABR) at 4 kHz, 12 kHz, and 16 kHz. Both CLRN1 N48K KI and CLRN1 KO rabbits showed elevated ABR thresholds. CLRN1 N48K KI rabbits showed milder hearing loss compared with the KO counterparts, with the largest increase at 16 kHz. At 14 months, all CLRN1 mutant rabbits had profound hearing loss at all frequencies tested.

Conclusions: This novel animal model mimics the progressive hearing loss in USH3A patients and is therefore useful for developing d rug and gene therapy as well as gene editing therapy for this form of deaf-blindness genetic disease.

4. Early-Onset Hearing Loss in a Mouse Model Lacking the Neuronal AP3B Complex
Category: Genetics B: General

Carlos Aguilar*[1], Sherylanne Newton[1], Andrew Parker[1], Gareth Banks[1], Sophie Boucher[2], Christine Petit[2], Steve DM Brown[1], Michael R Bowl[3]
[1]*MRC Harwell Institute*, [2]*Institut Pasteur*, [3]*MRC Harwell Institute, UCL Ear Institute*

Background: Adaptor-related protein (AP) complexes play a crucial role in the transport of proteins between organelles. The heterotetrameric AP3 complex consists of four subunits: β (Ap3b), δ (Ap3d), μ (Ap3m) and σ (Ap3s). Depending on the subtype of β and μ subunits present, it is found ubiquitously (AP3A – contains Ap3b1-Ap3m1) or specifically in neurons (AP3B – contains Ap3b2-Ap3m2). Evidence that the AP3 complex is important for hearing comes from the mocha mouse mutant (Ap3d null allele), which exhibits deafness and vestibular dysfunction. However, the Ap3d subunit is present in both AP3A and AP3B complexes. The neuronal-specific AP3B complex is reported to be important for the generation of synaptic vesicles and the sorting of cargo proteins to nerve terminals. While Ap3m2 is reported to be neuronal-specific, data available on the gene Expression Analysis Resource (gEAR) portal show that in the developing cochlea, Ap3m2 transcripts are predominantly found in hair cells.

Methods: In order to investigate the requirement of the AP3B complex for hearing and hair cell function, we have generated an Ap3m2 loss-of-function mutant. Using CRISPR/Cas9, we generated an Ap3m2 allele in which there is a frameshifting, single-nucleotide insertion causing an early truncation in the encoded protein that lacks the critical cargo-protein recognition domain.

Results: We find that homozygous mutants exhibit an early-onset hearing loss phenotype, with moderately elevated ABR thresholds (between 10-30 dB SPL) by 4-weeks of age. However, the hearing loss remains stable up to six-months of age when the animals begin to develop spontaneous seizures, precluding further aging.

In addition, Ap3m2 null mice show hyperactivity away from their home-cage and anxiety-like behaviour in open field and marble burying phenotyping platforms.

Meta-analysis of the ABR waveforms suggests a large synaptic component to the phenotype, with young animals exhibiting severely reduced Wave I amplitudes. However, DPOAE and ultrastructural studies (SEM) indicate no OHC dysfunction or bundle defects, while histological analyses reveal normal spiral ganglion neuron density. Likewise, we found no difference in number or matching, of Ribeye/GluR2 punctae at the IHC ribbon synapse. Furthermore, no difference in number or size of IHC synaptic vesicles is evident on TEM images. However, there is a difference in the combined size/spatial distribution of these vesicles.

Conclusions: Our results demonstrate a role for the neuronal AP3B complex in hearing and behaviour, and identifies AP3M2 as a gene that should be considered when assessing patients with idiopathic hearing loss. Studies are ongoing to fully elaborate upon the requirement of the AP3B complex for mammalian hearing.

5. Strial Pathology in Alport Mice is Likely Influenced by Aberrant Signaling Through Collagen and Laminin Receptors Resulting in Injury to Strial Marginal and Intermediate Cells
Category: Genetics B: General

Dominic Cosgrove*[1], Daniel T. Meehan[1], Jacob Madison[1], Gina Samuelson[1], Michael Anne Gratton[2]
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Background: Alport pathology is manifest in the glomerulus of the kidney and the stria vascularis in the inner ear. We have previously shown that extracellular matrix (ECM), including laminin α2 and collagen α-1(III), progressively accumulate in the strial capillary basement membranes of Alport mice. This results in thickening of

the strial capillary basement membranes and reduced vascular permeability associated with hypoxia and metabolic stress. Ultimately this leads to a drop in the endocochlear potential and susceptibility to noise-induced hearing loss. In the renal glomerulus, we have established that these same ECM components accumulate in the glomerular basement membranes, where they directly injure podocytes via specific receptor mediated signaling. Here we explore whether similar receptor-mediated signaling events contribute to Alport strial pathobiology.

Methods: We identified cells expressing specific receptors for laminin α2 and collagen α-1(III) using immunohistochemistry either on mid-modiolar cross sections or whole mounts of stria vascularis. We developed and qualified cell lines from strial explants derived from immortomice corresponding to basal, intermediate, and marginal cells. We performed transcriptome analysis on wild type and Alport stria vascularis and glomeruli from 7-week-old mice and performed comparative analysis for significantly elevated and suppressed transcripts.

Results: In glomerular podocytes we found the laminin α2 receptors to be integrin α7β1 and α-dystroglycan, while the collagen α-1(III) receptors were Discoidin Domain Receptor Tyrosine Kinase 1 (DDR1) and integrin α2β1. All these receptors are robustly expressed in the stria vascularis based on transcriptome analysis. Immunofluorescence analysis reveals integrin α2 expression exclusively on intermediate cells while integrin α7 and DDR1 were expressed on marginal cells and basal cells. Alpha-dystroglycan localization was observed in strial marginal cells. Expression specific mRNAs for each of these receptors in strial cell lines was consistent with the IF findings. Alignment of transcriptome data for stria vascularis and glomeruli from Alport mice relative to wild type mice reveals significant dysregulation of several genes implicated in cellular injury and important for strial/glomerular function.

Conclusions: We have shown previously that accumulation of laminin α2 and collagen α-1(III) injures podocytes both in vivo and in vitro. The receptors for these matrix molecules are present on glomerular podocytes and strial marginal or intermediate cells. Transcriptome data supports the hypothesis that the strial injury shares characteristics of glomerular injury. Thus, it is likely that these matrix molecules are injuring the stria vascularis through aberrant receptor signaling as they do in glomerular podocytes.

6. Generation of an Innovative Double-Humanized Mouse Model for Dominantly Inherited Hearing Impairment and Vestibular Dysfunction DFNA9
Category: Genetics B: General

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Background: DFNA9, caused by mutations in COCH, is a highly progressive form of dominantly-inherited adult-onset hearing impairment and vestibular dysfunction. COCH encodes cochlin, an important extracellular matrix protein that is produced by fibrocytes of the spiral ligament and spiral limbus. DFNA9-associated mutations were previously shown to affect post-translational processing of cochlin, and to lead to the formation of cytotoxic cochlin aggregates. However, the exact molecular mechanism underlying DFNA9 remains obscure. To increase our understanding of DFNA9 pathology, and investigate the therapeutic efficacy of novel treatments for DFNA9, we developed an innovative genetically humanized DFNA9 mouse model for the frequent Dutch/Belgian c.151C>T (p.(Pro51Ser) founder mutation.

Methods: In view of sequence specific genetic therapies, such as our recently developed mutant allele-specific silencing, we humanized murine Coch exons 3-6, including the intronic sequence. This approach will allow us to investigate therapeutic strategies that target the pathogenic variant or target the previously annotated mutant allele-specific (intronic) variants. The genetic humanization of several murine exons possesses several risks that may affect usability of the model. We describe the steps that we have taken to design our genetically humanized mouse model, and to ensure that the introduction of human-specific genetic information does not lead to unforeseen adverse effects.

Results: Fifteen codons present within the introduced human exons encode different amino acids as compared to the natural situation in mouse. To avoid the risk of potential damaging effects of the introduction of human-specific amino acids on the function of mouse cochlin, we recoded all human-specific amino acids to their murine counterparts. Next, we investigated pre-mRNA splicing of the hybrid Coch gene in mouse cochlear UB-OC1 cells. We initially identified skipping of human COCH exon 4 in murine cochlear cells, which we resolved by introducing several nucleotide substitutions to strengthen the exon 4 splice acceptor and donor sites. After that, correct hybrid Coch pre-mRNA splicing was seen in mouse embryonic stem cells that were generated to create the mutant and wildtype humanized mice. Finally, both hybrid Coch and endogenous wildtype Coch appeared

correctly spliced and expressed at equal levels in inner ears of heterozygous humanized mice. Preliminary data on the humanized wildtype strain shows that humanization did not lead to any adverse effects on auditory function. **Conclusions:** Further breeding is ongoing to remove the Cdh23ahl allele from the genetic background of the mice, to obtain the ultimate DFNA9 mouse model for the c.151C>T (p.(Pro51Ser) variant. The humanized DFNA9 mouse model is likely to be the first mouse model worldwide that allows to investigate the specificity and efficacy of a tailored allele-specific genetic therapy for inherited hearing loss. Insights obtained from this model will improve our understanding of DFNA9 pathology, and boost the development genetic therapies for other mutations underlying dominantly-inherited hearing loss.

7. Reversal of Hearing Loss in Spinster Homolog 2 Mutant Mice
Category: Genetics B: General

Elisa Martelletti*[1], Neil J. Ingham[1], Karen P. Steel[1]
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Background: Progressive hearing loss is common but we have no medical treatments to slow down, stop, or reverse it. In this study, we asked if progressive hearing loss in a mouse mutant can be reversed after it has developed as a proof-of-concept.
Spinster homolog 2, Spns2, is a sphingosine-1-phosphate (S1P) transporter, and Spns2tm1a mutant mice were previously described by our group (Chen et al., 2014) showing a rapidly-progressive hearing loss associated with a decline in endocochlear potential (EP). As EP appears to develop normally at first in mutants, we considered ways of restoring it to normal levels after the onset of hearing loss.
Methods: We used a genetic approach to initiate expression of the Spns2 gene, using tamoxifen injection to activate Flp recombinase which recognises FRT sites in the Spns2tm1a allele, removing the targeted insertion and leading to restoration of Spns2 gene activity. Tamoxifen was injected at 4 different ages (Postnatal day (P)14, P17, P21 and P28) and ABRs were recorded at intervals before and after injection up to 8 weeks old when the EP was also measured.
Results: By comparing pre and post tamoxifen ABR thresholds in the same mouse, we observed that injection of tamoxifen at P14 led to development of near-normal thresholds at 6, 12, 18 and 24kHz frequencies in the mutants. At P17, mutants already show raised ABR thresholds but tamoxifen injection reversed this hearing loss at 6, 12 and 18 kHz to near-normal thresholds. For frequencies of 18kHz or over, P21 and P28 injections were too late to improve thresholds, but for 12kHz some improvement was found with injection as late as P28. The reversal of hearing loss was stable up to 8 weeks old. EP levels at 8 weeks old were generally higher in mutants injected at younger ages than in those injected at P21 or P28, and lower ABR thresholds correlated with higher EP levels. Histological analysis of the marginal cells of the stria vascularis showed normal morphology in the mice injected at P14 and P17.
Conclusions: Overall, our results show that hearing loss due to the Spns2 mutation can be reversed and the earlier the reactivation of the Spns2 gene, the more extensive is the reversal. This study provides a proof of concept that certain forms of hearing loss can be reversed after the loss has occurred, which is an important support for the development of new treatments for humans.

8. Treatment of Autosomal Recessive Hearing Loss via in Vivo CRISPR/Cas9-Mediated Optimized Homology-Directed Repair in Mice
Category: Genetics B: General

Yilai Shu*[1], Xi Gu[1], Daqi Wang[1], Xingde Hu[2], Zhijiao Xu[1], Fang Wang[1], Genglin Li[1], Erwei Zuo[2], Huawei Li[1]
[1]*Eye and ENT Hospital, Shanghai Medical College, Fudan University,* [2]*Institute of Neuroscience, State Key Laboratory of Neuroscience, Key Laboratory of Primate Neurobiology, CAS Center for Excellence in Brain Science and Intelligence Technology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences*

Background: Most cases of genetic hearing loss arise from autosomal recessive loss-of-function mutations that require repair, rather than disruption, of the mutant allele. CRISPR/Cas9-mediated homology-directed repair (HDR)-based therapies have the potential to cure many genetic diseases because this class of therapeutics can achieve arbitrary base changes as well as the insertion or deletion of DNA fragments. Efficient HDR in non-dividing mammalian cells is a critical issue that needs to be solved. We previously designed a homology-mediated end joining (HMEJ)-based strategy using the CRISPR/Cas9 system to implement efficient transgene integration in postmitotic cells, and here we have used this new strategy with dual adeno-associated viruses (AAVs), to treat

Klhl18lowf mice that carry a recessive loss-of-function point mutation (g.9:110455454C>A; resulting in the p.V55F substitution) in the Klhl18 gene that causes progressive hearing loss by inner hair cells disfunction.
Methods: We designed and screened single guide RNA (sgRNA) for the point mutation in Klhl18 in cultured skin fibroblast cells, and we used dual AAVs, with one carrying the SaCas9-KKH sequence and the other carrying the sgRNA and donor sequences, to deliver the HMEJ-based system in vivo. We measured the editing efficiencies, including indels and corrections, with next generation sequencing. After injection of the dual-AAV system into the inner ears of homozygous mutants at P1, the phenotypic outcomes were evaluated using immunofluorescence staining, scanning electron microscopy, whole-cell patch-clamp electrophysiological recording, and measurement of the ABR thresholds and the peak amplitudes and latencies of ABR wave 1.
Results: We first screened and validated a highly efficient single-guide RNA (sgRNA) that specifically targets the Klhl18lowf site in cultured skin fibroblast cells. No obvious indel mutations were detected in homozygous Klhl18lowf fibroblasts transfected with SaCas9-KKH+sgRNA, indicating high fidelity of this CRISPR/Cas9 system. The AAV–sgRNA–donor transduced more than 93% of the IHCs on average in vivo at 10 weeks after injection. After injection into the inner ears of neonatal Klhl18lowf mice, this therapeutic system successfully corrected the C>A point mutation in Klhl18 in vivo. We observed clear recovery of the stereocilia morphology in an average of 16% of the inner hair cells in the apical and middle turns of the cochlea by scanning electron microscopy and restored sustained exocytosis in inner hair cells by patch-clamp electrophysiological recording. Auditory function study showed significant hearing preservation in Klhl18lowf mice up to 24 weeks of age.
Conclusions: These findings demonstrate the effective hearing preservation in mice via the HMEJ-based repair of a point mutation in vivo, and they support further development of this strategy for the treatment of recessive hearing loss and other recessive genetic diseases.

7:00 a.m. - 9:00 a.m.
Podium Session #14 – Auditory and Cognitive Processing
Moderators: Nima Mesgarani, Ph.D. & William Sedley, Ph.D.

1. Arousal State-Dependence of Interactions Between Short- and Long-Term Auditory Novelty Responses in Human Subjects
Category: Auditory Cortex and Thalamus: Human Studies

Kirill Nourski*[1], Mitchell Steinschneider[2], Ariane Rhone[1], Rashmi Mueller[1], Matthew Banks[3]
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Background: Predictable sensory stimuli are generally not ecologically informative. By contrast, novel or unexpected stimuli may signal ecologically salient changes in the environment. According to the predictive coding hypothesis, efficient sensory encoding minimizes neural activity associated with predictable stimuli and emphasizes detection of changes in the environment. In everyday life, the brain must resolve multiple unexpected sensory events occurring over multiple time scales. The local/global deviant experimental paradigm (Bekinschtein et al., Proc Natl Acad Sci U S A. 2009 106:1672-7) examines auditory predictive coding over both short and long time scales. Short-term novelty (hundreds of milliseconds; local deviance, LD) is created by presenting sequences of identical sounds (/xxxxx/) interspersed with sequences that contain deviants (/xxxxy/). Long-term novelty (several seconds; global deviance, GD) is created using either (a) frequent /xxxxx/ and infrequent /xxxxy/ sequences, or (b) frequent /xxxxy/ and infrequent /xxxxx/ sequences. In scenario (a), there is both an LD and a GD effect (LDGD, 'double surprise'). In (b), the global deviant is a local standard (LSGD). Cortical responses reflecting LD and GD originate in different brain areas, have a different time course, and are differentially sensitive to general anesthesia (Nourski et al., J Neurosci. 2018 38:8441-52). Neural processes underlying LD and GD interact, reflecting overlapping networks subserving the short- and long-term auditory novelty detection (Witon et al., Cereb Cortex 2020 30:5204-17).
Methods: This study examined local-by-global novelty interactions using intracranial electroencephalography in neurosurgical patients. Stimuli were quintuplets of vowels, presented in a GD target detection task. The task was performed both before and during induction of anesthesia with propofol. Recordings were made from the auditory cortex, surrounding auditory-related and prefrontal cortices in awake, sedated, and unresponsive states. High gamma activity was used to measure LD and GD effects and their interactions. Positive interaction was defined as a greater response to the double surprise LDGD condition compared to LSGD. Negative interaction was defined as a stronger response to LSGD.

Results: The LDGD condition typically provided an advantage for the performance of the GD detection task compared to the LSGD target condition in the awake state. Sedation decreased this advantageous behavioral effect. Positive interaction was primarily found in auditory cortex and was more frequent than negative interaction. Negative interaction typically occurred in prefrontal cortex and was more sensitive to general anesthesia. Auditory-related areas in temporal and parietal cortex exhibited both types of interaction.

Conclusions: Local-by-global interactions provide insight into how the brain simultaneously analyzes multiple levels of novelty. These interactions, identified in the local-global paradigm, may have translational relevance in clinical settings, serving as biomarkers of conscious perception in the assessment of depth of anesthesia, sleep and disorders of consciousness.

2. Behavioral and Electrophysiological Measures of Central Interaction Between Electric and Acoustic Stimulation in Cochlear Implant Users
Category: Auditory Cortex and Thalamus: Human Studies

Hanna Dolhopiatenko*[1], Waldo Nogueira[2]
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Background: The growing group of cochlear implant (CI) users includes subjects with preserved acoustic hearing on the opposite side to the CI. These subjects receive electric acoustic stimulation where electric stimulation is provided by the CI and acoustic stimulation is provided to the normal acoustic hearing or through amplification by the hearing aid (HA) on the contralateral side to the CI. The use of both listening sides results in improved speech perception in comparison to listening with one side. However, large variability in the measured benefit is observed. It is possible that this variability is associated with interaction between the electric and acoustic sides. However, there is a lack of established methods to assess interaction and consequently to adequately program the devices. For this reason, the first goal of this study is to design an electrophysiological paradigm based on electroencephalography (EEG) to investigate interaction between electric and contralateral acoustic stimulation. The second goal is to investigate if central interaction as measured electrophysiologically can predict the speech understanding performance benefit of combining electric and contralateral acoustic stimulation.

Methods: Five CI users with normal contralateral acoustic hearing and three CI users with a HA on the contralateral side participated in the study. In the speech understanding test, subjects were asked to repeat words to a target speaker in the presence of a competing voice with the same presentation level through the CI side, the acoustic side (AS) or to both listening sides (CI+AS). Two electrophysiological measures of central interaction were designed. The first one consisted of cortical auditory evoked potentials (CAEPs) to broadband clicks. The second one consisted of a selective attention (SA) decoding paradigm in which a target and a competing speech stream were presented to the subjects using the three listening modalities (CI, AS and CI+AS). The speech envelope was reconstructed using least mean square estimation. Correlation coefficients between reconstructed and attended speech envelope and between reconstructed and unattended speech envelope were calculated and compared across listening modalities.

Results: The average speech understanding performance score across subjects was similar for CI+AS and AS and higher than with the CI listening condition. Consistent with this result, the analysis of CAEPs presented higher amplitudes for the CI+AS and AS listening conditions. Moreover, SA decoding was possible with all three listening modes and the difference between the attended and the unattended correlation coefficients was larger with CI+AS than with CI but similar to the AS alone condition.

Conclusions: The result demonstrates the possibility to decode selective attention in CI users even if continuous artifact is present. This work shows that CAEPs and SA decoding can potentially be used as an electrophysiological measure interaction effects such as fusion or interference between CI electric stimulation and contralateral acoustic stimulation.

3. A System-Identification Approach to Disentangle the Sources Contributing to Electroencephalographic Correlates of Temporal Modulation Processing in Active and Passive Listening
Category: Auditory Cortex and Thalamus: Human Studies

Ravinderjit Singh*[1], Hari Bharadwaj[1]
[1]*Purdue University*

Background: Many studies have investigated how subcortical temporal processing, measured via brainstem evoked potentials (e.g., ABRs and FFRs) may be influenced by aging, hearing loss, musicianship, and other auditory processing disorders. However, human studies of cortical temporal processing are often restricted to the 40 Hz steady-state response or onset responses. One possible reason for the limited investigation is the lack of a fast and easy method to characterize temporal processing noninvasively in humans over a range of modulation frequencies. Moreover, the cortex exhibits highly nonlinear behavior in that measures of temporal processing depend strongly on the stimulus statistics. Finally, multiple sources can contribute to the overall response, interacting constructively or destructively at the level of the EEG sensors, further complicating the interpretation. Here, we present an approach to quantify cortical temporal coding that is (1) efficient, (2) uses ongoing stimuli that mimic the statistics of sounds encountered in the real world, and (3) enables source separation.
Methods: A novel extended maximum-length sequence was used to modulate a white noise carrier to yield an aperiodic stimulus with nearly flat modulation spectrum up to 75 Hz. We employed this stimulus in conjunction with EEG to obtain a modulation temporal response function (mTRF) using a simple cross-correlation analysis. To characterize the effect of attention and task demands on the individual sources contributing to the mTRF, measurements were made in one passive condition, and two active conditions with different attentional and temporal processing demands. To test whether the mTRFs were repeatable, the passive condition was measured twice, months apart, on each individual participant. Finally, to compare the mTRFs to responses obtained with conventional periodic stimuli, envelope-following responses (EFRs) were measured with sinusoidally modulated noise across eight different modulation frequencies.
Results: Although the mTRF exhibited large individual differences, they were repeatable across two measurements months apart, in the passive state. Further, we found that the overall mTRF is composed of five components that can be distinguished by virtue of latency, scalp topography, the frequency range that each component spans within the overall temporal modulation transfer function, and sensitivity that each component exhibits to manipulations of attentional and/or task demands.The mTRF also shows nonlinear behavior, in that the relative magnitudes of the constituent components are different when measured using broadband modulations versus a series of sinusoidal modulations.
Conclusions: The mTRF shows promise as an efficient and robust measure of cortical temporal processing that may be used to characterize the different sources contributing to the overall EEG response, and discover their respective functional correlates under various listening conditions and in various clinical populations.

4. Children With Amblyaudia Show Less Flexibility in Auditory Cortical Entrainment to Periodic Non-Speech Sounds

Category: Auditory Cortex and Thalamus: Human Studies
Sara Momtaz Bokharaei*[1], Deborah Moncrieff[1], Meredith Ray[1], Gavin Bidelman[1]
[1]*The University of Memphis*

Background: We investigated auditory temporal processing in children with amblyaudia (AMB), a subtype of auditory processing disorder, via cortical neural entrainment.
Methods: Evoked responses were recorded to click-trains at slow vs. fast (8.5 vs. 14.9/sec) rates in n=14 children with AMB and n=11 age-matched controls. Source and time-frequency analyses decomposed EEGs into oscillations (reflecting neural entrainment) stemming from the bilateral auditory cortex.
Results: Phase-locking strength in AMB depended critically on the speed of auditory stimuli. In contrast to age-matched peers, AMB responses were largely insensitive to rate manipulations. This rate resistance was seen regardless of the ear of presentation and in both cortical hemispheres.
Conclusions: Children with AMB show a stark inflexibility in auditory cortical entrainment to rapid sounds. In addition to reduced capacity to integrate information between the ears, we identify more rigid tagging of external auditory stimuli. Our neurophysiological findings may account for certain temporal processing deficits commonly observed in AMB and related auditory processing disorders (APDs) behaviorally. More broadly, our findings may inform communication strategies and future rehabilitation programs; increasing the rate of stimuli above a normal (slow) speech rate is likely to make stimulus processing more challenging for individuals with AMB/APD.

5. Speech-In-Noise Ability is Predicted by Neural Responses in Auditory Cortex of Cochlear Implantees

Category: Auditory Cortex and Thalamus: Human Studies
Joel Berger*[1], Phillip Gander[1], Laura Ponto[2], Jae-Hee Lee[3], Jean Hong[3], Camille Dunn[3], Bruce Gantz[3], Bob McMurray[4], Inyong Choi[5], Timothy Griffiths[6]

[1]*Dept. Neurosurgery, University of Iowa Hospitals and Clinics, [2]Dept. Radiology, University of Iowa Hospitals and Clinics, [3]Dept. Otolaryngology – Head and Neck Surgery, University of Iowa Hospitals and Clinics, [4]Dept. Psychological and Brain Sciences, University of Iowa, [5]Dept. Communication Sciences and Disorders, University of Iowa, [6]Biosciences Institute, Newcastle University Medical School

Background: Cochlear implants have proven to be an invaluable technology in restoring hearing to moderately-to-severely deafened individuals. Despite their success, there is still considerable variability in outcomes following surgery, particularly in the perception of speech in a noisy environment. Indeed, this is the most common complaint across the hearing-impaired population and can lead to social withdrawal due to avoidance of difficult hearing situations. We sought to understand the neural mechanisms underlying this variability, towards a greater aim of accounting for other factors that act as covariates.
Methods: We used O-15-water positron emission tomography in 27 cochlear implantees to examine neural activity whilst subjects performed either a speech-in-noise task or a level detection task, designed to control for the effects of attention.
Results: Contrasts between the two conditions across subjects showed greater activity across auditory cortex for the speech-in-noise task, the peak of which was frequently localized towards the lateral temporal convexity on an individual subject basis. Linear regression analyses demonstrated that peak beta values in auditory cortices of individual subjects were strongly predictive of behavioral scores on the speech-in-noise task (r = 0.58, p = 0.002). Inferior frontal gyrus activity was inversely correlated with speech behavioral scores (r = 0.50, p = 0.009), wherein better performance was associated with less of a difference between the two conditions.
Conclusions: These results highlight robust neural correlates of variability in speech-in-noise performance in cochlear implantees. The wider study of a larger group (n = 100) will examine various factors that contribute to this variability.

6. Retinotopy of Cross-Modal Plasticity in the Auditory Cortex in Prelingually Deaf Adults
Category: Auditory Cortex and Thalamus: Human Studies

Darin Zwaan*[1], Mathijs Raemaekers[2], Rosanne Timmerman[1], Feng Lin[1], Ralf Boerboom[3], Louise Straatman[3], Huib Versnel[3]
[1]UMC Utrecht, [2]UMC Utrecht Brain Center, [3]University Medical Center Utrecht

Background: Deprivation of auditory input may trigger the auditory cortex to be repurposed for processing information from other sensory modalities, in an attempt to compensate for functional loss. This cross-modal plasticity has been previously shown in deaf subjects for both visual and tactile stimulation. The aim of the present study was to assess whether the auditory cortex in deaf individuals responds to meaningless visual stimuli in central visual field, and whether there are certain regions in the auditory cortex that become activated only when a specific part of the visual field is stimulated, as is the case in the primary visual cortex, where retinotopic organization exists.
Methods: We gathered 7-Tesla fMRI data of 10 prelingually deaf, and 10 normal hearing adults (serving as reference) using visual stimuli, that previously revealed retinotopic organization in the visual cortex. Region of interest (ROI) determination of the auditory cortex was done by providing the hearing subjects with pure tone stimuli, normalizing the results to the same coordinate system and isolating voxels that showed significant correlation to the presence of pure tone stimuli (ROI 1). An additional region of interest was created by finding voxels which had statistically significant more response to one of the tone stimuli, as compared to the others (ROI 2).
The mean percent signal change of the BOLD response during visual stimulation in both auditory and visual cortex was compared between the normal hearing and the deaf individuals.
Results: Kruskal-Wallis analysis of the mean signal change during stimulation showed no significant differences in (primary) visual cortex between groups. The prelingually deaf group showed negative BOLD-changes in response to the stimuli in the right side of ROI 2, compared to the normal hearing group (p=0.027). The same trend was observed in the right side of ROI 1, but was not found significant (p=0.06). The left auditory cortex followed the same trend, but no significant effect was found (0.14<p<0.20) .
Conclusions: Our results suggest inhibition in right side of auditory cortex in deaf subjects in response to visual central-field retinotopic mapping stimuli. Such results have, to our knowledge, not yet been published. In most studies using visual stimuli during fMRI, a significant increase in activity was found in the auditory cortex of prelingually deaf subjects. This might be due to the difference in used stimuli. These studies mostly stimulated the entire field of view at once and/or used speech related stimuli such as recordings of lip movement during spoken

language. Another reason might be our determination of ROI 1, which targets tonotopic parts of the auditory cortex. This region of the auditory cortex might respond differently than higher order regions, required for complex processing.

7. Neural Mechanisms Underlying Speaker Identity-Based Selective Attention During Speech-In-Noise Perception

Category: Auditory Cortex and Thalamus: Human Studies
Hwan Shim*[1], Olivia Sourwine[1], Jae-Hee Lee[1], Inyong Choi[1]
[1]*University of Iowa*

Background: Noisy environments can make it difficult to understand what you are listening to. However, it may be easier to hear the familiar voice of a friend than to hear the unfamiliar voice of a stranger. Previous studies support this phenomenon with evidence for the presence of stronger speech-evoked brain activity when listening to familiar speakers. However, it has been unclear what neural substrates are involved in tracking speaker identity in speech and noise. Based on the predictive coding theory, this study aimed to investigate what neuro-cognitive processes are utilized while listeners prepare to recognize a target word spoken by a known voice or unknown. We sought two critical components of predictive coding using EEG: The internal representation of the prediction and the incoming sensory inputs that update the prediction. Given that gamma-band oscillations reflect bottom-up processing for sensory sampling while beta-band oscillations reflect the top-down flow of prediction information, we hypothesized that tracking a target word spoken by a known voice will recruit stronger beta oscillation in auditory cognitive regions and weaker gamma oscillation in auditory sensory regions.
Methods: To test this hypothesis, we measured 64-channel cortical EEG data from participants with normal hearing in a word-in-noise task with an auditory cue that either did or did not identify the upcoming speaker who will say a target word. The carrier phrase ("Choose the word") containing the auditory cue was spoken 1.5 seconds before the onset of the multi-talker babble noise that was continued for 2 seconds. In each trial, one of two types of auditory cues was randomly given: Either spoken by the same speaker (female or male) who will say a target word (henceforth referred to as "Cued" condition) or a mixture of both (female AND male) voices (i.e., "No-cue" condition). A target word was presented 1 second after the onset of the noise, which was 3 seconds after the auditory cue. After listening to all the sounds, four minimal-pair options appeared on the computer screen as the participant will have to select a key that corresponds to the target word.
Results: We analyzed event-related spectral perturbation during the period after the auditory cue and before the target word. The Cued condition exhibited stronger beta oscillations in the left inferior frontal gyrus than the No-cue condition, whereas the No-cue condition exhibited stronger gamma oscillations in the superior temporal gyrus of both hemispheres.
Conclusions: The combination of these results supports the predictive coding theory and provides a potential neurophysiological substrate of speaker tracking during speech-in-noise recognition.

8. Measuring Auditory Attention and Working Memory in Human Prefrontal Cortex Using fMRI

Category: Auditory Cortex and Thalamus: Human Studies
Eli Bulger[1], Barbara Shinn-Cunningham[1], Abigail Noyce*[1]
[1]*Carnegie Mellon University*

Background: Prefrontal cortex (PFC) has often been considered a general-purpose cognitive resource, recruited across tasks regardless of their specific computational demands. However, recent work has confirmed that human PFC can be parcellated into many smaller regions, some of which are specialized for auditory cognition (Michalka 2015, Glasser 2016, Noyce 2017, 2021). These regions are reliably recruited for auditory attention and working memory (WM), but their specific contributions are not understood. Here, we developed a functional magnetic resonance imaging (fMRI) study to measure brain activity, particularly in frontal cortex, during carefully-matched attention and WM tasks.
Methods: We first collected fMRI (TR = 2 s, TE = 30 ms) while subjects (N=10) performed a PFC auditory localizer task (2-back memory for animal vocalizations contrasted against 2-back memory for faces), and while the same subjects performed a language processing task (Fedorenko 2013). Then, the same subjects performed 8 runs of our attention and WM paradigm. Each block (25 s) featured two competing spatialized streams of 4-note melodies. Subjects were cued to attend to one stream, and either perform a challenging perceptual task or a WM task. To recruit auditory attention, subjects listened for occasional amplitude-modulated notes ("warbles") and responded when one was detected; to recruit WM, subjects listened for repeated 4-note melodies ("repeats")

within the cued stream. All stimuli were matched between attention and working memory conditions. Each run consisted of 4 attention blocks, 4 working memory blocks, and 2 passive listening blocks; the order of conditions was fully counterbalanced across the 10 runs.

Results: Using the auditory PFC localizer task, we labeled group-constrained subject-specific regions of interest in transverse gyrus intersecting precentral sulcus (tgPCS), caudal inferior frontal sulcus/gyrus (cIFS/G), frontal operculum (FO), and central medial superior frontal gyrus (cmSFG). Preliminary results suggest that the language task activates a small region that overlaps with, but is slightly anterior to, the tgPCS. We also observe that tgPCS, cIFS/G, and FO are recruited more strongly during WM than during attention, despite performance suggesting that the attention task was more difficult for subjects.

Conclusions: Our preliminary results suggest that the cortical language network overlaps but does not exactly coincide with the frontal portions of the auditory attention network. Further, despite careful stimulus matching between attention and WM tasks, we see differential recruitment within the frontal auditory network, suggesting that these regions have different specialization for different cognitive tasks.

7:00 a.m. - 9:00 a.m.
Podium Session #15 – Auditory Brainstem: New Molecular and Functional Insights
Moderators: Kirupa Suthakar, Ph.D. & Sima Chokr, B.S.

1. An Intrinsic Oscillator Drives Spontaneous Activity in Lateral Olivocochlear Neurons
Category: Brainstem: Structure and Function

Hui Hong*[1], Larry Trussell[2]
[1]*Oregon Health and Science University,* [2]*Oregon Health and Science University, Oregon Hearing Research Center*

Background: The auditory efferent system plays a crucial role in hearing. However, much of our understanding of these efferents derives from studies of the medial olivocochlear (MOC) system, whereas much less is known about lateral olivocochlear (LOC) system. We report that LOC neurons in juvenile and young adult mice exhibit robust spontaneous electrical activity of an unusual nature. By using calcium imaging, cell-attached and whole-cell patch-clamp recordings, we showed that this activity is characterized by low-frequency firing of long bursts of Na+ action potentials driven by slow, calcium dependent waves. Burst firing was resistant to synaptic receptor antagonists, suggesting an intrinsic drive. Nevertheless, excitatory or inhibitory inputs to LOC neurons were able to alter the temporal pattern of burst firing.

Methods: A Cre-dependent adeno-associated virus (AAV) was injected into the lateral superior olive (LSO) of ChAT-IRES-Cre transgenic mice at postnatal days 21-23, and brain slices were made 1-2 weeks after surgery. LOC neurons were identified by mRuby expression, and GCamp6f signals were collected using a two-photon microscopy. We also crossed ChAT-IRES-Cre transgenic mice with a tdTomato reporter line. Cell-attached and whole-cell patch-clamp recordings were then conducted on LOC neurons identified as fluorescent somata within the LSO.

Results: Of 214 imaged LOC neurons, 89% exhibited robust spontaneous bursts of calcium signals in vitro. Each burst lasted for seconds. Among these neurons, 77% showed an obvious periodicity with an average burst frequency of 0.11 Hz. This spontaneous activity was not blocked by NBQX or mecamylamine, glutamatergic and nicotinic acetylcholine receptor antagonists, respectively. Consistent with calcium imaging results, cell-attached recordings showed that LOC neurons generated spikes in a burst pattern, with an average spike frequency of 9 Hz. This spontaneous activity persisted with NBQX in the bath. In neurons recorded in current-clamp mode, application of TTX blocked the fast Na+ spikes, but revealed an underlying pattern of slow, regular depolarizations decorated with small broad spikes resembling Ca2+ spikes described in other systems. Eliciting excitatory or inhibitory postsynaptic potentials (EPSPs and IPSPs, respectively) by electrical stimulation in the slice was able to move the neuron between burst and quiescent states. EPSPs and IPSPs simulated using conductance clamp were also found to control the burst activity.

Conclusions: Taken together, we suggest that LOC neurons are driven by a potent intrinsic oscillator, and synaptic input controls the features of these oscillations. Such patterned activity could be important in driving release of diverse transmitters from LOC nerve terminals in the cochlea.

2. Auditory Brainstem Responses to Continuous Peaky Speech Systematically Change With Number of Simultaneous Talkers

Category: Brainstem: Structure and Function

Melissa Polonenko*[1], Ross Maddox[2]
[1]University of Minnesota, [2]University of Rochester

Background: Everyday communication often involves listening to someone talk while several other people are also speaking. The neural underpinnings of this natural, continuous speech-in-speech communication have largely been restricted to studies of behavior and auditory cortex. The contributions of early stages of continuous multi-talker processing have yet to be explored. We recently created a "peaky speech" paradigm that allows us to measure canonical brainstem responses that reflect distinct stages of subcortical speech processing. This initial work presented only one talker at a time. Here we aimed to systematically (1) evaluate the behavioral recognition of peaky speech versus unaltered speech in multi-talker noise, and (2) compare how the peaky speech brainstem responses change with increasing numbers of competing talkers.

Methods: We recruited 25 adults (16 females and 9 males) aged 23.4 ± 5.5 years with normal hearing thresholds. Unaltered and peaky speech stimuli were created for 5 audiobooks openly available on Librivox, as well as the Coordinate Response Measure (CRM) sentences. The stories had fundamental frequencies varying from 123 to 157 Hz and the number of glottal pulses per second varied from 66 to 77. First, we measured behavioral speech recognition thresholds for CRM sentences in the presence of 4 competing stories. Then we measured peaky speech brainstem responses for 4 randomly presented conditions that each lasted a total of 30 minutes: 1, 2, 3, and 5 simultaneously and diotically presented talkers (corresponding to signal-to-noise ratios, SNRs, of infinity, 0, -3, and -6 dB respectively).

Results: Behavioral target-to-masker thresholds were very similar for the unaltered and peaky speech. By presenting up to 5 talkers simultaneously, we collected up to 5 times the brainstem response data to analyze for a given condition. This was reflected in the pre-stimulus residual noise (comes before what happens to the waveforms themselves), which decreased on average by the square root of the number of talkers despite the same total recording time. However, response wave V amplitudes also significantly decreased – and latencies increased – with increasing number of talkers. Consequently, the grand average response SNRs also slightly decreased with more talkers, although they were above 19 dB for all conditions. The behavioral speech-in-speech thresholds did not correlate with the wave V amplitudes or latencies, nor the differences in amplitudes and latencies across number of talkers (i.e., the individual slopes).

Conclusions: The peaky speech method provides similar behavioral thresholds to unaltered speech and evokes canonical brainstem responses that are sensitive to changes in the number of simultaneously presented talkers. Responses with good SNRs are measurable for up to 5 talkers within 30 minutes – an important consideration that will facilitate future subcortical experiments that compare different multi-talker conditions, such as attending a talker, spatially separating talkers, or audio-visual speech.

3. Long-Term Inhibition of CSF1R Signaling Impairs Auditory Brainstem Function

Category: Brainstem: Structure and Function

Sima Chokr*[1], Giedre Milinkeviciute[1], Karina Cramer[1]
[1]University of California, Irvine

Background: Specialized sound localization circuit development requires orchestrated functions of neurons and glia. Neural circuit formation requires synapse strengthening, refinement, and pruning. Many of these functions are carried out by microglia, immune cells that aid in regulating neurogenesis, synaptogenesis, apoptosis, and synaptic removal. We previously showed that postnatal treatment with BLZ945 (BLZ), an inhibitor of colony stimulating factor 1 receptor (CSF1R), eliminates microglia in the auditory brainstem and results in impaired elimination of excess synaptic inputs and maturation of astrocytes in the medial nucleus of the trapezoid body (MNTB). BLZ treatment during the first two postnatal weeks also results in elevated hearing thresholds and delayed signal propagation as measured by auditory brainstem recordings (ABR) at four weeks. However, when microglia repopulate the brain following the cessation of CSF1R inhibition, calyceal pruning levels are restored by four weeks of age, astrocyte maturation marker levels are comparable to control by seven weeks of age, and hearing thresholds and delayed peak latencies largely recover by seven weeks of age. It is unknown whether this recovery of auditory brainstem development is achievable without the return of microglia.

Methods: Here, we developed a novel method in which we induced sustained elimination of microglia during postnatal development. Mice were treated using a two-drug approach optimized for delivery route. Subcutaneous

injections of BLZ945 were given from postnatal days 2-12 and the nursing dam was fed a CSF1R-inhibiting chow containing PLX5622 (PLX) during the entirety of the BLZ treatment period until weaning. After weaning the pups were placed on PLX until testing at either four or seven weeks of age. In these BLZ/PLX-treated mice, microglia were fully eliminated throughout the developmental of hearing onset, microglia-dependent calyceal pruning, and auditory circuit maturation. We investigated whether recovery of the auditory brainstem can occur despite the long-term loss of CSF1R signaling.

Results: Using standard immunohistochemical and fluorescent imaging techniques, we found that in seven-week-old mice, the synaptic protein marker synaptophysin was significantly diminished compared to age-matched vehicle controls. Further, click and pure tone ABRs revealed that at both four and seven weeks of age, BLZ/PLX treated mice had significantly elevated hearing thresholds, diminished peak amplitudes, and delayed peak latencies and inter-peak latencies.

Conclusions: Together, these data indicate that auditory function did not recover and suggest that microglia are required to repopulate the brain in order to rectify deficits from their ablation. This study emphasizes the importance of microglia during the development and maturation of auditory circuitry.

4. Serotonergic Modulation of Medial Olivocochlear Neurons
Category: Brainstem: Structure and Function
Kirupa Suthakar*[1], Catherine Weisz[2]
[1]*NIH/NIDCD,* [2]*National Institutes of Health*

Background: Medial olivocochlear (MOC) efferent neurons are located in the superior olive of the brainstem and form a sound evoked feedback loop that inhibits cochlear amplification via suppression of OHC electromotility. Previous work has identified a functional role for MOC neurons in protection from acoustic trauma, and signal extraction in noisy environments. In addition to primary auditory input via T-stellate neurons of the cochlear nucleus, MOC neurons are putatively modulated by synaptic inputs from various auditory and non-auditory brain regions, as identified anatomically using immunohistochemical and neuronal tract tracing methods. However, due to the difficulty in accessing neurons embedded in this ventrally located, myelin dense, and cellularly heterogeneous brain region, little is known of the functional connectivity afforded by a diverse repertoire of inputs. Given the proposed role of these neurons in context dependent tasks, we are interested in investigating the non-auditory modulation of MOC activity. While previous literature has identified the presence of serotonergic terminals in close apposition to retrogradely labelled MOC cells, there is a scarcity of physiological data. Thus, the question of how serotonin affects hearing via effects on MOC neurons remains to be answered.

Methods: Recent work from our lab and others has demonstrated that the combined use of transgenic mouse lines and in-vitro whole-cell patch-clamping techniques is a robust and reliable method of investigating the synaptic physiology of MOC neurons. To anatomically demonstrate the existence of serotonergic terminals in close apposition to MOC efferent neurons in mouse (existing data is limited to rat and guinea pig), we used a combination of neuronal tract tracing and immunohistochemistry in the same transgenic mouse lines used for physiology experiments. To electrophysiologically characterize the effect of this serotonergic innervation, we used in-vitro patch clamping methods coupled with bath application of drugs and more spatially precise optogenetic stimulation of serotonergic terminals.

Results: Retrograde tracer injections into the cochlea of adult ChAT-IRES-Cre X tdTomato (Ai14) mice, followed by immunohistochemistry for anti-serotonin or anti-TPH2 (tryptophan hydroxylase 2, the rate limiting step in serotonin synthesis) in sequential brain tissue sections confirmed the presence of serotonergic terminals apposing confirmed cholinergic MOC neurons. Preliminary results from bath application of 10μM serotonin are suggestive of an increase in excitability. Additionally, 'plateau potentials' qualitatively similar to those described in spinal motoneurons have also been observed.

Conclusions: Preliminary evidence suggests serotonin may play a role in modulating MOC efferent excitability; however, interpretation of these results is complicated by potential non-specific serotonergic responses of local circuitry to cells of interest. These data will aid in our understanding of central auditory processing and how factors such as mood and attention are involved in modulating MOC responses in complex listening situations such as in the presence of background noise.

5. Test-Retest Reliability of Subcortical EEG Responses to Running Speech
Category: Brainstem: Structure and Function
Florine L. Bachmann*[1], Jens Hjortkjær[1]
[1]*Technical University of Denmark*

Background: Recent research has employed linear regularized regression models to measure electroencephalographic (EEG) auditory brainstem responses to running speech (speech-ABR), with potential clinical applications. For clinical use, however, test-retest reliability is a prerequisite that has not yet been addressed. While traditional click-evoked auditory brainstem responses (click-ABRs) and event-related potentials (ERPs) have been found to be reliable across recording sessions, studies of their reliability in the most numerous target group for hearing intervention, older hearing-impaired listeners, are sparser.

Methods: We investigate test-retest reliability of subcortical responses to running speech, pure-tone evoked ERPs, and click-ABRs with scalp EEG in eleven (5 female) hard-of-hearing (PTA of 40.96 ± 7.88 dB HL) older (aged 68.45 ± 7.19 years) bilateral hearing aid users (8.73 ± 5.77 years of use) across six measurement sessions (S) on different days (3.13 ± 53.29 days between sessions). ERP stimuli were identical across sessions. Narrated stories served as running speech stimuli, calibrated to 65 dB SPL. Apart from S5, speech was linearly amplified based on individuals' audiograms using the Cambridge Method for Loudness Equalization (CAMEQ). In S1 and S5, the speech material was congruent and only differed in whether amplification was provided. In S2 and S6, we presented identical amplified speech material as well as clicks at a high level (110 dB ppeSPL). Clicks were presented at lower levels during all other sessions (65 dB ppeSPL with or without amplification). In total, we presented amplified speech by the same male narrator in three sessions (S1, S2, S6), and by the same female speaker in two sessions (S3, S4). The speech-ABR was computed via temporal response functions (TRFs), using forward regularized regression.

Results: The subcortical response to running speech showed a distinct positive peak at a latency corresponding to click-ABR wave V. For amplified speech, this response peak showed high intraclass correlation in amplitude across identical or similar sessions, and in latency across sessions with identical speech material. Click-ABR wave V measured at high levels and ERP components P1, N1, and P2 were highly reliable in both latency and amplitude across sessions.

Conclusions: Measures to running speech enable naturalistic listening experiments and are potentially more transferrable to real life. Subcortical measures to running speech were found to be reliable across sessions where identical speech materials were presented. ERP components P1, N1, and P2 and ABR wave V to clicks presented at high levels prove excellent test-retest reliability in older hard-of-hearing listeners.

6. The Role of Fragile X Mental Retardation Protein in Cellular and Synaptic Dynamics of Developing Auditory Circuits

Category: Brainstem: Structure and Function

Xiaoyan Yu*[1], Yuan Wang[1]
[1]*Florida State University*

Background: The establishment of central sensory systems is influenced by afferent experience during development. In the auditory system, hearing loss at young ages leads to neuronal cell loss and circuit reorganizations, yet the responsible molecular players have not been identified. We addressed this question by investigating the neuropathology of fragile X symptom (FXS), a leading genetic cause of neurodevelopmental disorders with prominent auditory dysfunction. FXS results from a loss of fragile X mental retardation protein (FMRP) encoded by Fmr1 gene. Studies of Fmr1 knockout (KO) rodents revealed abnormal circuit development and altered neuronal plasticity in the auditory system. However, little is known about how FMRP regulates neuronal integrity and synaptic connectivity in response to changes in afferent input. This study aimed to determine the consequences of FMRP absence in auditory processing, with and without intact afferent input, during early development.

Methods: We first examined afferent-regulated neuronal integrity in the ventral cochlea nucleus (VCN), the first brain station of auditory afferent input. Next, we examined synaptic development and plasticity in the medial nucleus of the trapezoid body (MNTB), which receives the excitatory input from the VCN and serves as an inhibitory station in the auditory brainstem. Finally, we use auditory brainstem response (ABR) to examine how FMRP regulates hearing sensitivity and function during early development.

Results: In wildtype (WT) mice, VCN exhibited a critical period (CP) of neuronal cell loss following afferent deprivation, showing a significant cell loss only when the deprivation occurred within the first postnatal (P) 11 days. We found the presence of a CP and a comparable level of cell loss within the CP in Fmr1 KO mice. However, the timing of CP closure was significantly delayed in Fmr1 KO mice, evident by substantial cell loss following afferent deprivation at P14, an age beyond the CP in WT. Next, FMRP loss had significant effects on the development of the MNTB circuit. In WT mice, a distinct topographic gradient in the level of several key

synaptic proteins was found across the MNTB before P7 and this gradient was not significant after hearing onset at P10-12. Fmr1 KO mice exhibited delayed progress of this developmental event. Following afferent deprivation, WT mice showed a significant increase in the density of inhibitory synapses on MNTB neurons. This response was diminished in Fmr1 KO mice, demonstrating a requirement of FMRP for inhibitory synaptic plasticity. Finally, using ABR, we revealed an impaired hearing sensitivity showing as a significantly increased threshold in response to auditory stimuli in Fmr1 KO mice at P14.

Conclusions: Together, these findings demonstrate that FMRP is required for selective aspects of afferent-regulated neuronal dynamics in the auditory brainstem at the cellular, connective, and functional levels by regulating their precise timing and/or sensitivity to afferent experience.

7. Leveling Up: A Glycinergic Olivogeniculate Projection That Circumvents the Inferior Colliculus
Category: Brainstem: Structure and Function
Alyson Burchell[1], Yusra Mansour[1], Randy Kulesza*[1]
[1]Lake Erie College of Osteopathic Medicine

Background: The medial nucleus of the trapezoid body (MNTB) plays essential roles in sound source localization and processing complex sounds. The MNTB is composed predominantly of principal neurons that are calbindin positive (CB+) and have round/oval cell bodies. Principal neurons receive glutamatergic input from the contralateral cochlear nucleus globular bushy cells via the Calyx of Held. MNTB principal neurons have known local and ascending glycinergic projections. Locally, through the short-range glycinergic projections to the surrounding superior olivary complex nuclei, the MNTB creates offset responses and encodes interaural timing and intensity differences. Additionally, there are ascending projections from the MNTB to the ventral and intermediate nuclei of the lateral lemniscus (INLL). However, a number of tract tracing studies indicate the MNTB does not innervate the dorsal nucleus of the lateral lemniscus or inferior colliculus (IC). Preliminary work raised suspicion that MNTB principal neurons might send a direct projection to the auditory thalamus.

Methods: To explore this novel projection to the MG, we employed stereotaxic retrograde tract tracing using fluorogold (FG), anterograde tracing using biotinylated dextran (BD) and immunohistochemistry for calbindin (CB, the glycine receptor and the vesicular inhibitory amino acid transporter.

Results: Large injections of FG into the inferior colliculus revealed FG labeling in <1% of MNTB neurons. However, injections of FG that included the ventral (vMG), dorsal (dMG) and medial nuclei of the MG resulted in robust retrograde labelling in the MNTB. Smaller FG injections restricted to the vMG resulted in the highest number of FG+ MNTB neurons. Combining FG+ injections with CB immunolabeling, revealed nearly all MNTB neurons projecting to the vMG are CB+. Injections of BD into the MNTB resulted in labelled axons through the lateral lemniscus and numerous en passant boutons in the vMG.

Conclusions: Together, these findings support the existence of a long-range, glycinergic projection from the MNTB to the vMG that bypasses the IC.

8. Characterization of Affective Hyperacusis and Nociceptive Sensitization Following Noise Overexposure in Mice
Category: Brainstem: Structure and Function
Lorraine Horwitz*[1], Susan Shore[1], Bo Duan[1]
[1]University of Michigan

Background: Noise exposure can result in cochlear damage, contributing to hearing loss, fullness in the ear, tinnitus, and hyperacusis. Hyperacusis is a debilitating auditory hypersensitivity disorder which is characterized by decreased tolerance to environmental sounds. This disease can be further classified as loudness hyperacusis (moderate-intensity sounds are perceived as too loud), avoidance hyperacusis (negative emotional reaction to sounds) and pain hyperacusis (sound-induced pain) (Baguley et al., 2011). Pain is a distressing feeling often caused by intense or damaging stimuli which motivates the individual to withdraw from a dangerous situation, protect a damaged body part while it heals, and/or to avoid similar experiences in the future. Pain hyperacusis can be particularly traumatic as pain within and/or surrounding the ear and parts of the face can begin almost immediately after moderate-intensity noise exposure or develop slowly over several hours (Hayes et al., 2014). Furthermore, patients with hyperacusis report widespread skin hypersensitivity (Fioretti et al., 2016). How pain sensitivity is altered following noise exposure is not well understood. This study examines changes in nociceptive sensitivity following acute noise exposure in mice.

Methods: Awake mixed C57B16 x 129 mice of both sexes were administered binaural noise over exposure (8-16kHz, 100dB SPL, 1 hour) producing temporary hearing threshold shifts (TTS). Age- and gender matched sham-noise exposed controls were used. Several acute somatosensory behavioral tests (rotarod, von Frey, facial von Frey, Hargrave's assay, hot plate, and cold plate) were used to measure locomotion, mechanical pain thresholds, and noxious thermal pain sensitivity (hot and cold) respectively (Bourance et al., 2015, Cheng et al., 2017, Duan et al., 2014). Prior to testing, three 'habituation' sessions (30 min per day) in the behavioral testing apparatus were performed. To measure affective hyperacusis (avoidance and pain), mice were tested in a conditioned place preference assay as well as the facial-grimace-response-to-noise assay.

Results: Noise exposure leading to TTS resulted in mechanical pain hypersensitivity without alterations to thermal (hot/cold) pain measurements or changes in locomotor ability. Furthermore, mice that displayed acute somatosensory sensitization displayed increased aversion and pain in response to moderate intensity noise presentation.

Conclusions: The results suggest the increases in mechanical pain sensitivity may be linked to hyperacusis generated by noise over exposure. Future studies will be undertaken to determine the neuronal mechanisms underlying these associations.

11:00 a.m. - 1:00 p.m.
Symposium #16

Auditory Cognition in Cochlear Implants: Scene Analysis, Attention, Effort, and Real-World Communications
Chair: Inyong Choi, *University of Iowa*
Co-Chair: Andrew Dimitrijevic, *Sunnybrook Hospital ENT University of Toronto*

Outcomes for cochlear implants (CIs) have improved tremendously over the last decades. Yet, one of the biggest complaints expressed by CI users is that they have to "shut down" in noisy situations. For years, CI research has attempted to capture this difficulty of real-world communications through laboratory tests. However, data collected in the control of a laboratory often include a great deal of heterogeneity that cannot be fully explained by subject factors. Further, individuals with identical speech-in-noise performance in the lab often exhibit huge variability in how they function in real-world settings.

The overarching goal of this symposium is to discuss basic and cognitive neuroscience methodologies to address this critical question about how to predict CI outcomes in noisy real-world environments. Cognitive science in the normal auditory system has established speech-in-noise processing models that incorporate multiple functional stages from the periphery to the cortex. Such functions include encoding of spectrotemporal features, auditory grouping, selective attention, and rapid mapping of sound to meaning. Presentations in this symposium will introduce recent advances in the assessment of the above auditory cognitive functions in CI users. Equally important, this symposium will also discuss human ecology — factors in the person and in the environment that can mediate or impede successful communication. To do this, this symposium will bring in new perspectives from clinical scientists and engineers who have established novel methodologies for the in-situ recording of the real-world performance and satisfaction of CI users.

There have been a few similar Symposia in recent ten years: One in 2011, another in 2012, and then in 2014. More recently, in 2017 and 2021, four Symposia discussed peripheral and central mechanisms of CI and future directions of auditory implants. However, none of the precedent Symposia focused on how to integrate the assessment of auditory cognitive functions and reliable in-situ measures of real-world communication outcomes. This symposium will be appealing to diverse audiences with various backgrounds including basic neuroscience, cognitive science, and clinical science since it will attempt to bridge the well-known gap between laboratory measures of auditory neural mechanisms and the real-world performance of hearing-impaired listeners.

Abnormal Binaural Fusion of Speech Across Fundamental Frequency: An Alternative Explanation for Difficulties With Speech in Background Talkers
Lina Reiss, *Oregon Health and Science University*

Hearing-impaired individuals often exhibit abnormal binaural fusion, fusing sounds differing in pitch by up to 3-4 octaves across ears. Here we present evidence that this abnormal binaural fusion extends to speech sounds. Listeners with broad binaural fusion fuse dichotically presented vowels, even for large fundamental frequency differences. Similarly, in complex listening environments, listeners with broad binaural fusion need higher signal-

to-noise ratios to understand target speech. Together the findings suggest that broad binaural fusion leads to spectral blending across ears, even for different fundamental frequencies, and may hinder the stream segregation and understanding of speech in the presence of competing talkers.

Hearing Emotions With Cochlear Implants
Monita Chatterjee, *Boys Town National Research Hospital*

As voice pitch cues are a dominant source of information about emotional prosody (the tone and manner of speaking signifying emotions), cochlear implant (CI) users, who have limited access to voice pitch cues, also show impaired identification of emotional prosody. In this presentation, I will describe our findings on emotional communication by CI users, including developmental effects in prelingually deaf children with CIs; senescent decline in postlingually deaf adults; predictors of individual variability in outcomes; and the consequence of these perceptual deficits for the production of emotional prosody by children with CIs.
[Support: NIH R01 DC014233, American Hearing Research Foundation]

Speech Perception and Listening Effort With Background Speech Maskers Among Cochlear Implant Users With Single-Sided Deafness
Lukas Suveg, *University of Wisconsin*

Cochlear implants (CIs) are becoming available to adult patients with single-sided deafness (SSD), allowing listeners to hear bimodally, with one acoustic ear and one CI (SSD-CI). These listeners' hearing is characterized by profound asymmetry. We were interested in two related phenomena: (a) whether the CI improves speech understanding in the presence of background noise, and (b) whether listening effort, or cognitive load, is expended to a different degree when target speech is presented in quiet (0 deg, front), or with maskers that are either co-located (target and masker 0 deg) or spatially separated (target 0 deg, maskers □90 deg). We compared these effects under two important listening configurations: with the acoustic ear only prior to the CI being implanted, and in the acoustic+CI (bimodal) condition, one year after CI activation. All testing was conducted at the University of Wisconsin-Madison, and surgeries were conducted at the Massachusetts Eye and Ear Infirmary as part of a clinical trial.
Data on speech understanding and listening effort were obtained during a single task. Percent correct for IEEE sentences (spoken by a male talker) were measured with stimuli presented from a loudspeaker. During each masking condition (co-located and spatially separated), participants heard two AzBio sentences spoken by two distinct male talkers. During the speech perception task, we monitored participants' task-evoked pupillary response using an EyeLink 1000 eye tracker as a measure of listening effort. We tracked pupil dilation before, during, and after presentation of each sentence. On each trial, we quantified baseline (pre- stimulus), and peak pupil dilation during the two second post-stimulus window prior to the participant's response.
As in prior investigations on benefits of spatial cues, benefit would be evidenced by improved speech understanding in the spatially separated condition compared with the co-located condition. The novel assessment is whether improved speech understanding is accompanied by reduced pupil dilation. Further, we asked if the CI provides an added benefit, resulting in greater effects in bimodal than the acoustic ear only listening mode.
Results suggest that, with maskers, speech understanding is better in the bimodal than acoustic ear only listening mode. Spatial separation induced improved speech understanding in both listening modes. However, on average, listeners expended more listening effort in the bimodal than acoustic ear only listening mode. These findings suggest that integration of acoustic and electric hearing is possible and leads to improved outcomes but can be "costly" in the listening effort domain.
This work was supported by NIH-NIDCD grant R01DC003083 to Ruth Y. Litovsky, and in part by a core grant from the NIH-NICHD (U54 HD090256 to Waisman Center). In addition, funds from Med-EL provided partial travel support for participants. This study was part of a clinical trial sponsored by Med-El (https://www.clinicaltrials.gov/ct2/show/NCT02532972?cond=cochlear+implant+single+sided and draw=2 and rank=7). Thank you to our wonderful participants who joined us at the Waisman Center for these experiments.

Grouping Mechanisms for Real-World Listening in CI Users
Timothy Griffiths, *Newcastle University*

We have developed an 'audiogram for scene analysis' to assess cross-frequency grouping mechanisms relevant to real-world listening, especially speech-in-noise perception. Performance in this task predicts speech in noise

ability in normal listeners and the neural basis has been characterized in humans and a non-human primate model: at the system and neural levels in both. Work on CI patients has shown that the ability of subjects to carry out cross-frequency grouping using their electrical hearing also predicts speech in noise ability. Ongoing work is assessing how the cortical system is used in CI patients.

Neural Correlates of Auditory Streaming in CI Users Are Related to Everyday Listening Experiences
Andrew Dimitrijevic, *Sunnybrook Hospital ENT University of Toronto*

Cochlear implant users often struggle with everyday listening in noisy environments. These everyday concerns are not typically assessed in standard clinical batteries. There appears to be a weak relationship between measures of quality of life and measures of clinical speech perception. We propose that an auditory scene analysis framework is related to everyday listening concerns in CI users. We will show that electrophysiological measures of everyday listening ranging from attention to speech in multitalker noise to selective attention of specific instruments in multi-instrument music are related to measures of quality of life and to listening effort in cochlear implant users.

Ecological Momentary Assessment in Hearing Research: Psychometric Characteristics, Limitations, and Opportunities
Yu-Hsiang Wu, *The University of Iowa*

This presentation will focus on the use of Ecological Momentary Assessment (EMA) to assess hearing device outcomes within the auditory ecology framework, which aims to characterize the interactions between listeners' unique perceptual demands, their daily listening environments, and hearing devices. We will summarize the evidence that supports the validity, reliability, and sensitivity of EMA. We will also discuss the limitation of EMA, as well as how EMA can be used in future hearing research.

Real-World Outcomes of Cochlear Implantation Using Ecological Momentary Assessment
Camille Dunn, *The University of Iowa*

Most cochlear implant (CI) research focuses on determining the effect of intervention on laboratory outcomes, such as speech perception. The overall goal of this presentation is to demonstrate the impact of CI on hearing-related functions and disability in the user's natural environments. In this talk, I will discuss the effect of CI from a group of listeners who utilized our smartphone-based ecological momentary assessment (EMA) system to capture their real-time experiences in situ (i.e., in natural environment). Data will be characterized in domains of speech understanding, satisfaction, social isolation, depression, and anxiety as they are situated in various listening contexts.

Effects of COVID-19 Lockdowns on Real World Listening Environments in Children Using Cochlear Implants: Evidence From Datalogging Systems
Karen Gordon, *The Hospital for Sick Children*

Changes in children's real-world auditory environments during COVID-19 restrictions including school closures were assessed. Decreased social interactions could reduce language exposure with implications for development of language. This is particularly concerning for children using cochlear implants (CIs) who are already at risk for language impairment. Validated datalogging CI systems were used to measure daily sound environments before and during pandemic restrictions. During initial lockdowns in 2020, children showed consistent CI use but reduced exposure to speech. As the lockdowns continued into 2021, children with single-sided deafness showed reduced daily CI use. These data and potential developmental consequences will be discussed.

11:00 a.m. - 1:00 p.m.
Symposium #17

Twenty-One Years of Prestin
Chair: Joseph Santos-Sacchi, *Yale University School of Medicine, Surgery, Neuroscience, Cellular and Molecular Physiology*

It is 21 years since prestin's discovery (SLC26a5; Zheng et al, 2000), the protein that drives the unique length changes (electromotility, eM; (Brownell, 1985)) of outer hair cells (OHC) from Corti's organ. The identification refocused our efforts on understanding how this protein could account for OHC characteristics previously determined, and how the protein could underlie the OHC's ability to enhance audition. In fact, just this year, our group (Butan et al., 2021) and two others (Ge et al, 2021; Bavi et al., 2021) have solved the cryo-EM structure of prestin. Here, we put together a team that has focused their careers on understanding prestin. Dominik Oliver will present on structure-function of prestin, including chloride interactions, crucial in understanding the switch from ion transporter to molecular motor. Prestin sits in a sea of lipid, having an enormous impact on its function. Rob Raphael will discuss the role of lipids in confining and directing prestin activity. Of course, cytoskeletal interactions are important to couple prestin's activity to the OHC soma. Kumar Navaratnam will discuss the interactions of prestin with sub-plasmalemmal structural proteins. It is important to investigate the influence of prestin variants that alter hearing. Kazu Homma will discuss an interesting human variant that has corresponding influences in the mouse, providing insight into prestin's role in vivo. In an interesting twist, Jing Zheng will highlight non-motor roles of prestin, showing its influence in the cell biology of the OHC. The last two speakers will remark on the dogmatic role of OHCs in high frequency hearing. Joe Santos-Sacchi will provide evidence that prestin electromechanics are not quite as fast as thought, questioning the influence of voltage in driving very high frequency OHC influences. Furthermore, Marcel van der Heijden will present high frequency OCT data of OHC activity in vivo, requiring new theories on how OHCs provide enhanced hearing. The information provided by these speakers indicates that we do not have the final answer on how the ear's works work.

Prestin and the Cytoskeleton
Dhasakumar Navaratnam, *Yale University School of Medicine*

Dhasakumar Navaratnam, Yale University
The activity of prestin (SLC26A5) in generating outer hair cell motility is established. Prestin's gating charge movement (NLC) has served as a surrogate measure for electromotility. It is widely accepted that electromotility is a membrane-based phenomenon. Using a number of cytoskeletal mutants, we demonstrate that electromotility has a significant cytoskeletal element. These mutants reduce hearing and DPOAEs while leaving NLC relatively intact. In contrast, electromotility is reduced and could account for decrements in DPOAEs. Collectively, our data argue that cytoskeletal elements in the sub-plasmalemmal structures are integral to electromotility.

Structural Dynamics of Prestin Underlying Electromotility
Dominik Oliver, *Philipps University Marburg*

The identity of prestin (SLC26A5) as an anion transporter homolog has important implications for understanding the molecular events underlying OHC electromotility, which is particularly evident from the impact of anions on prestin's function. Thus, the recent emergence of high-resolution structures of SLC26 homologs has provided a straightforward avenue to learn about prestin from transport mechanisms. Here, we will present our approach probing molecular mechanisms of prestin by integrating mutagenesis, chemical modification, electrophysiology, fluorescence spectroscopy, and molecular dynamics simulations. Mapping the results onto experimental and computational structures reveals details of anion binding, voltage control, and conformational dynamics of prestin.

What Role Does Prestin Play in OHC Function and Survival?
Jing Zheng, *Northwestern University*

OHCs are innervated by both MOC efferents and type II afferents, which also innervate supporting cells to form a local neural network around OHCs. Prestin is the molecular basis for OHC somatic electromotility that amplifies organ of Corti movements. However, early-onset OHC loss was found in two prestin-transgenic mouse models that either lack Prestin (prestin-KO mice) or lack electromotility (499-KI mice). 499-KI mice express mutant prestin with mutant amino acids (V499G/Y501H) located at the end of prestin's last membrane-spanning helix. To uncover molecular pathways that evoke OHC death, we profiled the coding transcriptome of OHCs from wildtype (WT), prestin-KO, and 499-KI mice using single cell RNA sequencing (scRNA-seq). scRNA-Seq transcriptomics and pathway analyses did not reveal common pathways associated with OHC loss observed in both prestin-KO and 499-KI mice. Enrichment clustering analysis showed that increased gene expression in OHCs from prestin-KO mice was associated with lipid metabolic processes and cell death pathways. These mRNA profiles likely contribute to OHC loss observed in prestin-KO mice and support the notion that Prestin is also a structural protein

required for normal plasma membrane compartmentalization, which is essential to establish OHC-MOC synapses. In contrast, the mRNA profile of OHCs from 499-KI mice does not provide a rational explanation of the early-onset OHC loss in this mutant. OHCs from 499-KI mice appear to have normal plasma membrane compartmentalization and normal OHC-MOC contacts. However, 499 Prestin appears to change the local neural network around OHCs as more synaptic markers were found near neighboring supporting cells when compared to WT and prestin-KO mice. Thus, OHCs in prestin-KO (no Prestin) and 499-KI (Prestin protein present, no electromotility) may influence local neuronal networks in different ways. Collectively, our data suggest that Prestin and its motile properties are also important for OHC survival and the maintenance of local afferent/efferent circuits.

The Pathological Mechanism of the p.R130S Prestin Missense Variant
Kazuaki Homma, *Northwestern University*

Two SLC26A5 (prestin) variants, p.W70X (c.209G>A) and p.R130S (c.390A>C), were found in compound heterozygous patients with moderate to severe hearing loss (Mutai et al., 2013). The p.W70X nonsense variant is almost certainly pathogenic, as it results in complete loss of the transmembrane and the C-terminal cytosolic domains. However, the pathological significance of the p.R130S variant had remained ambiguous. We generated an R130S-prestin knock-in mouse model and demonstrated that this missense variant is pathogenic. The purpose of this presentation is to provide mechanistic insights into how R130S-prestin affects hearing.

Revisiting the Role of Outer Hair Cells
Marcel Van Der Heijden, *Erasmus MC*

Recent experimental data on the frequency limits of outer hair cell (OHC) motility undermine their putative role as mechanical amplifiers of sound-evoked vibrations. For instance, just-audible 20-kHz tones evoke an AC receptor potential of only a few microvolts. Amplification based on OHC motility would be far too noisy to improve high-frequency sensitivity. I will review the commonly stated claim that losses from fluid resistance hamper cochlear sensitivity and show that this claim is unfounded. I will then discuss what the actual OHC behavior observed in OCT vibrometry may tell us about the potential mechanisms by which OHCs control sensitivity.

Prestin in the Membrane Environment
Rob Raphael, *Rice University*

As a transmembrane protein, the organization and activity of prestin depends on the composition and mechanics of the plasma membrane. We have established through fluorescence recovery after photobleaching (FRAP), fluorescence lifetime imaging (FLIM) and single-molecule imaging that prestin exhibits confined diffusion and localizes in cholesterol-rich membrane domains. Prestin activity is also modulated by biologically active molecules that incorporate into the membrane (alcohols and NSAIDs). This talk will summarize our current knowledge of how the biophysical properties of the membrane tune the activity of prestin and highlight directions for future research.

Prestin Electromechanics is Not so Fast (15 min)
Joseph Santos-Sacchi, *Yale University School of Medicine, Surgery, Neuroscience, Cellular aand Molecular Physiology*

OHC electromechanical activity has been viewed as ultrafast, a requirement if the OHC is promoting cochlear amplification at very high acoustic frequencies. Here we present our recent work that challenges this concept. Specifically, we find that electromotility cannot follow voltage changes in the cell with high fidelity, and this is related to the slow multifarious time constant of its membrane, and more importantly the unusually low-pass nature (non-Lorentzian) of prestin charge movement that evokes electromotility. We acknowledge the important influence of OHCs for hearing, but its role in very high frequency hearing is questionable.

11:00 a.m. - 1:00 p.m.
Podium Session #18 – New Molecular, Structural and Functional Perspectives of the Inner Ear

Moderators: Michael Deans, Ph.D. & Selina Baeza Loya, B.A.

1. Cochlear Supporting Cells Function as Macrophage-Like Cells Against Pathogens
Category: Inner Ear: Anatomy and Physiology

Yushi Hayashi*[1], Hidenori Suzuki[2], Wataru Nakajima[3], Tatsuya Katsuno[4], Shin-ichiro Kitajiri[4], Naoto Koyanagi[5], Yasushi Kawaguchi[5], Koji Onomoto[6], Mitsutoshi Yoneyama[6], Hiroki Kato[7], Takashi Fujita[7], Nobuyuki Tanaka[3]
[1]*Harvard Medical School, Department of Otolaryngology,* [2]*Nippon Medical School, Division of Morphological and Biomolecular Research,* [3]*Nippon Medical School, Department of Molecular Oncology,* [4]*Kyoto University, Department of Otolaryngology,* [5]*The University of Tokyo, The Institute of Medical Science,* [6]*Chiba University, Division of Molecular Immunology,* [7]*Kyoto University, Institute for Virus Research*

Background: Virus infection such as cytomegalovirus, rubella, mumps, measles, and herpes simplex virus is considered to cause sudden sensorineural hearing loss (SSHL) and congenital SHL. While mouse cytomegalovirus-infected mice show SHL due to reduced hair cell (HC) synapses and spiral ganglion neurons without HC and supporting cell (SC) missing (Sung et al., 2019), human temporal bone histopathology of SSHL exhibits HC and SC missing (Merchant et al., 2005).
We previously reported that SCs express type I interferon (IFN) through retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated protein 5 (MDA5) against virus infection (Hayashi et al., 2013). The aim of this project is to further understand immune response of the cochlea against virus infection, then to elucidate the mechanism of SHL caused by virus infection.
Methods: The organ of Corti of postnatal day 2 (P2) mice was cultured overnight for stabilization. Then Theiler's murine encephalomyelitis virus (TMEV), which belongs to picornavirus (RNA virus) and has high affinity to nerve tissues, was administered to the cultured tissue (3.0 x 107 pfu/ml). The virus-infected tissue was subjected to protein assay and gene expression assay.
Results: Ifnar1 and Ifnar2, subunits of IFN receptors, were expressed in both HCs and SCs under steady-state conditions. The Ifnar1 KO cochlea showed significantly more TMEV-infected outer and inner HCs than the WT cochlea. These observations indicate type I IFNs secreted from SCs protect HCs from virus infection.
Transcripts of cytokines such as Il6, Il1b, Cxcl11, and Il10 were up-regulated in TMEV-infected cochleae as well as those of type I IFNs (Ifna4 and Ifnb1). Moreover, expression level of macrophage markers such as F4/80, Mac-1, and Iba1, proinflammatory M1 macrophage markers such as Irf5, and anti-inflammatory M2 macrophage markers such as Jmjd3 was also up-regulated by virus infection.
Surprisingly, virus-infected SCs and greater epithelial ridge cells (GERCs) detached their cell layers, then started to migrate to the HC layer. SCs and GERCs strongly expressed F4/80 during virus infection. SCs also expressed both Irf5 and Jmjd3 in response to virus infection, indicative of both M1 and M2 macrophage types. Conversely, GERCs expressed Jmjd3, but not Irf5 during virus infection, indicative of M2 macrophage phenotype. Both SCs and GERCs activated as macrophages exhibited phagocytosis.
In the Irf5 KO cochlea where M1 macrophage polarization is suppressed, induction of the genes encoding F4/80, Mac-1, and Iba1 macrophage markers and migration of SCs in response to virus infection were impaired, but virus-induced migration of GERCs was still observed, which indicates that SC activation as macrophages is regulated by Irf5.
Conclusions: · Virus-infected SCs and GERCs show phenotype of macrophages, of which role is to protect HCs from virus infection by secreting type I IFNs.
· Irf5 plays a significant role to induce SC function as macrophage-like cells during virus infection.

2. Developmental Spontaneous Activity Establishes Sensory Domains and Proper Gain in Central Auditory Circuits
Category: Inner Ear: Anatomy and Physiology

Calvin Kersbergen*[1], Travis Babola[2], Jason Rock[3], Dwight Bergles[2]
[1]*Johns Hopkins University School of Medicine,* [2]*Johns Hopkins University,* [3]*Genentech*

Background: Neurons in the developing auditory system fire periodic bursts of action potentials that are generated in the cochlea in the absence of sound. This intrinsically generated activity is thought to promote maturation of sound processing circuitry; however, the precise roles of the highly stereotyped correlated firing of auditory neurons during development remain poorly understood. Addressing this question requires a means to selectively disrupt the correlated burst firing of auditory neurons prior to hearing onset, while preserving the later

ability of the inner ear to transduce sound. Our previous studies indicate that inner supporting cells (ISCs) within the cochlea initiate neuronal burst firing by spontaneously releasing ATP, initiating a cascade of events that culminates in activation of ISC calcium-activated chloride channels (TMEM16A/ANO-1). Chloride efflux through these channels provides the ionic force necessary to draw potassium out of ISCs, triggering depolarization of nearby inner hair cells.

Methods: Here, we genetically inactivated Tmem16a within the cochlea and assessed the consequences for both developmental spontaneous activity and response patterns of neurons to sound after hearing onset.

Results: Whole cell recordings from ISCs in cochleae isolated from Tecta-Cre;Tmem16afl/fl (Tmem16a cKO) mice indicated that loss of TMEM16A abolished spontaneous inward currents in ISCs throughout the postnatal pre-hearing period. Consistent with the critical role of ISCs in triggering neuronal burst firing, in vivo widefield Ca2+ imaging of the inferior colliculus (IC) revealed that spontaneous neuronal burst firing in neonatal mice was dramatically reduced and less spatially restricted in Tmem16a cKO mice; however, cochlear structure was preserved and no hair cell or spiral ganglion neuron loss was detected. At hearing onset, these mice also retained normal auditory brainstem response (ABR) thresholds, with only subtle changes in ABR waveform amplitude and timing. Nevertheless, neurons in Tmem16a cKO mice were more sensitive to pure tones, responding with larger Ca2+ increases elicited to a given sound stimulus, and the area activated within IC to each sound was broader, indicating that response gain in the central auditory pathway was enhanced. In addition, macroscopic imaging of neuronal responses to a range of sound frequencies revealed that the tonotopic map in IC was profoundly compressed in Tmem16a cKO mice, with sound-responsive neurons restricted to a smaller portion of the IC. Two-photon imaging revealed that individual neurons in the IC of these mice exhibited larger Ca2+ increases to a given tone, particularly at suprathreshold sound levels, and were more broadly tuned, responding to a larger range of frequencies than neurons in control mice.

Conclusions: These results indicate that intrinsically driven, correlated firing of auditory neurons within sensory domains is necessary to both establish isofrequency domains and set the proper sensitivity of auditory circuitry within sound processing centers of the brain.

3. FGF10, Together With FGF20, Regulates Auditory Sensory Progenitor Development Through FGFR1
Category: Inner Ear: Anatomy and Physiology
Pooja Roy*[1], Michael Ebeid[1], Sung-Ho Huh[1]
[1]*University of Nebraska Medical Center*

Background: The mammalian organ of Corti within cochlea of the inner ear harbors a mosaic arrangement of sensory cells crucial for sound detection. Defective sensory progenitor cell (SPC) development perturbs patterning of these cells and manifests as congenital sensorineural hearing loss (SHNL). Uncovering etiology warrants a detailed understanding of signaling pathways regulating SPC development. Signaling through FGFR1 is required for SPC development. We previously identified that FGF20 binds to FGFR1 to promote SPC differentiation but the phenotypic severity of Fgf20 mutant mouse cochlea is not as severe as that of Fgfr1 mutant. We recently identified FGFR1 is also required for SPC specification where SPCs are primed for differentiation by exiting cell cycle. Specification is independent of FGF20 as FGFR1, but not FGF20, regulates specification markers such as SOX2 and p27. Thus, the ligand controlling specification remains unknown. Through genetic screening, we propose that FGF10 is a potential candidate. We analyzed cochlear phenotypes of Fgf10 and Fgf20 compound mutant mice. In addition, FGF10 and FGF20 bind different alternative splice variants of FGFR1, FGFR1b and FGFR1c respectively. Hence, we determine whether both Fgfr1b and Fgfr1c transcripts are expressed in the cochlear epithelium.

Methods: We generated Fgf10 and Fgf20 compound mutant mouse lines. Cochleae harvested from mutant embryos (Fgf10-KO, Fgf20-KO, Fgf10/20-DKO and heterozygous controls) were prepared for whole-mount immunofluorescence with phalloidin to visualize hair bundles of sensory hair cells. Hair cells present in each group were counted in $100\ \mu m$ of the cochlear duct (n=3-5). Molecular detection of the Fgfr1b and Fgfr1c mRNA transcripts was accomplished using a colorimetric dual mRNA detection system called BaseScope. This was performed on paraffin sections prepared from wild-type embryos undergoing specification at embryonic day 13.5 and 14.5.

Results: Immunostaining revealed that cochlear phenotype of Fgf10/20 double knockout closely recapitulates the severe hair cell loss of Fgfr1 knockout cochlea. This suggests that FGF10 functions with FGF20 to activate FGFR1 during SPC development. Additionally, we identified that both Fgfr1b and Fgfr1c transcripts are expressed in cochlear epithelium during specification. Identification of signaling molecules controlling

specification-differentiation switch during SPC development is key from a regenerative medicine perspective of restoring SNHL.

Conclusions: Fgfr1 mutant phenotype was recapitulated with Fgf10/Fgf20 deletion and both Fgfr1 splice transcripts were detected in the cochlear epithelium. We conclude that FGF10 signals through FGFR1b to regulate SPC development. Going forward, we will determine whether Fgf10 regulates SPC specification independent of Fgf20, whether Fgf10 functions together with Fgf20 to regulate SPC differentiation and explore role of Fgfr1 splice variants during SPC development. This will uncover the unconventional manner by which FGF10 and FGF20 activate their cognate FGFR1 splice variants. Our work will elucidate critical molecular mechanisms of SPC development and help determine etiology of SNHL caused by defects of SPC development.

4. Omega-3 Fatty Acids in Combination With L-Carnitine Have an Effect on the Metabolic Activity of the Human Neuroblastoma (SH-SY5Y) and the Murine Organ of Corti (HEI-OC1) Cell Lines

Category: Inner Ear: Anatomy and Physiology

Magnus Teschner*[1], Wolfgang Nuss[1], Gudrun Brandes[2], Thomas Lenarz[1], Kirsten Wissel[1]
[1]*Hannover Medical School, Department of Otolaryngology,* [2]*Hannover Medical School, Institute for Neuroanatomy and Cell Biology*

Background: Omega-3 fatty acids such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are essential nutrients that have anti-inflammatory effects on neurons and support neuronal repair. So far, their influence on the auditory neurons have not been investigated. In this study, the effects of DHA and EPA on an in vitro model of neuroblastoma (SH-SY5Y) and organ of Corti (HEI-OC1) cell lines are characterized. In addition, the effects of L-carnitine hydrochloride (L-Car) which is well known to support the intracellular uptake of long-chain fatty acids is examined.

Methods: SH-SY5Y and HEI-OC1 cells were cultivated with DHA and EPA as well as in the combination of fatty acids with L-Car in concentrations between 5 and 100 µM. Changes in metabolic activity compared to untreated cells were quantified using Resazurin. Furthermore, electron microscopic examinations (SEM, TEM) were carried out on the morphology of the cells.

Results: After administration of 50 µM DHA and EPA each, an increase in the biological activity of the SH-SY5Y cells of about 10% and 22%, respectively, was found. In contrast, 100 µM of these omega-3 fatty acids had a cytotoxic effect, which could be confirmed with an electron microscope. In the HEI-OC1 cells, however, DHA and EPA led to a reduction in cell metabolism. L-Car alone had no effect in either cell line. However, the administration of DHA or EPA together with L-Car showed a significant increase in cell metabolism in both cell lines.

Conclusions: DHA, EPA and L-Car alone showed no effect or a worsening of the metabolic activity in the cells compared to the reference. Significant increases in SH-SY5Y and HEI-OC1 cell activity after administration of L-Car with DHA or EPA allow the conclusion that L-Car influences the interactions of DHA and EPA with the cell membrane and, thus, also changes the intracellular signaling pathways. The administration of omega-3 fatty acids together with L-Car could induce repair mechanisms of the mechanical damage associated with the CI implantation and support the functionality of the CI.

5. Prox1 Expression in the Organ of Corti is Required for Proper Innervation by Type II Spiral Ganglion Neurons

Category: Inner Ear: Anatomy and Physiology

Shubham Kale*[1], Thomas Coate[2], Michael Deans[1]
[1]*University of Utah,* [2]*Georgetown University*

Background: Outer Hair Cells (OHC) are innervated by Type II Spiral Ganglion Neurons (SGNs) which resemble nociceptors and extend peripheral axons towards the OHC rows that always turn towards the cochlear base. While the functional significance of this axon turning event is not known, our lab has shown that turning is regulated in part by non-autonomous Planar Cell Polarity (PCP) signaling from the organ of Corti. The transcription factor Prox1 is also necessary for guiding peripheral axon turning though it was previously proposed to function exclusively within the SGNs. Since Prox1 is also expressed by cochlear supporting cells we re-evaluated the potential that Prox1 makes an additional contribution to axon guidance from supporting cells. Moreover, we hypothesized that Prox1 may function upstream of PCP signaling in this context. To evaluate this, we tested innervation and cochlear development in organ of Corti restricted Prox1 conditional knockout mice (CKO).

Methods: Organ of Corti restricted Prox1 CKOs were generated using Emx2-Cre and a Prox1 line in which LoxP sites flank two exons encoding the Prox1 DNA binding domain, thereby restricting gene deletion to the cochlea while leaving expression in the SGN intact. Phenotypes were evaluated by immunofluorescent labeling of micro-dissected cochleae using antibodies against NF200 to visualize neurons, Sox2 and Prox1 to visualize supporting cells, and phalloidin to mark filamentous actin and stereocilia. All images were captured by structured illumination microscopy using the Zeiss Apotome system.

Results: In the absence of Prox1 expression in cochlear supporting cells, 34% of Type II SGNs turn incorrectly towards the cochlear apex. In-Situ Hybridization confirmed selective loss of Prox1 mRNA from the organ of Corti. While Prox1 deletion did not alter stereociliary bundle orientation, the patterning of cochlear supporting cells was disrupted.

Conclusions: Based on these findings, we conclude that Prox1 is required in cochlear supporting cells to guide peripheral axon turning. These findings complement prior work that also showed axon guidance defects when Prox1 was removed from the neurons. Though the nature of the non-autonomous mechanism in supporting cells has not been resolved, the absence of stereociliary bundle orientation defects suggests that Prox1 functions independent of PCP signaling. Instead, the disrupted patterning of supporting cells suggests that the incorrect turning might be secondary to organizational changes in the environment through which the growth cone navigates.

6. Resurgent and Persistent Sodium Currents Enhance Spiking Excitability in Mouse Vestibular Ganglion Neurons

Category: Inner Ear: Anatomy and Physiology

Selina Baeza Loya*[1], Ruth Anne Eatock[1]
[1]*University of Chicago*

Background: The vestibular inner ear transmits head-motion information to the brain via two populations of primary vestibular ganglion neurons (VGNs), which differ in the regularity of action potential (AP) timing. The two kinds of AP timing (regular and irregular) represent rate and temporal encoding, respectively (Jamali et al., Nat Comm 7:13229, 2016). Studies of isolated VGNs have shown that irregular neurons are less excitable than regular neurons, producing transient and sustained spiking in response to current steps, respectively. Although voltage-gated sodium (NaV) currents drive the rising phase of APs, their contributions to excitability differences are not fully understood. Rodent vestibular ganglia express all NaV channel α and β subunits. NaV currents in VGNs are dominated by transient (inactivating) currents but can include persistent (noninactivating) and/or resurgent currents (which flow after relief from inactivation block).

Methods: We use biophysical and computational approaches to assess the impact of different NaV current modes on patterns of AP firing. Whole-cell patch-clamp electrophysiological recordings were taken from mouse VGNs (postnatal days, P, 3-25) that were isolated and cultured overnight. To model the effects of NaV currents, we modified the Kalluri group's conductance-based VGN computational model (Ventura and Kalluri, J Neurosci 39:2860, 2019) by adjusting and adding expressions for transient, persistent, and resurgent NaV components with properties based on our data (Venugopal et al., PLoS Comput Biol 15(6): e1007154, 2019).

Results: In a sample of 100 VGNs, all had large transient NaV currents blocked by 1 μM TTX; 62 had persistent current (P3-25), 6 of 81 had resurgent current (P11-20), and 4 (P11-20) had both persistent and resurgent currents. NaV1.6 blocker 4,9-anhydro-tetrodotoxin (100 nM 4,9-ah-TTX) blocked 70% of transient current and all persistent and resurgent currents, indicating that a substantial portion of each is carried through NaV1.6 channels. In current clamp, 4,9-ah-TTX decreased neuronal excitability: increasing current threshold for spiking in all VGNs and decreasing resting membrane potential in sustained (regular) VGNs.

We lack ways to experimentally isolate the effects of transient, persistent, and resurgent currents on spiking because they flow through the same channel subunits. Therefore, we used a conductance-based VGN model to test effects of different NaV components on firing patterns evoked by current steps and trains of simulated EPSCs. Adding persistent and resurgent components had a negligible effect on firing by the model transient (irregular) VGN, where the effects of KLV channels are over-riding. In contrast, for the model sustained (regular) VGN, adding resurgent currents decreased first-spike latency (delay relative to EPSC onset), reduced interspike intervals (increased rate), and hyperpolarized voltage threshold, and reduced spike accommodation.

Conclusions: These results suggest that by increasing NaV channel availability in the after-spike interval in regular VGNs, persistent and resurgent NaV1.6 currents may enhance neuronal excitability and regularity.

7. The Creation of a High-Resolution Shareable Digital Archive of Human Temporal Bones Celloidin Sections
Category: Inner Ear: Anatomy and Physiology
Ivan Lopez*[1], Gail Ishiyama[1], Akira Ishiyama[1]
[1]*David Geffen School of Medicine at UCLA*

Background: The NIDCD National Temporal Bone Laboratory at UCLA houses one of the largest collections of archival temporal bones (approximately 1200 pairs) in USA. Hematoxylin and eosin (H and E) slides of each temporal bone contains the cochlea, vestibular endorgans, endolymphatic sac and eustachian tube. The objective of this study is to create high-resolution images collections using a high-speed digital system to be shared with the NIDCD Registry and Database and basic and clinical inner ear researchers.
Methods: Each H and E stained celloidin section is digitized using a digital system called Thunder Imager System (TS) as follow: Tissue section are placed in the microscope holder to identify the ear structures to be scanned using the 20x-magnification objective. The image is projected on the computer screen at a final magnification of 200x. The software used by the TS creates individual tiles of the area to be scanned and then using a navigator function to automatically digitize the image. The final image created is made of approximately 100 tiles. The digital image created is saved as a Tiff file. Each high-resolution image is about to 2 gigabytes in size. Snapshots of smaller file size are also created.
Results: The image acquired using this TS allows panoramic visualization of the middle and inner ear components in a single projection on the screen. The digital image can be zoomed, and remarkably, the hair cells and spiral ganglia neurons can be visualized without loss of resolution among other structures. Publications from our laboratory using this system (Noonan et al., 2020 and Stephenson et al., 2021) demonstrate the ability of our temporal bone laboratory to use the TS system for superb visualization of the human temporal bone histopathology. Serial images obtained of a whole temporal bone allow the creation of high resolutions 3D imaging. One mid-modiolar image for each temporal bone will be uploaded to the NIDCD Registry and Database, with the remainder available upon request to the Registry.
Conclusions: The creation of this shareable library of H and E sections of the human cochlea will allow basic inner ear researchers to request unstained celloidin sections from a specific inner ear region to be used for cellular and molecular biology. Additionally the images will be stored in the UCLA Cloud storage system and will be available upon request to ear basic and clinical researchers. The images will be uploaded into the National Temporal Bone registry and database. Funding: Supported by NIDCD U24DC 015910 grant (AI).

8. The Development of the Microscopic Anatomy of the Inner ear: From Light to Electron Microscopy
Category: Inner Ear: Anatomy and Physiology
Robert Ruben*[1]
[1]*Albert Einstein College of Medicine*

Background: There was a substantial development of histological knowledge of the inner ear from the end of the 19th and into the 20th century.
Methods: The primary sources of the pertinent publications were obtained and when needed translated into English for this study.
Results: Light microscopic techniques increase greatly during the latter part of the 19th century. These advances were dependent upon better optics, better preservation, new staining techniques, development of the microtome for serial sections, and the advent of photo microscopy. These advances supplied detailed descriptions of the inner ear and were reported in several landmark publications. One of the most important in these was the publication of Retzius in 1884 followed by Politzer's compilation of microscopic techniques in 1889 and then the report of the vasculature of the inner ear by Siebenmann in 1894. The 20th century reported the existence, in mammals, of the cupola of the ampullae of the semicircular canals in 1911 by Kolmer. Rasmussen first notes the presence of efferent nerve fibers from the olivary complex in 1942 and this was confirmed in 1946.
 These technical advances were then furthered with the development of transmission and scanning electron microscopy during the 1930's and utilized for biological studies beginning in the 1940's. The earliest transmission electron microscopic reports of the new year occurred 1953 several publications by Carlstrom, D., Engstrom, H, and Wersall, J. The pattern of afferent and efferent cochlear innervation was reported in several articles by Engstrom, Wersall, Smith, Spoendlin and Gacek during the 1960's. The scanning electron microscope was first utilized to show sensory epithelium of the statocyst of a cephalopod mollusc in 1967 by Barber and

Boyde. Mammalian studies were reported by Lim and Lane in 1969. Since then, there have been numerous detailed reports of electron microscopic

Conclusions: The use of magnification during 317 years since Du Verney's 1683 Traite de l'organ de l'ouie; contenant la Structure, les Usages et les Maladies de toutes les parties de l' Oreille to 2000 has produced the knowledge for understanding of the physiology and pathology of the inner ear.

1:15 p.m. - 3:15 p.m.
Podium Session #19 – Speech Perception: From Sensory Processing to Language
Moderators: Aaron Moberly, M.D. & Joaquin Valderrama, Ph.D.

1. Speech Categorization Reveals the Role of Early-Stage Temporal-Coherence Processing in Auditory Scene Analysis
Category: Speech Perception

Vibha Viswanathan*[1], Barbara Shinn-Cunningham[2], Michael Heinz[3]
[1]*University of Pittsburgh,* [2]*Carnegie Mellon University,* [3]*Purdue University*

Background: Temporal coherence of sound fluctuations across different frequency channels is thought to aid auditory grouping and scene segregation, as in comodulation masking release. Although most prior studies focused on the cortical bases of temporal-coherence processing, neurophysiological evidence suggests that temporal-coherence-based scene analysis may start as early as the cochlear nucleus (the first auditory region supporting cross-channel processing over a wide frequency range). Accordingly, we hypothesized that aspects of temporal-coherence processing that could be realized in early auditory areas may shape speech understanding in noise.

Methods: We explored whether physiologically plausible computational models could account for results from a behavioral experiment that measured consonant categorization in different masking conditions. Specifically, we tested whether within-channel masking of target-speech modulations predicted consonant confusions across the different conditions, and whether predictions were improved by adding across-channel temporal-coherence processing mirroring the computations known to exist in the cochlear nucleus. Consonant confusions provide a rich characterization of error patterns in speech categorization, and are thus crucial to rigorously test models of speech perception; however, to the best of our knowledge, they have not been utilized in prior studies of scene analysis.

Results: We find that within-channel modulation masking can reasonably account for category confusions, but that it fails when temporal fine structure cues are unavailable. However, the addition of across-channel temporal-coherence processing significantly improves confusion predictions across all tested conditions.

Conclusions: Our results suggest that temporal-coherence processing strongly shapes speech understanding in noise, and that physiological computations that exist early along the auditory pathway may contribute to this process.

2. The Effects of Language Experience and Inhibitory Control on Spoken Word Recognition for Cochlear Implant Users
Category: Speech Perception

Sarah Colby*[1], Francis Smith[1], Kristin Rooff[1], Bob McMurray[1]
[1]*University of Iowa*

Background: Cochlear implant (CI) users must learn to cope with the increased ambiguity in speech caused by the reduced quality of input received through their device. While speech in unfolding, listeners must quickly map the incoming signal to lexical representations by comparing potential candidates. For example, at the onset of "sandwich" (e.g., "san-"), many possible candidates will compete for activation (e.g., "sandal", "sandbag", "sandwich") until enough of the word has been heard to activate the correct target. Degraded input from the CI changes how this lexical competition is resolved during spoken word recognition (e.g., Farris-Trimble et al., 2014). Language experience – whether CI users received their implant before or after language development – further impacts the nature of lexical competition. Earlier CI implantation may lead listeners to use a wait-and-see approach to word recognition, where decisions about the target word are held off until more of the signal has unfolded, rather than the immediate, incremental processing seen in normal hearing (NH) listeners and to some

extent in postlingually deafened CI users (McMurray, Farris-Trimble, and Rigler, 2017). While lexical competition is usually thought to be language specific, we asked whether domain-general inhibitory control helps resolve lexical competition in the face of signal degradation.

Methods: Using eye-tracking, we asked if the dynamics of lexical access in NH listeners and CI users relates to non-linguistic inhibitory control. A group of NH listeners (N=68) and of post- (N=50) and pre-lingually (N=21) deafened adult CI users completed a Visual World Paradigm task and a Spatial Stroop task.

Results: NH listeners were faster than CI users to activate targets. Within the CI users, postlingual users were faster to activate targets compared to prelingual users. The prelingual CI users showed a wait-and-see pattern of competition, unlike the incremental processing seen in the NH listeners. Postlingual CI users who performed well on Stroop (low interference) were faster committing to the target word than those performing poorly. However, prelingual CI users showed the opposite pattern; those with better Stroop performance were slower to activate targets compared to prelingual users with poorer performance.

Conclusions: Activation of lexical targets is impacted by language experience in CI users. Individuals who receive their implant before adolescence are slower to activate targets, showing a wait-and-see approach, compared to those who receive their implant later in life. Our results also suggest that domain-general inhibitory control plays a meaningful role in spoken word recognition for CI users, but not NH listeners. How inhibitory control is deployed varies depending on the listener's language experience, suggesting that the competition differences between the two groups (incremental vs. wait-and-see) are distinct cognitive strategies. Prelingual CI users with better domain-general inhibitory control may strategically delay commitment under conditions of signal degradation.

3. Interaction Between Voice-Gender Difference and Spatial Separation in Release From Masking in Multi-Talker Listening Environments
Category: Speech Perception

Yonghee Oh*[1], David Eddins[2], Frederick Gallun[3], Lina Reiss[3]
[1]*University of Florida,* [2]*University of South Florida,* [3]*Oregon Health and Science University*

Background: In multi-talker listening situations, there are two major acoustic cues that can enhance speech segregation performance: 1) differences in voice characteristics between talkers (e.g., male versus female talkers); 2) spatial separation between talkers (e.g., co-located versus spatially separated talkers). This enhancement is referred to as release from masking. In this study, we systematically investigate potential interactions between voice-gender difference and spatial separation cues to explore how they influence the relative magnitude of masking release in normal-hearing (NH) listeners, and how they differ in hearing-impaired (HI) listeners including hearing aid (HA) and/or cochlear implant (CI) users.

Methods: Forty-seven adult subjects (11 NH listeners: 36 to 67 years; 12 bilateral HA users: 30 to 75 years; 12 bimodal CI users: 30 to 80 years; 12 bilateral CI users: 20 to 66 years) participated in this study. Speech recognition thresholds in competing speech were measured. One target and two masker phrases were drawn from the Coordinate Response Measure speech corpus (Bolia et al., 2001). For the spatial separation cue, the target phrase was fixed at 0° azimuth, and the presentation of the two masker phrases was either collocated with the target phrase (0°) or symmetrically separated at ±60° in the horizontal plane. For the voice-gender difference cue, four different voice-gender target-masker combinations were tested: male (or female) target and male (or female) maskers. The target level was fixed at 60 dBA, and the masker levels were adaptively varied.

Results: Two masking release metrics, the masking release based on target-masker voice-gender difference (VGRM) and the masking release based on target-masker spatial separation (SRM), were calculated. Averaged results from NH listeners show a distinct trend of weighting between VGRM and SRM with different spatial separations and one clear point of interaction where the magnitude of masking release was the same for the two cue types. That is, voice-gender difference cues are the more dominant cue to receive a benefit for speech segregation at spatial separations less than the intersection point. Conversely, the spatial cues become the more dominant cue rather than the voice-gender difference cues at spatial separations larger than the intersection point. However, both HA and bimodal CI users show greater perceptual weighting on VGRM than SRM without clear equally weighted intersection points. Bilateral CI users showed neither VGRM nor SRM.

Conclusions: The current data illustrate parameter-specific changes in the weighting of voice and spatial cues as a function of spatial separation. The results demonstrate that NH listeners have relatively equal perceptual weights between talkers' gender differences and spatial separation for their speech segregation performance. However, for HI listeners, especially for HA and bimodal CI users, it appears that they rely on more talkers' gender differences than talkers' spatial separation for speech segregation in multitasker listening situations.

4. Listening and Spoken Language Data Repository (LSL-DR): Design and Sample Characteristics
Category: Other

Ivette Cejas*[1], Christina Sarangoulis[1], Mona Oster[2], Ronda Rufsvold[3], Jennifer Coto[1]
[1]*University of Miami Miller School of Medicine,* [2]*Listen and Talk,* [3]*CCHAT Sacramento*

Background: Children with hearing loss are at-risk for speech, language, and academic delays. However, with early identification and intervention these children are able to achieve outcomes commensurate with their hearing peers. Current literature reports variability in outcomes, which may be due to demographic and audiological factors, or intensity and duration of intervention. The current study presents demographic, audiological, and sample characteristic data for a large, multisite, longitudinal database, the Listening and Spoken Language Data Repository (LSL-DR).

Methods: The LSL-DR is maintained by OPTION Schools, which is an international, non-profit organization comprised of listening and spoken language programs and schools for children who are deaf or hard of hearing (DHH). The LSL-DR is a robust source of speech, language, and educational outcome data on children who are DHH who have attended an OPTION program. Demographic, medical, audiological, speech and language outcome data are entered on a yearly basis, from the timepoint that children are enrolled in an OPTION program till they graduate or transfer to another program.

Results: The current LSL-DR includes 5,417 children enrolled in 38 programs across the United States, South America, and Canada. The sample consists of children with a mean age of 23.4 months (SD = 24.1), 46.3% are female, and most (49%) identify their race as White, 10.3% as African American, 8.2% as Asian, 1% as American Indian/Alaskan Native, and 0.3% as Native Hawaiian/Pacific Islander. Nineteen percent identified their ethnicity as Hispanic. The majority of children have sensorineural hearing loss (68.4%), 8.6% have a conductive hearing loss, and 3.5% have a mixed hearing loss. In terms of degree of hearing loss, 13.8% have a mild hearing loss, 16.8% a moderate hearing loss, 14% a moderate to severe hearing loss, 10.4% a severe hearing loss, and 28.9% have a profound hearing loss. The average hearing loss onset of the sample is 8.13 months (SD = 14.4) and these children were amplified on average at the age of 13.2 months (SD = 15.6). Most children in the LSL-DR wear hearing aids (48%) while 20% wear cochlear implants, and 7% wear bone conduction devices. Currently, of those children entered, 26% have two years of follow-up data, 19% have three years, and 12% have four years. Data will also be presented on the comprehensive list of assessments that are entered into this dataset, as well as the process for obtaining the LSL-DR for investigators that are interested in collaborating.

Conclusions: The LSL-DR is a free and publicly available database that can be used by investigators interested in outcome research for children with hearing loss with a range of severity and etiology. It provides a comprehensive assessment of children's overall functioning and may be used as a comparison group for ongoing research studies.

5. Acute Impact of Anterior Temporal Lobe Disconnection on Speech Prediction and Frontal-Auditory Neural Signals
Category: Speech Perception

Zsuzsanna Kocsis*[1], Rick Jenison[2], Thomas Cope[3], Peter Taylor[4], Bob McMurray[1], Ariane Rhone[1], McCall Sarrett[5], Yukiko Kikuchi[4], Phillip Gander[1], Christopher Kovach[1], Ryan Calmus[4], Inyong Choi[1], Jeremy Greenlee[1], Hiroto Kawasaki[1], Timothy Griffiths[4], Matthew Howard[1], Christopher Petkov[4]
[1]*University of Iowa,* [2]*University of Wisconsin,* [3]*Cambridge University,* [4]*Newcastle University Medical School,* [5]*Villanova University*

Background: The strongest level of causal evidence for the neural role of a brain hub is to measure the network-level effects of its disconnection. Here we present rare data from two patients who underwent surgical disconnection of the anterior temporal lobe (ATL) as part of a clinical procedure to treat intractable epilepsy.

Methods: During the surgery, we obtained pre- and post-resection intraoperative electrocorticographic (ECoG) recordings while the patients were awake and performing a speech-sound perceptual prediction task. We also obtained pre- and post-operative magnetic resonance imaging (MRI) including T1 and T2 structural and diffusion-weighted scans.

Results: Diffusion MRI tractography from ATL seed regions confirmed disconnection of the temporal pole from other cortical areas. Post-disconnection neurophysiological responses to the speech sounds showed a striking dissociation from the pre-disconnection signal in the form of, 1) magnified responses in auditory cortex (Heschl's gyrus) across oscillatory frequency bands (3-150 Hz); and, 2) disrupted oscillatory responses in prefrontal cortex (Inferior Frontal Gyrus, IFG). Moreover, after the disconnection auditory cortical mismatch responses and theta-

gamma coupling to the speech sounds were disrupted, and neural responses to different speech sounds became less segregable (i.e., more similar). State-space conditional Granger Causality analyses revealed substantial changes in neural information flow between auditory cortex and IFG post disconnection.

Conclusions: Overall, we demonstrate diaschisis, whereby the loss of ATL neural signals results in an immediate change in activity and connectivity in intact frontal and auditory cortical areas, potentially reflecting incomplete compensation for processing and predicting speech sounds.

6. Neural Tracking of Target Speech in Multi-Talker Speech Perception Reflects Speaker's Intelligibility
Category: Speech Perception

Xiaomin He*[1], Vinay Raghavan[1], Nima Mesgarani[1]
[1]*Columbia University*

Background: Neural responses in the auditory cortex track the acoustic envelope of speech, and this tracking is enhanced by attention to a speaker in multi-talker speech. Recent research has shown that neural speech tracking can be used to predict speech intelligibility and is closely linked to intelligibility through a range of influences, such as SNR, speech rate, visual cues, and prior knowledge. However, our current understanding of neural speech tracking is limited either by a narrow set of experimental conditions or a lack of behavioral data. Further, neural speech tracking can be leveraged for auditory attention decoding, a system used in neurally-assisted hearing devices to improve speech understanding in challenging multi-talker acoustic environments. Therefore, it is important to characterize neural speech tracking in a variety of multi-talker experimental conditions and compare it with behavioral intelligibility data.

Methods: We recruited 15 native English speakers for a 1.5h EEG experiment. Subjects were presented with 160 trials of 35s stories from 2 speakers spatially separated at $\pm 45°$ azimuth with background noise. Subjects were instructed to focus on a specific speaker under different types and volumes of noise. Repeated words were inserted throughout the speech. The intelligibility was measured by the accuracy of capturing the repeated words in the target speaker. Decoders were then trained to reconstruct the acoustic envelopes of the attended and unattended speakers from 64-channel EEG using regularized linear regression and were tested using leave-one-trial-out cross-validation. Neural speech tracking for both attended and unattended speakers was assessed by computing the correlation between the predicted and true envelopes under each experimental setting.

Results: (1) We identified a minimum threshold of intelligibility for each subject above which the neural tracking of the attended speaker exceeded that of the unattended speaker, and this threshold was consistent across noise types.

(2) When intelligibility reaches 100%, the difference in accuracy between attended and unattended speakers' reconstruction is not correlated with SNR.

Conclusions: There is an intelligibility threshold for successfully tracking the attended speaker in a multi-speaker scenario. Below this threshold, the neural tracking level for attended/unattended speakers was similar due to the competition of top-down attention on the target speech and bottom-up speech saliency of the surroundings. These results support the idea that there is a threshold of intelligibility for successful neural speech tracking and this threshold is not dependent on the type of background noise. Further, when speech is fully intelligible, increasing the SNR for attended speakers will not improve the neural tracking level of the attended speaker. These findings deepen our understanding of the relationship between intelligibility and speech tracking and crucially constrain the implementation of auditory attention decoding systems.

7. Audiovisual Speech Perception is Supported by the Inferior Frontal Occipital Fasciculus in Younger and Older Adults
Category: Speech Perception

James Dias*[1], Carolyn McClaskey[1], Jeffrey Rumschlag[1], Kelly Harris[1]
[1]*Medical University of South Carolina*

Background: Compared to younger adults, older adults typically have more difficulty identifying auditory and visual (lipread) speech. Even with these age-related unisensory deficits, some older adults exhibit a preserved ability to identify audiovisual speech. Behavioral and neurophysiological evidence suggests that this preservation of audiovisual speech identification by older adults may be explained by an improved ability to integrate information available across the senses. Frontal cortex activity can predict cross-sensory influences in audiovisual speech perception in both younger and older adults, and older adults often exhibit more frontal cortex activity than younger adults when processing audiovisual speech. This frontal cortex involvement is thought to reflect the role

of attentional and motor mechanisms in audiovisual speech processing. It has been hypothesized that frontal cortex involvement in audiovisual speech processing is facilitated by the inferior frontal occipital fasciculus (IFOF), a white-matter association tract that connects inferior frontal cortex to occipital cortex. Here we examined the extent to which IFOF microstructure predicts individual and age-related differences in auditory, visual, and audiovisual speech identification.

Methods: Older (n=24, 55+ years of age) and younger (n=31, 18-30 years of age) adults with clinically normal hearing up to 4 kHz and normal or corrected-to-normal sight completed an audiovisual speech-in-noise identification task and diffusion imaging. Whole-brain streamlines tractography was performed on each participant's diffusion-weighted image using pyAFQ. Kurtosis-derived scalars for white-matter microstructure, including fractional anisotropy (FA), mean diffusivity (MD), and mean kurtosis (MK), were calculated for each participant's IFOF, arcuate fasciculus (AF), and inferior longitudinal fasciculus (ILF), as well as for their whole brain white matter.

Results: Replicating past findings, older adults exhibited deficits in auditory and visual (lipread) speech identification but identified audiovisual speech at a rate similar to that of younger adults. Audiovisual speech identification was predicted by IFOF FA in both younger and older adults, even after accounting for whole brain white matter microstructure. Age-related differences in IFOF microstructure did not modulate the relationship between the IFOF and audiovisual speech identification.

Conclusions: The results suggest that age-related preservation of audiovisual speech identification may be facilitated, at least in part, by a greater reliance on frontal-cortical mechanisms that modulate cross-sensory influences on speech perception. Greater reliance on mechanisms for cross-sensory processing may compensate for age-related unisensory declines. These findings are important for identifying targets for intervention to help older adults with unisensory declines communicate more effectively in complex multisensory environments.

8. The Neural Response at the Fundamental Frequency of Speech is Modulated by Linguistic Information
Category: Speech Perception

Mikolaj Kegler*[1], Hugo Weissbart[2], Tobias Reichenbach[3]
[1]Imperial College London, [2]Radboud University, [3]Friedrich-Alexander-University Erlangen-Nürnberg

Background: Comprehension of spoken language requires rapid and continuous integration of incoming acoustic information. Up to date, the majority of studies investigating neural correlates of natural language processing focused on comparatively slow cortical activity, usually below 30 Hz. However, fast neural activity in subcortical and cortical areas can also track the fundamental frequency of voiced speech. Whether this fast neural tracking plays a role in linguistic aspects of speech processing remains unclear. Here, we investigated whether linguistic cues influence this neural response.

Methods: We measured EEG responses while participants listened to audiobooks. Using a language model, we computed linguistic features related to the words from the stories. Each word was characterized by its frequency out of context, as well as by its context-dependent surprisal and precision. We used a linear model to find a mapping between the fundamental waveform, which oscillated at the fundamental frequency of the speech signal, and the EEG. The model allowed to quantify the neural tracking of the fundamental waveform through a reconstruction score. To assess the modulation of this neural response by the linguistic cues, we established a multiple regression model that predicted the reconstruction score for each word from its associated linguistic features.

Results: The response estimated by the linear model had a low latency of 11 ms and a high frequency above 50 Hz, characteristic of the neural tracking of the fundamental frequency of the voice. We found that this neural tracking was strongest when the data was segmented according to the word onsets, but not short segments of a fixed duration. The coefficients of the multiple regression model indicated that the single-word neural phase-locking to the fundamental frequency of the voice was significantly influenced by the contextual linguistic features, word precision and surprisal.

Conclusions: We showed that the neural response to the fundamental frequency of continuous speech in naturalistic narratives is modulated by context-dependent linguistic cues. Due to the low latency, the response is likely under top-down control from higher processing areas. Our results suggest that the early neural tracking of the fundamental frequency plays an active role in the rapid and continuous processing of the spoken language.

1:15 p.m. – 3:15 p.m.
Podium Session #20 – All Things Tinnitus

Moderators: Fan-Gang Zeng, Ph.D. & Donald Caspary, Ph.D.

1. Too Blind to See the Elephant? Why Neuroscientists Ought to Be Interested in Tinnitus
Category: Tinnitus
Marlies Knipper*[1], Pim Van Dijk[2], Birgit Mazurek[3], Holger Schulze[4]
[1]*Hearing Research Center Tübingen, University of Tübingen,* [2]*University Medical Center Groningen,* [3]*Charite University Medical Center Berlin,* [4]*University of Erlangen-Nuremberg*

Background: A curative therapy for tinnitus currently does not exist. One may actually exist but cannot currently be causally linked to tinnitus due to the lack of consistency of concepts about the neural correlate of tinnitus. Depending on predictions, these concepts would require either a suppression or enhancement of brain activity or an increase in inhibition or disinhibition. Although procedures with a potential to silence tinnitus may exist, the lack of rationale for their curative success hampers an optimization of therapeutic protocols.
Methods: We discuss here six candidate contributors to tinnitus that have been suggested by a variety of scientific experts in the field and that were addressed in a virtual panel discussion at the ARO round table in February 2021. In this discussion, several potential tinnitus contributors were considered: (i) inhibitory circuits, (ii) attention, (iii) stress, (iv) unidentified sub-entities, (v) maladaptive information transmission, and (vi) minor cochlear deafferentation. Finally, (vii) some potential therapeutic approaches were discussed
Results: The results of this discussion is reflected here in view of potential blind spots that may still remain and that have been ignored in most tinnitus literature.
Conclusions: We strongly suggest to consider the high impact of connecting the controversial findings to unravel the whole complexity of the tinnitus phenomenon; an essential prerequisite for establishing suitable therapeutic approaches.

2. The Role of Tinnitus in Hearing Loss- Induced Cognitive Decline
Category: Tinnitus
Yasmeen Hamza*[1], Fan-Gang Zeng[1]
[1]*University of California Irvine*

Background: Hearing loss, a highly prevalent condition, in the elderly, is a leading modifiable risk factor for cognitive decline. Tinnitus, a frequent comorbid condition of hearing loss, is often presumed to impair cognition, largely due to this high comorbidity. However, tinnitus is highly heterogeneous, with different tinnitus attributes and types potentially affecting groups and cognitive domains differently. The present cross-sectional study aimed to delineate the interaction of tinnitus and cognition in age-related hearing loss, after adjusting for covariates in the race, age, sex, education, pure tone average, hearing aids, and physical well-being.
Methods: Data was analyzed from a biannual US-representative cross-sectional study (NHANES 2011-2012), and a multicenter US community-based prospective study in Hispanic/Latino populations (HCHS 2008-2011). Participants included 643 adults (60-69 years old; 51.3% females) from NHANES and 1716 (60-69 years old; 60.4% females) from the HCHS. Multivariable linear and binary logistic regression was used to assess the association between tinnitus and cognition in two sub-cohorts of normal hearing (NHANES, n= 508; HCHS, n= 1264) and hearing loss (NHANES, n= 135; HCHS, n= 453). A composite z-score from four cognitive tests was used as the outcome measure: The Consortium to Establish a Registry for Alzheimer's Disease (CERAD)-word learning, CERAD-animal fluency, CERAD-word list recall, and the digit symbol substitution test (DSST) in NHANES, and a comparable Hispanic version of these four tests in HCHS (the Spanish-English Verbal Learning Test (SEVLT), Word Frequency Test, SEVLT recall, and DSST).
Results: There was no association between tinnitus and cognition, using multivariable linear regression, except for the NHANES (non-Hispanic) participants with hearing loss, where the presence of tinnitus was associated with improved cognitive performance (Mean= 0.3; 95% CI, 0.1– 0.5; p, 0.018). Additionally, for that specific group, the absence of tinnitus increased the risk for poor cognitive performance (OR=5.6, 95% CI, 1.9– 17.2; p, 0.002) defined as a score at or below the 25th percentile of the control (i.e., normal hearing and no tinnitus). Sensitivity analysis found a positive correlation between tinnitus duration and cognitive performance in the NHANES cohort (F (4,140), 2.6; p, 0.037).
Conclusions: The present study challenges the present assumption that tinnitus impairs cognitive function. On the contrary, in the non-Hispanic elderly with hearing loss, tinnitus was associated with improved cognitive performance. Future longitudinal and imaging studies are needed to validate the present findings and understand

their mechanisms. Moreover, race should be considered as an important and relevant factor in the experimental design of tinnitus research.

3. nAChRs Partial Desensitizing Agonists Ameliorate Behavioral and Tinnitus-Altered A1 nAChRs Pathology in a Rat Model of Tinnitus
Category: Tinnitus

Madan Ghimire*[1], Rui Cai[1], Lynne Ling[1], Kevin Brownell[1], Kurt Wesner[1], Thomas Brozoski[1], Donald Caspary[1]
[1]*SIU School of Medicine*

Background: Incidence of chronic tinnitus has progressed to impact more than 20 percent of the global population. Individuals most disturbed by their tinnitus, show bimodal abnormalities of selective attention. These individuals are bound to their tinnitus percept while also being distracted by their tinnitus, resulting in impaired attentional function. Present studies are focused on understanding tinnitus-related changes in primary auditory cortical (A1), nicotinic cholinergic receptors (nAChRs). The goal is to examine drugs for patients most impacted by their tinnitus, those who are unable to divert their attention away from the percept in their heads. While numerous treatments to ameliorate tinnitus have been tried, no therapeutic approach has been clinically accepted. Here we posit nAChRs partial desensitizing agonists, Sazetidine-A (Saz-A) and Varenicline (VCL), as therapeutic agents with a focus on normalizing tinnitus-related cholinergic/nAChRs pathology at the level of A1 VIP+ neurons and L5 pyramidal neurons (PNs).
Methods: Chronic tinnitus was induced by a single unilateral exposure to 116 dB (SPL) 1/3rd octave band limited noise for one hour in anaesthetized rats. Tinnitus was assessed using an established operant conditioned-suppression paradigm. Once tinnitus score was established, a set of animals were used for in vitro whole cell patch clamp studies and a second set of animals were used for operant tinnitus testing with systemic drug (Saz-A and VCL) administration. nAChRs responses were isolated in in vitro whole cell recordings with bath applied atropine.
Results: In vitro whole cell patch-clamp studies showed significant dysregulation of nAChRs signaling in A1 L5 pyramidal neurons (PNs) and VIP+ interneurons from animals with behavioral evidence of tinnitus. L5 PNs showed significant decreases in nAChRs evoked current to puffed Ach while, A1 VIP+ neurons were found to be highly depolarized resulting in significant increases in excitability in response to electrical stimulation and puffed Ach. L5 tinnitus pathology in A1 was reflected by significant increases in spontaneous glutamatergic currents (sEPCs) and loss of spontaneous GABAergic currents (sIPCs). Bath application of 1µM acetylcholine for 5 minutes was found to normalize tinnitus-related glutamatergic and GABAergic pathology. Acetylcholine significantly increased sIPC amplitude and frequency while hyperpolarizing L5 pyramid neurons in animals with behavioral evidence of tinnitus. Like Ach, bath applied Saz-A and VCL decreased tinnitus-related differences in sIPCs and sEPCs suggesting that selective nAChRs desensitization could be normalizing A1 tinnitus pathology. Intraperitoneally injected Saz-A or VCL, an hour prior to behavioral testing, resulted in a significant dose-dependent reduction in tinnitus score in animals with behavioral evidence of tinnitus.
Conclusions: Collectively, behavioral and A1 cellular studies support potential therapeutic benefits of nAChRs partial desensitizing agents in tinnitus pathology in rat model. Further clinical investigations of these drug candidates will be essential to our understanding of their potential therapeutic benefits in the management of severe tinnitus in humans.

4. A Population-Based Study of Plasma Metabolomic Biomarkers of Persistent Tinnitus
Category: Tinnitus

Oana Zeleznik[1], Raji Balasubramanian[2], D. Bradley Welling[3], Konstantina M. Stankovic[4], Gary Curhan[1], Sharon Curhan*[1]
[1]*Brigham and Women's Hospital/Harvard Medical School,* [2]*University of Massachusetts,* [3]*Massachusetts Eye and Ear Infirmary, Harvard Medical School,* [4]*Stanford University School of Medicine*

Background: There is a critical need to unravel the heterogeneous etiologies that underlie tinnitus and use data-driven approaches to identify targets for tailored treatments. Metabolomics is a powerful tool to investigate comprehensive arrays of metabolites, encompassing those that are endogenous and those related to the metabolism of dietary intake, medications, lifestyle factors and environmental exposures. Analyzing metabolite profiles in relation to tinnitus may provide insights into its underlying pathophysiology and reveal potential therapeutic targets for alleviation of tinnitus symptoms. Although plasma metabolite alterations have been associated with tinnitus, studies were small and data are scant. Therefore, we conducted the first large-scale study using metabolic

profiling in human plasma samples to identify novel tinnitus metabolic biomarkers and pathoetiologic metabolic pathways.

Methods: We conducted a cross-sectional investigation of the association of plasma metabolite profiles and persistent tinnitus among 4207 female participants in the Nurses' Health Study (NHS) and Nurses' Health Study II (NHSII). This study sample included 659 cases of persistent tinnitus, defined as daily tinnitus lasting ≥5 minutes. Information on tinnitus at the time of the blood collection and demographic, medical, diet and lifestyle factors was collected on biennial questionnaires. Liquid-chromatography mass spectrometry was used to measure 548 metabolites, including lipids, free fatty acids, bile acids, amines, cationic metabolites, sugars and organic acids. We used per-metabolite logistic regression models adjusted for potential confounders to estimate odds ratios for tinnitus [OR (95% CI)] per 1 standard deviation increase in metabolite levels and to identify individual metabolite biomarkers of persistent tinnitus. We used the number of effective tests to account for testing multiple correlated hypotheses (adjusted-p<0.05). We used Metabolite Set Enrichment Analysis (MSEA) and the false discovery rate (adjusted-p<0.05) to identify metabolite classes enriched for concordant associations with tinnitus.

Results: After multiple testing correction in fully adjusted models, we identified several individual plasma metabolites and metabolite classes that were significantly associated with persistent tinnitus. Compared with participants with no tinnitus, three metabolites were inversely associated with persistent tinnitus: urobilinogen, a by-product of bilirubin metabolism in the gut, [OR = 0.82 (0.73-0.91)]; glycolithocholic acid, the glycine-conjugated form of the secondary bile acid lithocholic acid, [OR = 0.69 (0.56-0.86)]; and the neurosteroid pregnenolone sulfate [OR = 0.70 (0.56-0.86)]. Homocitrulline was positively associated with persistent tinnitus [OR = 1.13 (1.13-1.50)]. Among metabolite classes, significant inverse associations were observed for steroid and steroid derivatives, lysophosphatidylethanolamines, lysophosphatidylcholines, and ceramides, while fatty acyls and phosphatidylethanolamine plasmalogens were significantly positively associated with persistent tinnitus.

Conclusions: This first large population-based metabolomics study of tinnitus identified several plasma metabolites and metabolite classes that were significantly associated with persistent tinnitus.
These findings provide insight into metabolic pathways involved in tinnitus etiology and contribute to the discovery of novel therapeutic targets for effective treatment.

5. Functional Changes in the Auditory Cortex and Associated Regions Caused by Presbycusis and Tinnitus
Category: Tinnitus

Oliver Profant*[1], Jaroslav Tintěra[2], Antonín Škoch[2], Jakub Fuksa[3], Veronika Svobodová[4], Diana Tóthová[4], Josef Syka[5]
[1]IEM CAS, [2]Institute of Clinical and Experimental Medicine, [3]University Hospital Královské Vinohrady, [4]University Hospital Motol, [5]Institute of Experimental Medicine, ASCR

Background: Presbycusis and tinnitus are two of the most common hearing related pathologies. Although both presumably originate in the inner ear, there are several reports about their central components. Interestingly, the onset of presbycusis coincides with the highest appearance of tinnitus. The aim of our project is to identify age, hearing loss and tinnitus related functional changes within the auditory system and associated structures.

Methods: Eighty-nine participants were selected based on their age, hearing and tinnitus and divided into six following groups: young controls (YC), mild presbycusis (MP), expressed presbycusis (EP), young with tinnitus (YT), mild presbycusis with tinnitus (TMP) and expressed presbycusis with tinnitus (TEP). An MR functional study was performed with a 3T MR system using event related design (with different types acoustic and visual stimulations and their combinations).

Results: The amount of activation of the auditory cortices (Acs) depends on the complexity of the stimulus; higher complexity leads to increased size of the activated cortex. Auditory stimulation leads to greater activation (especially of the right AC) in the elderly, especially those with mild presbycusis. Combination of auditory and visual stimulations does not change the amount of activity based on the relation between both signals (congruent vs. discongruent). The sensory unclarity (word->bubble noise->word like sound->speech in noise) changes the laterality index (LI) of AC involvement from the right toward the left dominance. The presence of tinnitus increases the activation of the AC, specifically in the aged population and tinnitus also bolsters the shift toward the left AC asymmetry.

Conclusions: Overall, we can conclude that tinnitus causes changes in the involvement of the left and right Acs, on the other hand doesn't lead to changes in the activation of limbic structures. Similar outcome is related also to unclarity of the acoustic stimulus and suggests that the presence of tinnitus decreases sensory clarity of acoustic stimulation. Complexity of the acoustic stimulus plays a major role in the activation of AC, however its support by

visual stimulation leads to minimal changes. The degree of presbycusis does not play a significant role, yet ageing itself leads to increased activity in the right AC.

6. Neural, Autonomic, and Behavioral Signatures of Excess Central Gain in Individuals With Hyperacusis and Tinnitus

Category: Tinnitus

Kelly Jahn*[1], Jenna Sugai[1], Kenneth Hancock[1], Daniel Polley[1]
[1]*Massachusetts Eye and Ear*

Background: Two of the most common and debilitating hearing disorders arise from the inability to suppress an awareness of phantom sounds (tinnitus) and the perception that moderate-intensity everyday sounds are unbearably loud, aversive, or painful (hyperacusis). Hyperacusis and tinnitus often occur together, with some coincidence estimates as high as 80%. Whether they occur simultaneously or in isolation, these conditions often have a profoundly negative impact on quality of life, leading to loss of employment, social isolation, and severe psychiatric co-morbidities, to name a few. Decades of research on acoustic injury in animal models suggests that neural hyperactivity in the central auditory and limbic systems (i.e., excess central gain) may be a root cause underlying complaints of sound intolerance in tinnitus and hyperacusis. Clinicians lack objective biomarkers for excess central gain or other physiological markers that would provide a point of translation between mechanistic insights from animal models and future treatment avenues in human subjects. To address this shortcoming, we developed a comprehensive battery of psychophysical and electrophysiological assessments that can quantitatively dissociate complaints of enhanced loudness perception and sound-evoked distress in humans, and which have the potential to evolve into a new class of clinical tools for evaluating sound intolerance. In this study, we hypothesized that individuals with hyperacusis and tinnitus would exhibit steeper neural sound-level growth functions and greater behavioral and autonomic signatures of sound-evoked arousal compared with age-matched neurotypical controls.

Methods: To date, 24 subjects with normal hearing, 11 with tinnitus, and 4 with hyperacusis have participated. Neural sound-level growth is characterized by recording cortical electroencephalography (EEG) responses to a swept-intensity stimulus that is customized to encompass each subject's unique auditory dynamic range. To quantify sound-related distress, we assess complementary behavioral and objective indices of arousal including changes in pupil diameter and skin conductance in response to emotionally evocative and neutral acoustic stimuli.

Results: Across subjects, we find that neural sound-level growth is steepest for individuals with tinnitus and in those who report subjective hypersensitivity. We also show that sounds that elicit negative emotional reactions facilitate elevated physiological arousal (e.g., larger changes in pupil diameter and skin conductance responses) relative to neutral sounds. Preliminary findings suggest that this sound-induced arousal is largest in subjects who experience hyperacusis.

Conclusions: The present study demonstrates that central auditory gain secondary to hyperacusis and tinnitus may be elucidated through the application of clinically feasible EEG, pupillometry, and skin conductance recordings. The development and validation of a comprehensive battery to quantify the multifaceted aspects of sound tolerance will prove valuable in diagnosing and managing disorders with core auditory hypersensitivity phenotypes.

7. Speech Intelligibility in Tinnitus and Ageing: Exploring the Role of Impaired Peripheral and Brainstem Processing

Category: Tinnitus

Sarah Verhulst*[1], Sarineh Keshishzadeh[1], Hannah Keppler[1], Ingeborg Dhooge[1]
[1]*Ghent University*

Background: Tinnitus and age-related hearing difficulties can occur while audiometric hearing sensitivity remains normal, and therefore cochlear synaptopathy (CS) was named as a possible trigger mechanism for either etiology. However, the exact mechanisms underpinning ascending neural pathway adaptation after CS are poorly understood and this makes it difficult to tie CS to functional consequences for degraded sound perception and tinnitus. In this study, we investigate to which degree the presence of tinnitus in age-matched older and younger groups can be attributed to CS and related to speech intelligibility declines. We pose that if CS triggered tinnitus, CS-markers should be more prominent in the tinnitus groups (irrespective of their age) and brainstem gain should be more pronounced.

Methods: The hearing profile included standard clinical measures (audiogram, tympanogram, tinnitus questionnaires) as well as high-frequency thresholds (HFT, up to 20 kHz), envelope-following-response (EFR) markers of CS and brainstem gain markers. EFR stimuli were 120-Hz rectangularly amplitude-modulated (RAM) pure tones (120-Hz, 70 dB SPL, 4-kHz carrier) and brainstem gain was determined as the ratio between the wave-I and V amplitudes of the auditory brainstem response (ABR; stimulus: 80 and 100 dB peSPL, 11 Hz clicks). Speech intelligibility was quantified using the speech reception threshold (SRT) derived from a 5-word Matrix sentence test presented in quiet or in 70-dB stationary noise. We computed the SRT in different frequency bands and investigated its relation to the other sensorineural hearing loss (SNHL) markers. We collected data from two age-matched younger (22.7 ± 1.1 y/o, PTA = 4.2 ± 2.9 dB, N=31) and older (48.8 ± 5.6 y/o, PTA = 13.8 ± 4.7 dB, N=23) subject-groups with or without sustained tinnitus.

Results: RAM-EFR and ABR wave-I markers of CS were significantly smaller in the older than younger group and did not differ between tinnitus and non-tinnitus subjects within the age groups. This supports the view that CS is associated with ageing rather than with tinnitus. Elevated SRTs were observed in older listeners, and in those with weaker RAM-EFRs or elevated hearing thresholds. Tinnitus did not affect these outcomes, demonstrating a stronger importance of SNHL than tinnitus in predicting speech perception difficulties. Possible alterations in brainstem gain after CS were evaluated by studying the effects of age, tinnitus, the RAM-EFR or HFTs on the ABR wave-I/V ratio. We found no systematic dependencies, and thus no straightforward connection to this brainstem gain marker.

Conclusions: Our findings support the view of age-related SNHL (including CS) with a functional connection to reduced speech intelligibility. In our cohort, tinnitus had no influence on this relationship and was not associated with CS-induced brainstem gain alterations.

Work supported by ERC 678120 RobSpear and Ugent BOF-IOP EarDiMon

8. Replication and Progression of the Intensity Mismatch Asymmetry (IMA) Method to Objectively Measure Tinnitus
Category: Tinnitus
Ekaterina Yukhnovich*[1], Kai Alter[1], Timothy Griffiths[1], William Sedley[1]
[1]*Newcastle University*

Background: Chronic tinnitus appears to have a number of different causes (Baguley et al. 2013);(Langguth et al. 2019). A biomarker is needed that would indicate the presence of tinnitus, including all potential subtypes that may exist. This would need to relate to mechanisms forming part of a 'final common pathway' for tinnitus. Such a biomarker might help to better understand tinnitus mechanisms and allow treatment studies to determine effectiveness across tinnitus groups. A new EEG biomarker termed 'Intensity Mismatch Asymmetry' (IMA), using Mismatch Negativity responses (MMN) to deviants in sound intensity, was suggested, based on the Sensory Precision Integrative Model of Tinnitus, which encompasses the variety of causes of abnormal neural activity (Sedley et al. 2016, Sedley et al. 2019). IMA showed that 26 participants with tinnitus had larger MMN responses to upward intensity deviants, but smaller MMN responses to downward deviants, compared to the hearing-matched control group, at frequencies close to the tinnitus of the participant. The aim of the current experiment was to replicate the original study (Sedley et al. 2019), with adjustments to address potential drawbacks, such as gender matching, hyperacusis measures, and a 1 kHz control frequency in addition to near-tinnitus frequency.

Methods: The sample included 14 pairs of tinnitus and control participants (7 females, 7 males in each group). Participants were matched based on gender and pure tone audiometry results. Tinnitus Handicap Inventory and Hyperacusis Questionnaire answers were also collected.

A roving intensity paradigm was used to generate MMN responses, which included two types of standard 300 ms stimuli. Deviants were defined as pseudo-random transitions between one standard type and the other. The high intensity standard was interrupted by a quieter (downward) deviant, while a low intensity standard was interrupted by a louder (upward) deviant.

Results: At the time of submission, analysis was carried out on 8 pairs out of 14. Despite a trend at the downward deviant in tinnitus frequency, where MMN responses in controls seemed stronger than in tinnitus group, this difference did not reach significance in ANOVA (p=0.336).

Conclusions: The results obtained so far support the findings of the original study and appear on track to successfully replicate these. However, results appear smaller than previously reported in terms of effect size. Possible contributors include tighter matching for subject characteristics such as gender than previously, a smaller sample size, and/or chance. The next steps will be to increase the statistical power of the paradigm as we aim to produce a paradigm to 84nderlyin presence of tinnitus on an individual basis. Additional studies currently being

conducted include exploring whether attentional states, or presence of hyperacusis in both participant groups, would alter MMN responses in this paradigm (Sussman et al. 2014, Cederroth et al. 2020).

1:15 p.m. – 3:15 p.m.
Podium Session #21 – From Peripheral Auditory Encoding to Central Processing
Moderators: Yi Yuan, Ph.D. & Dyan Ramekers, Ph.D.

1. Auditory Perceptual Shifts From Non-Speech to Speech Enhance Subcortical Auditory Processing
Category: Auditory Nerve
Fan-Yin Cheng*[1], Can Xu[1], Lisa Gold[1], Spencer Smith[1]
[1]*University of Texas at Austin*

Background: The efferent auditory nervous system may be a potent force in shaping how the human brain responds to behaviorally significant sounds. Efferent-induced functional changes in the human auditory subcortex have been observed "online" and over short- and long-term time scales using the frequency following response (FFR). However, a contemporary understanding of FFR generation presents new questions about whether previous effects were constrained solely to the auditory subcortex. In this experiment, FFRs were evoked using an acoustically sparse speech stimulus in which only the first two formants were represented by time-varying sine waves ("sine wave speech", SWS). The advantages of using SWS in this context were that: 1) SWS contains relatively high frequency content, thus biasing FFRs to reflect caudal brainstem generators, and 2) SWS is not perceived as speech without additional context, allowing FFRs to be collected from the same listeners before (i.e., naïve) and after (i.e., non-naïve) a perceptual shift has occurred. The purpose of this experiment was to determine if FFRs were enhanced following a perceptual shift from non-speech to speech.
Methods: Naturally-spoken minimal pair speech tokens, /bɔ/, /bu/, and /bo/, were used to create SWS stimuli in which only the first two formants were represented by time-varying sine waves. These stimuli were presented to "Training" and "Control" groups in three different experimental blocks. SWS FFRs were first passively recorded from both groups of naïve listeners. The Training group then underwent a short training session in which each SWS token was heard at the end of a SWS carrier phrase ("The word is   ."); listeners were then required to choose which SWS token was heard. The Control group was exposed to the same stimuli as the Training group, but was asked to complete a visual task. Lastly, FFRs were again passively recorded from both groups. Pre- and post-training FFR amplitude were compared using a Fourier Analyzer. Machine learning classification of pre- and post-training FFRs were also conducted using a linear support vector machine.
Results: Perceptual reaction time and accuracy of SWS discrimination indicated rapid perceptual shifts from non-speech to speech in Training group listeners. SWS FFRs were significantly enhanced in the Training group post-training, whereas the Control group FFR amplitudes remained stable across the experiment. Machine learning classification significantly improved in the Training group after training, whereas it remained stable in the Control group.
Conclusions: In the first study using SWS to evoke FFRs, we observed that rapid perceptual shifts from non-speech to speech were concomitant with FFR enhancement. These results offer insight into how rapidly and potently the auditory brainstem can be modulated through the efferent system when higher-order speech comprehension networks are engaged.

2. Distortion Modulations Derived From Inner Hair Cells Identified Using Electrocochleography
Category: Auditory Nerve
Douglas Fitzpatrick*[1], Kendall Hutson[1], Meredith Hamby[1], Raymond Haggerty[1], Paul Manis[1]
[1]*University of North Carolina at Chapel Hill*

Background: Distortion product otoacoustic emissions (DPOAEs) are used to assess the functional status of outer hair cells. Similar distortions are also seen using electrocochleography (EcochG), where peaks in Fourier transforms of the recorded responses for the difference (F2-F1) and cubic (2F1-F2) distortion tones, as well as others, are prominent. These peaks are typically assumed to be from traveling waves to the low frequency parts of cochlea. Here, we show that some large peaks associated with distortions to low intensity sounds are derived from modulations within inner hair cells at the CF locations of the primary tones, which in turn modulate the rates of auditory nerve fibers that are observed in the EcochG.

Methods: Stimuli consisted of single tones or a multitone complex with six equal-level tones from 4100-7700 Hz. The tones in the complex were spaced such that unique combinations of DPs were produced by interactions among each pair. EcochG from the round window was performed in untreated (normal-hearing) urethane-anesthetized gerbils. A neurotoxin (kainic acid, 60 mM in artificial perilymph) was then applied to the round window, and continuous recordings were made to the multitone complex and single tones. Analysis consisted of Fourier analysis of the recorded responses for the DP and N1-P1 measurement and of the CAP to assess the loss of neural activity to different frequencies as the neurotoxin spread from base to apex.

Results: Prior to KA application, large peaks at the difference and cubic difference tones of each pair were present to each stimulus. The F2-F1 peaks to low frequencies within the phase-locking range of the gerbil were particularly large to low intensities, exceeding the sizes of the peaks to the primaries, for levels of 0-30 dB SPL. These peaks were absent after one hour of treatment with the neurotoxin, indicating they were of neural origin, while the hair cell responses to the primaries remained. The time-course experiment showed that the low-frequency peaks disappeared at the same time as the CAPs to the frequencies associated with primaries that produced them. An auditory nerve model showed that modulations of high spontaneous rate nerve fibers produced by two-tone interaction within inner hair cells would be phase-locked to the modulation frequencies at low sound levels.

Conclusions: The results indicate that modulations of nerve firing to low frequency DPs to low-intensity stimuli are produced at the CF site of the primary tones. The peaks to DP frequencies are therefore not due to traveling waves to low frequency parts of the cochlea. The source is likely to be distortions produced within inner hair cells that then modulates the neural firing observed.

3. Effects of Auditory-Nerve Loss on Behavioral Sensitivity to Envelope Statistics
Category: Auditory Nerve
Kenneth Henry*[1], Kristina Abrams[2]
[1]*University of Rochester,* [2]*Universtiy of Rochester*

Background: Auditory-nerve loss is a common cochlear pathology in humans with unclear and controversial effects on auditory perception. While previous studies have focused primarily on moderate to high stimulus presentation levels and found mixed results, sensitivity to low-level narrowband sounds could be more impacted by auditory-nerve loss considering that these stimuli evoke only limited neural excitation even in the healthy ear. The present animal-behavioral study tested the hypothesis that auditory-nerve loss adversely impacts discrimination of low level, narrowband signals based on differences in envelope statistics.

Methods: Auditory-nerve loss was induced in budgerigars (a small parrot species) using bilateral kainic-acid infusions, which permanently reduced click-evoked compound auditory-nerve activity by 40-85% across animals. Budgerigars were trained through operant conditioning to discriminate standard noise, which has intrinsic temporal-envelope fluctuations, from 'low-noise' noise processed to have a flat envelope. Noise bandwidth was 100 Hz. Discrimination performance was assessed using a single-interval, two-alternative task and two-down one-up adaptive tracking procedures. Noise center frequencies of 1-3 kHz and sensation levels of 10-30 dB were tested. Thresholds quantified the minimum stimulus duration at which noise discrimination (i.e., standard vs. low-noise envelopes) was ~70.7% correct.

Results: Noise discrimination thresholds based on envelope statistics decreased (improved) with increasing sensation level from several hundered ms for the 10-dB sensation level to several tens of ms for the 30-dB level, and were similar across center frequencies. Furthermore, budgerigar thresholds were similar to those of human subjects from a previous study. Thresholds overlapped considerably between control and kainic-acid exposed animals for all test conditions, with no clear associations found between the degree of auditory-nerve loss and behavioral performance on the envelope discrimination task.

Conclusions: These animal-behavioral results suggest that the impact of auditory-nerve loss, if any, may be smaller than the typical range of inter-animal variability. Within-animal experimental designs may be required to assess effects of auditory-nerve loss, which may be small compared to those associated with all-too-common hair-cell pathologies. This research was supported by grant R01-DC017519

4. Estimation of Cochlear Frequency Selectivity Using a Convolution Model of Forward-Masked Compound Action Potentials
Category: Auditory Nerve
Francois Deloche*[1], Satyabrata Parida[2], Andrew Sivaprakasam[1], Michael Heinz[1]
[1]*Purdue University,* [2]*University of Pittsburgh*

Background: Frequency selectivity is a fundamental property of the peripheral auditory system, but the invasiveness of auditory nerve (AN) experiments limits its study in the human ear. Compound Action Potentials (CAPs) associated with forward-masking have been suggested as a less invasive means of assessing cochlear frequency tuning. Previous methods relied on an empirical comparison of AN and CAP tuning curves in animal models, arguably not taking full advantage of forward-masked CAPs to provide an accurate estimate of frequency selectivity. In this work, we propose a new method based on a convolution model of forward-masked CAPs, seeking to directly estimate the quality factor of the underlying auditory filters. The model is inspired by convolution models of the CAP that have been around for decades, but not used in conjunction with the masking paradigm that provides a lot of additional information (e.g., place-latency relationship, masking input-output curves).

Methods: We recorded click-evoked CAPs at the round window of anesthetized chinchillas in the presence of Gaussian noise forward maskers. 150 masker conditions were used, corresponding to high-passed noise or notched-noise maskers with various notch widths, center frequencies and attenuations. The model was primarily based on the determination of the amount of masking as a function of place-specific response intensity. The forward-masked responses were approximated as the convolution product of masked excitation patterns and a unitary response. The minimization of the approximation error by gradient descent led to the estimation of the model parameters, including the quality factor characterizing frequency tuning.

Results: The convolution model was found to be highly accurate for the forward-masking of CAP responses, with the model accounting for about 90% of the variance in CAP release-of-masking signals corresponding to the notched-noise maskers. The estimated quality factor $Q10$ as a function of center frequency was shown to closely match the quality factor of AN fibers on average.

Conclusions: This work demonstrates that cochlear frequency selectivity can be estimated with a convolution-based model of forward-masked CAPs without the need for an empirical correction factor. Beyond frequency selectivity, the good fit between the forward-masked responses and the model estimates motivates future developments of the model to study more complex aspects of cochlear signal processing (e.g., compressive nonlinearities and suppression), in animal or human models. The proposed method could therefore become a valuable tool to investigate the compound response of auditory nerve fibers, specifically for the human ear.

5. Interactions Between Peripheral and Central Measures of Temporal Coding in a Chinchilla Model of Noise-Induced Cochlear Synaptopathy

Category: Auditory Nerve
Jonatan Märcher-Rørsted*[1], Jens Hjortkjær[2], Gerard Encina-Llamas[1], Torsten Dau[1], Michael Heinz[3]
[1]*Technical University of Denmark,* [2]*Danish Research Centre for Magnetic Resonance,* [3]*Purdue University*

Background: Steady-state electrophysiological responses phase-locked to the carrier or modulation frequencies of an auditory stimulus are reduced with age. Age-related reductions in frequency following responses (FFRs) have been attributed to a decline in temporal processing in the central auditory system. Yet, age-related cochlear synaptopathy may reduce synchronized activity in the auditory nerve, which may also contribute to reduced FFR responses. Here, we investigate the effect of noise-induced cochlear synaptopathy on peripheral and brainstem temporal coding (i.e., the FFR) in a chinchilla model of temporary threshold shift (TTS) by simultaneously recording electrocochleography (EcochG) and (brainstem) FFR responses.

Methods: We collected simultaneous electroencephalography (EEG) and EcochG responses from anesthetized chinchillas. Half the chinchillas were exposed to two hours of 100 dB SPL octave-band noise (centered at 1 kHz), producing a significant TTS measured one day post exposure. This exposure has been shown previously to create a broad region of significant (up to 50%) cochlear synaptopathy in chinchillas, with minimal permanent threshold shift. Electrophysiological responses in exposed chinchillas were measured at least two weeks post exposure. The remaining animals were treated as controls. FFRs to the carrier frequency of 10-ms tone bursts at low (516 Hz), mid (1032 Hz) and high (4064 Hz) frequencies, presented at levels ranging from 40 to 80 dB SPL, were recorded to examine potential level- and frequency-dependent effects. Additionally, responses to tonal frequency sweeps from 0.2-1.2 kHz at 80 dB SPL were collected. Evoked responses to clicks from 0-80 dB SPL were also recorded to quantify level-dependent latencies of different sources in the auditory pathway.

Results: Reduced FFR and EcochG responses to the carrier of low (516 Hz) and mid (1032 Hz) frequency tones were observed in exposed animals. In peripheral neurophonic EcochG responses, we observed more pronounced reductions at higher levels. Low-frequency tones showed the largest reductions, whereas high-frequency (4064 Hz) tones showed no effect of noise exposure, suggesting a presynaptic origin of the response (i.e., cochlear

microphonic, which is not affected by synaptopathy). Brainstem FFR responses to lower-level low-frequency tones (516 Hz at 60 dB SPL) showed more pronounced reductions compared to the peripheral neurophonic response in this condition, suggesting a level-dependent interaction between peripheral and central responses to low-frequency tonal stimuli. Reductions of the phase-independent second harmonic of the tonal carrier (twice the fundamental frequency) were also observed in both peripheral and central measures, consistent with a neural origin of the reduced response.

Conclusions: Disentangling peripheral and central responses is crucial for our understanding of the underlying generation mechanisms of the FFR, and its connection to peripheral neural degeneration. These results suggest level- and frequency-dependent interactions between peripheral and central generators in both normal and noise-exposed TTS animals, which may guide further advancements in diagnostics for peripheral neural degeneration.

6. Optimizing Stimulus Parameters for Ultrafast Cochlear Optogenetic Encoding
Category: Auditory Nerve
Antoine Huet*[1], Artur Mittring[1], Tobias Moser[1]
[1]*Institute for Auditory Neuroscience, University Medical Center Göttingen*

Background: Spatially confined optogenetic stimulation of the spiral ganglion neurons (SGNs) represents a prospective alternative to electrical stimulation currently used in cochlear implants (CI). The reduced spread of excitation promises to increase the number of independent stimulation channels in future optical cochlear implants. Toward the development of clinical optical Cis, designing the coding strategy requires to identify optimal stimulation parameters (light pulse duration and repetition rate) and to computationally model the biophysical parameters of optogenetically driven SGN firing in vivo. Those parameters can be empirically measured by in vivo single SGN juxta-cellular recordings in response to optical stimulation delivered to the cochlea. Nonetheless those recordings are limited in time and, hence, measuring all combinations of interests per SGN using deterministic stimulation is challenging.

Methods: Therefore, we developed and validated an optical non-deterministic stimulus paradigm containing the combination of pulse duration and repetition rate of interest. The validation was done by recording in vivo from mice single SGNs expressing targeting optimized Chronos (Keppeler et al., 2018) and transduced using a viral approach (AAV-PHP.B, human synapsin promoter, titer = 3.3-8.4 x 10^12 GC/ml, early postnatal injection).

Results: This stimulus paradigm allowed to identify optimal illumination parameters of SGNs in minutes.

Conclusions: Our results will be used to analyze neural network function in the auditory brainstem and to design coding strategies of the future optical CI.

7. Temporal Processing of the Human Auditory Nerve and Brainstem
Category: Auditory Nerve
Skyler Jennings*[1]
[1]*University of Utah*

Background: Temporal processing defines how the auditory system encodes changes in sound amplitude over time. Temporal processing of the human auditory system has been evaluated by evoked-potential studies sensitive to brainstem function; however, the temporal processing of the human auditory nerve (AN) – the foundation of all auditory information – is poorly understood, as is the relationship between AN and brainstem temporal processing. Here we compare temporal processing estimated from evoked-potentials sensitive to AN function to those sensitive to brainstem function using data from simultaneous measurement of the compound action potential (CAP) and the auditory brainstem response (ABR).

Methods: Our measurements include responses to the temporal envelope (i.e., CAPenv, and the envelope following response [EFR]) elicited by long (i.e., 2.25 sec.) amplitude-modulated probes in quiet and the time course of such responses to noise gated in the ipsilateral or contralateral ears. Further, we compare CAPs and ABRs elicited by upward frequency chirps, which are expected to reveal the temporal processing of sound onsets in terms of synchrony across a population of AN fibers of differing characteristic frequencies (CFs).

Results: Our preliminary data show 1) that phase locking to the temporal envelope extends to higher frequencies for CAPenv compared to EFR, consistent with the upper limits of phase locking for neurons in the AN compared to the brainstem, 2) a release from masking during the presentation of ipsilateral noise for EFRs but not CAPenv, consistent with a time-varying change in gain for neural circuits in the auditory brainstem, and 3) suppression of EFR, but not CAPenv, amplitudes in the presence of gated contralateral noise, consistent with central masking, 4) larger CAPs for chirps than clicks at low sound pressure levels (SPLs); however, this chirp benefit is absent for

high SPLs due to adaptation and upward spread of excitation, and 5) a large chirp benefit at high SPLs when the original chirp is modified to reduce the low-frequency components of the stimulus.

Conclusions: These preliminary findings suggest our approach has the potential to provide a detailed understanding of the temporal processing of the human AN, and how this processing compares to that of the brainstem. This contribution is significant because AN and brainstem temporal processing form the neural foundation upon which hearing is mediated, and altered temporal processing is central to hearing difficulties experienced by older adults with normal hearing and hearing loss.

8. The Role of the Calcium Channel Blocker Verapamil on Hearing
Category: Auditory Nerve
Selin Yalcinoglu*[1], Rod Braun[1], Amaar Wattoo[1], Avril Genene Holt[1]
[1]*Wayne State University School of Medicine*

Background: Hearing loss affects approximately 48 million Americans. Increased spontaneous neuronal activity often occurs in auditory pathways following hearing loss. One of the leading hypotheses is that after hearing loss the reduction in the afferent input to the ear leads to central hyperactivity to compensate for the decrease of input (Schrode et al., 2018). Voltage-gated calcium channels regulate neuronal activity. Therefore, we examined the effect of the L-type calcium channel blocker, verapamil, in normal hearing and noise-exposed rats. The response of the auditory nerve and the inferior colliculus during auditory brainstem response (ABR; wave I and wave V respectively) was tested.

Methods: Twenty-five male Sprague-Dawley rats were divided into four groups (n = 5 – 7/group) and given either verapamil (30 mg/kg) or saline solution intraperitoneally. The treatment groups were: a) no noise exposure plus verapamil (n=5), b) no noise exposure plus saline (n=6), c) noise exposure plus verapamil (n=7), and d) noise exposure plus saline (n=7). The noise groups were unilaterally exposed to a 16 kHz, 106 dB SPL tone for one hour, while no noise control groups were maintained in ambient noise conditions for an equal amount of time. For ABR analysis both amplitudes (wave I and V) and thresholds were evaluated. The assessment was performed at two different frequencies (12 kHz and 20 kHz) and time points (one and five days after treatment).

Results: Verapamil administration did not have any negative effect on the hearing threshold. In fact, when the noise groups had a temporary threshold shift (TTS), verapamil decreased the recovery time. The administration of verapamil had an effect on the amplitudes of both ABR waves assessed. In no noise conditions, administration of verapamil (n=5) caused a significant increase in wave V amplitude compared to the saline group (n=6) one day after treatment (8.4% at 12 kHz, $p<0.05$). The wave V/I ratio in the no noise saline group at 12 kHz one day after treatment was 0.393 and in the no noise verapamil group at 12 kHz one day after treatment was 0.667 ($p<0.12$). The wave V/I ratio in the no noise saline group at 20 kHz one day after treatment was 0.523 and in the no noise verapamil group at 20 kHz one day after treatment was 0.667 ($p<0.13$). The wave V/I ratio for the verapamil no noise group was significantly increased (95% at 12 kHz, $p<0.02$) five days after verapamil treatment.

Conclusions: Our results demonstrate that verapamil may increase gain in the inferior colliculus. Future studies should focus on further understanding the relationship among changes in neuronal activity, voltage-gated calcium channels, and susceptibility to noise-induced hearing loss and tinnitus.

Tuesday, February 8, 2022

7:00 a.m. – 9:00 a.m.
Symposium #22

WITHDRAWN

7:00 a.m. – 9:00 a.m.
Podium Session #23 – From Sterocilia to Mechanostransduction and Electromotility
Moderators: Marcos Sotomayor, Ph.D. & Sandrine Vitry, Ph.D.

1. Single-Molecule Mechanics of a Deafness Mutation in Protocadherin 15
Category: Hair Cells: Anatomy and Physiology

Camila Villasante*[1], Ahmed Touré[2], Tobias Bartsch[2], A. J. Hudspeth[3]
[1]*Rockefeller University,* [2]*The Rockefeller University,* [3]*HHMI/The Rockefeller University*

Background: One in a thousand children is born with profound hearing loss, 50 % of which stems from genetic causes such as mutations within the inner ear's mechanotransduction machinery. Transduction begins when a stimulus deflects a hair bundle towards its tall edge, tensing the gating springs atop each stereocilium on a hair cell. These gating springs control the precise and sensitive opening of transduction channels by modulating the tension that reaches their molecular gates. Although the exact molecular identity of the gating spring remains uncertain, a candidate is the filamentous tip link that connects each stereocilium to its tallest neighbor. This link comprises two parallel molecules of protocadherin 15 (PCDH15) and two of cadherin 23 that interact through a "handshake." Mutations in PCDH15 can result in both syndromic and non-syndromic deafness, underscoring its role in hearing and other processes. For the mutations associated with non-syndromic deafness alone, the higher Ca2+ concentration in the vestibular system might allow PCDH15 to function, whereas the lower Ca2+ level in the cochlea precludes this. This hypothesis suggests that the structure and function of non-syndromic deafness variants are critically dependent on Ca2+. Because little is known about the pathogenic mechanism of such variants, we chose to study a point mutation in PCDH15 that results in non-syndromic deafness.
Methods: We investigated the monomeric form of PCDH15 V507D, the murine homolog of the human V528D variant associated with non-syndromic autosomal recessive deafness type 23 (DFNB23). In our optical-trap apparatus, a single PCDH15 molecule was tethered between a pedestal bead covalently attached to a coverslip and a probe bead diffusing in solution. While a weak laser beam measured the position of the probe bead, a strong laser beam exerted controlled forces on the bead, and thus on the tethered molecule. By increasing the force at a constant rate, we evaluated the extension of the protein as a function of forces within the physiological range.
Results: Our data acquired at a Ca2+ concentration of 3 mM—a high value meant to saturate the Ca2+-binding sites of PCDH15—revealed that PCDH15 V507D existed in a wide variety of structural states compared to wildtype monomer at the same Ca2+ concentration. Several large unfolding events, many of which corresponded to the expected length of an unfolded cadherin domain, occurred in individual force-extension traces.
Conclusions: Our findings suggest that, even at a high Ca2+ concentration, PCDH15 V507D is unable to convey appropriate tension to the mechanotransduction channel due to numerous unfolding events. We will next study the behavior of this mutation at a physiological Ca2+ concentration, for which we expect to see an even greater degree of structural heterogeneity that will give further insight into the cause of DFNB23.

2. Molecular Mechanisms Underlying CIB Function in Inner-Ear Mechanotransduction
Category: Hair Cells: Anatomy and Physiology

Wei-Hsiang Weng[1], Jonathan Montgomery[1], Sanket Walujkar[2], Jeffrey Lotthammer[1], Arnaud Giese[3], Mark Foster[1], Zubair Ahmed[4], Marcos Sotomayor*[2]
[1]*Ohio State University,* [2]*The Ohio State University,* [3]*Sensorion,* [4]*University of Maryland School of Medicine*

Background: In inner-ear mechanotransduction, forces from sound and head movement are converted into nerve impulses to facilitate hearing and balance perception. At the apical surface of inner-ear sensory hair cells are actin-filled protrusions called stereocilia. Successively ascending rows of stereocilia form hair-cell bundles that are deflected by force to open transduction channels for cation influx. This causes hair-cell depolarization and initiates signaling cascades for sensory perception. Transmembrane channel-like proteins (TMCs) 1 and 2 have been established as the pore-forming components of hair-cell transduction channels. Recent studies have shown that calcium- and integrin-binding proteins (CIBs) 2 and 3 function as essential auxiliary subunits for TMC1 and TMC2. Electrophysiology experiments also suggest that CIB2 and CIB3 are functionally redundant in cochlear hair cells, and CIB2 mutations associated with deafness have been reported to disrupt binding to TMC1/2 and abolish mechanotransduction currents to varying degrees. Additionally, two studies have reported that CIB proteins bind to either the N-terminal (NT) or to the intracellular loop-1 (IL1) domains of TMC1. However, the structural details of how CIB proteins interact with TMC1/2 intracellularly to modulate mechanotransduction remain unclear.
Methods: Here, we use AlphaFold 2 models, molecular dynamics simulations, and nuclear magnetic resonance (NMR) experiments to explore the molecular mechanisms underlyinng function of CIB proteins in TMC-mediated inner-ear mechanotransduction.

Results: The AlphaFold 2 models of TMC1 proteins alone and in complex with CIB proteins suggest a 'clamp-like' interaction involving CIBs and the TMC1 NT and IL1 domains. Molecular dynamics simulations of these models provide additional insights into possible mechanisms underlying CIB function in mechanotransduction. We also present data from nuclear magnetic resonance (NMR) experiments that test the interaction between full-length [15N]-labeled CIB2 with TMC1-IL1 and TMC1-peptides. The NMR spectra suggest that TMC1-NT and TMC1-IL1 peptides bind [15N]-CIB2 non-competitively, consistent with the 'clamp-like' ternary complex predicted by AlphaFold 2.

Conclusions: These data and models explain seemingly inconsistent experimental results about the interactions between TMC and CIB proteins and suggest how the complex may function in inner-ear mechanotransduction.

3. Centrin-2 Promotes Ca2+-Dependent Assembly of Myosin 15 Biomolecular Condensates
Category: Hair Cells: Anatomy and Physiology
James Heidings*[1], Zane Moreland[1], Elli Hartig[2], Basile Tarchini[2], Jonathan Bird[1]
[1]*University of Florida,* [2]*The Jackson Laboratory*

Background: Stereocilia are required for the detection of sound and are packed with a para-crystalline array of actin filaments that determine their size and shape. Myosin 15 (MYO15A) traffics molecules critical for the growth and maintenance of the stereocilia actin core, in addition to directly regulating actin polymerization. Mutations in MYO15A cause human hereditary hearing loss, DFNB3. The motor domain of MYO15 is an ATPase with three IQ domains that bind light chains to form the lever arm necessary for force production. Understanding how the MYO15 motor is modulated by its light chains is thus critical for elucidating the mechanism of DFNB3 deafness. Essential and regulatory light chains (MYL6, MYL12B), in addition to calmodulin (CALM) bind to IQ domains 1 and 2 in vitro, whilst the protein(s) binding to IQ domain 3 (IQ3) remain unknown. Here, we show that the centrosome – associated protein, centrin-2 (CETN2) specifically binds to IQ3 and imparts unique biochemical properties to MYO15.

Methods: To identify potential light chains binding to IQ3, we performed a co-IP using pituitary AtT20 cells expressing an EGFP-tagged MYO15-3IQ-FLAG construct. Proteins were captured via affinity chromatography and eluted peptides analyzed using LC-MS2. We used baculovirus and the Sf9 insect cell system to express the mouse MYO15 motor domain with 3 IQ domains (MYO15-3IQ) and 2 IQ domains (MYO15-2IQ), along with candidate light chain proteins. Proteins were purified using affinity, ion exchange and size exclusion chromatography. MYO15 with novel lights chains were characterized using steady-state ATPase assays, low-speed centrifugation, and total internal reflection fluorescence microscopy (TIRFM).

Results: Mass spectrometry of MYO15-bound peptides revealed CETN2, a calcium-binding protein related to CALM, that is involved in the function of the microtubule organizing center and cilia basal body. We show that CETN2 specifically binds to IQ3 of MYO15 and is a bona fide light chain. No significant differences in ATPase activity were detected between MYO15-2IQ (without CETN2) or MYO15-3IQ (with CETN2 bound). At higher protein concentrations (1 µM MYO15-3IQ) we discovered that CETN2 specifically drives the calcium-dependent assembly of MYO15 into condensates that sediment during low-speed centrifugation (10k x g). Analysis of these condensates using TIRFM reveal that CETN2 drives the assembly of MYO15-3IQ into a polymer-like network in the presence of Ca2+. We are presently investigating the properties of this motor polymer network.

Conclusions: CETN2 specifically binds to the 3rd IQ domain of MYO15 as an accessory light chain, and in the presence of Ca2+ ions drives the biomolecular condensation of MYO15 into larger mesoscopic structures. We speculate these structures are critical for MYO15 to move within stereocilia and ultimately regulate actin polymerization at the stereocilia tips. Our data further suggest that CETN2 may be a candidate gene for studies of human hereditary hearing loss.

4. Mechanotransduction and Synaptic Activity Shape a Hair Cell-Specific Mitochondrial Phenotype
Category: Hair Cells: Anatomy and Physiology
Andrea McQuate*[1], David Raible[1]
[1]*University of Washington*

Background: Disruption of mechanosensory hair cells in the cochlea causes irreversible hearing impairments, and current therapeutic interventions are limited. Hair cells are particularly sensitive to changes in their mitochondria, subcellular organelles necessary for energy production in all eukaryotic cells. There are over thirty mitochondrial deafness genes, and mitochondria are implicated in hair cell death following noise exposure, aminoglycoside

antibiotic exposure, as well as in age-related hearing loss. However, little is known about the basic aspects of hair cell mitochondrial biology.

Methods: I use hair cells from the zebrafish lateral line as a model for cochlear hair cells, and serial block-face scanning electron microscopy (SBFSEM), to reconstruct hair cells and their mitochondria in three dimensions with ultrastructural resolution.

Results: I have shown that hair cells have a unique mitochondrial phenotype distinct from surrounding support cells. This phenotype includes (1) a high mitochondrial volume, and (2) specific mitochondrial architecture: multiple small mitochondria apically, and a reticular basolateral mitochondrial network. The phenotype develops gradually with hair cell maturation. Disruption of this mitochondrial phenotype impacts synaptic ribbon formation and calcium buffering in hair cells. I have further shown that hair cell mechanotransduction and synaptic activity shape the mitochondrial architecture.

Conclusions: These results demonstrate the high degree to which hair cells regulate their mitochondria for optimal physiology and provide new insights into mitochondrial deafness.

5. AAV-Mediated Clrn2 Gene Therapy Prevents Progressive Hearing Loss in Mice
Category: Hair Cells: Anatomy and Physiology

Sandrine Vitry*[1], Pranav Patni[2], Thibault Peineau[3], Maureen Wentling[1], Pierrick Bordiga[1], François Simon[4], Audrey Maudoux[1], Carlos Aguilar[5], Sylvie Nouaille[1], Andrea Lelli[1], Steve Brown[5], Paul Avan[1], Mathieu Beraneck[4], Michael R Bowl[5], Sedigheh Delmaghani[1], Didier Dulon[3], Aziz El-Amraoui[2]
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Background: To date, we still know very little about the pathways key to post-natal progressive hearing/balance impairments. Clarin-1 is a tetraspan-like glycoprotein that is essential for post-lingual hearing and variable vestibular and vision deficits in humans. Recent collaborative work has established that, like clarin-1, clarin-2 is also essential for hearing in zebrafish, mice and humans. Together, our findings support evolutionary conserved key role(s) of these clarins in the inner ear. Here, we further characterize the function of clarin-2 in hair cells and assess the efficacy of gene therapy in clarin-2 deficient mice.

Methods: Utilizing the Clrn2clarinet mouse model, we have employed molecular, cellular, imaging, physiological, and behavioral approaches to further elucidate the pathogenesis of deafness, and to assess the potential beneficial outcome of gene therapy. Confocal imaging was used to monitor distribution of appropriate markers for the hair bundle (e.g. F-actin, harmonin, PDZD7) and synaptic substructures (Ribeye, GluR2). Scanning electron microscopy (SEM) was used to monitor the architecture of stereocilia bundles. ABRs, DPOAEs, along with Mechano-electrical transduction (MET) and capacitance measurements were used to assess hearing sensitivity, hair cell MET activity, and synaptic exocytosis. Vestibular-evoked potentials (VsEPs) and vestibulo-ocular responses (VORs), along with balance behavioral assays, were used to document vestibular function. For gene replacement therapy, control and Clrn2clarinet mice were injected at P0-P1 through the round window membrane using AAV9-PHP.eB-Clrn2.

Results: Our findings establish that in addition to contributing to MET function and ensuring bundle stereocilia integrity, clarin-2 is also required for auditory inner hair cell synapses. Capacitance measurements in the absence of clarin-2 reveals defective calcium currents and decreased inner hair cell (IHC) exocytosis. VsEPs responses show decreased amplitudes of wave 1, which remains stable at P30, P60 and P90. However, morpho-molecular and behavioral follow-up studies show normal bundle structure and function, and confirms a lack of an overt balance deficit in Clrn2clarinet mice. Importantly, all the clarin-2 mediated structural and functional hearing deficits are prevented upon AAV9-PHP.eB-Clrn2 mediated gene replacement. Specifically, re-expression of clarin-2 prevented the MET current decrease and the loss of the 'transducing' short row stereocilia. Furthermore, ABRs, DPOAEs, MET, SEM and capacitance recordings confirm: the restoration of hearing; normal transduction currents; typical hair bundles; and, normal calcium currents and IHC synaptic exocytosis.

Conclusions: Our study reveals a hitherto unrecognized role of clarin-2 in IHC synaptic function. Furthermore, in spite of decreased VsEPs starting at 1-month, no overt behavioral balance deficit was observed regardless of age analyzed. Unlike in Clrn1−/− knockout mice, the post-natal progressive hearing impairment caused by the Clrn2clarinet allele can be durably prevented. In summary, our findings support the applicability of gene replacement therapy for progressive hearing impairment, which critically is a disorder that has a post-natal time window for therapeutic intervention.

6. A Novel Population of Short Actin Filaments at Stereocilia Tips

Category: Hair Cells: Anatomy and Physiology
Xiayi Liao*[1], Chun-Yu Tung[1], Benjamin Perrin[1]
[1]*Indiana University - Purdue University Indianapolis*

Background: Hair cell stereocilia size and shape are dictated by its actin core, which is comprised of parallel actin filaments (F-actin) that are oriented with their fast-growing barbed ends towards the tip and their slower-growing pointed ends towards the stereocilia base. F-actin in the stereocilia core is highly stable, but actin at stereocilia tips turns over more rapidly. It is not known if the dynamic actin is part of the stereocilia core or a separate population of F-actin.

Methods: To define actin populations at stereocilia tips, we permeabilized postnatal mouse cochlear tissue with the detergent saponin to allow exogenous proteins to access and bind within unfixed stereocilia. We then used purified His-CapZ or fluorescent actin monomers to label barbed ends and purified His-tropomodulin (Tmod1) to label the pointed ends of actin filaments. CapZ and Tmod1 are well characterized proteins and are known to bind only the barbed and pointed ends of actin filaments, respectively.

Results: Tmod1 labeled the tips of stereocilia in all rows before postnatal day 6 (P6). After P6, Tmod1 staining decreased at the tips of stereocilia in shorter rows before also declining in stereocilia in the tallest row. Thus, levels correlate with when stereocilia are lengthening and widening. Since F-actin in the stereocilia core presents only barbed ends at stereocilia tips, we propose that the pointed ends detected by the Tmod1 probe reflect short actin filaments (tip filaments), which are not part of the core. Correspondingly, pretreating cochlear explants with the actin-depolymerizing drug Latrunculin A (LatA) dramatically reduced the level of tip filaments while the actin core itself remained intact. Following LatA washout, tip filaments returned within a short time, suggesting they are rapidly turning over in vivo. Finally, tip filaments are decreased in EPS8 knockout mice, which have abnormally short stereocilia, suggesting that the EPS8 dependent elongation complex makes or stabilizes tip filaments, and that tip filaments consequently contribute to stereocilia growth.

Conclusions: Our data show that F-actin at stereocilia tips has both barbed and pointed ends when only barbed ends would be expected based on current models of stereocilia structure. We propose that there is a population of short actin filaments at stereocilia tips, which we call tip filaments, that likely contribute to stereocilia growth.

7. Tip Links Regulate Stereocilia Dimensions and Row Identity in Inner Hair Cells

Category: Hair Cells: Anatomy and Physiology
Jocelyn Krey[1], Paroma Chatterjee[1], Jennifer Goldsmith[1], Peter Barr-Gillespie*[1]
[1]*Oregon Hearing Research Center and Vollum Institute, OHSU*

Background: Hair bundles' staircase architecture optimizes mechanotransduction. During development, stereocilia in each row differentially widen and lengthen in specific phases, which are regulated by emergence of transduction. Tension in tip links, which interconnect stereocilia rows, elicits transduction currents but may independently regulate stereocilia structure. Here we used mutant mice to examine the role of stereocilia links on bundle development and the localization of tip-protein complexes in inner hair cells (IHCs).

Methods: Using scanning electron microscopy and super-resolution fluorescence imaging, we examined stereocilia dimensions in postnatal IHC hair bundles from Cdh23v2J, Pcdh15av3J, and Myo7a8J mice ("tip-link mutants"), as well as in TmieKO and Myo15ash2 mice, which lack transduction or row 1 tip complexes, respectively.

Results: Compared to controls, stereocilia in homozygous-null mice from all five mutant lines had thinner row 2 and thicker row 3 stereocilia. Although stereocilia length in all three rows become more uniform in TmieKO/KO and Myo15ash2/sh2, stereocilia lengths of tip-link mutants became more variable.

We correlated changes in stereocilia dimensions with localization of row-specific tip proteins. In tip-link mutants, like in TmieKO/KO mutants, row 1 proteins EPS8 and WHRN distributed to all stereocilia tips; unlike in TmieKO/KO, however, all rows of tip-link mutants contained similar, high amounts of EPS8/WHRN at their tips. While GNAI3 was substantially reduced at row 1 tips in TmieKO/KO, GNAI3 remained only at row 1 tips in tip-link mutants, like in controls. Row 2 proteins, including EPS8L2, CAPZB, and TWF2, exhibited the same localization pattern in both transduction and tip-link mutants, with EPS8L2 concentrating at both row 1 and 2 tips and CAPZB and TWF2 disappearing from all stereocilia tips. In Myo15ash2/sh2, which lack row 1 tip complexes, CAPZB was present at all stereocilia tips but EPS8L2 failed to accumulate at tips, instead remaining along the stereocilia shaft.

We created double knockout Pcdh15av3J/av3J;TmieKO/KO¬ (DKO) mice, and found that while IHCs had increased row 3 stereocilia widths similar to those in TmieKO/KO, stereocilia lengths became more variable like

in Pcdh15av3J/av3J. While GNAI3 failed to accumulate in DKO stereocilia, EPS8/WHRN uniformly distributed to all stereocilia tips in DKO bundles, as in Pcdh15av3J/av3J.

Conclusions: GNAI3 localization to row 1 tips thus depends on transduction-channel proteins but not transduction currents or tip links. The EPS8/WHRN/MYO15A complex localizes to tips independently of GNAI3 and its accumulation at tips is inhibited by the presence of tip links at the tips of the shorter rows, but not necessarily by transduction alone. Localization of row 2 tip proteins like CAPZB and TWF2, however, is regulated by both transduction and tip links. Finally, MYO15A complexes may inhibit the localization of CAPZB at stereocilia tips and prevent actin filament capping there.

8. Coupling Between Outer Hair Cell Electromotility and Prestin Sensor Charge Depends on Voltage Operating Point
Category: Hair Cells: Anatomy and Physiology

Joseph Santos-Sacchi*[1], Winston Tan[2]
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Background: Outer hair cells (OHCs) play a critical role in cochlear amplification, a process driven by the membrane motor protein prestin (SLC26a5), which undergo voltage-induced conformational changes that couple into cell length changes termed electromotility (eM). The frequency response of nonlinear capacitance (NLC), which is a ratiometric measure of prestin's voltage-sensor charge movement (dQp/dVm), depends on the location of AC voltage excitation along prestin's operating voltage range, being slowest at the voltage (Vh) where NLC peaks. Here we aimed to directly investigate the coupling between prestin charge movement (Qp) and eM across frequency (up to 6.25 kHz).

Methods: Isolated guinea pig OHCs were whole-cell voltage clamped in ionic blocking solution and stimulated with a voltage protocol designed to elicit nonlinear eM and associated nonlinear membrane (displacement) currents. Contiguous repetitions of multi-frequency voltage epochs (5.12 ms duration epoch with summed sinusoids of 195,390, 780, 1560, 3120 and 6238 Hz at 20 mV pk) were superimposed on a ramp voltage (150 ms duration), with a sampling rate of 10 µs. eM was simultaneously recorded with a fast video camera (Phantom 310, Vision Research) at a frame rate of 25 kHz and measured by tracking the cuticular plate using a shape tracking algorithm that provides sub-pixel resolution of movements.

Results: We find that coupling between prestin charge movement and eM is dependent on prestin's voltage operating point. There is tight correspondence between Qp and eM across frequency (up to our highest interrogation frequency of 6.25 kHz) at operating voltages away from Vh. However, near Vh, a disparity in coupling between Qp and eM develops at high frequency, with eM showing a slower frequency response than Qp.

Conclusions: We reason that coupling is more susceptible to molecular and cellular loads at maximal eM gain (Vh), where prestin compliance is expected to be maximal. Recent cryo-EM studies have begun to shed light on structural features of prestin that impact its performance against loads.

7:00 a.m. - 9:00 a.m.
Podium Session #24 – Auditory Perception and Effort in Listening to Sounds, Speech and Music
Moderators: Samira Anderson, Au.D., Ph.D. & Gabriella Musacchia, Ph.D.

1. Does Cognition Contribute to Performance on a Psychophysical Measure of Spectro-Temporal Processing?
Category: Psychoacoustics

Aaron Moberly*[1], Kara Vasil[1], Terrin Tamati[1]
[1]*The Ohio State University*

Background: Cochlear implants (CIs) provide degraded auditory input, and speech recognition abilities of adult CI users remain highly variable. This variability has been partially attributed to differences among listeners in the spectro-temporal fidelity of the signal, which is impacted by the electrode-neural interface and higher level auditory processing. Non-linguistic psychophysical measures have increasingly been developed and used to assess individual listeners' spectro-temporal processing abilities. In particular, performance on a three alternative forced-choice spectral-temporally modulated ripple test (SMRT, Aronoff and Landsberger, 2013) has shown strong

correlations with speech perception outcomes in both children and adults with CIs. However, a concern that has arisen is that psychophysical measures like the SMRT likely are not pure measures of spectro-temporal processing. Rather, cognitive functions (e.g., fluid intelligence) may contribute to SMRT performance. The goal of this study was to determine the degree to which cognitive factors contribute to SMRT performance among younger and older adults with normal-hearing and older adults with hearing loss. The main hypothesis was that the three groups would demonstrate differing performance on SMRT, based on their relative cognitive and hearing abilities. That is, if relatively poorer cognitive and hearing abilities negatively contribute to SMRT performance, then younger normal-hearing adults would obtain the best SMRT thresholds, followed by older normal-hearing adults, and then older adults with hearing loss.

Methods: Data were collected from three groups: 40 young normal-hearing (YNH) listeners, 42 older near normal-hearing (ONH) listeners, and 38 adults with bilateral severe-to-profound sensorineural hearing loss who were CI candidates (CICs). Participants were tested using pure-tone audiometry (pure-tone average, PTA, of 500, 1000, and 2000 Hz), sound-field presentation of the SMRT to assess spectro-temporal processing, and the visual Raven's Progressive Matrices to measure fluid intelligence. Regression analyses were performed to assess relative contributions to SMRT performance of hearing and cognitive functions in the three groups of listeners.

Results: Expected differences were found among the three groups on PTA and cognitive functions. The hypothesized group differences were found in SMRT performance among the three groups (YNH > ONH > CIC), which were partially explained by both PTA and Raven's performance. SMRT performance in ONHs was independently predicted by both PTA and Raven's; In CICs, SMRT was independently predicted by only PTA; in YNHs, neither PTA nor Raven's significantly predicted SMRT scores.

Conclusions: Performance on the SMRT task of spectro-temporal processing is impacted by cognitive functions, namely fluid intelligence, but to a relatively small degree, particularly in our clinical population of interest: adults with severe-to-profound hearing loss. Findings suggest that cognitive functions should indeed be considered during interpretation of SMRT scores, but that these functions play a limited role compared with the contributions of hearing abilities.

2. Pitch Discrimination is Better for Synthetic Timbre Than Natural Musical Instrument Timbres, Despite Familiarity

Category: Psychoacoustics

Emma Holmes*[1], Timothy Griffiths[2], Ingrid Johnsrude[3]
[1]UCL, [2]Newcastle University; UCL, [3]University of Western Ontario

Background: Pitch discrimination is better for complex tones than for pure tones, but how more subtle differences in timbre affect pitch discrimination is not fully understood. Here, we compared pitch discrimination thresholds of flat-spectrum harmonic complex tones with those of natural sounds played by musical instruments of three different timbres (violin, trumpet, and flute).

Methods: To investigate whether high natural familiarity with sounds of particular timbres affects pitch discrimination thresholds, we recruited musicians who were trained on one of the three instruments (violin, trumpet, or flute), but neither of the other two instruments. We also recruited a group of non-musicians. To measure pitch discrimination thresholds, we presented participants with two bars of a 4-tone sequence that contained a deviant-pitch tone in the second bar. Participants had to respond whether the second or third tone was 'mistuned' in a two-alternative forced-choice procedure. We adapted pitch for each timbre (violin, flute, trumpet, artificial complex tone) in four separate, but interleaved, runs. Violin, trumpet, and flute tones were obtained from the Philharmonia Orchestra sound sample database. Artificial flat-spectrum harmonic complex tones were created by summing sine waves (with 0° phase) at integer multiples of the fundamental frequency up to 20,000 Hz.

Results: We found that flautists and trumpeters could discriminate smaller differences in pitch for artificial flat-spectrum tones than for sounds played by musical instruments—despite the unfamiliar timbre of flat-spectrum tones compared with musical instrument sounds, which are regularly heard in everyday life. Furthermore, thresholds were no better for the instrument a musician was trained to play than for other instruments. This suggests that even extensive experience listening to and producing sounds of particular timbres does not reliably improve pitch discrimination thresholds for those timbres.

Conclusions: The results show that timbre familiarity provides minimal improvements to auditory acuity. Physical acoustics (i.e., the presence of equal-amplitude harmonics) determine pitch-discrimination thresholds more than does experience with natural sounds and timbre-specific training.

3. The Impact of Frequency Equalization on Musical Sound Quality Perception in Cochlear Implant Users

Category: Psychoacoustics

Jonathan Mo*[1], Nicole Jiam[2], Mickael Deroche[3], Patpong Jiradejvong[2], Charles Limb[2]
[1]*University of Pennsylvania,* [2]*University of California - San Francisco,* [3]*Concordia University*

Background: Frequency equalization (FEQ) is a process that adjusts the energies of specific frequency bands within an audio signal. In this study, we examined how the amplification and reduction of these bands may be used to improve musical sound quality in cochlear implant (CI) users. Because bass frequencies are not well represented by CIs and CI users prefer vocals in musical stimuli, we hypothesized that musical sound quality would be improved by amplifying lower and mid-range frequencies. Furthermore, we hypothesized that prior musical training yields greater sensitivity to FEQ manipulations.

Methods: This was a prospective study involving 33 adult CI users who participated in an online study between November 2020 and February 2021. Participants listened to 80 auditory tokens derived from ten Billboard magazine top songs of the year between 1970-2015 and assessed the musical quality of acoustic stimuli using the MUltiple Stimulus with Hidden Reference and Anchor for CI users (CI-MUSHRA) rating scale. For each song, 8 versions of the real-world stimulus were created: 6 made by amplifying (+9 dB) or reducing (-9 dB) low frequencies (20-500 Hz), mid frequencies (500-2,000 Hz), and high frequencies (2,000-20,000 Hz), a composite stimulus containing a 1,000 Hz low-pass filter and white noise ("anchor"), and an unaltered version ("hidden reference"). Stimuli were RMS normalized and research participants were kept blind to manipulations. Subjects listened to altered music clips and provided sound quality ratings (SQR, the primary outcome) based on a 100-point scale that reflected the perceived sound quality difference of the clip relative to the unaltered reference excerpt.

Results: In general, amplifying low and mid frequency ranges led to higher SQR compared with reducing them, but no condition led to significantly higher ratings versus the reference clip. Only reduced mid frequencies had significantly lower SQR when compared with the reference ($p<0.001$). FEQ manipulations had a greater impact on musical sound quality for CI users with prior musical training compared to those without it. More specifically, trained individuals showed greater preference for amplified mid ($p=0.018$) and high frequency ranges ($p=0.012$) versus non-musically trained individuals. CI users without musical training were less sensitive to manipulations and rated SQR to be roughly the same across conditions.

Conclusions: Significantly lowered SQR due to a reduction in mid frequencies may be explained by attenuated vocal or melodic lines, each of which are core components of lyrical music stimuli. Amplified low and mid frequencies trended towards significantly higher SQR ratings compared with the reference clip, suggesting the need for higher powered studies and further exploration of the degree to which amplification in these ranges may improve musical sound quality. Furthermore, these results demonstrate that CI-mediated musical sound quality appraisals of FEQ are significantly impacted by prior musical training.

4. The Effects of Social Observation on Cardiovascular and Pupil Responses to Speech Perception

Category: Speech Perception

Adriana Zekveld*[1], Hidde Pielage[2], Bethany Plain[2], Michael Richter[3], Gabrielle Saunders[4], Tanveer Bhuiyan[5], Niek Versfeld[2], Thomas Lunner[6], Sophia Kramer[2]
[1]*Amsterdam University Medical Center,* [2]*Amsterdam UMC - location VUmc,* [3]*Liverpool John Moores University,* [4]*University of Manchester,* [5]*Demant A/S,* [6]*Eriksholm Research Centre*

Background: An important aspect of speech perception in daily life is the social context in which conversations take place in part because besides speech perception performance, the listening effort and stress evoked by speech perception difficulties have social implications. In the current study, we investigated whether being observed while performing a standard speech perception test influences sympathetic and parasympathetic cardiovascular parameters and the pupil response during listening. We hypothesized that being observed would increase the importance of performing the listening task successfully, and thereby the motivation of the listener. We expected that being observed would result in increased performance, especially in difficult conditions, and in higher cardiovascular activity, larger pupil response and higher levels of subjectively rated listening effort as compared to performing the task alone.

Methods: Twenty-nine hearing-impaired participants (mean age 65 years) performed a speech reception task both alone and in the presence of two observers. Auditory stimuli consisted of Danish Hearing in Noise Test (HINT) sentences spatially masked by four-talker babble. Sentences were presented at two signal-to-noise ratios, corresponding to 50% and 80% of sentences correct. We measured the pupil dilation response and the change in pre-ejection period, two indices of heart rate variability, heart rate and blood pressure relative to a baseline

condition. After each condition, participants rated their effort investment, stress, tendency to give up and preference to change the situation to improve audibility.

Results: The presence of an observer did not influence performance and did not significantly affect the peak or mean pupil dilation response (listening effort) during listening. However, it resulted in larger baseline pupil size and a larger second half of the evoked pupil response, as revealed by a post-hoc exploratory analysis. Furthermore, systolic, diastolic, and mean arterial blood pressure increased while observed compared to when the task was performed alone. The additional cardiovascular measures were not affected by observation state or signal-to-noise ratio. Finally, participants' subjective ratings were sensitive only to signal-to-noise ratio, but not the observation state.

Conclusions: The current results indicate higher cardiovascular stress levels when participants were being observed during a speech perception task as compared to being alone. In addition, the effects shown with pupillometry might indicate that monitoring the observers might require effortful processing. This study showed that social context can lead to increased mental effort or stress during listening. This emphasizes the need for ecological test settings as these provide a more comprehensive insight into the daily life difficulties of people with hearing impairment.

5. Audiological and Demographic Factors That Impact Phonetic Categorization by Cochlear Implant Users
Category: Speech Perception

Sarah Colby*[1], Michael Seedorff[1], Bob McMurray[1]
[1]*University of Iowa*

Background: The ability to adapt to variable acoustic input is a necessary skill for successful speech perception. In broad terms, cochlear implant (CI) users tend to benefit from the addition of residual acoustic hearing. However, previous studies tend to compare CI users in different listening conditions within-subjects (i.e., in their typical Acoustic + Electric configuration compared to Acoustic-only or Electric-only configurations) and comparisons among different groups of CI users do not always reflect an Acoustic + Electric benefit. McMurray et al. (2016) suggest that CI users with residual acoustic hearing perform similar to electric-only listeners on phonetic voicing contrasts and unexpectedly poorer with fricative contrasts which have little energy in the range of the Acoustic + Electric listeners' acoustic hearing. To further investigate how residual acoustic hearing impacts adaptation to phonetic ambiguity, we examined whether device configuration, age, and device experience influenced phonetic categorization in a large individual differences study.

Methods: CI users with various device configurations (Electric-only: 18 unilateral, 23 bilateral; Acoustic + Electric: 25 hybrid, 43 bimodal, 27 single-sided deafness; N=136) categorized tokens from five /b-p/ and five /s-ʃ/ minimal pair continua (e.g., bet-pet; sock-shock). We investigated age, device experience (time since implantation), and when applicable, residual acoustic hearing (pure tone hearing thresholds) as predictors of categorization.

Results: Acoustic + Electric users were better able to categorize along the voicing contrast (steeper categorization slope) compared to Electric-only users, but there was no group-level difference for fricatives. There were differences within the groups for fricatives: bilateral users showed better categorization than unilateral users and bimodal users had better categorization than hybrid users. Age was significant factor for voicing, while device experience was significant for fricatives. Critically, in the Acoustic + Electric group, CI users with better residual acoustic hearing (better pure tone hearing thresholds) had shallower slopes than those with poorer residual hearing

Conclusions: Our findings suggest residual acoustic hearing is beneficial for categorizing voicing, but not frication. Age and device experience play different roles across different contrasts. Within the group CI users with residual acoustic hearing, those with better hearing thresholds may be over-relying on their acoustic hearing rather than extracting as much information as possible from their CI, and thus have worse fricative categorization.

6. The Use of Binaural Beamforming to Reduce Listening Effort
Category: Speech Perception

Joaquin Valderrama-Valenzuela*[1], Angela Wong[1], James Galloway[1], Jorge Mejia[1], Brent Edwards[2], Nicholas Herbert[3]
[1]*National Acoustic Laboratories,* [2]*National Acoustic Laboraotories,* [3]*Sonova Group*

Background: People with hearing loss often complaint about the amount of concentration required to follow a conversation in noisy venues. This research aimed to investigate whether a binaural beamformer designed to enhanced directivity – known as 'StereoZoom' (SZ) reduced the amount of cognitive effort required to understand

speech relative to a soft beamformer aiming to replicate the natural pinna effect (known as 'Real Ear Sound' (RE) in the devices used) in a realistic, noisy scenario. It was hypothesized that the acoustic advantage provided by SZ would reduce the listening effort required to understand a speech stream coming from the front.

Methods: 20 hearing-impaired adults fitted with Phonak Marvel M90 hearing aids using Target software (v7.0.5) participated in an experiment that recreated a realistic cafeteria in the anechoic chamber of the Australian Hearing Hub. Listening effort was compared between RE and SZ via a novel dual-task which provided behavioural measures based on reaction time – with the hypothesis that longer reaction times would be associated with increased effort; physiological measures based on electroencephalography – aiming to characterise brain activation patterns associated with listening effort; and self-reported measures based on a questionnaire.

Results: Results showed that, relative to RE, (1) intelligibility improved with SZ from 83.8% to 88.9% (p-value = $1 \cdot 10\text{-}14$) at signal-to-noise ratio corresponding to around 80% intelligibility (i.e. SRT-80), and from 90.9% to 93.4% (p-value = $2 \cdot 10\text{-}7$) at SRT-95; (2) reaction times decreased with SZ by 75 ms (p-value = 0.003) at SRT-80 and by 50 ms (p-value = 0.02) at SRT-95; and (3) participants reported lower levels of self-perceived effort with SZ at the two SRTs – on a scale of 1 (No effort) to 7 (Extreme effort), self-perceived effort decreased with SZ from 4.72 to 4.22 at SRT-80 (p-value = $2 \cdot 10\text{-}16$) and from 4.16 to 3.96 at SRT-95 (p-value = 0.0005). Electrophysiology data showed brain activation patterns consistent in the two evaluated SRTs, which were in accordance with previous literature of listening effort.

Conclusions: Together, results demonstrate that SZ reduces listening effort in a realistic noisy scenario and validate the sensitivity of the proposed methodology to small changes in listening effort. The outcomes of this research have the potential to inform clinicians of technologies that can reduce the amount of concentration required to communicate in noisy environments. It also facilitates the management of expectations with regard to the possibilities of these technologies.

7. An Irrelevant Task Enhances Mandarin Tone Recognition Training With Cochlear Implant Simulation
Category: Speech Perception

Seeon Kim*[1], Qi Gao[2], Fei Chen[2], Xin Luo[1]
[1]*Arizona State University,* [2]*Southern University of Science and Technology*

Background: Mandarin tone recognition with cochlear implant (CI) simulation can be improved via targeted training (Kim et al., 2021). However, Mandarin vowels carry not only tone but also vowel information that is important for sentence recognition. Listeners may also experience sound exposure without targeted tone recognition training in daily life. Studies have shown that additional exposure to similar acoustic stimuli while doing an irrelevant auditory or written task may enhance the performance of target auditory task (Wright et al., 2010). As such, this study investigated whether adding Mandarin vowel recognition training or adding sound exposure to Mandarin vowels during a written task may improve the efficacy of Mandarin tone recognition training and the generalization to Mandarin sentence recognition with CI simulation. This study also tested whether combined Mandarin tone and vowel recognition training may lead to greater improvements in Mandarin vowel and sentence recognition than Mandarin vowel recognition training alone.

Methods: Native Mandarin-speaking young adult normal-hearing listeners were randomly assigned to five training regimens with 10 participants each. For all the training regimens, six Mandarin single vowels in four lexical tones produced by two groups of five native Mandarin speakers were processed with a 4-channel noise-band vocoder and were used for training and testing, respectively. Mandarin Hearing-in-Noise Test sentence recognition was also tested before and after training. There were four 1-hour training sessions on four consecutive days. For the five training regimens, each training session consisted of tone recognition training interleaved with a written task in quiet (Tone-Silence), tone recognition training interleaved with a written task and sound exposure (Tone-Exposure), tone recognition training interleaved with vowel recognition training (Tone-Vowel), tone recognition training alone (All-Tone), and vowel recognition training alone (All-Vowel). Tone-Vowel, All-Tone, and All-Vowel training used the same number of training trials, while Tone-Silence and Tone-Exposure training used half the number of training trials.

Results: Preliminary data showed that Tone-Vowel and Tone-Exposure training led to greater improvements in Mandarin tone recognition than All-Tone training and in turn, than Tone-Silence training. All-vowel training had no effect on Mandarin tone recognition. Mandarin vowel recognition did not improve with Tone-Silence, Tone-Exposure, and All-Tone training, but improved similarly with Tone-Vowel and All-Vowel training. All training regimens resulted in similar improvements in Mandarin sentence recognition.

Conclusions: The results suggest that with the same total amount of training, combined tone and vowel recognition training improves both tone and vowel recognition, while tone and vowel recognition training alone

only improve the performance of target task. Adding sound exposure during an irrelevant task also brings benefits to tone recognition training without listeners' exertion. These results extend previous findings of normal hearing (Wright et al., 2010) to CI simulation and have important implications for auditory training with CIs.

8. A Data-Driven Approach to Assess Task Difficulty and Exerted Effort From Pupillary Recordings
Category: Speech Perception

Helia Relaño-Iborra*[1], Dorothea Wendt[2], Mihaela-Beatrice Neagu[1], Abigail Anne Kressner[1], Torsten Dau[1], Per Bækgaard[1]
[1]*Technical University of Denmark,* [2]*Eriksholm Research Centre Denmark*

Background: Task-evoked pupil dilation has become a common metric for measuring listening effort. However, most findings linking pupillometry and effort are contingent on data averaged across multiple listeners. Less is known about the association between an individual's evoked pupil response and their exerted effort. Here, we propose a data-driven analysis of pupil recordings that can classify pupil traces in terms of task demand and listeners' exerted effort.

Methods: Pupillometry data from 31 listeners were recorded during a speech-in-noise listening task. They were presented with Danish Hearing in Noise Test (HINT) sentences mixed with a four-talker babble noise. The task demand was manipulated using different signal-to-noise ratios (SNRs) ranging from -12 to 4 dB. For each SNR condition a block of 20 trials was recorded.

The recordings were preprocessed, whereby blinks and artifacts were removed, and trials deemed too noisy (i.e., >20% of blinks) were rejected. Trials were baseline-corrected and averaged to obtain one pupil trace per listener and SNR condition. Preprocessing metadata and derived metrics, such as information about gaze events (e.g., saccade duration, number of fixations and blink rate) and data validity (e.g., number of trials removed and amount of interpolated data) were extracted. Growth curve analysis was used to characterize the mean, slope, curvature, and delay of the pupil response. Additionally, the peak pupil dilation was extracted.

The final metric space, consisting of gaze information, validity data and pupil trace parameters, was subjected to a dimensionality reduction using principal component analysis (PCA) and the PCA factors were analyzed using a k-means clustering approach.

Results: The results from the PCA revealed four factors that accounted for 73% of the variance in the data. Importantly, the first factor (PC1) consisted of the validity metrics and explained 31% of the variance, and the second factor (PC2) consisted of all pupil trace characteristics.

The k-means analysis uncovered three underlying clusters in the dataset: a first cluster characterized by a low PC1 (i.e., good data quality) and high PC2 (i.e., increased PPD, mean and slope and reduced curvature and delay); a second cluster with similarly low PC1 but low PC2, and a third cluster characterized by high PC1 (i.e., poor data quality). An ANOVA analysis revealed significant differences across these three clusters in terms of the task demand (SNRs) and self-reported effort, thereby allowing for the classification of the individual traces into "low", "high" and "uncertain" exerted effort.

Conclusions: Our method uncovered underlying structures in a pupillometry dataset and found clusters that correlated well with task demand and self-reported effort. Including validity information in the metric space was key to separate listeners and conditions for which a reliable classification could not be performed. The proposed methodology opens the door for the automatic classification of individual pupil traces.

9:30 a.m. - 11:30 a.m.
Symposium #25

Comparative and Integrative Research in Directional Hearing
Chair: Elizabeth McCullagh, *Oklahoma State University*
Co-Chair: Catherine Carr, *University of Maryland*

Evolution of Ears, Hearing, and Acoustic Communication Among Frogs and Toads
Molly Womack, *Utah State University*

With more than 7,000 species known to locate and attract mates via acoustic communication, anurans provide unique opportunities for hearing and acoustic communication research. We combine neurophysiological, morphological, and developmental studies to understand how acoustic communication has evolved in anurans over 200 million years. We show surprising variation in hearing abilities among species, with mixed evidence for

matched filter hypotheses. Furthermore, we examine anuran species that lack tympanic middle ears to reveal atympanic hearing pathways and highlight undetermined mechanisms of 'earless' directional hearing. Here we collate these findings, raise new questions, and outline future directions for anuran hearing research.

Built-In Representation of Natural ITD Statistics Driving Adaptive Coding and Spatial Perception across Species

Jose Pena, *Albert Einstein College of Medicine*

Anticipating the statistical structure of sensory cues may optimize perception. We tested this hypothesis in binaural hearing, investigating whether natural statistics of interaural time difference (ITD) determine spatial discriminability in birds and humans. Avian sound driven orienting behavior and human auditory spatial perception show correlation with natural ITD statistics, supporting this hypothesis. Further investigation showed that spatial novelty detection is also correlated with anticipated ITD statistics across species. Recent in vivo recordings in the avian midbrain provide evidence of population response properties supporting the coding of anticipated and ongoing ITD statistics, and the effect of early experience on this coding.

Bonehead Solutions for a Couple of Ears: Prospects for Leveraging Transcranial Crosstalk to Improve Directional Hearing via Bone Conduction

Andrew Brown, *University of Washington*

Comparative studies of directional hearing have revealed an impressive diversity of solutions to the same fundamental problem. Whereas a majority of vertebrates are "acoustically small" and leverage mechanisms built to overcome limited interaural separation, large-headed terrestrial vertebrates, including humans, benefit from comparatively large external interaural disparities. An interesting exception is that of bone conduction hearing, in which the ears are coupled by the bones and tissues of the head. Inspired by comparative work on coupled ears, this talk will consider prospects for leveraging transcranial crosstalk to generate effective interaural disparities, toward improved directional hearing outcomes in users of bone conduction devices.

Leveraging the Across-Species "natural ablation" of Brainstem Nuclei to Understand Mechanisms of Binaural Hearing in Mammals

Daniel Tollin, *University of Colorado School of Medicine*

The size and number of neurons comprising the brainstem nuclei that encode binaural spatial cues, the medial (MSO) and lateral (LSO) superior olive vary. It is posited that since physiological responses that rely on a particular nucleus would also "scale" with its size across species so too would behavior – this approach was dubbed "natural ablation". We review across species studies to link brainstem morphology to physiological and behavioral aspects of sound localization. The relative size of the MSO and LSO nuclei determine which binaural cues are used for localization and reveal the neural generator of binaural sound evoked potentials.

Bone Conduction Enables Directional Auditory Sensitivity in Atympanate Salamanders

Grace Capshaw, *Johns Hopkins University*

Inherently directional otolithic ears evolved early in vertebrate evolutionary history, revealing the significance of sound detection and localization to survival; however, fossil evidence indicates that the aquatically adapted ear of early terrestrial tetrapods lacked specializations for transducing aerial sound pressure (e.g., tympana) and therefore required extratympanic mechanisms for hearing. In this study, we investigated the ability of extratympanic pathways to support directional hearing in atympanate salamanders. We combined laser Doppler vibrometry with auditory brainstem response recordings to reveal that bone conduction supports a figure-eight pattern of directional sensitivity to airborne sound in the absence of a pressure-transducing tympanic ear.

Processing of Directional Information in the Tokay Gecko: A Population Code for Directionality Already in the Auditory Nerve.

Jakob Christensen-Dalsgaard, *SDU Odense University*

The ears of lizards, frogs and birds have a special configuration: the two eardrums are connected by air spaces, canals or permanently open, wide Eustachian tubes that allows sound to travel from one eardrum to the other. This

interaural coupling allows sound to reach both sides of the eardrum, producing binaural processing already at the level of the eardrum, and resulting in a strong peripheral directionality. This may simplify further neural processing in comparison to mammals with uncoupled ears, since a simple binaural comparison produces a robust directional response, as shown by models and biorobotic implementations.

Here, we report on a study of the response of auditory nerve fibers to directional sound stimulation (free field sound) in the Tokay gecko. Single unit responses are strongly directional in all fibers with CFs spanning most of the gecko's hearing range (CFs from 200-4000 Hz) and reflects the directional response of the eardrum. Analysis of mutual information in the nerve fibers show a strongly lateralized distribution with high Fisher information across the midline. Thus, a strong population code for sound source direction is already present in the auditory nerve. The directional response of the fibers is limited by the fibers' dynamic range, and simplified models of IE (ipsilateral input excitatory, contralateral input inhibitory) processing based on the individual neural responses suggest a role for IE in widening the directional response. In contrast, models of neural coincidence does not suggest a prominent role for coincidence processing.

The finding of a population representation of direction in the auditory nerve raises the question of what additional central processing can be expected in the gecko. We will discuss the implication for central processing in geckos based on simple models of binaural neural processing and robotics. These models will be a guideline for future studies of neural processing in the ascending auditory pathway of the tokay gecko, and the possible function of a population code in steering the animal towards sound sources.

Binaural Hearing across Rodent Species
Elizabeth McCullagh, *Oklahoma State University*

The ability to find where a sound is coming from in the environment is a critical behavior that enables foraging, avoidance of predators, engaging in social/affiliative behavior, and other vital tasks for survival. Are there other factors beyond external features (such as head size) that determine an animal's sound responsiveness? Recent work suggests that environmental and evolutionary pressures are some of the many factors that determine sound localization and hearing ability in mammals. Comparisons of anatomical markers in brainstem nuclei and auditory brainstem responses will be compared across Oklahoma rodent species with varying social structures.

9:30 a.m. - 11:30 a.m.
Podium Session #26 – Intercellular Regulation of Cochlear Development
Doris Wu, Ph.D. & Ronna Hertzano, M.D., Ph.D.

1. Notch1 is Required for Supporting Cell Survival During Cochlear Maturation
Category: Development: Cellular/Systems
Alison Heffer*[1], Felicia Gilels[2], Amy Kiernan[2]
[1]*University of Rochester Medical Center,* [2]*University of Rochester*

Background: Notch signaling is important in the formation and regulation of many aspects of inner ear development. Early in embryogenesis, Notch is required for development of the early sensory regions, a process known as lateral induction. Later, Notch plays a role in lateral inhibition where it is important in maintaining the supporting cell fate during sensory differentiation. However, little is known about the role Notch signaling plays after birth, during cochlear maturation.
Methods: To delete Notch1 in the organ of Corti, tamoxifen (37.5mg/kg) was administered by IP injection into Sox2CreER Notch1fl/fl pups at postnatal day 1 and 2 (P0/P1) and cochleae were collected and processed for experiments at several time points during cochlear maturation. Auditory function was measured at 6 weeks by Auditory Brainstem Response (ABR) and Distortion Product Otoacoustic Emissions (DPOAE), which tests the function of inner and outer hair cells, respectively. Ultrastructure analysis was done using scanning electron microscopy (SEM) imaging. Whole mount immunofluorescence and confocal imaging were performed to examine supporting cells and hair cells during cochlear maturation (P2-P14).
Results: We found that loss of Notch1 at birth resulted in profound hearing loss at 6 weeks, as evidenced by significantly raised ABR and DPOAE thresholds in mutants compared to controls. Additionally, whole mount confocal analysis and scanning election microscopy (SEM) imaging revealed a significant loss of supporting cells and outer hair cells in the organ of Corti, along with abnormalities in inner hair cell morphology and stereocilia. To further investigate when loss of these supporting cells and hair cells occurred, we injected tamoxifen at P0/P1

and analyzed cochlea at several timepoints during cochlear maturation. We found a significant loss of outer pillar and Deiters' supporting cells as early as P2 throughout all regions of the cochlea, with no increase in outer hair cell number. These results indicate a role for Notch1 in early survival of the supporting cells. Consistent with this, we found a significant increase in caspase3+ Deiters' cells. By P6 we see further outer pillar cell and Deiters' cell loss, with a significant increase in hair cells in the apical regions, consistent with either a loss in lateral inhibition or proliferation of hair cells (Ni et. al, 2016. J Neuro. 36(33) 8734-8745). Despite early loss of the supporting cells, outer hair cell loss did not occur until 2-3 weeks (P14-P21) after Notch1 deletion, suggesting hair cell loss is secondary to supporting cell loss.

Conclusions: Together, these results support an early requirement for Notch1 in the survival of cochlear supporting cells and indicate that outer hair cell survival is dependent on outer supporting cells, including Deiters' and outer pillar cells.

2. Wnt7b Regulates Hair Cell Planar Polarity via Non-Canonical Wnt/G-Protein/PI3K Signaling
Category: Development: Cellular/Systems

Andre Landin Malt*[1], Connor Smith[2], Diane Hwang[2], Xiaowei Lu[1]
[1]University of Virginia School of Medicine, [2]University of Virginia

Background: The V-shaped stereociliary hair bundle atop auditory hair cells is essential for hair cell's function as sound receptors. During development, hair bundle morphogenesis is tightly coupled with planar polarization of the apical cytoskeleton of cochlear hair cells. Multiple cell-intrinsic signaling pathways act in concert with intercellular planar cell polarity (PCP) signaling to control the polarized V-shape of the hair bundles and align their orientation along the medial-lateral axis of the cochlear sensory epithelium, the Organ of Corti(OC). We have recently discovered a non-canonical Wnt/G-protein/PI3K pathway that coordinates cell-intrinsic and tissue level PCP signaling to regulate cochlear elongation, kinocilium positioning, and asymmetric localization of a subset of core PCP proteins1,2. However, the identity of the Wnt ligands that initiate this signaling cascade is still unknown. Wnt5a, a prototypical non-canonical Wnt ligand, appears to be dispensable for this process 3, suggesting other Wnt ligands may be involved. Wnt7b is dynamically expressed in the OC during cochlear outgrowth and hair bundle morphogenesis. Therefore, we hypothesize that Wnt7b plays a role in the Wnt/G-protein/PI3K pathway during cochlear morphogenesis.

Methods: To test this, we deleted Wnt7b in the OC using Emx2Cre and Wnt7bflox alleles.

Results: Wnt7b conditional knockout (Wnt7bcKO) mutants had normal cochlear length, although four rows of OHCs were present in the apical turn of the cochlea. Moreover, mild hair bundle orientation and kinocilium positioning defects were observed. We also assessed asymmetric PCP protein localization in the OC. While asymmetric localization of Vangl2 and Dvl3 was unchanged, planar asymmetry of Fzd6 and Dvl2 was significantly diminished. To determine whether Wnt7b activates the G-protein/PI3K signaling, we examined the localization of Daple, a GEF for Gαi, as well as active Akt phosphorylated at serine-473 (pS473-Akt). We found that both Daple and pS473-Akt immunostaining were significantly decreased in the Wnt7bcKO OC. To further establish the role of Wnt7b in the Wnt/G-protein/PI3K pathway, we examined the effect of Wnt7b overexpression in the OC using a Cre-inducible Wnt7b transgene driven by Emx2Cre. We found that Wnt7b overexpression led to increased immunostaining of pS473-Akt. Together, these findings suggest that Wnt7b activates Wnt/G-protein/PI3K signaling in vivo.

The Wnt7bcKO cochlear phenotypes are milder compared to mutants deficient for Wntless, a gene required for the secretion of all Wnt ligands. Therefore, we hypothesized that Wnt7b and Wnt5a have redundant functions in non-canonical Wnt signaling in the cochlea. To test this, we deleted both Wnt5a and Wnt7b in the OC using Emx2Cre (dcKO). Remarkably, we found that cochlear lengths and hair cell numbers were significantly decreased in dcKO mutants, partially phenocopying Wntless mutants. We are now evaluating the PCP phenotypes of the dcKO mutants.

Conclusions: These experiments will identify the Wnt ligands that regulate cochlear morphogenesis through the Wnt/G-protein/PI3K pathway.

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2_Landin-Malt,A.etal.Non-Canonical_Wnt_Signaling_Regulates_Cochlear_Outgrowth_and_Planar-Cell-Polarity_via_Gsk3beta_Inhibition. FrontCellDevBiol(2021).
3_Najarro,E.H.etal.Dual_regulation_of_planar_polarization_by_secreted_Wnts_and_Vangl2_in_the_developing_mouse_cochlea. Development(2020).

3. The Novel Role of Transcription Factor Six2 in Vestibular Planar Cell Polarity (PCP)
Category: Development: Cellular/Systems

Sumana Ghosh*[1], Punam Thapa[1], Bradley Walters[1]
[1]*University of Mississippi Medical Center*

Background: Sine Oculis Homeobox Homolog (Six)2 is a transcription factor that belongs to an evolutionarily conserved Six family of genes which have been shown to be critical for the development and patterning of a number of tissues and organs such as eyes, heart and kidney. From RNAseq data it is known that Six2 is expressed in the developing otocyst and the resulting auditory and vestibular epithelia (VE). However, little is known about its function in this context. Here we sought to map Six2 expression during inner ear development and investigate Six2 function using germline Six2 knockout (KO) mice. The ancestral homolog of Six2, the sine oculis (so) gene, was first revealed in Drosophila loss-of-function mutations where loss of so not only severely affected the development of the compound eye, but the entire visual system. Loss of so also led to disorganization of the f-actin-based ommatidial bristles which are normally arranged with a very stereotypical planar cell polarity (PCP) organization reminiscent of cochlear and vestibular hair bundles which are also actin-based. Therefore, we hypothesize that Six2 plays a role in establishing PCP of the inner ear hair cells (HCs).
Methods: We performed RNAScope in-situ hybridization for Six2 in cryosectioned or whole-mounted inner ear tissues at various timepoints from embryonic day (E) 10.5 to postnatal day (P) 30. Samples were co-labeled by immunostaining of: SOX2 to identify pro-sensory cells or supporting cells, HuD or NF-H to label neurons, and MYO7A to label HCs. To investigate PCP organization in Six2 KO versus wildtype controls, we immunolabeled whole-mounted cochleae and utricles for VANGL2 and beta spectrin, and also labeled stereocilia bundles with phalloidin, at E17.5.
Results: Our data suggest that Six2 is expressed in the otic vesicle at least as early as E10.5 and continues to be expressed both in and around the pro-sensory domain at E12.5 and E14.5. In postnatal cochleae, Six2 expression becomes largely restricted to the outer hair cells (OHCs). In the utricle at E17.5, Six2 is expressed in both type I and type II HCs. Germline deletion of Six2 led to severe misorientation of hair bundles in the utricle. However, no obvious PCP defect or misorientation was readily apparent in the S2KO cochlea.
Conclusions: Six2 is expressed in the developing inner ear and regulates PCP organization of the utricular epithelium.

4. Wnt Signaling Promotes Cell Caudalization and Inner Ear Differentiation in Mouse Stem Cell-Derived Organoids
Category: Development: Cellular/Systems

Pei-Ciao Tang*[1], Li Chen[2], Sunita Singh[3], Andrew Groves[4], Karl Koehler[5], Xue Liu[1], Rick Nelson[2]
[1]*University of Miami School of Medicine,* [2]*Indiana University School of Medicine,* [3]*Baylor College of Medicine,* [4]*Baylor College of Medicine,* [5]*Boston Children's Hospital/Harvard Medical School*

Background: The stem cell derived-inner ear organoid system has shown potential in modeling disease, studying developmental process, and development of therapeutic treatments. However, low efficiency and inconsistent reproducibility have impeded the application using this system. In this study, we focused on the Wnt signaling pathway and its effects in the inner ear organoid induction during the early stage with the overarching goal of advancing our understanding of the early development of the inner ear. The otic placode is derived from posterior pre-placodal ectoderm (pPPE) and PPE locates at the neural plate border (NPB). We hypothesized that Wnt activation is required for the induction of the otic placode by enhancing the generation of the pPPE following caudalization of the NPB.
Methods: The mouse embryonic stem cell (mESC) derived-inner ear organoid system was used in this study. To precisely modulate Wnt signaling during early inner ear development, we tested various doses of the Wnt activator/GSK3 inhibitor CHIR99021 on the R1/E mESC line to activate the canonical Wnt pathway, while simultaneously reducing paracrine Wnt signaling activity by inhibiting the secretion of Wnt ligands with IWP2. The productivity of otic vesicle and expression of marker genes for PPE, aPPE, and pPPE were measured. Single cell RNA sequencing (scRNA-seq) analyses were conducted every 24-48 hr in organoids treated with various Wnt modulations.
Results: We identified that the optimal Wnt modulation produced inner ear organoids in ~ 90% of cultures. Moreover, reproducibility was improved by reducing the standard deviation from 31.9% in the DMSO control to 4.6% in samples treated with the optimal Wnt modulation. Expression of posterior marker genes, e.g., Gbx2, Irx2, and Pax8, was observed in samples treated with the optimal Wnt modulation. On the contrary, expression of

anterior marker genes increased while Wnt levels decreased. scRNA-seq data showed that the optimal Wnt modulation leads to a caudal microenvironment, i.e., similar to the rhombomere 5 region, for the inner ear organoid induction. Based on reconstructing the otic trajectory from scRNA-seq data (D4 to D10), it appears that the otic placode is derived from the pPPE following direct transition from the caudal NPB.

Conclusions: Wnt modulation during early induction therefore drastically improved the consistency and productivity of current inner ear organoid culture protocol. High yield of otic progenitor cells and later hair cells benefits the future applications, such as high throughput drug screening and cell therapy, using the inner ear organoid system. Furthermore, this study demonstrated that this organoid system recapitulates the A-P patterning based on the gradient of Wnt signaling during the development. Our data suggested that the inner ear organoid system exhibits multiple ectodermal derivatives and recapitulates early inner ear development.

5. Sonic Hedgehog Signaling Promotes Auditory Neuroblast Proliferation During Inner Ear Development
Category: Development: Cellular/Systems
Loksum Wong*[1], Doris Wu[1]
[1]*National Institutes of Health/NIDCD*

Background: Spiral ganglion neurons (SGNs) are auditory neurons that relay sound information received by the cochlear hair cells to the brain. These afferent neurons are highly susceptible to damages that can lead to permanent hearing loss. Currently, no treatments can replace their functions after damage. Sonic hedgehog (SHH) is a signaling molecule that has been shown to only express in the developing SGNs within the inner ear and thus, efforts to understand how SHH signaling pathway regulates the SGN formation during development may help design novel therapeutics that can replace these auditory neurons in the future.

Methods: To investigate how SHH controls SGNs development, I first examined how Shh expression is regulated in the SGNs through lineage tracing studies using a tamoxifen inducible ShhCreERT2/+ mouse line. Then I identified which cells are activated by the SHH signaling pathway using two key readouts: the receptor for SHH called Patched (Ptch1), and ARL13B-positive primary cilium where SHH signaling is transduced. Lastly, I generated Shh conditional knockout mutants with three different Cre lines (Foxg1Cre/+, NeuroDCre/+ and ShhCreER/+) to further dissect the mechanism by which SHH promotes SGN development.

Results: Fate-mapping studies demonstrate that Shh is expressed only in nascent post-mitotic SGNs and is downregulated in mature SGNs. Since earlier-born neurons are destined to become basal SGNs while the later-born ones are positioned at the cochlear apex, the expression of Shh within the SGNs follows a similar order of appearance from base to apical cochlea. Interestingly, Ptch1 is expressed in the dividing neuronal progenitors adjacent to the nascent SGNs. These Ptch1-expressing otic neuroblasts are primed to respond to SHH signaling as they are enriched in Arl13b expression. In contrast, nascent and mature SGNs show minimal Arl13b expression, suggesting that they do not respond to SHH stimulation. Analyses on the three hypomorphic Shh mutants show that Ptch1 expression in the neuroblasts is downregulated, and each of these Shh conditional mutants have various extent of SGN loss depending on the timing of Cre activation. Early Cre activation in Foxg1Cre/+; Shhlox/- embryos affect both early- and later-born neurons, whereas late activation of Cre in ShhCreER/lox animals spare those early-born neurons.

Conclusions: The primary function of SHH-expressing nascent neurons is to regulate otic neuroblasts. My results demonstrate that SHH in the SGNs is necessary for maintaining the pool of auditory neuroblasts, and loss of SHH signaling depletes this neuronal progenitor population and affects subsequent SGN production.

6. The Stage Dependent Expression of JAG1 is Regulated by MYBL2 via an Incoherent Feed-Forward Loop in the Developing Mammalian Cochlea
Category: Development: Cellular/Systems
Caryl Young*[1], Vidhya Munnamalai[2]
[1]*University of Maine,* [2]*The Jackson Laboratory*

Background: The precision of cochlear patterning is determined by the dynamic regulation of critical genes during development. This dynamic regulation generates an asymmetric sensory epithelium with one row of inner hair cells and three rows of outer hair cells. Cochlear progenitors differentiate into specialized cell types under the control of signaling molecules such as diffusible morphogens and receptor-ligands. Two examples of important signaling molecules that operate during early development are WNT morphogens and the Notch ligand, JAG1. We identified a novel transcription factor MYBL2 with unknown functions in cochlear development that is

downstream of the Wnt pathway. Here, we define a role for MYBL2 in the refinement of JAG1 expression, an important prosensory gene.

Methods: Between E12.5 to E14.5, the JAG1 domain is shifted from the medial edge to the centrally located, sensory domain that will give rise to the sensory epithelium, the organ of Corti. To test whether the dynamic regulation of JAG1 is mediated by the Wnt pathway, we temporally deleted β-catenin using Fbxo2CreER with tamoxifen induced on E10.0- E14.0 (early) and Isl1Cre on E13.0 (late). We hypothesize that there is a Wnt-mediated gene that spatially represses Jag1 expression. To identify this gene, we performed RNA-seq on E14.0 CHIR99021-treated (Wnt activator) cochleas for 6 hours. From this we identified Mybl2 that encodes a transcription factor. Like Jag1, Mybl2 is a predicted Wnt target gene. To investigate the role of MYBL2 on Jag1 regulation and cochlear patterning, we generated E14.5 Fbxo2CreER; Mybl2 cKO embryos and quantified JAG1 expression. Finally, we examined cochlear patterning on E18.5 Fbxo2CreER; Mybl2 cKO cochleas.

Results: The dynamic stage-specific regulation of JAG1 was found to be Wnt/ β-catenin-dependent. We confirmed that Mybl2 is regulated by the Wnt pathway. Our data showed that the loss of Mybl2 resulted in a two-fold expansion of the JAG1 domain on E14.5. This was accompanied by an expansion in the size of the SOX2-positive sensory domain. On E18.5, Mybl2 cKO cochleas showed extra inner hair cells. We also saw an increase in afferent innervation of the medial sensory domain.

Conclusions: We conclude that the increase in inner hair cells and afferent innervation were due to the expanded JAG1 domain that was observed on E14.5. Since both Jag1 and Mybl2 are predicted to be direct targets of the Wnt pathway, and MYBL2 spatially represses Jag1 to refine the medial sensory domain, this is referred to as an incoherent feed-forward loop. We identified a novel developmental gene, Mybl2, that plays a transient, but critical role in establishing sensory boundaries by regulating Jag1, a major prosensory and deafness gene. This advances our understanding how gene interactions, early in development, will give rise to patterning defects that can cause developmental auditory deficits in the cochlea

7. Feedback Between Notch Signaling and Mechanical Forces Drives Precise Patterning of the Inner Hair Cell Row

Category: Development: Cellular/Systems

Olga Loza*[1], Roei Cohen[1], Shahar Taiber[1], Shahar Kasirer[1], Shiran Woland[1], David Sprinzak[1]
[1]*Tel Aviv University*

Background: The mammalian hearing organ, the organ of Corti, consists of a precisely organized checkerboard-like pattern of four rows of hair cells (HCs) interspersed by non-sensory supporting cells (SCs). The inner hair cell row that contains a single line of alternating HCs and SCs is the first region specified during the development of the organ of Corti. While the differentiation into HCs and SCs has been associated with Notch signaling mediated lateral inhibition, it has been unclear how a single line of HCs and SCs is generated during embryonic development.

Methods: We use live imaging of cochlear explants from mice containing ZO1-EGFP (marking apical boundaries) and Atoh1-mCherry (marking differentiation of HCs) to track both morphological and differentiation dynamics. Laser ablation experiments are used to characterize the mechanical properties of the cells. Antibody staining of adhesion molecules at different developmental stages reveals dynamic expression patterns controlling adhesion rules. Finally, we use mathematical modeling to elucidate how feedback between Notch-mediated lateral inhibition and mechanical forces drive the patterning of inner hair cells.

Results: Quantitative analysis of 4D live imaging of cochlear explants reveals that an initially disordered pattern of HCs and SCs spread across 2-3 rows of cells is defined by a combination of two main morphological transitions: (i) A newly identified morphological transition termed 'hopping intercalation', where an Atoh1 positive cell sends a subapical protrusion that opens a second apical surface that expands until it merges with the first apical surface. (ii) Some Atoh1 positive cells that do not touch the pillar cells (PC) delaminate and removed from the apical surface. We also show that the heterotypic cell adhesion molecules, Nectin-1 and Nectin-3, accumulate at the boundary between inner HCs and PCs likely promoting enhanced adhesion. Finally, we show that inhibiting Notch signaling leads to significant morphological changes, in addition to the increase in the number of Atoh1 positive cells. Taking together our experimental data, we provide a hybrid model of lateral inhibition combined with mechanical modeling, explaining the generation of a precise line of HCs and SCs from an initially undifferentiated pattern.

Conclusions: Based on our experimental results and mechanical modeling we conclude that Atoh1 positive cells organize into a single ordered line of cells by refining an initially disordered salt-and-pepper pattern through a combination of a novel type of intercalation, the hopping intercalation, and delaminations of out-of-line Atoh1

positive cells. Thus, precise patterning of HCs is achieved through complex feedback between Notch mediated lateral inhibition and mechanical forces.

8. Transcription Factor IKZF2 is Required for Outer Hair Cell Development and Maintenance
Development: Cellular/Systems

Christopher Shults*[1], Elena Chrysostomou[1], Reza Amanipour[1], Beatrice Milon[1], Michael R Bowl[2], Ronna Hertzano[1]
[1]*University of Maryland School of Medicine,* [2]*UCL Ear Institute*

Background: The transcriptional cascade that drives outer hair cell (OHC) maturation is still heavily under investigation. Prosensory cells sequentially express transcription factors ATOH1, POU4F3, and GFI1 to achieve hair cell differentiation. Our lab has identified the transcription factor Helios, which is encoded by the Ikzf2 gene, as an essential regulator for hair cells to reach a mature OHC fate. Mice deficient for Helios suffer from early-onset hearing loss secondary to dysfunction of the OHCs, including a reduction in Prestin-dependent electromotility. Additionally, the ectopic expression of Ikzf2 in inner hair cells results in the downregulation of IHC-specific genes and a transcriptomic shift towards OHC genetic markers. These findings provide insight into the regulatory power of Helios in OHCs; however, little is still understood about the role of Helios post-OHC maturation. In this study, we assess the requirement of Helios in fully differentiated mature OHCs.
Methods: Ikzf2 floxed mice were crossed separately with either Gfi1-Cre (onset of cre-recombinase expression in cochlear HCs at ~E16.5) or Prestin-CreERT2 (tamoxifen-induced at P12/13) lines to conditionally deplete IKZF2 from HCs. The auditory function of these Ikzf2 cKO mice, along with littermate controls, was determined using auditory brain response (ABR) tests and measuring distortion product otoacoustic emissions (DPOAEs) at both 4- and 6-weeks of age. To evaluate OHC loss, whole mount cochlear immunohistochemistry was performed at 6-weeks of age utilizing phalloidin and an anti-Prestin antibody.
Results: Both the Gfi1-Cre and the Prestin-CreERT2 driven Ikzf2 cKO mice demonstrate a decrease in auditory function as assessed by ABR and DPOAE testing. Interestingly, both conditionally-deleted lines exhibit elevated ABR thresholds by 4-weeks of age compared to their normal hearing littermate controls. Histological analyses identify that in the Gfi1-Cre driven Ikzf2 cKO mice there is a profound decrease in OHC number. However, in the Prestin-CreERT2 driven Ikzf2 cKO mice fewer OHCs are lost and these are primarily from the first row. Detailed analysis of the histological findings is further discussed.
Conclusions: Our data reveal that IKZF2 is required both for OHC development, as well as OHC maintenance. These two lines exhibit distinct phenotypes related to the timing of Ikzf2 deletion. To gain insight to role of Helios in regulating OHC differentiation and their maintenance, transcriptomic studies are needed to identify upstream regulators of Ikzf2 and downstream target genes of Helios.

9:30 a.m. - 11:30 a.m.
Podium Session #27 – Hearing Loss: Consequences, Adaptation and Other Issues
Moderators: Matthew Fitzgerald, Ph.D. & Karen Banai, Ph.D.

1. Do Sudden Sensorineural Hearing Loss Patients With a Neural Pattern of Hearing Loss Have the Same Recovery Profile as Other Sudden Sensorineural Hearing Loss Patients?
Category: Hearing Loss: Consequences and Adaptation

Rebecca Xu*[1], Printha Wijesinghe[2], Grace Joshua[2], Aysha Ayub[1], Melissa Lee[1], Desmond Nunez[2]
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Background: Sudden Sensorineural hearing loss (SSNHL) is a condition of unknown aetiology in which the site of lesion along the axis from inner ear sensory cells to auditory nerve is undetermined. 30% or more of patients fail to recover with current treatments. In this study, audiometric findings at presentation were used to locate the site of lesion in SSNHL patients as neural or inner ear. The null hypothesis is that neural lesion SSNHL patients are as likely to recover as inner-ear lesion SSNHL patients.
Methods: The charts of SSNHL patients presenting to Vancouver General Hospital from November 2013 to June 2019 were retrospectively reviewed. Patient age and sex, initial and final Pure Tone Audiometric (PTA) average. Word Recognition Scores, and treatment were recorded. Patients with audiometric confirmed partial or complete

hearing recovery were classified as recovered. The inter-group differences in the proportion of patients with hearing recovery and pure tone audiometric gain were compared by Fisher's exact and independent samples t-test respectively using SPSS vs. 25.0.

Results: 166 charts were reviewed. 53 met inclusion criteria. 13 (mean age: 55.6±14.5) and 40 (mean age: 55.4±14.9) patients were classified as neural and inner ear, respectively. Recovery was demonstrated in 61.5% and 65.0% of neural and inner ear, respectively (p=1.0). Affected ear's mean PTA4 gain was 24.2±25.6 and 9.8±20 dB (p=0.043); mean WRS change was 45.3±28.9 and 3.0±24.5; (p=0.0003), respectively in neural and inner ear. Both were significantly different.

Conclusions: SSNHL patients with a neural type of hearing loss demonstrate greater hearing gain after treatment than those with an inner-ear type.

2. Effect of Liraglutide Treatment on Mitigating Hearing Damage Induced by Repeated Low-And Mild-Intensity Blasts: A Chinchilla Study
Category: Hearing Loss: Consequences and Adaptation

Shangyuan Jiang*[1], Sarah Sanders[1], Rong Gan[1]
[1]*University of Oklahoma*

Background: Blast-induced hearing loss is one of the most prevalent types of occupational diseases among Service members and Veterans. Our recent study revealed the therapeutic function of a GLP-1 receptor agonist (Liraglutide) to mitigate the auditory damage after multiple exposures to blast in the animal model of chinchilla (Jiang et al., JARO under review). This study aims at the effect of liraglutide on hearing restoration after repeated exposures to blasts at different intensity levels. The function and histology changes observed along the ascending auditory pathway from chinchillas exposed to low- and mild-intensity blasts were compared with each other to investigate the therapeutic function of liraglutide in relation to the blast intensity.

Methods: Chinchillas were divided into two groups by blast overpressure level: a low-intensity (G1) and a mild-intensity or mild-TBI group (G2). G1 chinchillas were exposed to 6 blasts at 3-5 psi or 21-35 kPa, while G2 animals were exposed to 3 blasts at 15-20 psi (103-138 kPa). Animals were then observed for 14 days and the auditory brainstem response (ABR), distortion product otoacoustic emission (DPOAE), and middle latency responses (MLRs) were measured. The 7-day-long liraglutide treatment was performed identically in G1 and G2 animals. Upon the completion of 14-day experiments, tissues along the auditory pathway of chinchillas were collected for immunofluorescence studies. Statistical analyses were conducted on all measured data and immunofluorescence images between G1 and G2 groups.

Results: The hearing function was ameliorated by the liraglutide treatment in both G1 and G2 chinchillas. G2 animals showed more severe post-blast hearing damage but greater post-blast recovery than G1s. Analyses of the ABR threshold, ABR waveform, MLRs, DPOAE, and immunofluorescence results indicated that the liraglutide treatment introduced more significant changes at multiple locations along the auditory pathway in G2 chinchillas.

Conclusions: The effect of liraglutide treatment on mitigating the blast-induced hearing damage was more significant in chinchillas exposed to blasts at the mild-TBI level. This feature suggests the liraglutide treatment could potentially be more effective for hearing loss patients with TBI or histories of high-intensity blast exposures. Acknowledgment: This work was supported by DOD W81XWH-19-1-0469.

3. Ensemble and Cellular Signatures of Cortical Hyperactivity Following Acoustic Trauma_x0000_
Category: Hearing Loss: Consequences and Adaptation

Matthew McGill*[1], Ariel Hight[2], Yurika Watanabe[3], Dongqin Cai[3], Kameron Clayton[3], Aravind Parthasarathy[4], Daniel B. Polley[3]
[1]*Harvard Medical School,* [2]*NYU Langone Medical Center,* [3]*Massachusetts Eye and Ear,* [4]*University of Pittsburgh*

Background: In an ever-changing sensory environment, cortical circuits continuously adapt their activity to changes in the overall levels of acoustic input. One such change is a sudden deprivation of peripheral input, which triggers a central compensatory process that often overshoots the mark, resulting in chronic neural hyperactivity and perceptual hypersensitivity. Many lines of research have converged on the idea that excess central gain and perceptual hypersensitivity arise from the combination of increased excitability and decreased inhibition that culminates in a state of persistent neural hyperactivity, hyper-synchrony, and hyper-responsivity at the level of the auditory cortex (ACtx). To date, there have no efforts to characterize ACtx dynamics before and after sudden loss of peripheral input at a cellular scale, across the complete tonotopic map, and at single-day resolution. Our aim is

to leverage single-cell, chronic imaging approaches and operant mouse behavior to inform both the manifestations of compensatory plasticity and the link between cortical hyperactivity and perceptual hypersensitivity._x0000_

Methods: Here, we induced sensorineural hearing loss in mice by exposure to an intense, high frequency noise band (n=16 noise, n=13 sham). We developed chronic two-photon calcium imaging of excitatory pyramidal neurons in ACtx (n = 11,201 cells) to track population and single-cell responses over weeks across the tonotopic map. To demonstrate cortical involvement in perceptual hypersensitivity, we developed new Go/NoGo (GNG) acoustic and optogenetic operant behavioral tasks._x0000_

Results: In acoustic GNG tasks, mice showed steepened detection functions for low frequency tones after acoustic trauma, suggesting a hypersensitivity to sound. In optogenetic GNG tasks, mice detected laser activation of thalamocortical inputs and similarly showed steepened detection functions, lending support to a cortical origin of perceptual hypersensitivity. We then found location-specific and stimulus-specific increases in neural gain, spontaneous activity, and correlated activity following acoustic trauma at the single cell level that match the time course of perceptual hypersensitivity. Using a model trained on neural activity to decode stimulus information, the ability of the model to detect sound presence steeply improved at supra-threshold intensities after acoustic trauma, thereby mirroring behavioral changes in the GNG task. _x0000_

Conclusions: Cortical hyperactivity and accompanying hypersensitivity are a cornerstone of many neurological disorders triggered by peripheral deprivation or injury. Through our approaches, we are able to characterize dynamic, single-cell changes and account for a large amount of heterogeneity across a wide population of neurons following sudden sensorineural hearing loss caused by acoustic trauma. We have found specific subsets of cells that show hyper-responsiveness following acoustic trauma that mirror the stimulus-specific manifestation of hypersensitivity to sound in mice. Identifying the neural signatures of hyperactivity following acoustic trauma may prove broadly useful for understanding the mechanisms underlying many forms of inherited and acquired neural hyperactivity disorders._x0000_

4. WITHDRAWN

5. A Spiking Neural Network for Masking Release: From Auditory Nerve Fibers to Cochlear Nucleus
Category: Other

Hyojin Kim*[1], Bastian Epp[1]
[1]*Hearing Systems Section, Health Tech, Technical University of Denmark*

Background: Computational auditory models can be helpful to develop hearing diagnostic methods. These models link sensory loss in the cochlea to degraded sound perception. However, while these models well incorporate the function of the periphery up to auditory nerve fibers (ANFs) with high physiological accuracy, the remainder of auditory processing is approximated by simple transformations like band-pass filters. As a result, these models are limited in reflecting the specific role of the auditory brain stem that can extract context-dependent sound features and often fail to explain impaired context-dependent sound perception, such as speech segregation from noise.

One such feature is coherent amplitude modulation across frequency (comodulation). Sound consists of multiple frequency components that overlap in time. The auditory system can use comodulation to segregate overlapping frequency components into separate sound streams. Enhancement in detection performance by comodulation is called comodulation masking release (CMR). Physiological studies showed that CMR is reflected as an enhanced neural response to the target signal in comodulated noise compared to noise with uncorrelated envelope fluctuations. However, the neural mechanism of CMR is not clear as these findings are from individual neurons, which do not show how they function as neuronal circuits. Moreover, the neural origin of the temporal effect on CMR in various time-scale is unknown.

Methods: Here, we propose a spiking neural network (SNN) consisting of various CN neural circuit types. We implemented the SNN with Brian2. The SNN takes the output from the recent phenomenological model of ANFs as an input. We implemented the following neuronal circuit types: feedforward excitation, feedforward inhibition, feedback inhibition, lateral inhibition, and mutual excitation. We designed the structure of the SNN based on physiological findings in animal models and validated the SNN with the same stimuli used in physiological studies. We explored the functional role of each circuit type in the auditory processing of comodulation. We investigated how the output of SNN changes as parameters vary, such as the convergence and strength of excitatory and inhibitory connections in neuronal circuits.

Results: As a result, we show that the previously suggested wide-inhibition mechanism could partially describe simple CMR (e.g., the tone in comodulated masker), indicating that other functional neural types may be needed

to account for context-dependent CMR phenomena (e.g., release from forward masking). We are currently investigating the role of feedback inhibition and mutual excitation in such context-dependent CMR.
Conclusions: Our SNN method for auditory modeling can provide insights into the neural mechanisms of CMR. With further development, our future goal is to use the impaired cochlea model as an input and investigate how such peripheral damage affects CMR processing on a neural basis.

6. Ebselen Mediated Improvements in Hearing Loss and Speech Perception in Meniere's Disease
Category: Speech Perception

E Emily Harruff[1], Shaun Nguyen[2], R Paul Lambert[2], Jonathan Kil*[1]
[1]*Sound Pharmaceuticals, Inc.,* [2]*MUSC*

Background: SPI-1005 is an investigational new drug that contains ebselen (novel anti-inflammatory) and was shown to prevent acute noise-induced hearing loss in a Phase2 randomized controlled trial (RCT) (Kil et al., Lancet, 2017) and to treat newly diagnosed Meniere's Disease (MD) in a Phase1b RCT (Kil et al., ARO, 2018) and active MD in a Phase2b RCT (Kil et al., ARO, 2020) where fluctuating hearing loss, tinnitus, and vertigo were documented and/or patient-reported. Ears were analyzed independently, averaged at baseline, at study day 28 and 56, and compared across treatment groups. Improvements in low-frequency hearing loss, speech discrimination using the Words-in-Noise (WIN) test, and tinnitus loudness were clinically relevant and/or statistically significant in some ebselen groups (200, 400 or 600 mg BID) vs placebo. Previous analyses did not specify affected ears (based on low frequency hearing loss) or correlate hearing loss to speech discrimination in the affected ears over time.
Methods: Randomized, double-blind, prospective, placebo-controlled, multicenter Phase1b/2b (N= 39/N=126) studies in adults (18-75) with Meniere's disease. Phase1b patients were randomized to SPI-1005 (200, 400 or 600 mg BID) or placebo (1:1:1:1 ratio). Phase2b patients were randomized to SPI-1005 (200 or 400 mg BID) or placebo (1:1:1 ratio). Outcome variables were specific hearing thresholds (250-8000 Hz), and WIN scores (0-35) in the affected ears over time (Baseline, Day 28 and 56). ANOVA followed by post-hoc analysis was used to compare between groups over time.
Results: In the Phase 2b MD-affected ears (with baseline hearing threshold $\geq$30 dB at 250, 500, or 1000 Hz), the 400 mg BID group (N=57) showed both clinically relevant and statistically significant improvements in low frequency hearing thresholds (250, 500, and 1000 Hz) of 4.3 ± 10.34 dB (p-value = 0.003) and WIN scores of 2.9 ± 4.68 words (p-value < 0.001) at Day 56 from baseline. Conversely, the placebo group (N=57) showed non-significant improvements of 1.6 ± 8.03 dB (p-value = 0.393) and 0.8 ± 4.94 words (p-value = 0.203), respectively. The differences in WIN score improvements were significant (p-value = 0.024) between the 400 mg BID group and placebo, while the difference in low frequency hearing threshold improvements were non-significant (p-value = 0.126).
Conclusions: Four weeks of SPI-1005 treatment resulted in specific improvements in low frequency hearing that correlated with clinically relevant and statistically significant improvements in speech discrimination over an 8-week follow-up. The 400 mg BID group showed the most durable auditory improvements at Day 56 (end of study) when compared to placebo. Improvements in the affected ears appeared to be the most clinically relevant and statistically significant. These additional findings have influenced the study design and statistical analysis plan of the pivotal Phase3 RCT in active Meniere's disease.

7. Cross-Modal Effects at Sensory Borders in Congenitally Deaf Mice: Combined EEG and Intra-Cortical Recordings
Category: Hearing Loss: Consequences and Adaptation

Rüdiger Land*[1], Sarah Sentis[2], Andrej Kral[1]
[1]*Department of Experimental Otology, Hannover Medical School,* [2]*Center of Brain, Behavior and Metabolism, University of Lübeck*

Background: Congenital deafness affects the development of the brain. Absent auditory input during development leads to intra-modal and cross-modal changes in the auditory system, likely affecting the border regions between auditory cortex and the remaining functional sensory areas. Importantly, such changes may have detrimental effects on subsequent cochlear implant stimulation in deafness.
Methods: Here we studied the sensory border between auditory and somatosensory cortex of congenitally deaf Otof(deaf5jcs/Kjn) mice and hearing wild types (n=14/14). We tested, how congenital deafness influences sensory border delineation and whether this can be related to non-invasive EEG markers. For this, we combined 30-

channel epicranial EEG with simultaneous intra-cortical 32-channel multi-electrode array recordings, spanning the border between somatosensory and auditory cortex. We then measured responses to whisker stimulation in deaf and hearing mice and responses to acoustic acoustic stimulation in hearing mice.

Results: In hearing mice, the functional intra-cortical border between auditory and visual cortex was marked by a clear change in multiunit responsiveness to whisker vs. acoustic stimulation. In deaf mice, auditory multiunit responses were obviously absent, and somatosensory responses were slightly shifted postero-laterally towards the auditory cortex. Interestingly however, we did not observe responses to whisker stimulation within the core (primary) auditory fields of deaf mice. In the simultaneous recorded EEG, the topology of somatosensory evoked EEG responses was more spread out and responses were larger in congenital deaf mice in comparison to hearing mice. However, general characteristics were surprisingly similar between hearing and deaf mice.

Conclusions: This indicates, that after sensory deprivation in congenital deaf mice, cross-modal changes occur around the border between auditory and somatosensory areas. Further, although some technical challenges remain, it demonstrates that combined EEG and intra-cortical recordings is a valuable approach to bridge between different measurement levels studying cross-modal or intra-modal effects in deafness, to be paired in future steps with cochlear implant stimulation.

8. The Hair Bundle and Ribbon Synapse Integrity of Cochlear Hair Cells is Vulnerable to ER Stress
Category: Hearing Loss: Consequences and Adaptation

Kuu Ikaheimo*[1], Tuuli Lankinen[1], Antti Aarnisalo[1], Maria Lindahl[1], Mart Saarma[1], Ulla Pirvola[1]
[1]*University of Helsinki*

Background: Previous work from our lab has revealed that the endoplasmic reticulum (ER)-resident, chaperone-like protein Manf (Mesencephalic astrocyte-derived neurotrophic factor) promotes hair cell survival and proper hearing function. We found that the effect of Manf was strongly dependent on the mouse genetic background (Herranen et al., Cell Death Dis, 2020). Manf is known to modulate the cellular response to perturbations in ER proteostasis and thereby Manf is considered as a pro-survival factor in ER-stressed cells. Our previous work did not address if hair cell structure and function are also affected by ER stress. In the current study, we demonstrate that Manf inactivation triggers early-onset, progressive hearing deficit coupled with structural defects in the key functional compartments of hair cells.

Methods: We studied conditional Manf knock out (cKO) mice under the C57BL/6J (B6) genetic background, from juvenile to adult stages. We measured ABR and DPOAE thresholds and studied the hair bundle and ribbon synapse morphology by light and electron microscopy. We focused on the stereocilia taper region proteins PTPRQ and Radixin, on F-actin in the cuticular plate, and on neuroplastin and PMCA2 in stereocilia. In the ribbon synapse, we focused on the presynaptic ribbon protein CtBP2, the postsynaptic scaffolding protein Homer 1 and the Ca2+ sensor otoferlin. We analysed structural and molecular changes in the ER-stressed hair cells undergoing progressive hearing loss due to Manf inactivation. In addition, we analysed audiological data from a human patient with loss-of-function variant of MANF.

Results: Loss-of-function MANF mutations triggered early-onset and severe sensorineural hearing loss in mice and human, as measured by functional audiometry. Manf cKO B6 mice displayed disorganized outer hair cell (OHC) hair bundles. A mild disarray pattern was found at the onset of hearing and it progressed to extensive fusion of stereocilia at adulthood. This was accompanied by molecular changes in the stereocilia taper region and the cuticular plate. Inner hair cell (IHC) stereocilia were unaffected, but these cells displayed robust synaptopathy at young adulthood. Both the hair bundle and synaptic degradation progressed in severity along the high-to-low-frequency axis of the cochlea.

Conclusions: Analysis of the Manf cKO B6 mouse model allowed us to conclude that the structural integrity of the OHC hair bundle and IHC ribbon synapse are strongly dependent on the maintenance of the ER proteostasis network. The background-related genetic causes may predispose hair cells of B6 mice to ER stress. Therefore, a functional ER proteostasis network is critical for these cells, reflected as major problems when a component of this network, Manf, is depleted. It appears that the high-frequency hair cells are the most vulnerable hair cells to ER stress. In all, we present MANF as novel gene whose mutations cause severe sensorineural hearing loss.

12:00 p.m. - 2:00 p.m.
Podium Session #28 – Non-Auditory Factors in Auditory Processing
Moderators: Yi Zhou, Ph.D. & Brian Monson, Ph.D.

1. Loss of Synchronization Between Supragranular and Infragranular Cortical Layers in Congenital Deafness
Category: Primary Auditory Cortex

Prasandhya A. Yusuf[1], Aly Lamuri[1], Peter Hubka[2], Jochen Tillein[3], Martin Vinck[4], Andrej Kral*[2]
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Background: Previous studies have documented a reduction of interareal couplings between primary and secondary auditory areas in congenital deafness, particularly pronounced in top-down direction (Yusuf et al., 2017, Brain; Yusuf et al., 2021, Front Neurosci). Furthermore, an anatomical study documented a dissociation in the effect of deafness between supragranular and infragranular layers (Berger et al., 2017, J Comp Neurol), suggesting that their interaction is substantially modified in deafness. In the present study we directly investigated spike-field coherence between supragranular and infrangranular layers of field A1 of hearing cats under acoustic stimulation and cochlear implant (CI) stimulation. The data between hearing cats and congenitally deaf cats (CDCs) were compared.

Methods: The stimulation was using a train of three condensation clicks (50 µs) or three biphasic charge-balanced pulses (200 µs/phase) applied through a CI in wide bipolar configuration to the contralateral ear. A1 activity was recorded in the most responsive spot (defined by a functional mapping as in Kral et al., 2009, J Neurosci). Neuronal activity was recorded with Neuronexus 16-channel probes. Spike-field coherence was analyzed using pairwise phase consistency (PPC; Vinck et al., 2012 J Comput Neurosci). Both the resulting magnitude as well as the preferred phase of synchronization was analyzed.

Results: Spike-field coherence was significantly reduced in CDCs than in controls in the alpha and beta bands. In CDCs there was a large difference in the preferred phase between supragranular and infragranular layers that was not found in hearing animals.

Conclusions: These results suggest a loss of synchrony and thus a decoupling of these layers in congenital deafness. Together with the anatomy of their connections this observation explains why the effects of deafness differ between supragranular and infragranular layers.

2. Effect of Noise Exposure Designed to Cause Synaptopathy on Putative Efferent Function in Nonhuman Primates
Category: Hearing Loss: Consequences and Adaptation

Jane Burton*[1], Catherine Alek[2], Leslie Liberman[3], Charles Liberman[3], Troy Hackett[2], Ramnarayan Ramachandran[2]
[1]*Vanderbilt University,* [2]*Vanderbilt University Medical Center,* [3]*Mass. Eye and Ear*

Background: Sensory systems contain dynamic feedforward and feedback pathways that support complex signal processing mechanisms. Damage to sensory organs alters neuronal encoding in these pathways, affecting physiological responses and perceptual abilities. In the auditory system, exposure to high-level noise can result in hearing loss with a wide range of associated physiological changes and perceptual deficits, especially in background noise. These clinical presentations have been attributed to different sites of cochlear and retrocochlear pathology, including the olivocochlear efferent system. Here, we describe physiological and perceptual assays that may probe the medial olivocochlear reflex (MOCR) in macaque monkeys with normal hearing and following noise exposure designed to cause cochlear synaptopathy.

Methods: Rhesus macaques (Macaca mulatta, n=8; 4 male) performed a reaction-time Go/No-Go lever release task to detect short duration pure tones (0.5-32kHz, 12.5 ms) in a 200 ms gated broadband noise under diotic, open field testing conditions. Tone thresholds were obtained for different signal-to-masker onset asynchronies (0, -50, -100, and -150 ms) in order to estimate overshoot (threshold difference between 0 and non-zero onset asynchrony conditions), which is thought to arise in part from MOCR mechanisms. While macaques were awake and head-fixed, transient-evoked otoacoustic emissions (TEOAEs) were measured alone and with a contralateral broadband noise (40, 50, or 60 dB spectrum level) to assess TEOAE suppression, which is mediated in part by the MOCR. Data were obtained before and after bilateral exposure to a 120 dB SPL band of noise (2-4kHz) for four hours. Following noise exposure, TEOAE suppression was measured monthly and overshoot was probed bimonthly to assess changes over time.

Results: Noise exposure caused temporary threshold shifts that recovered in 1-3 weeks. Overshoot was greatest (6.8-9.0 dB) for the -100 ms signal-to-masker onset asynchrony and for mid-frequency tones (4-8kHz). Following noise exposure, overshoot was unchanged for 2-3 months, then decreased for frequencies near the exposure band (5.6-8kHz) by 10-11 months post-exposure. Contralateral TEOAE suppression ranged from 1.7-5.7 dB, and was greatest for higher noise levels and low frequency TEOAE components. Compared to young normal hearing humans, nonhuman primates showed greater TEOAE suppression across all frequencies and noise levels. TEOAE suppression was unchanged up to 3 months post-exposure, and ongoing longer-term measurements are in progress to track expected changes. Cochlear histology suggests that noise exposure caused significant ribbon synaptopathy in some animals. Efferent innervation density is also being quantified.

Conclusions: Following noise exposure, putative efferent-mediated measures remain stable early, and at least some show decline at later post-exposure time points. These data suggest delayed degeneration of olivocochlear efferent pathways secondary to noise-induced damage, implicating an additional mechanistic contribution to difficulties understanding speech in background noise. [Supported by R01DC015988 (MPI: R. Ramachandran and B. Shinn-Cunningham) and F32DC019817 (PI: J. Burton)].

3. Dual Vector Gene Therapy Rescues Hearing in a Stereocilin Deficient Mouse Model
Category: Other

Madeline Barnes[1], Quyn-Anh Fucci[1], Sarah Cancelarich[2], Tian Yang[1], Vickie Nguyen[1], Danielle Velez[2], Tyler Gibson[1], Leah Sabin[2], Meghan Drummond[2], Ning Pan[1], Lars Becker*[1]
[1]*Decibel Therapeutics,* [2]*Regeneron Pharmaceuticals, Inc.*

Background: In humans Stereocilin (STRC) deficiency leads to autosomal recessive deafness (DFNB16) with patients showing mild to moderate hearing loss. DFNB16 has been estimated to be the second most prevalent genetic auditory deficiency in the US and EU5.

The cochlea amplifier relies on a functional connection between the tallest row of outer hair cell stereocilia and the overlying tectorial membrane. Several proteins have been identified in forming an attachment complex between the stereocilia and the tectorial membrane, with Stereocilin being an essential component. Stereocilin is a 193 kDa large GPI anchored protein with several ARM-like repeats facilitating binding to extracellular glycoproteins. Stereocilin previously has been shown to form lateral side connectors in outer hair cell stereocilia and attachment crowns in the tallest row, facilitating attachment to the tectorial membrane.

Methods: To recapitulate the human phenotype and study the feasibility of AAV gene therapy for DFNB16, we generated two Stereocilin knockout mouse models utilizing CRISPR/Cas9 technology.

To re-express full-length Stereocilin in the knockout mice we utilized dual vector technology.

To achieve precise targeting of transgenes, we identified potential regulatory elements that were selective for outer hair cells and validated them in organ of Corti explants and in vivo mouse studies.

Mice were injected via the round window membrane or through the posterior semicircular canal.

We characterized pathophysiology and efficacy after treatment by ABR, DPOAE, SEM, and immunohistochemistry.

Results: The animal models show highly elevated ABR thresholds and DPOAEs are absent. Inner and outer hair cells are maintained over time, outer hair cell bundles show the characteristic W shape, but the stereocilia are splayed compared to wildtype animals. Attachment crowns, lateral side connectors and tectorial membrane imprints are absent in the knockout models.

Stereocilin was re-expressed in outer hair cell stereocilia and Stereocilin could be detected on the tectorial membranes of treated ears.

Testing of potential outer hair specific regulatory elements revealed defined sequences specific to restrict expression to just outer hair cells in both organ of Corti explants and adult mice in vivo.

Conclusions: Our results indicate that dual vector AAV gene replacement therapy re-expressing full length Stereocilin can lead to meaningful auditory threshold improvements by allowing the attachment of the tectorial membrane to the outer hair cell.

4. Orbitofrontal Cortex Activity Modulates Auditory Cortex Responses and Sound Detection and Correlates With Perceptual Sensitivity
Category: Primary Auditory Cortex

Matheus Macedo-Lima*[1], Lashaka Jones[1], Rose Ying[1], Melissa Caras[1]
[1]*University of Maryland – College Park*

Background: It is commonly accepted that, when it comes to learning to dance or ride a bike, "practice makes perfect." However, our senses also benefit from training, a process termed perceptual learning. During auditory perceptual learning, practice is thought to strengthen the connection between a top-down brain network and auditory cortex, thereby enhancing cortical responses to sound, and improving perceptual detection capabilities; however, the top-down brain regions involved in this process are unknown. One promising candidate is the orbitofrontal cortex (OFC). The OFC sends direct projections to the auditory cortex and pairing sounds with OFC stimulation improves neural discrimination.

Methods: To test OFC's involvement in auditory perception and perceptual learning, we first determined whether bilateral OFC inactivation with muscimol affected performance of Mongolian gerbils on an amplitude modulation (AM) detection task. Then, we performed extracellular recordings from chronically implanted electrode arrays into auditory cortex to assess the downstream effects of bilateral OFC muscimol infusions on AM neural responses. Finally, we recorded extracellular activity from the OFC of freely moving gerbils as they underwent auditory perceptual learning.

Results: Bilateral muscimol infusions into OFC before the task significantly impaired AM detection. Extracellular recordings from chronically implanted electrode arrays revealed that OFC inactivation abolished the top-down modulation of auditory cortical neurons during task performance. Finally, we we found that the activity of OFC neurons increased over the course of perceptual learning. Specifically, OFC firing rates significantly correlated with perceptual thresholds, but did not correlate with training day, which suggests that OFC activity is associated with learning and not task familiarity or repetition.

Conclusions: We hypothesize that the OFC provides a top-down signal to facilitate practice-dependent improvements in auditory cortical and perceptual sensitivity.

5. Behavioural and Neural Measures of Auditory Regularity Detection in Ferrets
Category: Primary Auditory Cortex

Katarina Poole*[1], Maria Chait[2], Jennifer Bizley[2]
[1]University College London, [2]UCL Ear Institute

Background: Acoustic stimuli that transition from sequences of randomly drawn tones to regularly repeating sequences have been employed to investigate the involvement of regularity within auditory scene analysis. Previous studies, in humans, have highlighted a network of brain regions that are involved in this process such as auditory cortex and hippocampus (Barascud et al., 2016). Here we seek to understand how the neurons within auditory cortex detect acoustic patterns and encode the transition from random to regular tone sequences.

Methods: We trained ferrets (n = 6) on a go/no-go task to detect the transition from a random sequence of tones to a repeating pattern of tones. We recorded from primary and secondary fields of auditory cortex using multi-electrode arrays during task performance (n = 2), and in naïve passively listening animals (n = 4).

Results: In the behavioural task, when presented with a pattern of 3 tones, all animals performed significantly above chance ($p < 0.001$, d' range across animals = 1.87 to 2.33). Performance decreased but was maintained above chance for the majority of animals across increasingly complex auditory patterns ($p < 0.05$, mean d' across animals for pattern repeat lengths 3, 5 and 7 respectively: 2.12, 1.50, and 0.87). For a pattern length of five, performance was also above chance for matched stimuli in which both the random and regular sequence were generated from the same unique frequencies ($p < 0.05$, mean d' across animals: 0.86).

To identify whether units adapted or increased their response to repeated tones in the context of a repeating pattern, the firing rates of single units were calculated for each tone and entered into a generalized linear model. Tone frequency (expressed as distance from best frequency) and the number of tone repetitions in a pattern were used as predictors. In the resulting models, a negative coefficient for the tone repetition number indicates adaptation across multiple pattern repeats, whereas units whose rate increased yields positive coefficients. Preliminary neural analysis of data from behaving animals shows predominantly positive coefficients that increase in magnitude with the length of the pattern (mean coefficients ± standard error; 0.0164±0.002, 0.0254±0.0111, 0.0277±0.0014 for pattern lengths 3, 5 and 7 respectively).

Conclusions: In summary – we show that ferrets are capable of detecting statistical regularities across increasingly complex patterns of tones. This provides us an animal model to directly probe auditory cortex and how its neurons encode this information to guide the animal's behaviour. Preliminary analysis of the neural data suggest that, during regularity, the firing rate of single-units within auditory cortex are facilitated and that this facilitation may grow with increasingly complex patterns. Ongoing analysis will relate the time course of facilitation to behavioural detection and compare behaving and naïve auditory cortical responses.

6. Medial Olivocochlear Efferent Activity During an Auditory and Visual task: Otoacoustic Emissions and Event-Related Potentials Study

Category: Otoacoustic Emissions

W. Wiktor Jedrzejczak*[1], Krzysztof Kochanek[2], Edyta Pilka[2], Malgorzata Ganc[2], Rafal Milner[2], Henryk Skarzynski[2]

[1]*Institute of Physiology and Pathology of Hearing, Warsaw, Poland,* [2]*Institute of Physiology and Pathology of Hearing*

Background: The medial olivocochlear (MOC) system is thought to be responsible for modulation of peripheral hearing through descending (efferent) pathways. This study investigated the connection between peripheral hearing function and conscious attention during two different modality tasks – auditory and visual.

Methods: Peripheral hearing function was evaluated by analyzing the amount of suppression of transiently evoked otoacoustic emissions (TEOAEs) by contralateral acoustic stimulation (CAS), a well-known effect of the MOC. Simultaneously, attention was evaluated by event-related potentials (ERPs). For extracting the main components of the TEOAE signal, a method based on the matching pursuit (MP) algorithm was used. There were 20 normally hearing adults (age 25–40 years, 14 females) who participated in the study.

Results: Although the ERPs showed clear differences in processing of auditory and visual tasks, there were no differences in the levels of TEOAE suppression. We also analyzed TEOAEs for the highest magnitude resonant mode signal detected by the MP method, but again did not find a significant effect of task, and no difference in noise level or number of rejected trials. However, for auditory tasks the amplitude of the P3 cognitive wave negatively correlated with the level of TEOAE suppression.

Conclusions: There seem to be no difference between the effect of visual and auditory attention on MOC as measured by TEOAEs. It seems that when attention is switched from one modality to another it is done by the cortex alone without the help of the periphery, in this case the ear. On the other hand, the negative correlation between suppression of TEOAEs and the P3 amplitude during the auditory task seems to suggest that the cortex compensates for lower synchronization at the MOC level.

7. Directional Hearing in the Barking Gecko (Ptenopus Garrulus)

Category: Binaural Hearing and Sound Localization

Jakob Christensen-Dalsgaard*[1], Michael Cherry[2], Grace Capshaw[3], Catherine E. Carr[3]

[1]*SDU Odense University,* [2]*Stellenbosch University, South Africa,* [3]*University of Maryland – College Park*

Background: Lizards have highly directional ears, owing to acoustical coupling of their eardrums, but behavioral implications of the acute directionality have not been studied. The lizard with the most robust calling behavior is the barking gecko Ptenopus garrulus, a small burrowing lizard from South Africa that call regularly and frequently from their burrows during the mating season, and it is therefore a promising species to study in relation to sound localization behavior. To investigate the consequences of this tympanic directionality, we measured auditory and directional sensitivity in the barking gecko, Ptenopus garrulus and here report on the preliminary results of the investigation.

Methods: We investigated barking geckos in the field in Kuruman River Reserve, South Africa using auditory brainstem responses (ABR) to sound, laser vibrometry, anatomy and behavioral observations. The ABR and laser measurements were undertaken on animals that were captured in the field and anesthetized using ketamine. For ABR, animals were placed in a sound-insulated box and the response to sound emitted from a loudspeaker was recorded using subdermal electrodes. For laser experiments, the animals were placed in the center of a boom with a loudspeaker, and directional responses to sound were recorded using a portable laser vibrometer. Also, the interaural transmission was measured using local sound stimulation and laser vibrometry. Behavioral responses were recorded on small video cameras under infrared illumination. The cameras were placed directly above the burrows of calling geckos and recording throughout the night. Finally, we investigated the anatomy using CT-scans.

Results: The barking geckos are very sensitive to sound (lowest ABR thresholds around 20 dB SPL) in a frequency range up to 10 kHz with peak sensitivity at 3 kHz. The ear is strongly directional from 2 to 4 kHz (the peak frequencies in their calls), where interaural transmission gain is close to 0 dB, showing strong interaural coupling. Nighttime video recordings of calling males revealed chorus site burrow activity. Also, we have recorded evidence of directional responses in the video recordings (head turning towards sound sources and neighboring calls), but still need to get more quantitative measurements. Morphometric measurements revealed features of the otic endocast and ear.

Conclusions: The strong directionality of the barking gecko is similar to that of other lizards, but the robust sound communication behavior allows us directly to relate the directionality to behavior and to processing of the communication calls, and we have shown that we can record behavioral responses to directional sound. This is information that has not been available previously and further studies of behavioral directional responses will be highly informative for our understanding and modelling of directional sensitivity in lizards and of the neural processing of directional sound.

8. Factors Influencing the Minimum Audible Change in Head Orientation of a Talker
Category: Speech Perception
Brian Monson*[1], Brendan Moriarty[1]
[1]*University of Illinois at Urbana-Champaign*

Background: Humans are able to detect changes in the head orientation (a.k.a. facing angle) of a talker using only auditory cues. This ability can be useful for determining when one is the intended recipient of a vocalization or warning call, particularly when visual cues are obscured or absent. Head orientation cues are beneficial for segregating a target talker from background talkers. Because human talkers are directional sound sources, binaural cues, such as interaural level differences, and monoaural spectral cues can both contribute to talker head orientation perception.
Methods: In this study, we assessed listeners' sensitivity to talker head orientation changes using only monaural cues. This was accomplished by measuring listeners' ability to detect differences between speech recordings made simultaneously at multiple angles around a talker. We tested methodological factors (bandlimited vs. full-band; headphone vs. sound field) and speech characteristics (male vs. female; speech vs. singing) that could influence the minimum audible change in head orientation.
Results: Listeners performed more poorly when stimuli were bandlimited at 8 kHz or even 10 kHz, relative to the full-band (20 kHz) condition. Sound field presentation led to better performance compared to headphone presentation. Performance with speech was better than that with singing.
Conclusions: The results suggest that monaural spectral cues, particularly at extended high frequencies, are useful for detecting talker head orientation. However, different methodological approaches can greatly affect assessment of the utility of monaural cues.

12:00 p.m. – 2:00 p.m.
Podium Session #29 – Elephant to Mouse Middle Ears
Moderators: Alessandra Carriero, Ph.D. & Melissa Castillo-Bustamante, M.D.

1. Anatomical and Mechanical Features of the Elephant's Low-Frequency Middle Ear
Category: Middle and External Ear
Caitlin O'Connell-Rodwell*[1], Jodie Berezin[2], Anbuselvan Dharmarajan[3], Michael E. Ravicz[2], Xiying Guan[4], Yihan Hu[5], Rachel Chen[6], Kevin O'Connor[3], Sunil Puria[7]
[1]*Eaton Peabody Lab, Massachusetts Eye and Ear Infirmary,* [2]*Eaton Peabody Lab, Massachusetts Eye and Ear, Harvard Medical School, Boston, MA,* [3]*Eaton-Peabody Lab, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA,* [4]*Department of Communication Sciences and Disorders, Wayne State University,* [5]*IQ-Analog Corporation, San Diego, CA,* [6]*Eaton Peabody Lab, Massachusetts Eye and Ear, Harvard Medical School, Boston, MA,* [7]*Harvard Medical School, Mass. Eye and Ear Infirmary*

Background: The elephant ear is much bigger than the human ear and elephants can hear lower-frequency sounds with greater sensitivity than humans. This increased sensitivity is believed to facilitate the elephant's ability to communicate over long distances. We hypothesize that this greater lower-frequency sound sensitivity could be explained by differences in middle-ear anatomy and function. To date, very little is known about elephant middle-ear function, particularly at low frequencies.
Methods: We measured the malleus, incus and stapes velocities in response to ear canal sound stimuli in thawed, unfixed cadaveric temporal bones of African (N=1) and Asian (N=2) elephants, as well as humans (N=3). Retroreflective tape mounted with glass beads was placed at multiple locations on the ossicles, and velocity was measured using a 3D laser doppler vibrometer (3D LDV) in the 6 Hz to 12,000 Hz frequency range. A probe-tube microphone measured the ear-canal sound pressure to normalize the measured velocities. The orientations of the tympanic ring and stapes footplate were measured relative to the 3D LDV axes to transform the measured 3D

velocities of the ossicles to umbo velocity perpendicular to the plane of the tympanic ring (to represent middle-ear input) and stapes velocity in the piston direction (to represent middle-ear output). We computed the lever ratio from these representative stapes and umbo velocities.

Results: The elephant stapes velocity in all three specimens was at least an order of magnitude greater than that of humans below 1000 Hz, the frequency range in which elephant hearing sensitivity is greater than human. Above 1 kHz, elephant and human stapes velocities are similar.

Conclusions: The elephant eardrum is the largest among terrestrial mammals, approximately seven times the area of the human eardrum. This greater area alone could provide increased sound collection at the umbo. A higher lever ratio in elephants could further contribute to the difference in stapes velocity between elephant and human middle ears. The remaining difference below 1 kHz could be due to differences in ME stiffness. The similarity in stapes velocities above 1 kHz fits the similarity in hearing thresholds but is surprising given that the ossicular mass of elephants is nearly ten times that of humans. These comprehensive ossicular-motion measurements in a low-frequency-hearing species provide additional insight into the role of the middle ear in hearing.

2. WITHDRAWN

3. Using Machine Learning to Determine the Probability Distribution of Middle-Ear and Cochlear Model Parameters

Category: Inner Ear: Cochlear Mechanics

Hamid Motallebzadeh*[1], Michael Deistler[2], Florian M. Schönleitner[3], Jakob H. Macke[2], Sunil Puria[4]
[1]*Harvard Medical School*, [2]*Machine Learning in Science, Excellence Cluster Machine Learning, University of Tübingen, Germany*, [3]*Elite Master's Program in Neuroengineering, Department of Electrical and Computer Engineering, Technical University of Munich*, [4]*Harvard Medical School, Mass. Eye and Ear Infirmary*

Background: Computational models are powerful tools used to interpret and predict system behavior. Finite-element (FE) models are built using accurate anatomical representation obtained for example from micro-CT imaging. One of the key challenges in developing FE models is to assign parameter values to the different model components and then 'tuning' them to obtain a reliable 'baseline' model. Such tuning to obtain 'general agreement' with characteristic features of averaged empirical data has generally been a subjective endeavor. Machine Learning is a powerful approach for objective parameter identification of FE modes.

Methods: We implemented simulation-based inference (SBI) by neuronal density estimators (Gonçalves et al., 2020) to identify the parameters of FE models of the human middle-ear (ME) and a gerbil cross-sectional cochlea model. SBI has three essential ingredients: (1) a mechanistic model (e.g., FE model), (2) prior knowledge of plausible or constrained parameter values (material and geometric parameters), and (3) a reference objective which is the experimental data. The NN learns the association between model parameters and simulation outcomes so that it can infer a posterior probability distribution of parameter values when presented with experimental data. For the ME model we performed 10,000 simulations with 7 material parameters (e.g., eardrum, ligaments, joints stiffness and damping and cochlear load) randomly varying within plausible ranges, reported in the literature and trained the inference NN for WBT outputs (impedance and absorbance) and stapes velocity. A similar approach was used to tune the gerbil cochlea slice model with 4,000 simulations of randomly varying 10 parameters of the material parameters with available basilar-membrane radial motion (Cooper and Heijden 2017) and organ of Corti motion (Dong et al. 2018).

Results: A key output of SBI is a full posterior probability distribution for each selected model parameter. The posterior captures all model configurations that can reproduce experimental data using plausible parameters. This approach differs from previous methods where only a single parameter set is typically reported for a given model. To evaluate the SBI performance, new simulations with sampled values from the posterior were simulated and the outcome was compared with experimental data. SBI is able to to reveal interactions and dependencies between model parameters and metrics for parameter sensitivity.

Conclusions: SBI is a powerful tool for objective parameter identification of mechanistic models and can be easily adopted to any computational models such as FE. It can replace current subjectively tuning practices based on a limited sensitivity analysis. The outcome of the SBI model is robust and we are performing sensitivity analysis of the SBI parameters such as its architecture, density estimator, summary statistics, and number of input simulations.

4. Mechanical Effects of Material and Geometrical Properties of Medical Devices Attached to Tympanic Membrane

Category: Middle and External Ear
Arash Ebrahimian*[1], Nima Maftoon[1]
[1]*University of Waterloo*

Background: There are several treatment methods that rely on attaching a medical device to the tympanic membrane. Examples of these treatment methods include but are not limited to the tympanostomy tube insertion and contact-based device attachment. In addition to these existing methods, new treatment procedures may be developed that rely on attaching medical devices to the tympanic membrane for drug delivery to the cochlea or vestibule. In this work, we studied the mechanical effects of attaching medical devices to the tympanic membrane on sound conduction in the middle ear.

Methods: We studied the effects of material and geometrical properties of medical devices attached to the tympanic membrane on middle-ear vibrations. We developed a finite-element model of the human middle ear and validated our model against existing middle-ear measurements in the literature. We then attached a cylindrical medical device to the anterior inferior quadrant of the tympanic membrane. We simulated the middle-ear vibration responses by considering three different materials for the device: silicone rubber, Teflon, and stainless steel. We compared the results of each simulation with the results of the baseline model (the middle-ear model without any medical devices). Additionally, in order to study the effects of variations of the geometrical properties of the device, we varied the diameter and height of the device and studied their impacts on the umbo and stapes footplate vibrations.

Results: We quantified the effects of combination of changes in materials (silicone rubber, Teflon, and stainless steel) and geometries on the umbo and stapes footplate response at different frequencies. Our simulations showed that varying the material properties while keeping the geometry constant can significantly change these outputs at some frequencies. Additionally, we also observed that the variations of the diameter and height of the device can have significant effects on the middle-ear vibration outputs (umbo and stapes footplate responses).

Conclusions: Based on our results, the variations of both material and geometrical properties of a medical device that is attached to the tympanic membrane can result in considerable variations in the middle-ear vibration outputs and the sound conduction to the inner ear. Our study suggests that these parameters should be carefully chosen while designing medical devices to be attached to the tympanic membrane. This study enables optimizing existing and new treatment methods to have the least adverse effects on hearing. Further studies on the other aspects of the medical device attachment to the tympanic membrane are in progress.

5. Middle Ear Ossicles Mineralization in the Osteogenesis Imperfecta Murine Model

Category: Middle and External Ear
Faisal Patel[1], Irene Liu[1], Maialen Ugarteburu[2], Asier Muñoz[2], Lu Xi Li[3], Evan R. Maxey[3], Olga Antipova[3], Stuart R. Stock[4], Xiaodong Tan[5], Alessandra Carriero[2], Claus-Peter Richter*[5]
[1]*Illinois Mathematics and Science Academy,* [2]*The City College of New York,* [3]*Advanced Photon Source, Argonne National Laboratory,* [4]*Department of Cell and Developmental Biology, Northwestern University,* [5]*Northwestern University Medical School*

Background: Osteogenesis Imperfecta (OI) is a group of inherited genetic disorders characterized by collagen defects that affect in the United States roughly 25,000 people annually. People with OI have a greater risk of bone fractures, impaired bone growth, and other body impairments such as hearing loss. The hearing loss can be conductive, sensorineural or mixed and typically worsens with age. To date, there is no treatment to cure or ameliorate hearing loss in OI. To identify potential mechanisms for the hearing loss, this study determines differences in concentrations of biologically essential elements in the middle ear ossicles of a mouse model for OI the osteogenesis imperfecta murine (oim/oim) model of OI, and their wild type (WT) mice using X-ray fluorescence microscopy (XFM). Elements included calcium, phosphorus, sulfur, zinc, copper, and iron.

Methods: The middle ear ossicles (malleus, incus, and stapes) originated from 14 weeks old adult mice. After euthanizing the animals, we harvested both temporal bones containing the middle ears and cochleae. After the bullae were removed, they were instantly frozen in liquid nitrogen and subsequently transferred into a -80 degrees Celsius freezer. For imaging, we mounted the three middle ear ossicles on Ultralene® XRF Pre-Cut Window Film, 4 µm. At beamline 8-BM at the Advanced Photon Source (APS), the samples were scanned at 10 keV photon energy. The samples were scanned initially at a step size of 20 µm for a low-resolution overview. From those initial scans, we selected regions of interest to be scanned at 25 µm spatial resolution. With MAPS, software available through Argonne National Laboratories, the XFM scans were analyzed, and the concentration for each element of interest was determined.

Results: The results of our study show that the oim/oim mouse had higher iron and calcium and lower phosphorus concentrations than WT mice. The other elements investigated were similar in concentration in the oim/oim and wild-type mice.

Conclusions: The increased calcium and iron content in bone may contribute to hearing loss in people with OI by increasing the bone tissue stiffness and therefore affecting the biomechanics of the hearing. Treatment of the hearing loss in OI would need to reduce their bone porosity and increase flexibility but also aim to decrease the bone tissue mineralization by regulating the calcium uptake.

6. Smaller and Deformed Ossicles in Oim/Oim Mouse Model of Osteogenesis Imperfecta
Category: Middle and External Ear

Maialen Ugarteburu*[1], Annalisa De Paolis[1], Anxhela Docaj[1], Michael Doube[2], Christoph Rau[3], Luis Cardoso[1], Claus-Peter Richter[4], Alessandra Carriero[1]
[1]*The City College of New York,* [2]*City University of Hong Kong,* [3]*Diamond Light Source,* [4]*Northwestern University*

Background: Progressive hearing loss affects 70% of people with Osteogenesis Imperfecta (OI), a genetic disorder generating mutations in collagen type I of connective tissues. Hearing loss in OI has early onset and is either conductive, sensorineural or mixed. There is no cure for OI, and treatments for hearing loss rely on therapies for the general population, with scarce success in OI ears. To date, the mechanisms of hearing loss in OI are still unknown. This study examines the morphometric analysis of middle ear ossicles in the oim/oim mouse model of OI, suffering from hearing loss.

Methods: The ears of 14-week-old oim (n = 6, 3 females, and 3 males) and wild type (WT) (n = 6, 2 females, and 4 males) mice were imaged using synchrotron microtomography. Volumetric-morphometric analysis was conducted for the auditory ossicles. For each ossicle, we determined volume, thickness, and intracortical canal porosity. Additional parameters calculated for the malleus included head volume (MhV), body volume (MbV), lamina's enclosed area, and length of the manubrium (Mm). For the incus, we measured functional length (IfL), angle of the facet for the malleus, and area of the disk. For the stapes, we assessed total height (Sh), footplate height (SfH), crura and head height (SchH), footplate's 3D surface area (SfA), and smallest and biggest head-heights. The lever arm ratio and an estimation of the area convergence ratio were also calculated. Finally, the presence of fractures in the ossicles was quantified.

Results: The oim mice ossicles are significantly ($p<0.05$) smaller (total volume of the ossicles WT = 0.185 ± 0.005mm^3, oim = 0.168 ± 0.012mm^3) than WT counterparts, with a smaller malleus (malleus volume WT = 0.13 ± 0.003mm^3, oim = 0.11 ± 0.009mm^3) with a smaller head (MhV WT = 0.018 ± 0.0009mm^3, oim = 0.015 ± 0.0009mm^3) and body volume (MbV WT = 0.11 ± 0.003mm^3, oim = 0.098 ± 0.008mm^3), smaller footplate-height (SfH WT = 0.148 ± 0.004mm, oim = 0.133 ± 0.007mm) and 3D surface area (SfA WT = 0.176 ± 0.011mm^2, oim = 0.163 ± 0.005mm^2), and larger SchH:SfH ratio (WT = 3.34 ± 0.08, oim = 3.72 ± 0.27). Besides, half of the oim incudes were fractured at the facet for the malleus. WT ossicles had no fractures.

Conclusions: Our results report for the first time on a volumetric-morphometric analysis of the ossicles in OI and indicate a series of morphological differences between the auditory ossicles of WT and oim mice that may affect the biomechanics of the middle ear in oim mice, further contributing to their hearing loss. This knowledge will aid in the understanding of hearing loss in OI and clinical care of the same.

7. Endoscopic Versus Microscopic Stapes Surgery: An Anatomical Feasibility Study
Category: Middle and External Ear

Esther Blijleven*[1], Koen Willemsen[2], Ronald L.A.W. Bleys[3], Robert J. Stokroos[4], Inge Wegner[5], Hans G.X.M. Thomeer[6]
[1]*University Medical Center Utrecht,* [2]*3D Lab, University Medical Center Utrecht,* [3]*Department of Anatomy, University Medical Center Utrecht, Utrecht, the Netherlands,* [4]*Department of Otorhinolaryngology – Head and Neck Surgery, University Medical Center Utrecht,* [5]*Department of Otorhinolaryngology – Head and Neck Surgery, University Medical Center Groningen,* [6]*Department of Otorhinolaryngology – Head and Neck Surgery University Medical Center Utrecht*

Background: This study aims to investigate the feasibility of the endoscopic approach versus microscopic approach during stapes surgery, focusing on the visualization of important anatomical structures of the middle ear, the volume of the resected scutum and chorda tympani nerve (CTN) injury.

Methods: An anatomical feasibility study was performed in a tertiary referral center. Fresh frozen human cadaveric heads, which showed no signs of trauma, previous middle ear surgery or anatomical abnormalities of the

middle ear, underwent two stapes surgeries using a surgical microscope on one ear and an endoscope on the other ear. Postoperatively, the surgeon documented the visualization of the middle ear landmarks, such as the stapes, and the exposure and injury of the CTN. The amount of resected scutum was evaluated using cone beam computed tomography scanning and three-dimensional imaging.

Results: In total, 21 fresh frozen ears were included. The endoscope with an angle of 30 degrees provided good visualization of the middle ear landmarks and the placement of a piston was performed in all ears. The microscope provided less visualization of the middle ear landmarks compared to the endoscope. The volume of resected bony scutum differed significantly between the endoscopic group (median = 2.20 mm3, IQR = 4.17) and the microscopic group (median 13.25 mm3, IQR = 8.71). In two endoscopic ears, no scutum was removed. Scutum removal was performed in all microscopic ears. There was no statistically significant difference in CTN trauma between the endoscopic and the microscopic approach.

Conclusions: This study showed that the endoscopic stapes surgery procedure is feasible and might potentially be less invasive compared to the microscopic procedure. Future prospective and functional studies will be needed to support our findings.

8. Microbiota Associated With Cholesteatoma Tissue in Chronic Suppurative Otitis Media
Category: Middle and External Ear

Regie Lyn Santos-Cortez*[1], Daniel Frank[1], Jose Pedrito Magno[2], Karen Joyce Velasco[2], Jacob Ephraim Salud[2], Kevin Jer David[2], Eljohn Yee[2], Heather Dulnuan[2], Jan Alexeis Lacuata[2], Jeric Arbizo[2], Beatrice Guce[2], Kevin Michael Mendoza[2], Gabriel Martin Ilustre[2], Alessandra Nadine Chiong[2], Charles Robertson[1], Erik Tongol[2], Nicole Sacayan[2], Shi-Long Lu[1], Talitha Karisse Yarza[3], Charlotte Chiong[4]
[1]*University of Colorado Anschutz Medical Campus,* [2]*Philippine General Hospital,* [3]*Philippine National Ear Institute,* [4]*University of the Philippines College of Medicine*

Background: Otitis media (OM), defined as infection or inflammation of the middle ear (ME), remains a major public health problem worldwide. Cholesteatoma is a non-cancerous, cyst-like lesion in the ME that may be acquired due to chronic OM and cause disabling complications. Surgery is required for treatment, with high rates of recurrence. Current antibiotic treatments have been largely targeted to previous culturable bacteria, which may lead to antibiotic resistance or treatment failures. For this study, our goal was to determine the microbiota of cholesteatoma tissue in comparison with other ME tissues in patients with long-standing chronic suppurative OM.

Methods: ME samples including cholesteatoma, granulation tissue, ME mucosa and discharge were collected from patients undergoing tympanomastoidectomy surgery for chronic suppurative OM. Bacteria were profiled by 16S rRNA gene sequencing in 103 ME samples from 53 patients. Respiratory viruses were also screened in 115 specimens from 45 patients.

Results: Differences in bacterial profiles (beta-diversity) and the relative abundances of individual taxa were observed between cholesteatoma and ME sample-types. Additionally, patient age was associated with differences in overall microbiota composition while numerous individual taxa were differentially abundant across age quartiles. No viruses were identified in screened ME samples. Biodiversity was moderately lower in cholesteatoma and ME discharge compared to ME mucosal tissues. We also present overall bacterial profiles of ME tissues by sample-type and age, including prevalent bacterial taxa.

Conclusions: Our findings will be useful for fine-tuning treatment protocols for cholesteatoma and chronic OM in settings with limited health care resources. Additionally the identified bacterial profiles of ME tissues by sample-type and age, including prevalent bacterial taxa, underline the need to perform bacterial studies and antibiotic sensitivity tests for cholesteatoma and/or chronic OM samples in order to customize post-operative antibiotics to the specific patient and maximize the benefit of surgical intervention.

12:00 p.m. – 2:00 p.m.
Podium Session #30 – Regeneration & Progenitor Cells
Moderators: Ksenia Gnedeva, Ph.D. & Jennifer Stone, Ph.D.

1. Deciphering the Role of Islet-1a in Zebrafish Hair Cell Regeneration
Category: Regeneration

Paloma Meneses-Giles*[1], Tatjana Piotrowski[1]
[1]*Stowers Institute for Medical Research*

Background: Mechanosensory hair cell loss leads to permanent hearing deficiencies in humans. However, our understanding of how to trigger hair cell regeneration is still limited. Zebrafish possess sensory hair cells and support cells within mechanosensory organs called neuromasts that share a striking resemblance to mammalian hair cells and support cells in the inner ear. Unlike mammals, zebrafish robustly regenerate hair cells throughout life. We previously identified central support cells as the direct hair cell progenitors in zebrafish. However, the molecular mechanisms that trigger central support cell proliferation and their differentiation into hair cells after injury are not well understood. Isl1a is a transcription factor specifically expressed in central support cells with a dynamic response to hair cell death in zebrafish. Isl1 is expressed in developing chicken and mouse inner ear hair cells and support cells but is downregulated in hair cells as they differentiate. Its overexpression in concert with other transcription factors aids in hair cell production during regeneration, but its mechanism of action and how it is regulated is poorly understood.

Methods: We generated an isl1a whole gene deletion mutant and using transgenic lines we analyzed the hair cell regeneration capacity. We used time lapse and EdU incorporation analyses to measure changes in proliferation. To identify downstream targets and isl1a interactions we performed transcriptome analyses and in situ hybridization of candidate genes.

Results: We discovered that homeostatic mutant larvae possess significantly bigger mechanosensory organs with more hair cells and support cells. Time lapse and EdU incorporation analyses revealed that this increase is driven by an increase in proliferation. However, the increase in these cell populations is proportional to the neuromast size demonstrating that isl1a loss leads to an increase in proliferation in all cell types but does not affect cell type specification during homeostasis. In contrast, during regeneration isl1a mutants generate a disproportionately higher number of hair cells, showing that isl1a normally inhibits the hair cell fate. Transcriptome analyses and in situ hybridization of candidate genes showed that isl1a acts downstream of Fgf and Notch signaling pathways in homeostasis. Isl1a loss results in the upregulation of multiple central support cell genes, suggesting that it plays a repressive role in this population. Isl1a downstream targets are currently under investigation.

Conclusions: Even though isl1a is necessary for proliferation in other tissues, our data suggest that isl1a negatively regulates central support cell proliferation and further differentiation into hair cells in the neuromast. Isl1 is a promising candidate for improving regeneration in mammals and understanding how isl1a is dynamically regulated and identifying its downstream targets is essential to optimize its therapeutic effectiveness.

2. Characterization of Otic Progenitor Cells in GER-Derived Organoids
Category: Regeneration

Marie Kubota*[1], Paul Lee[1], Angela Ling[2], Taha Jan[2], Stefan Heller[1]
[1]*Stanford University School of Medicine,* [2]*UCSF*

Background: In mammals, sensory hair cell loss is irreversible and caused by exposure to loud noise and certain drugs, some viral infections, and aging. Nevertheless, the neonatal mouse cochlea shows limited and transient regenerative potential in vivo and in vitro. We previously showed that the greater epithelial ridge (GER), a cell population that disappears during the postnatal period, has a high capacity to proliferate and differentiate into hair cell-like cells in vitro (Kubota et al., Cell Rep. 2021). However, open questions remain: How do GER cells enter the cell cycle? And why does direct cell-to-cell contact promote proliferation?

Methods: GER cells were isolated at >90% purity from cochlear duct cells from postnatal day 2 (P2) Fgfr3-tdTomato/Sox2-GFP transgenic mice using FACS (Kubota et al., STAR Protoc. 2021). They were cultured for seven days in media that efficiently supports organoid growth. At days 1, 3, and 7, GER-derived organoids were individually harvested, dissociated, and subjected to single-cell RNA-Seq.

Results: Clustering analysis revealed that the organoid cells form distinct groups of cells that are different from the original GER cells, which were directly sequenced from isolated GER cells without culture. In a parallel set of experiments, we performed single-cell RNA-Seq analyses on organoids and organoid-derived colonies grown from whole cochlear duct cells of P2 mice at days 3, 8, 14, and 21, cultured in conditions that give rise to myosin 7a-positive hair cell-like cells. Computational analysis and comparison of the various datasets identified proliferating hair cell progenitors.

Conclusions: Identified hair cell progenitors expressed genes suggesting the activity of pathways related to cell-cell adhesion, cytoskeletal rearrangement, and interaction with the extracellular matrix. We hypothesize that the active production of extracellular matrix proteins in organoids contributes to cell adhesion and activation of growth-promoting signaling cascades. Ongoing experiments aim to validate our hypotheses and ultimately provide insight into the mechanism of GER cells' strong ability to proliferate and generate hair cell-bearing organoids.

3. Defining the Human Otic Progenitor Niche
Category: Regeneration

Jingyuan Zhang*[1], Matthew Steinhart[2], Wouter van der Valk[3], Karl Koehler[3]
[1]*Childrens Hospital Boston,* [2]*Boston Children's Hospital,* [3]*Boston Children's Hospital/Harvard Medical School*

Background: Inner ear development involves the assembly of cells from a variety of different embryological origins. There is an unmet need for developing a human inner ear model in vitro to mimic both the complex structure and the function of the inner ear outside of the body. Because the human inner ear is difficult to biopsy, a major challenge is to faithfully recapitulate the embryonic development of the inner ear from hPSCs. However, our limited understanding of the microenvironmental niche in which human otic progenitors emerge and develop is a key barrier to progress. This project builds on our previous discovery of a 3D culture system that uses hPSCs to generate inner ear (otic) organoid containing sensory epithelium, neurons, and mesenchymal cells.
Methods: The culture system contains three stages. In Stage-I, we induce inner ear progenitors by modulating TGF, BMP, and FGF signaling. In Stage-II, the premature otic organoids (otic vesicles) appear after WNT activation. In Stage-III, the organoids mature into multi-chambered cysts containing sensory and non-sensory epithelium surrounded by mesenchyme. Drawing upon recent single-cell genomic studies in our lab, we have gained deeper insight into the early progenitors that form otic organoids. We identified a founding population of otic placode/vesicle-like epithelial cells, which are defined by the expression of TBX2, PAX2/8, SOX2, and OC90. We also identified a population of mesenchymal cells, reminiscent of the pharyngeal or lateral plate mesoderm, that co-develop with otic organoid epithelia and expresses markers of the peri-otic mesenchyme, such as OTOR and POU3F4. We then used in silico analysis and immunostaining to define a set of surface markers, which could be used to isolate these critical progenitor cell populations for subculture and expansion.
Results: Here, we will present our progress toward isolating human otic epithelial and mesenchymal progenitor cells using FACS- or MACS-based approaches. In addition, we will outline a test screen to determine the responsiveness of these cells to various otic patterning factors.
Conclusions: The goal of this project is to elucidate the critical microenvironmental cues that contribute to the expansion, specification, and maturation of otic progenitor cells. Long-term, this defined model of the inner ear will be integral to pre-clinical studies for therapies to treat congenital deafness and balance-related disorders.

4. Characterization of Trim71 in Hearing and Hair Cell Regeneration
Category: Regeneration

Xiaojun Li*[1], Waldemar Kolanus Kolanus[2], Angelika Doetzlhofer[3]
[1]*Johns Hopkins University School of Medicine,* [2]*University of Bonn,* [3]*Johns Hopkins University School of Medicine*

Background: Mechano-sensory hair cells within the inner ear cochlea are essential for sound detection and their loss is among the leading causes for hearing impairments in humans. Cochlear supporting cells in newborn mice have some limited capacity to regenerate hair cells. However, such plasticity is lost within the first postnatal week. We recently showed that reactivation of the RNA binding protein LIN28B in postnatal day 5 (P5) cochlear cells/tissue restores the ability of supporting cells to re-enter the cell cycle and generate hair cells. Interestingly, LIN28B overexpression in supporting cells lead to the re-activation of the progenitor-specific gene Trim71, which during otic development is highly expressed in neural-sensory progenitor cells. The evolutionary highly conserved TRIM71 (also referred to as LIN41) protein is a well-known positive regulator of pluripotency, self-renewal and cell reprogramming. TRIM71 regulates gene expression by two distinct mechanisms. It can directly bind to mRNAs/miRNAs, a function that is mediated by the C-terminal NHL domain and it possess E3 ubiquitin ligase activity mediated by the N-terminal Tripartite motif (RING domain, B-box and coiled-coil regions).
Methods: To address the function of Trim71 in cochlear development and hair cell regeneration, we deleted Trim71 at various stages using Trim71 floxed mice. To provide temporal control the mice also carried a tetracycline-inducible cre transgene, which becomes activated in the presence of doxycycline. For our gain-of-function experiments, we infected cochlear organoids with lentiviral particles containing full length Trim71, or constructs with deletions of Trim71's RING, coiled-coil or NHL domain.
Results: Our analysis revealed that knockout of Trim71 gene at embryonic day E9.5 (E9.5) or E10.5 causes hair cell and supporting cell progenitors (pro-sensory cells) to drop out of the cell cycle prematurely and to initiate hair cell differentiation earlier than normal. Consistent with Trim71's developmental role, we found that deletion of Trim71 in early postnatal cochlear epithelial cells reduced the ability of supporting cells to proliferate form hair cells, while overexpression of Trim71 promoted supporting cell proliferation and hair cell formation in organoid

culture. Moreover, our overexpression experiments revealed that the coiled-coil and NHL domains but not the RING domain were required for Trim71's postnatal function in hair cell regeneration.

Conclusions: Firstly, our developmental studies in mice indicate an essential role for Trim71 in maintaining auditory pro-sensory cells in a proliferative, undifferentiated state. Secondly, our 122ongolia experiments at early postnatal stages indicate that Trim71 functions as a positive regulator of supporting cell plasticity and that such function requires TRIM71's ability to bind RNA.

5. Regeneration of Hair Cells in the Mature Mouse Cochlea Following Reprogramming With Atoh1-Gfi1-Pou4f3
Category: Regeneration

Melissa McGovern*[1], Sumana Ghosh[2], Ken Nguyen[1], Bradley Walters[2], Andrew Groves[1]
[1]*Baylor College of Medicine,* [2]*University of Mississippi Medical Center*

Background: In the mature mammalian cochlea, hair cells (HCs) detect sound from the environment. These HCs do not regenerate, and any HC loss is permanent thus leading to hearing loss. In contrast, non-mammalian supporting cells (SCs), which surround HCs, regenerate lost HCs throughout the life of the animal. Additionally, some immature SCs in the neonatal mammalian cochlea can spontaneously regenerate into HCs after damage. Furthermore, neonatal SCs and other non-sensory cells adjacent to inner HCs respond to the ectopic expression of the HC transcription factor Atoh1 by differentiating into HCs. In the mature cochlea, however, Atoh1 alone does not induce the conversion of SCs into HCs. In neonatal mice as well as non-mammalian species, the regenerative ability of SCs depends on the removal of regulator signaling from HCs. Unfortunately, little is known about how mature HCs and SCs communicate. It is possible that mature HCs regulate mature SCs and that the loss of the HCs can improve the response to reprogramming factors.
Methods: To determine whether mature SCs can convert into HCs, we have targeted the ROSA locus with three conditional alleles that drive expression of one of three combinations of HC transcription factors: Atoh1 alone (Rosa26-A), Gfi1 and Atoh1 (Rosa26-GA), or Gfi1, Atoh1, and Pou4f3 (Rosa-GAP). When combined with Lfng-CreER, these lines express their respective transcription factors in SCs. Expression was induced at three weeks of age and cochleae were analyzed at 4, 5, and 6 weeks of age. In addition to reprogramming in intact cochlea, we ablated HCs in Lfng-CreER::Rosa26-A, GA, or GAP mice by using the Pou4f3DTR mouse. Reprogramming and HC killing were induced at 3 weeks of age and cochleae were examined at 5 and 6 weeks of age.
Results: Expression of Gfi1, Atoh1, and Pou4f3 reprogrammed SCs into HC-like cells in the apex of the intact cochlea of 5- and 6-week-old mice but was limited to the abneural edge of the organ of Corti. However, after HC damage robust regeneration was observed at 5 weeks of age throughout the length of the cochlea with inner and outer HCs identifiable by location. Examination of regenerated HC-like cells revealed neural connections and phalloidin labeled immature stereocilia bundles that were also detected via SEM. Further work is ongoing to characterize regenerated HCs and investigate the expression profile of these cells.
Conclusions: Currently, the best therapeutic for hearing loss are hearing aids and cochlear implants. While these enable users to re-gain some hearing, they provide incomplete recovery. Understanding the response of SCs to HC death and reprogramming, provides insight into the molecular pathways regulating SC identity. As clinical trials for gene therapies increase, identifying genes that can produce new HCs in the mature cochlea will provide therapeutic approaches for hearing restoration in humans.

6. A Small-Molecule Cocktail Targeting Pou4f3 and p27Kip1 Induces Adult Mouse Cochlear Supporting Cells to Express a Hair Cell-Like Phenotype
Category: Regeneration

Rene Vielman Quevedo*[1], Fred Millan V[1], Joseph Frank[1], Yan Zhang[2], Yuju Li[2], Jackson Diers[1], Jian Zuo[2]
[1]*Creighton University,* [2]*Creighton University School of Medicine*

Background: Mammals, unlike birds or reptiles, are unable to regenerate lost cochlear hair cells, making hearing loss (HL) beyond a temporary threshold shift permanent. There are strategies to overcome this, mainly in the form of hearing aids and bionic implants of various types covering a large spectrum of etiologies and degrees of HL. All of these come with their own set of limitations, however, and none can deliver the granularity of natural hearing. Over 1.5 billion worldwide experience varying degrees of permanent hearing loss with an accompanying loss in quality of life, so any successful regenerative therapy would be of great benefit to the hearing field. Previous work has shown that Atoh1 overexpression can drive cochlear supporting cell (SC) to hair cell (HC) conversion in neonatal mice, and that p27Kip1 inhibition can potentiate this effect and replicate it in adult mice as well (Liu et

al, 2012; Cox et al, 2014; Walters et al, 2017). It has also been found that Pou4f3 overexpression has a greater effect than Atoh1 alone, and coupled with the need to turn off Atoh1 to obtain a mature HC phenotype, we decided to pursue a Pou4f3 overexpression/ p27 inhibition paradigm. We have previously identified a Pou4f3 agonist known to us as compound #18 (C18), and we used it in conjunction with a previously identified p27 inhibitor, Alsterpaullone, 2-cyanoethyl (A2CE).

Methods: Adult (P28) FVB mice of both sexes were treated via transtympanic injection into the left middle ear with a cocktail consisting of 500µM C18 plus 5mM A2CE in 15% DMSO vehicle. Controls consisted of contralateral uninjected ears and vehicle-only injected mice. Mice were euthanized 4 weeks after injection, and their cochleae harvested, decalcified, immunostained (Pou4f3, Myosin VI, Sox2 and DAPI), and analyzed via confocal microscopy. Using Imaris software (Bitplane, USA), SCs (Sox2+) were analyzed for both Pou4f3 expression and HC-like phenotype (Myo6). Triple positive cells were interpreted as converted HCs (cHC). The experiment will be repeated using Sox2-CreER; TdTomato mice for lineage tracing.

Results: In vivo intratympanic treatment with small molecules targeting Pou4f3 and p27Kip1 causes cochlear supporting cells in wild-type adult FVB mice to exhibit a hair cell-like phenotype 4 weeks after injection in a significant manner compared to uninjected and vehicle-only injected controls.

Conclusions: Here we demonstrate that existing small molecules screened to target key factors involved in hair cell fate can be successfully repurposed and delivered locally to induce supporting cell-to-hair cell conversion in adult cochleae.

7. Functional Properties of Regenerated Synapses Between Hair Cells and Auditory Nerve Fibers in Vitro
Category: Regeneration

Philippe Vincent*[1], Eric Young[1], Albert S. B. Edge[2], Elisabeth Glowatzki[1]
[1]*Johns Hopkins School of Medicine,* [2]*Massachusetts Eye and Ear*

Background: The loss of ribbon synapses between inner hair cells (IHCs) and type I spiral ganglion neurons (SGNs) contributes to noise-induced and age-related hearing loss. Therefore, strategies to promote ribbon synapse regeneration have been sought, including in vitro models (for example Tong et al., 2013; Brugeaud et al., 2014). In such in vitro studies, denervated organs of Corti have been plated with isolated SGNs and 'regenerated' ribbon synapses have been identified based on the juxtaposition of pre- and postsynaptic markers in immunolabeling. These culture systems have been proposed for testing compounds that have the potential of improving synapse regeneration (Seist et al., 2020). Here, we are functionally testing properties of regenerated ribbon synapses in culture, to gain an understanding of how similar/different their properties are compared to native hair cell synapses. Second, we test the hypothesis that the age of the plated denervated organ of Corti (hair cells) affects the level of maturity of the synaptic activity at the newly formed synapse.

Methods: At three different postnatal age ranges (P3-5; P7-8 and P10-11), denervated organs of Corti from Gfi1-Cre; Ai32 mice expressing channelrhodopsin-2 in hair cells were co-cultured with P0-2 SGNs isolated from separate animals expressing a fluorescent marker (GFP or TdTomato) in neurons. After 10 to 12 days in culture, co-cultures were tested for new functional synapses by recording from SGN somata and characterizing excitatory postsynaptic currents (EPSCs(P3-5HCs), EPSCs(P7-8HCs), EPSCs(P10-11HCs)) induced by optogenetically stimulating hair cells.

Results: Excitatory postsynaptic currents (EPSCs) induced by hair cell stimulation at newly formed synapses were found in 13% of recorded SGNs (43/323 SGNs, all age ranges combined). EPSCs were blocked by NBQX, an AMPA/123ongoli receptor blocker. In comparison to EPSCs from native synapses recorded in P4-5 acutely isolated organs of Corti, EPSCs(P7-8HCs) and EPSCs(P10-11HCs), but not EPSCs(P3-5HCs), from regenerated synapses exhibited significantly larger amplitudes and faster decay times. Deconvolution analysis was performed to model the EPSC waveforms via a sum of underlying transmitter release events (see Young et al., JNP 2021). Based on this analysis, there was a trend for EPSC properties to more closely resemble those of mature native synapses, when older organs of Corti were cultured to create new synapses.

Conclusions: Hair cells from more mature organs of Corti form more mature regenerated ribbon synapses with SGNs in vitro. We therefore suggest using more mature organs of Corti for culture approaches that are aimed at finding compounds that improve hair cell synapse regeneration.

8. Repair of the Murine Tympanic Membrane Displays Hallmarks of Regeneration
Category: Regeneration

Sonia Scaria*[1], Stacey Frumm[1], Amar Sheth[2], Aaron Tward[1]
[1]*UCSF,* [2]*Yale University*

Background: The tympanic membrane has a remarkable ability to repair itself, with perforations typically closing in days to weeks in all mammalian species studied. The cellular and molecular mechanisms underlying this repair ability remain largely unknown, however. This study looked to thoroughly characterize the repair process of the injured TM and uncover mechanisms of repair that could serve as future targets to inform treatments for disorders of the tympanic membrane.

Methods: Cells from TMs perforated on adult mice 14, 7, 3, and 1 day prior to sacrifice were enzymatically dissociated and submitted for single-cell RNA sequencing using the 10x Genomics scRNA-seq platform. Using Seurat clustering and the CellFindR algorithm, unbiased clusters of cells were generated along with matrices of differentially expressed genes for each cluster, allowing for transcriptional characterization of each identified cluster. Protein detection via immunohistochemistry (IHC) and RNA detection via RNAscope were used to biologically validate and define the anatomic locations of distinct cellular populations that were computationally identified by the scRNA-seq data. The K5CreERT2 promoter was utilized in mouse models to genetically manipulate and fluorescently label keratinocyte populations. The mTmG and Confetti reporters were utilized as fluorescent labels.

Results: RNA expression data from all timepoints of perforation were merged and analyzed, revealing 15 distinct clusters with 97 total sub-populations of cells in the cumulative data and revealing transcriptional shifts characteristic to each time point. From both cross-sectional and whole-mount views, the TM shows a rapid, proliferative response to injury as early as 18 hours post-injury, predominantly in the keratinocytes. Three days after perforation, there are large transcriptional shifts in the immune, mesenchymal, and mucosal populations. The multi-layered tissue shows a large volumetric increase by day 7 but quickly remodels and restores the original volume of the TM by day 14. However, at longer timepoints, the radial and circular collagen patterning of the TM is restored, thus creating a scar-free structure. We identified a regeneration-induced "wounded epithelial" population, characterized by a combination of distinct marker genes. A K5Cre;Confetti mouse model shows that the population is first localized at known stem cell regions of the organ and migrates to the site of injury. Based on expression values and immunostaining, EGFR signaling is upregulated throughout the regenerative response corresponding with increased expression of EGFR ligands and processing co-factors. When EGFR is deleted in vivo, using a K5CreERT2;Egfr(fl/fl);mTmG mouse model, tympanic membranes no longer display the same rapid proliferative response post-injury and can no longer repair perforations.

Conclusions: Tympanic membrane perforation healing displays a complex and coordinated series of transcriptional, morphological, and patterning events, ultimately culminating in the restoration of normal tissue architecture to the TM. These characteristics are more typical of epimorphic regeneration, rather than more typical scar induced mammalian wound healing.

Wednesday, February 9, 2022

7:00 a.m. – 9:00 a.m.
Symposium #31

Hearing Loss and Social Accountability: How Can We Make a Difference?

Chair: Avril Genene Holt, *Wayne State University School of Medicine*
Co-Chair: Radha Kalluri, *University of Southern California*
Co-Chair: Karl Grosh, *University of Michigan*

Sensorineural hearing loss is a worldwide problem that affects people across age, race, and socioeconomic status. Inner ear gene therapies, cochlear implants, hearing aids, and more recent cutting-edge treatments are all effective in returning some level of function to hearing impaired individuals seeking restoration. However, these therapies are expensive, may be available in only certain zip codes, often require multiple visits to be effective, and therefore pose significant barriers to treatment. Recent events have made disparities in healthcare even more apparent. As we continue to research and provide improved treatments for hearing related conditions, who within our society will have little to no access and how does being hearing impaired limit access? How do we elevate our vigilance to change a system that favors successful treatment outcomes only for some members of society? There

continues to be a need for increased awareness and education surrounding social accountability and the role of academic medical centers. The symposium will provide an update on the current status of treatments for hearing loss and then discuss social determinants of health, and barriers to treatment.

Hearing Loss and Social Accountability: How Can We Make a Difference?
Karen Avraham, *Faculty of Medicine and Sagol School of Neuroscience, Tel Aviv University*

Karen Avraham will introduce the recent advances in hearing loss treatment (genetics) and what the future holds.

189.2 Hearing Health and Equity: The Role of Academic Medical Centers in Social Accountability
Howard Francis, *Duke University Health System*

Hearing impairment has fundamental implications for population health that are further shaped by its intersection with stigma in marginalized communities. We review the concept of social accountability of Academic Medical Centers (AMC), and how hearing status presents an additional and unique source of variability in health status particularly in aging populations. Intersectional stigma superimposed on structural inequities require engagement by hearing clinicians and researchers in evolving efforts by AMCs to fulfill their social accountability mandate. Components of a multi-pronged approach are presented.

Transforming Healthcare for Patients with a Hearing Loss Through an Intersectionality Framework
Michael McKee, *University of Michigan*

Nearly 20% of individuals report a hearing loss yet healthcare is ill equipped to care for them. Individuals with hearing loss report miscommunication, inaccessible health information, reduced awareness by healthcare providers, low patient satisfaction while struggling with inadequate health literacy, placing them at high risk for adverse health outcomes and inappropriate health care utilization. Those from disadvantaged backgrounds, including those with co-existing disabilities and language minorities, experience greater gaps in care. These factors contribute to existing health inequities that are exacerbated by the arrival of the COVID-19 pandemic. Rethinking and redesigning our health care, through the guidance of innovative clinics, accessible equipment and programs, is needed to address these inequities and care for these individuals effectively. The pandemic is providing opportunities for disruption but the engagement of individuals with hearing loss and hearing loss organizations are critical to ensure accessibility, inclusion and equity in health care remains prioritized for this group.

Hearing Loss and Social Accountability: How Can We Make a Difference?
Charlene Le Fauve, *NIH*

This presentation will address the science of scientific workforce diversity, reviewing the data demonstrating the impact of diversity, the opportunities for further enhancing diversity, and processes recently initiated at NIH via The NIH UNITE Initiative | National Institutes of Health (NIH).

7:00 a.m. – 9:00 a.m.
Podium Session #32 – Otoacoustic Emissions
Moderators: Sarah Verhults, Ph.D. & Wei Dong, Ph.D.

1. A New Mouse Model of DFNB7/11 Recovers Broad-Spectrum Auditory Sensitivity After TMC1 Gene Therapy

Irina Marcovich*[1], Nicholas Baer[2], Olga Shubina-Oleinik[3], Rachel Eclov[4], Clayton W. Beard[4], Jeffrey Holt[5]
[1]*Boston Children's Hospital - Harvard Medical School,* [2]*Boston Children's Hospital,* [3]*Boston Children's Hospital/ Harvard Medical School,* [4]*Audition Therapeutics,* [5]*Harvard Medical School/Boston Children's Hospital*

Background: Transmembrane channel-like 1 (TMC1) constitutes the pore-forming protein of the mechanosensory apparatus in mature cochlear hair cells (Pan et al., 2013; 2018). In humans, more than 70 TMC1 mutations have been described that underlie recessive nonsyndromic hearing loss, DFNB7/11. Some mutations are associated with congenital deafness, whereas other milder mutations produce moderate-to-severe hearing loss that

progresses during the first decade of life (Imtiaz et al., 2016). Generation of murine models harboring milder human mutations is important for understanding disease progression and for designing therapeutic strategies for patient treatment. We generated a mouse model with a Tmc1 p.N193I mutation, equivalent to the hypofunctional human TMC1 mutation p.N199I (c.596A>T), which causes progressive moderate-to-severe hearing loss during childhood. Subsequently, we designed a gene therapy approach with potential clinical translation to overcome the auditory deficiencies.

Methods: N193I mice were generated using a CRISPR/Cas9 strategy with homology directed repair (HDR) in collaboration with the Mouse Gene Manipulation Core at Boston Children's Hospital. Utricle injections (Lee et al., 2020) with AAV vectors that encoded TMC1 were performed at P1. Auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs) were used to monitor the auditory capacity of the animals, followed by immunohistochemistry of cochleas using phalloidin and antibodies to Myosin7a to assess hair cell survival. Hair cell mechanosensory transduction was evaluated with FM 1-43 permeation studies and electrophysiological recordings from hair cells.

Results: Tmc1 N193/N193I mice were profoundly deaf from postnatal day (P) 16, the earliest time point tested. Additionally, hair cells expressing only TMC1 p.N193I exhibited no FM1-43 uptake, indicating a lack of sensory transduction. Surprisingly, the number of surviving hair cells at P28 was similar to that of Tmc1 N193/+ mice, which have normal hearing, suggesting the possibility of a broader window for therapeutic intervention. Injection of viral vectors encoding TMC1 revealed remarkable recovery of ABR thresholds to near wild-type levels. The auditory recovery was found to be durable until six months post-injection, the latest time point tested. Wild-type and mutant animals injected with AAVdid not exhibit any sign of viral induced toxicity.

Conclusions: The Tmc1 N193/N193I mouse carrying the equivalent of the human p.N199I mutation constitutes an excellent model for studying moderate-to-severe DFNB7/11 hearing loss. We report unprecedented recovery of auditory thresholds by delivering optimized AAV constructs encoding TMC1. These results demonstrate that early and efficient gene therapy intervention can prevent hearing loss in mice, with potential translation for treatment of DFNB7/11 patients.

2. Lateral Line Ablation by Different Ototoxins Results in Distinct Rheotaxis Profiles in Fish
Category: Other

Kyle Newton[1], David Kacev[2], Simon Nilsson[3], Sam Golden[3], Lavinia Sheets*[1]
[1]*Washington University School of Medicine in St. Louis*, [2]*University of California San Diego*, [3]*University of Washington*

Background: Sensory hair cells of the lateral line transduce and transmit water flow stimuli and mediate rheotaxis in fishes, or the ability to orient with respect to flowing water. The zebrafish lateral line is an established model for understanding the cellular mechanisms of hair cell organ damage and repair; however, few studies link mechanistic disruptions to observable changes in biologically relevant behavior. Therefore, we used larval zebrafish to model how ototoxic drugs damage hair-cell organs and determine how this damage impacts positive rheotaxis, or the ability of fish to orient and swim into water flow.

Methods: Fish (6-7dpf) were exposed to CuSO4 (10 µM; n=204) or neomycin (50 µM; n=222) to disrupt lateral line function. Control fish (n=248) received no chemical treatments yet underwent the same procedures as the treatment fish. Then we exposed fish to water flow stimuli of constant velocity and recorded their swimming behavior with a high-speed camera under IR light (850 nm) to eliminate visual cues. DeepLabCut software was used to estimate and track the body pose and orientation angles of fish, then SimBA behavioral analysis software was used to annotate and classify rheotaxis behavior.

Results: Lateral line disrupted fish could successfully orient and swim into flowing water and thus perform rheotaxis, yet their responses were significantly different from that of intact fish and resulted in distinct ototoxin-specific behavioral profiles. Lateral line intact fish had mean body angles that were more tightly clustered around the flow vector, rheotaxis events that lasted longer, and they traveled a shorter overall distance during flow conditions compared to disrupted fish. The relative movement, velocity, acceleration, and mean body angle of intact fish had fluctuations of lower magnitude, but greater temporal variation compared to impaired fish. Furthermore, the direction and relative timing between changes in the linear and angular components of movement was progressively delayed in impaired fish in a treatment specific manner. Thus, intact fish could hold their station near the flow source and occupy the forward part of the arena, whereas impaired fish were pushed against the rear boundary and performed rheotaxis with maximum effort.

Conclusions: The accurate detection and response to water flow facilitates behaviors critical for individual fitness, such as foraging strikes, schooling, and predator evasion. Our data show that, while the lateral line is not essential

for rheotaxis behavior, it plays an important role in effectively performing rheotaxis with minimal effort. Notably, our analyses also reveal that pharmacological agents commonly used to ablate zebrafish lateral line have unique, predictable, and significant effects on natural behaviors. The ability of this assay to detect subtle variances in rheotaxis behavior will be leveraged in future studies to link hair cell damage and recovery to differences in fish behavioral profiles.

3. Characterizing Otoacoustics of the Anolis Lizard
Category: Otoacoustic Emissions
Rebecca Whiley*[1], Zena Khadour[1], Christopher Bergevin[1]
[1]*York University*

Background: Given the complexity of the mammalian cochlea and its associated biomechanics, lizards are emerging as valuable alternative models for hearing research. The green anole, Anolis carolinensis, has sensitivity and selectivity comparable to that of many mammals, despite its relatively simpler inner ear morphology (e.g., approximately 150 hair cells, most of which have free-standing bundles with no overlying tectorium [TM]). Additionally, anoles exhibit strong otoacoustic emissions (OAEs). Our purpose here is to characterize a broad range of their OAE features, in part to serve as a foundation for comparisons to other neurophysiological measures (e.g., auditory evoked potentials) and constrain theoretical models.
Methods: Here, we summarise key qualitative results of several OAE properties based on measurements in more than 20 green anoles.
Results: First, spontaneous emissions (SOAE) exhibit robust responses to swept tones, including idiosyncratic nonlinear interactions such as sub-harmonic facilitation. Second, although SOAE activity is typically observed above approximately 1 kHz, raising the lizard's body temperature can elicit the emergence of SOAE activity at low frequencies (approximately 0.2 kHz). These emissions presumably relate to the small fraction of hair cells covered by a TM. Third, stimulus frequency emissions (SFOAEs) evoked using low-level stimuli (e.g., 30 dB SPL) demonstrate a complex mixture of linear and nonlinear dependencies upon stimulus level. Further, such behaviour appears tightly linked to SOAEs, with non-monotonic growth in SFOAEs confined to frequency regions close to SOAE peaks. Fourth, upon using a suppression paradigm, SFOAE behaviour depends on the relationship between probe and suppressor frequencies. When evoked at a fixed probe frequency, SFOAE magnitude and phase are relatively invariant to changes in the suppressor frequency, at least at frequencies within a fraction of an octave above or below the probe frequency. However, when there is a fixed difference between the probe and suppressor frequencies, more complex behavior is observed across the spectrum, corresponding to SOAE-related peaks and valleys in responses. Fifth, distortion product emission (DPOAE) phase-gradients can show extensive regions of frequency-independence, similar to that reported for mammals and hypothesized to arise from "wave-fixed" sources, despite the absence of a canonical basilar membrane travelling wave in the anole.
Conclusions: Together, these observations further establish the anole as a key animal model and pave the way for future complementary directions (e.g., vibrometry, hair cell regeneration mechanics, binaural synchrony of two active ears).

4. Wide Bandwidth of Ear-Canal Otoacoustic Emission Signals Supports Fast Reverse Propagation From Organ of Corti Through Fluid Pressure
Category: Otoacoustic Emissions
Jonathan Siegel*[1]
[1]*Northwestern University*

Background: One of the unresolved questions about otoacoustic emissions is how they propagate from their intracochlear generation sites due to hair cell activity to the ear canal. Models have most commonly postulated reverse propagation as slow basilar membrane traveling waves, with fluid coupling from the basal end of the basilar membrane to the stapes (e.g., Shera and Guinan, JASA 1999; Venkovsky, et al, JASA 2020). However, attempts to directly test this hypothesis have not only failed to confirm reverse slow waves, but detected only forward propagation of the 2f1-f2 distortion product following initial stapes deflection, strongly suggesting that the initial reverse propagation was via fast pressure waves (He, et al, Biophys J 2010). Recently, the Ren group has reported that the spectrum of intermodulation distortion evoked by a pair of stimulus tones was much broader in the reticular lamina motion (RL) of gerbils than in the basilar membrane (Ren and He, Commun. Biol. 2020). I sought to examine the spectrum of distortion product otoacoustic emissions in data measured previously in my lab for another purpose.

Methods: Data presented here were collected from 11 male 128ongolian gerbils with stimulus levels of L1, L2 = 65 and 55 dB SPL, f2/f1=1.2 and f2 from 1 to 40 kHz.

Results: The absolute and relative levels of intermodulation distortion products in the gerbil ear canal pressure strongly resembled those reported by Ren and He in RL, but not detectable above the noise floor in the spectrum of basilar membrane motion. The distortion products f2-f1, 3f1-2f2 and 4f1-3f2 in our data were clearly present in the same frequency range where RL components at these frequencies were reported by Ren and He.

Conclusions: Our data add to the evidence presented by He, et al (2010) that intermodulation distortion products generated by hair cells are strongly coupled to the stapes through fast, direct coupling in the fluid pressure. Thus, previous reports of broad-spectrum nonlinear interactions in ear canal otoacoustic emissions (i.e., Martin, et al, 2010; 2016 and Charaziak and Siegel, JARO, 2015) also appear to be explained by this direct coupling. Given that direct measurements of such broad-band nonlinearity is absent from basilar membrane measurements, models of otoacoustic emissions appear to be largely in error and should be reevaluated. In particular, a new generation of models that explore the direct coupling from the organ of Corti to the stapes footplate through fluid pressure appear to be required.

5. Side Lobes in SOAE Suppression Are Not Observed in Psychophysical Tuning Curves
Category: Otoacoustic Emissions

Emile de Kleine*[1], Sina Engler[1], Etienne Gaudrain[2], Pim Van Dijk[1]
[1]*University Medical Center Groningen,* [2]*CNRS*

Background: Spontaneous otoacoustic emissions (SOAEs) can be suppressed by external tones. This allows for the evaluation of cochlear frequency selectivity by determining the suppression tuning curve (STC) of an SOAE. Interestingly, some STCs have additional side-lobes at the high frequency flank. These are thought to result from the interaction between the probe tone and the cochlear standing wave corresponding to the SOAE being suppressed. The aim of this study was to compare STCs and psychoacoustic tuning curves (PTCs), where the probe tone of the PTC was placed at the center frequency of an SOAE.

Methods: STCs and PTCs were measured in (1) subjects in which the STC had a side-lobe (n=8), and (2) subjects without STC side-lobes (n=9). Additionally, PTCs were measured in subjects without SOAEs (n=9).

Results: Across participant groups, the quality factor Q10dB of the PTCs was similar, independently from whether SOAEs were present or absent. Side lobes, as observed in STCs, were not visible in PTCs. Smaller irregularities in PTCs were observed in all three subject groups, but not in all subjects.

Conclusions: The presence of an SOAE does not provide enhanced frequency selectivity at the emission frequency. Side lobes in STCs are not related to PTC irregularities in a straightforward way. This suggests that different mechanisms cause these irregularities.

6. Finding a Distortion Product Otoacoustic Emissions Index for Detecting Noise-Induced Hearing Loss
Category: Otoacoustic Emissions

I-Fan Lin*[1], Perng-Jy Tsai[2], Jiunn-Liang Wu[3], Wei-Shan Chin[4], Cheng-Yu Lin[3], Yue Leon Guo[5]
[1]*Taipei Medical University/Shuang Ho Hospital,* [2]*National Cheng Kung University,* [3]*National Cheng Kung University/National Cheng Kung University Hospital,* [4]*National Taiwan University,* [5]*National Taiwan University/National Taiwan University Hospital*

Background: Noise-induced hearing loss (NIHL) is an important health issue among workers exposed to noise. This study aimed to identify an index based on distortion product otoacoustic emissions (DPOAEs) that can be used to detect NIHL. Since both age and noise exposure deteriorate hearing, we defined a suitable index for detecting NIHL as an index that is more affected by noise and less affected by age.

Methods: We measured DPOAEs for 316 male workers in a steel factory. Their median employment duration was 27.05 years. Based on their on-site noise level measurements and questionnaires, we were able to use the job-exposure matrix to calculate their cumulative noise exposure. Their median cumulative noise exposure was 88.20 dBA-year. Multivariate linear regression models were used to examine the effect of age and cumulative noise exposure on DPOAEs at individual frequencies, after adjusting for hypertension, dyslipidemia, tobacco use, and alcohol consumption.

Results: The results of multivariate linear regression models showed that the DPOAE levels at 2, 3, 4, 6 kHz were significantly affected by both age and cumulative noise exposure. Among them, the DPOAE levels at 3 and 4 kHz were more affected by cumulative noise exposure than those at 2 and 6 kHz. On the other hand, the DPOAE levels at 1 and 8 kHz were only significantly affected by age but not cumulative noise exposure. Moreover, the

difference between the DPOAE levels at 1 kHz and those at 3 or 4 kHz were only significantly affected by cumulative noise exposure but not age.

Conclusions: Based on our definition, the difference between the DPOAE levels at 1 kHz and those at 3 or 4 kHz could be suitable indices for detecting NIHL, because they were only affected by cumulative noise exposure but not age.

7. How Distributed is the Active Process in Generating Cubic Distortion Product Otoacoustic Emissions?
Category: Otoacoustic Emissions

Yi Shen*[1], Robert Withnell[2], Mackenzie Mills[2], Kevin Ohlemiller[3]
[1]*University of Washington,* [2]*Indiana University,* [3]*Washington University School of Medicine*

Background: Mechanical amplification in the cochlea provides negative damping to counteract fluid viscosity and enhance basilar membrane vibration. This amplification process is thought to be distributed all along the cochlea but effective only within a narrow spatial region impedance-matched to the frequency of amplification. For a stimulus consisting of two pure tones (at frequencies f1 and f2), Young et al. (2012) estimated the spatial extent of the active process (in a 1D transmission line model of the cochlea) for the intracochlear distortion product 2f1-f2 to be in a narrow region about the best place for f2. In this study, we examined the impact of varying the spatial extent of the active process in a transmission line model of the cochlea on 2f1-f2 traveling waves and the input/output functions of the distortion-product otoacoustic emissions (DPOAEs).
Methods: The outputs of a transmission-line model of DPOAE generation were fit to experimentally obtained DPOAE input/output functions from mice. Instantaneous nonlinearity was implemented in the transmission-line model and the velocity responses on the basilar membrane and at the stapes were solved in the frequency domain using a quasilinear approach. Once the model was fitted to data, the spatial extent of the active process was examined by artificially varying the source distribution without changing the local nonlinearity.
Results: The transmission line model 2f1-f2 input-output function match to the data was found to depend on the extent of the distribution of the active process. When the active process was enabled across the length of the entire cochlea, the transmission-line model was able to capture detailed features in DPOAE input/output functions, including the notch at a moderately high level characteristic to mouse DPOAE input/output functions. On the other hand, when the active process was constrained to a narrow region near the best place for f2, the model predicted a DPOAE input/output function that resembled the implemented local nonlinearity.
Conclusions: The current results provide support for the generation of distortion products from a widely distributed region along the basilar membrane with the effective source distribution being stimulus level dependent.

8. A Hair Cell Analysis Toolbox
Category: Other

Christopher Buswinka*[1], David Rosenberg[2], Artur Indzhykulian[2]
[1]*Harvard University,* [2]*Harvard Medical School/MEEI*

Background: The advent of widely used and readily available immunohistochemical fluorescence microscopy techniques has made biological imaging a uniquely powerful and commonplace method in the toolbox of auditory scientists. The tilescan functionality of confocal microscopes enables collection of Z-stacks of large cochlear regions gradually making it a common practice. Consequently, the limiting factor in many experimental designs becomes the data analysis, rather than data acquisition. To address this need, we have developed a collection of machine-learning based algorithms which help to automate many previously tedious analysis tasks.
Methods: This Hair Cell Analysis Toolbox (HCAT) offers two analysis solutions: 1) a detection algorithm which detects, classifies, and assigns a best frequency to hair cells along the cochlear spiral, and 2) a volumetric segmentation pipeline which allows to uniquely segment individual hair cells within a confocal z-stack.
Results: The hair cell detection pipeline, utilizing the faster R-CNN algorithm, offers highly accurate cell detection at speed (95% accuracy), analyzing a single cochlea under a minute. The segmentation algorithm, utilizing the U-Net architecture with a spatial embedding segmentation paradigm, enables volumetric hair cell segmentation with a subsequent volumetric fluorescence intensity analysis at a single-cell resolution (< 10 minutes per cochlea). For each analysis approach, each cell is assigned a best frequency with a novel method of cochlear path estimation via nonlinear curve fitting which leads to highly accurate frequency estimation via the greenwood function compared to conventional methods (maximum 0.1% octave error).

Conclusions: From these algorithms a wider array of experimental designs become accessible, including in-depth analysis of cochleograms, fluorescence labeling intensity assessment as a function of frequency, or high throughput drug screens of cochlear tissue. We hope these tools will significantly reduce the barrier to large cochlear data analyses and lead to even more productive and rigorous studies in the future.

7:00 a.m. – 9:00 a.m.
Podium Session #33 – All the Worlds A Stage: Molecular Players in Hair Cell Function
Moderators: Cata Velez Ortega, Ph.D. & Anthony Peng, Ph.D.

1. A Myosin 15 – Centrin 2 Phase Condensation Reaction Underlies Formation of the Tip Density
Category: Hair Cells: Anatomy and Physiology

Zane Moreland*[1], James Heidings[1], Elli Hartig[2], Juan Guan[1], Basile Tarchini[2], Jonathan Bird[1]
[1]*University of Florida,* [2]*The Jackson Laboratory*

Background: Stereocilia are actin-rich mechanosensory organelles responsible for the conversion of fluid motion into electrical impulses in the inner ear. A key protein in stereocilia development is the molecular motor myosin 15 (MYO15A); mutations of which cause hereditary human hearing loss, DFNB3. In vivo, MYO15A localizes to the tips of stereocilia where it delivers several distinct proteins, referred to as the elongation complex (EC), all of which are critical for stereocilia growth and architecture. Interestingly, an electron-dense plaque, called the tip density, has been demonstrated at the stereocilia tip using transmission electron microscopy. The presence of the tip density is dependent upon MYO15 (Rzadzinska et al, 2004). Here, we show that MYO15 and a newly identified EC protein, centrin-2 (CETN2), undergo a phase condensation reaction at the tips of actin-based structures that is dependent upon the motor activity of MYO15.
Methods: We used HEK293 cells to perform a host of cellular assays commonly used to characterize biomolecular condensates, in addition to monitoring filopodial dynamics using time-lapse microscopy. We utilized membrane-dye and fluorescence recovery after photo-bleaching (FRAP) experiments to probe the physical properties of cytoplasmic MYO15-CETN2 phase condensates, and used time-lapse confocal microscopy to understand their biogenesis. We perturbed the condensation reaction by testing mouse MYO15 isoform 2 (MYO15-2 / MYO15-S) mutant constructs, including the missense shaker-2 that ablates ATPase motor activity, and variants in the 3rd IQ light chain-binding domain that interfere with CETN2 binding.
Results: MYO15-2 is well-documented in heterologous cells to accumulate at the tips of actin-based filopodia, before undergoing retrograde flow back to the cell body. Whilst imaging filopodia retrograde flow, we discovered that large puncta of MYO15-2 were ejected into the cytoplasm, where they accumulated. Membrane-dye experiments revealed that these cytoplasmic puncta were membrane-less, consistent with MYO15 forming a phase condensate. To further characterize these, we used FRAP and found that condensates maintained a small mobile fraction representative of a solid-like assembly. Introduction of the shaker-2 mutation prevented phase condensation, demonstrating that active concentration of MYO15-2 at the filopodia tip was essential for their formation. Mutation of the MYO15-2 3rd IQ domain, to preclude CETN2 binding, did not affect trafficking to filopodia tips. However, loss of CETN2 binding to MYO15-2 disrupted the condensation reaction from occurring at filopodial tips, resulting in dissociation of the puncta during retrograde flow.
Conclusions: The MYO15-CETN2 complex can form membrane-less, solid-like phase condensates in vivo. Additionally, active concentration of the MYO15-CETN2 complex is a key condition required for the phase condensation reaction to proceed. We argue that a MYO15-CETN2 phase condensate contributes to the formation of the stereocilia tip density.

2. CIB2 is an Auxiliary Subunit of the Met Channel in Cochlear Hair Cells Regulating Channel Transport and Properties
Category: Hair Cells: Anatomy and Physiology

Xufeng Qiu*[1], Xiaoping Liang[1], Christopher Cunningham[1], Michele Pucak[1], Guihong Peng[1], Ye-Hyun Kim[1], Amanda Lauer[1], Ulrich Mueller[1]
[1]*Johns Hopkins Medical Institution*

Background: Mechanoelectrical transduction (MET) at hair cells converts mechanical stimuli into electrical signals, which is critical for sound perception. Hair cells are highly polarized with F-actin based stereocilia

protruding from the apical hair-cell surface. Sensory channels locate near the tips of shorter stereocilia are responsible for MET. Several proteins have been identified as integral components of the MET machinery including TMC1, TMC2, TMIE and LHFPL5. Recent studies have shown that the Calcium and Integrin binding protein 2 (CIB2) interacts with TMC1 and affects MET. Here we have investigated the mechanisms by which CIB2 regulates MET.

Methods: We used CRISPR to make Cib2 mutant mouse lines. Whole mount immunostaining in either acute dissected cochlea or cultured explants was applied to study the localization of TMC and CIB2 proteins in hair cells. Additionally, we used whole cell patch clamp recordings with stiff probe or fluid jet stimuli applied to hair bundle to record MET currents. Rescue experiments were performed by injectoporation of cultured cochlear explants.

Results: Taking advantage of TMC1-HA and TMC2-MYC knock-in mice, we detected the localization of endogenous TMC1 and TMC2 proteins in cochlear hair cells and showed that neither TMC1 nor TMC2 localize to stereocilia in CIB2 knock-out mice. In addition, we overexpressed TMC1 and TMC2 in CIB2 knock-out hair cells using injectoporation, and confirmed that TMC proteins cannot traffic to stereocilia in the absence of CIB2. Next, we engineered mouse lines carrying point mutations in Cib2 linked to deafness and showed those mutants differentially affect TMC1 and TMC2 localization to hair bundles and impair MET. Finally, detailed functional studies showed that CIB2 proteins carrying point mutations linked to deafness affect channel resting open probability but not adaptation, indicating that CIB2 directly affects MET channel function.

Conclusions: Our results show that CIB2 is required for the normal transport of TMC proteins into stereocilia and that it directly affects MET channel function within stereocilia.

3. Mechanotransduction in the Lateral Line is Radically Different From Cochlear Hair Cells
Category: Hair Cells: Anatomy and Physiology

Elias Lunsford*[1], Yuriy Bobkov[2], James Strother[2], James Liao[2]
[1]University of Florida, [2]University of Florida, The Whitney Lab for Marine Bioscience

Background: Hair cells fundamentally function by exchanging ions across their membrane to convert physical motion into electrical signals. Cochlear hair cells are bathed in an electrogenically maintained endolymph (K+ ~ 150 mM) to facilitate this exchange, whereas the homologous hair cells of superficial neuromasts in the zebrafish lateral line are naturally exposed to a freshwater environment (0.02 mM). Despite the stark difference in external media, their transduction mechanisms are assumed to both be driven by an inward K+ current. Freshwater K+ concentrations are not high enough (10-20 mM) to drive K+ influx into lateral line hair cells, yet zebrafish respond to fluid motion. It has been suggested lateral line hair cell activation is facilitated by a K+ rich microenvironment in the gelatinous cupula, however the mechanisms are unknown. By leveraging the zebrafish hair cell system, we took a multidisciplinary approach to characterize the ionic microenvironment surrounding the hair cell transduction zone and offer an alternative mechanism of depolarization in categorically freshwater.

Methods: The permeability of the larval zebrafish (5-7 dpf) cupula was quantified by measuring the movement of a negatively charged small molecule fluorophore through the gelatinous structure. We then systematically characterized the internal and external ionic environment using ion selective probes (K+, Na+, Ca2+, H+) to determine an ion gradient. We performed functional calcium imaging of hair cells and electrophysiological recording of afferent neurons during cupula deflection in media of varying ionic composition to determine the ions necessary for hair cell depolarization. Our findings motivated us to search neuromast single cell transcriptome data for over expression of alternative ion channels. Pharmacological and immunohistochemistry experiments verified the functional role and location of these channels within the hair cell.

Results: The cupula was exceedingly diffusive and there was no evidence of an ion gradient within the cupula. Functional calcium imaging and electrophysiology demonstrated hair cell depolarization persisted in cation deficient saline. Analysis of transcriptome data uncovered hair cell specificity in the expression profile of an abundant chloride (Cl-) calcium activated channel, ANO2. Hair cells depolarized when exposed to an ANO2 agonist and immunohistochemistry revealed Cl- associated channels localized in the stereocilia.

Conclusions: Our findings provide the first evidence of anion efflux to drive hair cell activation in freshwater. We maintain that by investigating zebrafish hair cell signal transduction in natural conditions has revealed Cl- efflux as a novel mechanism of mechanotransduction. These findings connect seemingly disparate sensory systems and may provide fundamental insight into the evolution of signal transduction. Anion efflux has previously been established as an efficient mechanism of signal amplification in olfactory and gustatory receptor neurons. We reason that chloride efflux may be a transduction mechanism across sensory modalities adapted to compensate for exposure to variable environmental conditions.

4. Single-Cell RNA Sequencing Reveals Age-Related Patterns of Expression of Hereditary Deafness Genes in Outer Hair Cells
Category: Hair Cells: Anatomy and Physiology

Miles Klimara*[1], William Walls[1], Ranum Paul[2], Tucker Trefz[1], Cameron Vannoy[1], Richard Smith[1]
[1]*University of Iowa Hospitals and Clinics,* [2]*Children's Hospital of Philadelphia*

Background: Age of onset of hearing loss is highly variable. A potential contributor to this variability may be the expression pattern of hearing loss genes over time. We sought to test this hypothesis using single-cell RNA sequencing (scRNA-Seq) to study gene expression in outer hair cells (OHCs) in the murine organ of Corti.
Methods: OHCs were isolated from cochlear tissue harvested from P15 and P70 mice maintained on a C3H/FeJ background using a manual micropipetting approach. scRNA-Seq was completed as we have described and differential gene expression was assessed using Model-based Analysis of Single-cell Transcriptomics (MAST) with Bonferroni correction for multiple tests.
Results: 65 P15 and 41 P70 OHCs were isolated for sequencing and passed all quality control filters. There were 8412 (mean) unique genes per cell, 310 of which were differentially expressed ($p < 0.001$). 11 genes associated with human hereditary hearing loss were differentially expressed, including Kmt2d ($p = 1.18 \times 10^{-10}$), Pjvk ($p = 1.21 \times 10^{-5}$), and Myo6 ($p = 6.0 \times 10^{-5}$). In addition, as compared to P15 OHCs, P70 hair cells showed greater expression of several genes involved in calcium binding, buffering and release, such as Ocm ($p = 1.5 \times 10^{-6}$), Cabp2 ($p = 1.2 \times 10^{-19}$), and Sri ($p = 0.0001$).
Conclusions: Over the time spectrum, the transcriptional profile of OHCs changes, with changes in expression of several deafness-associated genes and upregulation of several calcium-associated genes. Further studies clarifying the temporal patterns of gene expression in cochlear hair cells may provide insight into the biology of hearing and deafness and the mechanisms underlying age-related changes in cochlear function.

5. Repair of Noise-Induced Damage to Stereocilia F-Actin Cores is Facilitated by XIRP2
Category: Hair Cells: Anatomy and Physiology

Elizabeth Wagner[1], Jun-Sub Im[1], Maura Nakahata[1], Terrence Imbery[1], Sihan Li[1], Daniel Chen[1], Jung-Bum Shin*[1]
[1]*University of Virginia*

Background: Prolonged exposure to loud noise has been shown to affect inner ear sensory hair cells in a variety of deleterious manners, including damaging the stereocilia core. The damaged sites can be visualized as "gaps" in phalloidin staining of F-actin, and the enrichment of monomeric actin at these sites, along with an actin nucleator and crosslinker, suggests that localized remodeling occurs to repair the broken filaments.
Methods: Confocal microscopy and immunofluorescence were used to detect damage to stereocilia F-actin cores and the presence/absence of repair factors following noise exposures. Auditory brainstem response testing was used to measure hearing function.
Results: Herein we show that gaps in mouse auditory hair cells are largely repaired within one week of traumatic noise exposure through the incorporation of newly synthesized actin. Additionally, we report that XIRP2 is required for the repair process, as XIRP2 is enriched in gaps, Xirp2 knockout mice develop large numbers of gaps without exposure to loud noise, and gaps are not repaired following noise exposure in Xirp2 knockouts.
We found that XIRP2 interacts with monomeric actin and its LIM domain-containing C-terminus is required for the recruitment of gamma-actin to gaps, suggesting that gaps are not repaired in the absence of XIRP2 because actin repair factors are not recruited to damaged sites.
Finally, we showed that Xirp2 knockout mice are more susceptible to age-related and noise-induced hearing loss, potentially implicating gap repair in the preservation of hearing function.
Conclusions: Our study describes a novel process by which hair cells can recover from sub-lethal hair bundle damage and which may contribute to recovery from temporary hearing threshold shifts and the prevention of age-related hearing loss.

6. Structure-Function Analysis of Murine Inner Hair Cell Ribbon Synapses Using Optogenetics Combined With Electron Tomography
Category: Hair Cells: Anatomy and Physiology

Rituparna Chakrabarti[1], Lina Maria Jaime Tobon*[2], Loujin Slitin[1], Magdalena Redondo Canales[1], Gerhard Hoch[1], Marina Slashcheva[1], Elisabeth Fritsch[1], Kai Bodensiek[1], Özge Demet Özçete[3], Mehmet Gültas[4], Susann Michanski[1], Felipe Opazo[5], Jakob Neef[1], Tina Pangrsic[1], Tobias Moser[1], Carolin Wichmann[1]

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Background: Cochlear inner hair cells (IHCs) display remarkable rates of synaptic transmission. However, the precise mechanisms of synaptic vesicle (SV) release at the ribbon synapse remain unclear. Hypothesis of compound fusion vs uniquantal release of SVs have been mainly addressed using electrophysiological approaches. Yet, ultrastructural analysis of IHC ribbon synapses during defined functional states has been limited by the poor time resolution of the available stimulation techniques. Here, we combined optogenetics with electron tomography to capture the ultrastructural correlates of exocytosis occurring within milliseconds.
Methods: We characterized the responses of murine IHCs expressing channelrhodopsin-2 to light pulses of diverse durations and irradiances by performing patch-clamp recordings of the IHC or of a contacting postsynaptic bouton. We then combined comparable light stimulation paradigms with high pressure freezing (HPF) in order to capture early events of release. Internal and external sensors strategically placed in the HPF machine allowed us to precisely determine the length of the light pulse for each freezing. We studied ultrastructural changes in the membrane-proximal (MP) and ribbon-associated (RA) pools of SVs after short (~17-25 ms) and long (~48-76 ms) stimulation paradigms.
Results: Short light pulses of 10 ms at 7-16 mW/mm2 sufficiently depolarized the IHC to trigger exocytosis at individual synapses. Longer light pulses and/or higher light irradiances triggered sustained depolarizations with enduring release. Ultrastructurally, light stimulation brought the MP SVs closer to the membrane of the active zone and to the presynaptic density that anchors the ribbon. Particularly, we found a significant increase in the number of docked SVs, yet we did not observe omega-profiles or hemifusion states of SVs. Additionally, we did not observe morphological correlates of homotypic and or/ compound fusion of SVs, including largely unchanged numbers and diameters of MP and RA SVs upon light stimulation.
Conclusions: Our findings contrast the ultrastructural processes observed in conventional and retinal ribbon synapses, in which stimulation triggers a drastic decrease in docked SVs and an increase in the presence of omega-profiles. Whether the accumulation of docked SVs under our stimulation paradigms reflect "kiss-n-run", transient docking and/or limited clearance of the release site, remains to be determined.

7. The Role of MYO7A Isoforms in Tuning Hair Cell Function
Category: Hair Cells: Anatomy and Physiology

Sihan Li*[1], Andrew Mecca[2], Jeewoo Kim[1], Guisy Caprara[2], Elizabeth Wagner[1], Ting-Ting Du[1], Jonathan Bird[3], Anthony Peng[2], Jung-Bum Shin[1]
[1]*University of Virginia, [2]University of Colorado, [3]University of Florida*

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 the molecular motor that tensions the MET complex. We further discovered that MYO7A isoforms with unique N-terminal extensions are differentially expressed in inner and outer hair cells (IHCs and OHCs), 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: 5' RACE and RT-PCR was performed to identify MYO7A isoforms expressed in hair cells. Isoform-specific MYO7A deletion or affinity tagged mouse lines were generated by using CRISPR/Cas9. 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. ABRs and DPOAEs were measured to test hearing. Vestibular functions will be analyzed using animal pose estimation algorithms based on deep learning.
Results: Two isoforms of MYO7A are reported in the genomic database: a widely-studied canonical isoform (MYO7A-C) and an uncharacterized short isoform (MYO7A-S). Using 5'RACE, we identified an additional isoform with a unique N-terminal extension (MYO7A-N). To study the expression and functional differences of MYO7A isoforms, we generated mouse lines in which MYO7A isoforms are genetically deleted or tagged. Immunofluorescence analyses of these mice indicated that IHCs predominantly express MYO7A-C, and much lower levels of MYO7A-N. In OHCs, MYO7A-C and MYO7A-N are expressed in opposing gradients along the

tonotopic axis. The deletion of MYO7A-S did not affect overall MYO7A levels in the cochlea. Thus, we conclude that MYO7A-C and MYO7A-N are the major isoforms in the auditory system.

To further elucidate the function of MYO7A in auditory hair cells, we performed patch-clamp experiments on Myo7a-ΔC hair cells. Our results showed a significant reduction of resting open probability in IHCs, consistent with the proposed role of MYO7A in generating tip-link tension. Next, we will characterize the MET properties of hair cells in which the MYO7A-N isoform is specifically deleted. Finally, we will test whether MYO7A isoforms differ in their intrinsic motor properties, by measuring ATPase activity and in vitro sliding motility of recombinant head/neck domains of the three MYO7A isoforms.

Conclusions: Our studies reveal an unexpected isoform diversity of MYO7A in the cochlea, and highlight their essential roles in tensioning the MET complex. The differential expression of MYO7A isoforms with distinct motor properties might contribute to the tonotopic gradient of tip-link tension in OHCs, with potential importance for establishing the remarkable frequency range of mammalian hearing.

8. The Functional Role of Connectors in Outer-Hair-Cell Hair Bundles
Category: Hair Cells: Anatomy and Physiology
Zenghao Zhu*[1], Anthony Ricci[1], Daibhid O Maoileidigh[1]
[1]Stanford University

Background: Our hearing relies on outer hair cells, which amplify sound-induced vibrations in our ears. Each hair cell has a hair bundle composed of stereocilia, "hair-like" structures protruding from the cell's apical surface. Two classes of links link stereocilia, gating springs and connectors, also known as top or shaft connectors, side, lateral, or ankle links. Gating springs link neighboring stereocilia of different heights, while connectors link all neighboring stereocilia. Sound-induced gating-spring oscillations open and close mechanoelectrical transduction channels attached to the gating springs, causing oscillations in the hair cell's sensory current. High-intensity sound breaks gating springs, causing hearing loss. In contrast to gating springs, connectors are not attached to channels, and their functional role is unclear. We hypothesize that connectors facilitate the reformation of broken gating springs by limiting hair-bundle splaying, in which neighboring stereocilia separate.

Methods: To determine the role of connectors, we use a computational model of an outer-hair-cell hair bundle, which accounts for fluid forces on stereocilia, channel dynamics, and hair-bundle splaying. The model reproduces many experimental observations, including hair-bundle stiffness decreases caused by breaking gating springs or connectors, the hair-bundle damping decrease caused by breaking connectors, and the hair-bundle deflection caused by breaking gating springs.

Results: The model shows that increasing connector stiffness decreases the sensory current in response to oscillatory stimulation at the characteristic frequency of the hair cell and decreases hair-bundle splaying. If connectors are not sufficiently stiff, however, breaking the gating springs causes too much splaying for them to reform – neighboring stereocilia are further apart than the length of the gating spring's extracellular component, the tip link.

Conclusions: We find that outer hair cells benefit from connectors, which facilitate gating-spring reformation, but the cost is a decrease in their responses to stimuli.

9:30 a.m. - 11:30 a.m.
Symposium #34

Insights From Naturalistic Stimuli for Models of Human Speech Perception
Chair: Aysha Motala, *University of Western Ontario*

Using Naturalistic Audiovisual Stimuli to Understand Sensory Encoding in Pediatric Intracranial Electrophysiology
Liberty Hamilton, *The University of Texas At Austin*

Intracranial recordings in patients with intractable epilepsy have strongly advanced our understanding of speech perception in the brain. However, many tasks used in research experiments are not particularly interesting for patients to perform and require repeated presentations of stimuli. Here, I describe efforts by our lab to understand the development of neurophysiological responses to speech in children undergoing surgical monitoring for epilepsy. By combining controlled stimuli with more naturalistic and engaging annotated movie clip stimuli, we

show the potential that naturalistic tasks have to engage younger participants and improve knowledge of speech representations in the developing brain.

An Event-Based Hierarchy of Cortical Speech Prediction
Lea-Maria Schmitt, *University of Lübeck*

The comprehension of speech is proactive. With the advent of naturalistic experiments and machine learning, we have started to understand how the brain exploits semantic context of speech to inform predictions. Here, I present a study (N = 34) mapping hemodynamic responses onto prediction errors derived from artificial neural networks that predict upcoming words in a story at multiple timescales of context. We find a surprisal hierarchy in temporo-parietal cortex, with surprisal at longer timescales represented in parietal regions. Critically, updates to context representations are made only at event boundaries, thereby positing a computationally sparse architecture of predictive processing.

Using Pupillometry and Dual-Task Paradigms to Examine Highly-Intelligible Speech Materials
Drew McLaughlin, *Washington University in St. Louis*

Transcription tasks, in which the outcome measure is typically a proportion of keywords correctly transcribed, are widely used in speech perception research to examine effortful listening. However, this approach places constraints on experiment designs, requiring that stimulus intelligibility is not at ceiling. Thus, for comparing listening effort for highly-intelligible speech materials, an alternative approach is necessary. In the proposed talk, I will present a set of experiments that use pupillometry and dual-task paradigms to examine listening effort for highly-intelligible, naturalistic speech. Discussion will focus on the benefits of each methodological approach, and applications for other topics in speech perception research.

Generalizable EEG Encoding Models From Naturalistic Audiovisual Stimuli
Maansi Desai, *University of Texas at Austin*

Speech perception involves integrating multiple sensory cues, including both auditory and visual information. In this talk, we show that both highly controlled sentence stimuli without noise and acoustically rich audiovisual movie trailer stimuli can be used to model electroencephalography (EEG) responses to acoustic and phonological features. Furthermore, receptive fields derived from movie trailer stimuli generalize to more controlled data sets, such as speech sentences used in typical sensory neuroscience experiments. This work demonstrates how audiovisual movies can uncover similar tuning for acoustic and phonetic information while allowing investigation of complex scenarios including speech in noise and audiovisual integration.

Neural Markers of Speech Comprehension
Marlies Gillis, *KU Leuven Department of Neurosciences, ExpORL*

Recent evidence suggests when listening to natural speech, our brain responds to linguistic characteristics of speech, such as word surprisal. These responses are seen over and beyond responses to acoustic properties of speech, suggesting linguistic characteristics might be markers of speech comprehension. Currently, we are focussing on (a) the reliability of this neural marker for speech understanding and (b) whether the marker is robust in different clinical populations. Preliminary results indicate this neural marker is robust over different story content and speakers in normal-hearing participants. If this extends across different populations, it will allow a behavior-free evaluation of speech comprehension.

Exploring the Impact of Context and Intelligibility on the Neurophysiological Encoding of Natural Narrative Speech
Shyanthony Synigal, *Department of Biomedical Engineering, University of Rochester*

The use of continuous speech allows listeners to predict various features of incoming words and to use context to understand what is being said. These strategies are especially beneficial in noisy environments. This talk will discuss the use of electroencephalography and computational modeling to investigate the processing of clean and degraded speech. I will show how acoustic and linguistic speech feature encoding may vary with noise and

describe how contextual manipulations can alter how one processes different speech representations. Lastly, I will discuss how our work may lead to tools that can assess deviations in perceptual processing in clinical populations.

Attention Modulated Neuro-markers Extracted from Listening to Continuous Speech Stimuli
I.M Dushyanthi Karunathilake, *University of Maryland - College Park*

Selectively attending to speech in a noisy environment is crucial in day-to-day interactions and strongly depends on the extent to which one can filter relevant from irrelevant information. Continuous speech offers many advantages over simpler speech stimuli in understanding how the brain processes speech in complex environments, including the ability to separately estimate the time locked response contributions from both attended and unattended components of an auditory scene. Using Magnetoencephalography (MEG) to scan older and younger participants selectively attending to one speaker while ignoring a competing speaker in minute-long passages, we investigate how attended and unattended speech streams are represented in the cortex, using both stimulus reconstruction and temporal response function (TRF) methods. Estimated TRFs show three prominent peaks (M50TRF, M100TRF, and M200TRF) representing distinct cortical processing stages, at corresponding early (~50 ms), middle (~100 ms) and late (~200 ms) latencies. Results show that reconstruction accuracy, and M100TRF, and M200TRF TRF peak amplitudes, are stronger for attended speech compared to unattended speech in both age groups. Interestingly, TRF peak latencies can show different trends at the different processing stages: at the early processing stages both streams are processed with similar latencies, but for the late peak, attended speech is processed for a longer duration compared to the unattended speech. We show the feasibility of real-time tracking of these neuro-markers in continuous speech to investigate attentional build-up over time in a competing speaker environment. Such neuro-markers could be employed to assess attention-mediated hearing deficits, including those arising from age-related hearing impairment. Perhaps counterintuitively, older adults exhibit exaggerated TRF peak amplitudes and reconstruction accuracies compared to younger adults, likely due to different mechanisms at the different latencies. Differences between the attended and unattended TRF peak latencies also depended on age group: for older adults the unattended M200TRF peak was much earlier than attended M200TRF. Taken together, these findings are promising in that they can be used to decode the attentional state of the listener and show promise in understanding the neural mechanisms underpinning attentional mediated hearing deficits.

Exploring Shared Neural Synchronisation During Story Listening With Acoustic Masking
Aysha Motala, *University of Western Ontario*

Spoken speech is engaging, meaningful and follows a narrative. Speech comprehension in noise, however, is challenging, recruiting brain regions and networks beyond those recruited during comprehension of clear speech. Using fMRI, we explore brain regions functionally recruited to aid the comprehension of masked speech during naturalistic story listening. Listening to actively engaging narratives has been found to drive highly synchronised activity, with the extent of synchronisation dependant on engagement and measured as voxel-wise intersubject correlation (ISC). We use ISC to quantify responses to naturalistic speech and discuss advantages of using engaging stories to study the brain substrates of effortful listening.

9:30 a.m. - 11:30 a.m.
Podium Session #35 – Hearing Loss: Damage and Protection
Moderators: Marlan Hansen, M.D. & Suhrud Rajguru, Ph.D.

1. ERK 1/2 Inhibitor AZD-0364 Mitigates Noise-Induced Hearing Loss in Mice
Category: Inner Ear: Damage and Protection
Richard Lutze*[1], Matthew Ingersoll[1], Daniel Kresock[1], Emma Malloy[2], Tal Teitz[1]
[1]*Department of Pharmacology and Neuroscience, Creighton University School of Medicine,* [2]*Creighton University School of Medicine*

Background: Ten Percent of the world population experiences hearing loss from noise, aging, antibiotics, and chemotherapy, yet there are currently no Food and Drug Administration (FDA)-approved drugs to prevent any type of hearing loss. Our lab has recently shown that the mitogen-activated protein kinase (MAPK) pathway is activated with noise and cisplatin-induced hearing loss and demonstrated that dabrafenib, a BRAF kinase

inhibitor, protects against both insults in vivo while administered at clinically relevant doses. Here, we tested the molecular target ERK, a central node of the MAPK pathway, that to the best of our knowledge has not been targeted directly before for mitigating noise-induced hearing loss.

Methods: FVB mice age 6-8 weeks were used in all experiments. Auditory Brainstem Response (ABR) was recorded 7 days before noise challenge as the baseline hearing measurement. Mice were exposed to 100 dB sound pressure level (SPL) at 8-16 kHz octave band for 2 hours. Mice were then treated with AZD-0364, a third generation highly specific ERK 1/2 inhibitor, 24 hours after noise exposure. Mice were treated with 25 mg/kg, 5 mg/kg, 0.5 mg/kg, or 0.1 mg/kg of AZD-0364 twice a day for 3 days. ABRs were recorded again 14 days after noise exposure and threshold shifts were determined. The different treatment groups were carrier alone, AZD-0364 alone, noise alone, and AZD-0364 plus noise.

Results: Oral delivery of AZD-0364 mitigated noise-induced hearing loss in adult mice when delivered 45 minutes before noise exposure, indicating it most likely crosses the blood-labyrinth barrier and enters the inner ear. Mice were then treated with AZD-0364 24 hours after noise exposure and similar protection was shown compared to the mice that were pretreated with the compound. Furthermore, dose response of AZD-0364 in mice revealed that protection from noise-induced hearing loss occurred with a dose of AZD-0364 as low as 0.5 mg/kg body weight. Mice treated with 0.5 mg/kg of AZD-0364 24 hours after noise exposure had an average decrease in ABR threshold shifts of 22 dB at 8 kHz and 23 dB at 16 kHz. No toxicity was observed with all doses of AZD-0364 tested as measured by weight and behavior of the animals. The therapeutic index of AZD-0364 is at least 50 when given to FVB mice to protect from noise-induced hearing loss. Ongoing studies include quantifying the mice Organ of Corti Ctbp2 protein in the inner hair cells after noise exposure and AZD-0364 treatment, to examine for better function in synaptic ribbons where inner hair cells form synaptic contacts with neuronal fibers.

Conclusions: These results show that inhibiting ERK alleviates noise-induced hearing loss in vivo effectively, and AZD-0364 is a promising therapeutic candidate for noise-induced hearing loss.

2. Risks of Noise-Induced Hearing Loss During Cochlear Implant Insertion – Effects of Electrode Orientation

Category: Inner Ear: Damage and Protection

Nathaniel Greene*[1], Carolyn Chabuz[1], Kenny Rodriguez[1], Joseph Gonalez[1], John Peacock[1], Renee Banakis*[2], Stephen Cass[1]
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Background: Cochlear implants (CIs) have been an effective treatment for the profoundly deaf for decades and are increasingly offered to patients with residual (low frequency) acoustic hearing. This remaining acoustic hearing correlates with improved hearing outcomes; unfortunately, a large subset of patients lose this residual hearing either immediately or some time after CI implantation. Several mechanisms have been investigated as sources of the acute loss of residual hearing. In a previous study we showed that the presence of a CI array in the cochlea does not appear sufficient to cause the observed hearing losses, although stiffening of the round window due to the CI presence has been previously implicated.

Our group has identified a novel mechanism in recent reports involving generation of high amplitude pressure transients in the inner ear during CI insertion that may be sufficiently loud to cause noise induced hearing loss. It appears that these transients are present during typical scala-tympani insertions whenever the CI electrode contacts the cochlear wall, but it is not clear whether other factors may contribute. That prior work quantified electrode insertion depth; it remains unclear whether the orientation of the electrode shaft within the scala impacts pressures.

Methods: To determine whether electrode position and orientation within the scala impact generation of intracochlear pressure transients, cadaveric human heads were surgically prepared with a mastoidectomy and extended facial recess. Fiber-optic pressure sensors were inserted into the scala vestibuli and scala tympani near the oval and round windows to measure intracochlear pressures. CI electrodes (including both straight and perimodiolar styles) were inserted via a round window approach under time-synced fluoroscopy.

Results: CI electrode insertions produced pressure transients in the cochlea up to 160-170 dB SPL equivalent, consistent with more typical insertions reported in previous studies. The electrode position within the cochlea, design-related electrode dynamics, and poor surgical technique were associated with increased rates of insertion errors and pressure transients. Results suggest that appropriate 'soft' surgical techniques can minimize acoustic exposure during CI surgery.

Conclusions: Results suggest that appropriate 'soft' surgical techniques can minimize acoustic exposure during CI surgery. Furthermore, the implications of surgical tool use, electrode design, and anatomical considerations will be discussed.

3. Durability and Lubricity of Anti-Fouling and Anti-Fibrotic Thin Film Zwitterionic Hydrogel Coating for Cochlear Implantation

Category: Inner Ear: Damage and Protection
Nir Ben-Shlomo*[1], Adreann Peel[2], Douglas Bennion[1], Ryan Horne[1], Allan Guymon[2], Marlan Hansen[1]
[1]*University of Iowa Hospitals and Clinics,* [2]*University of Iowa Chemical and Biochemical Engineering*

Background: After cochlear implantation, many patients experience significant decrease in hearing outcomes due to intracochlear inflammation and fibrosis. Factors contributing to inflammation include damage due to insertional forces and the immune system's foreign body response (FBR) to inserted biomaterials. Thin film zwitterionic hydrogels (ZH) of sulfobetaine methacrylate (SBMA) polymers demonstrate favorable, ultra-low biofouling surface properties shown to decrease FBR and fibrosis both in vitro and in vivo. The following is an investigation assessing lubricity, durability, and feasibility of applying thin film zwitterionic hydrogel coatings for human cochlear implantation.

Methods: Tribometry was used for measuring friction coefficients along polydimethylsiloxane (PDMS) with and without ZH coating. Flat discs of uncoated and ZH-coated PDMS were submerged in saline and contact with the measurement probe was maintained at a constant speed while 1-Newton and 5-Newton normal forces were applied. Additional ZH-coated discs were tested without saline bath until friction force reached final plateau (failure). PDMS sheets coated with ZH underwent flexibility testing via mandrel bend curling around rods with progressively decreasing diameter (32mm to 2mm) with failure catalogued as visible or audible cracking of hydrogel coating. To assess impacts of desiccation, time to failure on mandrel bend was determined by bending on 5mm diameter rod every 5 minutes until fracture. Seven ZH coated mid-scala cochlear implant electrode arrays were implanted into one of four cadaveric temporal bones drilled with facial recess approach and subsequently explanted. Electrode arrays were dipped in 0.5% fluorescein solution for 5 minutes and coating integrity was assessed microscopically for defects pre- and post-implantation.

Results: Mean coefficient of friction (μ) of ZH was significantly lower than bare PDMS with both 1-Newton ($\mu=0.036$ coated vs $\mu=0.117$ bare PDMS, $p<0.015$) and 5-Newton normal forces ($\mu=0.056$ coated vs $\mu=0.218$ bare PDMS, $p<0.001$). Lubricity was maintained for an average 46.1 minutes before desiccation and plateau. No samples failed mandrel bend flexibility testing when ZH coating was hydrated at all diameters. Repeated bending on 5mm diameter rod failed after 65 minutes of desiccation. All electrode arrays were uniformly coated pre-implantation. After explantation, coating was incomplete in the tip region of one electrode. Coatings remained intact throughout the lengths of all remaining electrode arrays despite mechanical and sheer forces incurred with surgical insertion and explantation.

Conclusions: Thin film ZH have anti-fouling properties shown to decrease the host FBR associated with implanted biomaterials. Thin film ZH coatings are durable and flexible and can accommodate bending to the appropriate cochlear diameter without fracture. Hydrated ZH coatings increase lubricity and can decrease frictional insertion forces as compared to bare electrode arrays. The ZH coating is able to withstand mechanical forces encountered during cochlear implantation and the coating integrity is maintained after insertion and explantation into human cochleae via surgical approach.

4. A 'Double-Insult' Model of Cochlear Implantation in Noise-Exposed Cochleae

Category: Inner Ear: Damage and Protection
Christine Mei*[1], Rachele Sangaletti[2], Samantha Rincon Sabatino[3], Hillary Snapp[4], Michael Hoffer[5], Curtis King[6], Suhrud Rajguru[7]
[1]*University of Miami Miller School of Medicine,* [2]*Department of Otolaryngology, University of Miami,* [3]*University of Miami, Department of Biomedical Engineering,* [4]*University of Miami,* [5]*University of Miami School of Medicine, University of Miami Ear Institute,* [6]*Lucent Medical System,* [7]*Department of Otolaryngology and Biomedical Engineering, University of Miami*

Background: Noise-induced hearing loss (NIHL) is a global health burden resulting from thepathophysiological impact of noise overexposure, afflicting approximately 16% of the worldwide population. NIHL is the second most common form of sensorineural hearing loss and is increasingly recognized as a unique cause of inner ear trauma. At present, cochlear implants (CIs) are often the treatment for profound sensorineural hearing loss with more recently developed electroacoustic stimulation CIs (EAS-CIs) aimed at patients with residual low frequency hearing. However, CI surgery also results in a significant trauma to the inner ear that damages residual hearing (including a delayed loss of residual hearing). While effects of NIHL and CI trauma have been characterized

separately, phenotypes and mechanisms underlying CI trauma in patients with prior noise related damage to the peripheral auditory system remain to be detailed. Here, we developed a preclinical rodent model of the accumulated pathophysiology of the cochleae with high-level noise exposures that subsequently undergo CI surgeries. Our overall goal is to test the efficacy of mild therapeutic hypothermia (mTH) during CI surgery in this 'double-insult model' for the protection of residual hair cells, synaptic elements, and spiral ganglion neurons. **Methods:** Twenty juvenile Brown Norway rats were exposed to high level noise (110-120 dB, 1-2 hours, broadband noise). CIs were implanted one month after the noise trauma. Half the animals received CI under normothermic conditions while the other half received CI with mTH treatment. mTH treatment was applied with a one-hour cooling protocol using a patented probe and Peltier device during CI insertion. Auditory brainstem responses (ABR) were recorded prior to the noise exposure, post-noise exposure for one month, and post-CI at multiple time points up to one month. Histology was performed at the conclusion of experimentation. Statistical analysis of ABR analysis and histologic findings was performed comparing control and experimental groups. **Results:** Different levels of noise trauma generated temporary and permanent threshold shifts up to 28 days. Hearing threshold shifts, damage to hair cells, synaptic elements, and SGN counts allowed for characterization of the 'double-insult' group and efficacy and safety of the hypothermia treatment. **Conclusions:** Functional and anatomical outcomes in this model can generate new knowledge underlying pathology and mechanisms in patients and develop novel potential therapeutic interventions to preserve residual hearing.

5. Chronic Suppurative Otitis Media Causes Macrophage Associated Sensory Hearing Loss
Category: Inner Ear: Damage and Protection

Anping Xia*[1], Anthony Thai[1], zhixin Cao[2], Xiaohua Chen[3], Jing Chen[1], Brian Bacacao[1], Laurent Bekale[1], Paul Bollyky[1], Peter Santa Maria[1]
[1]*Stanford University*, [2]*Shandong Provincial Hospital Affiliated to Shandong First Medical University*, [3]*The First Affiliated Hospital of Zhengzhou University*

Background: Chronic suppurative otitis media (CSOM) is the most common cause of permanent hearing loss in children in the developing world. The mechanism of sensor hearing loss in CSOM has not been well studied, with most reports analyzing post-mortem human specimens or acute infection models that are not representative of human CSOM. In this report, we assess cochlear injury in a validated pseudomonas aeruginosa (PA) CSOM mouse model. **Methods:** In this report, we assess cochlear injury in a validated pseudomonas aeruginosa (PA) CSOM mouse model **Results:** We observed outer hair cell loss predominantly in the cochlear base at 14d after inoculation. Macrophages, not neutrophils are the major immune cells in the cochlea in CSOM displaying increased numbers and a distribution correlated with the observed cochlear injury. Their morphological changes occurred from monocytes into tissue macrophages following inflammation. PA did not enter the cochlear and live bacteria are required for cochlear injury. We also show abroad range of cytokines were upregulated actively in the CSOM cochlea. **Conclusions:** Taken together, this data shows a critical role for macrophages in CSOM-mediated sensory hearing loss.

6. Engineering Efferent Feedback to Protect Hearing
Category: Inner Ear: Damage and Protection

Yuanyuan Zhang[1], Hakim Hiel[2], Philippe F.Y. Vincent[3], Megan Wood*[1], Ana Belen Elgoyhen[4], Wade Chien[5], Paul Fuchs[6]
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Background: Previous studies have shown that hearing loss is exacerbated in mice lacking the hair cell acetylcholine receptor ligand-binding subunit, α9, and is mitigated by a gain-of-function variant, α9L9'T (Taranda et al., 2009; Boero et al., 2018; Boero et al., 2020). That is, stronger efferent feedback protects hearing. The present effort utilizes viral transduction of α9L9'T to restore efferent protection to α9 knockout mice.

Methods: α9L9'T cDNA was incorporated into a modified AAV (AAV2.7m8, Dalkara et al., 2013), shown to provide efficient transduction of cochlear hair cells (Isgrig et al., 2019). This was injected in combination with AAV2.7m8 expressing green fluorescent protein (GFP). Virus (1.3 µl, 1x1013 viral particles) was injected unilaterally into the posterior semi-circular canal of 0-to-2-day old (P0-P2) α9 knockout mice (C57BL/6J background). The auditory brainstem response (ABR) was measured at P28, followed at P30 by exposure to 90 dB SPL white noise for 2 hours. ABRs were again recorded 1 and 14 days after trauma.

Results: The efficacy of viral injection was shown initially by GFP expression, replicating the broad pattern of expression seen previously. Expression of the α9L9'T transgene was assessed by labeling hair cell AChRs with Cy3- conjugated RgIA-5727, a modified Conus peptide that binds to and blocks α9-containing AChRs with high affinity (Ellison et al., 2006; Fisher, Zhang, Vincent et al., 2021). Outer hair cells uniformly exhibited synaptic Cy3 puncta by P24.

Without viral injection, α9 knockout mice had ABR thresholds elevated by ~30 dB from 8 to 32 kHz one day after noise exposure (n=24) that was reversed at 14 days. Equivalent threshold shifts were found for mice injected with GFP-expressing virus only. A9 injected animals showed a temporary threshold shift of ~ 10 dB for higher, but not lower, frequency tones one day after noise exposure (n=29). Threshold at 8 and 12 kHz was unchanged, and smaller threshold shifts occurred at 16, 24 and 32 kHz. Two-way ANOVA comparing the combined α9L9'T virus plus GFP virus injection to GFP virus only for experimental condition across frequencies gave $p<0.0001$ ($F = 28.27$, $DFn = 1$, $DFd = 234$). That is, hearing loss was reduced as a specific condition of α9L9'T transduction.

Conclusions: Viral transduction of a gain of function AChR in cochlear hair cells mitigates the impact of noise-induced hearing loss. This strategy could provide prophylaxis for those whose family history or genetic make-up predict early onset age-related hearing loss. More generally, long-lasting enhancement of efferent protection could be a complementary therapy wherever acoustic exposure exacerbates hearing loss. A compelling argument is that efferent activity is itself regulated by the acoustic environment. Thus, genetic enhancement of efferent inhibition leverages the intrinsic protection of the inner ear.

7. Investigating the Impact of Synonymous and Missense Variants in SLC26A4 on RNA-Splicing
Genetics B: General

Joseph Chin*[1], Hela Azaiez[2], Kevin T Booth[3], Richard Smith[4]
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Background: Variants in SLC26A4 are a common cause of autosomal recessive non-syndromic hearing loss associated with an enlarged vestibular aqueduct (EVA) and Pendred syndrome. Within this subset of persons with hearing loss (HL), in ~15-30% of cases, biallelic mutations in SLC26A4 are not identified, suggesting the presence of an undetected second pathogenic variant. We hypothesized that in some cases, the second variant is a splice-altering synonymous or missense change.

Methods: We completed in vitro splicing assays on eight variants of uncertain significant that recur in persons with HL and EVA using a pre-constructed pET01 Exontrap vector (MoBiTec) encoding a 5' and 3' exon separated by a multiple cloning site.

Results: Two pathogenic SLC26A4 variants were identified. The first variant, a chr7:107323721:C>T (NP_000432.1:p.Val280Val) variant results in exon skipping. The second variant, a chr7:107329499:T>C (NP_000432.1:p.Phe335Leu) variant results in partial exon skipping.

Conclusions: In the absence of biallelic pathogenic or likely pathogenic variants, the impact of presumed benign synonymous and missense variants on splicing must be considered in persons with SLC26A4-related hearing loss.

8. COL6A5 Modifies Severity and Progression of Hearing Loss in TECTA Mutant Mice
Category: Genetics B: General

Monique Weaver*[1], Kevin T Booth[2], Hela Azaiez[1], Guy P Richardson[3], Richard JH Smith[1]
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Background: The tectorial membrane (TM) is a narrow band of extracellular matrix lying above the sensory hair cells of the organ of Corti. Its morphology and mechanical properties are changed by mutations in genes encoding

TM proteins, the consequence of which is hearing loss. One such gene, TECTA, encodes alpha-tectorin, the major non-collagenous component of the TM. Variants in TECTA are implicated in both autosomal dominant (DFNA8/12; ADNSHL) and recessive (DFNB21; ARNSHL) non-syndromic hearing loss.

Methods: To characterize DFNA8/12 TECTA-related hearing loss, we studied strain-specific variation in two Tecta mouse mutants (TectaC1619S and TectaC1837G) on FVB/NJ, CBA/J and C57BL/6 backgrounds.

Results: Auditory brainstem responses (ABRs) of one-month-old heterozygous mice showed background-dependent hearing loss, and by six months of age, variability was also noted in its progression. Specifically, hearing in FVB/NJ.TectaC1619S mice was worse in the middle frequencies and progressed over a six-month period as compared to hearing in CBA/J.TectaC1619S mice; and FVB/NJ.TectaC1837G mice had poorer hearing thresholds than C57BL/6.TectaC1837G mice. To identify possible genetic modifiers underlying these differences, we filtered whole genome sequence data on FVB/NJ, CBA/J and C57BL/6 mice for differences in TM genes. Variants' rank-ordering identified differences based on CADD and SIFT deleteriousness prediction scores. Four variants were predicted to be damaging by both tools—three of which are in Col6a5. We confirmed COL6A5 expression in P3 and P15 mice by RT-PCR and in P10 mice by immunofluorescence. We then investigated possible effects of this putative modifier by crossing FVB/NJ and CBA/J strains and measuring ABR thresholds of the F2 generations at one month of age. Mice homozygous for the Col6a5 haplotype originating from the FVB/NJ background had increased hearing thresholds as compared to littermate controls homozygous for the CBA/J haplotype, replicating the strain-specific hearing loss pattern observed in the parental generation.

Conclusions: Using CRISPR editing, we have generated two Col6a5 mutants—an out-of-frame deletion that results in a null allele and a missense variant–to further refine our understanding of the role of COL6A5 in auditory function. We anticipate that identification of TECTA modifiers may facilitate the development of personalized treatments for individuals with TECTA-related deafness.

9:30 a.m. - 11:30 a.m.
Podium Session #36 – Of Pitch and Space
Moderators: Nathan Higgins, D.Phil. & Erol Ozmeral, Ph.D.

1. Pitch Perception of Complex Tones: Predictions Based on the Neural Fluctuation-Place Model
Category: Psychoacoustics

Laurel Carney*[1], Afagh Farhadi[2], Braden Maxwell[3]
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Background: Most stimuli used to study pitch perception elicit complex temporal responses in auditory-nerve (AN) fibers. AN responses tuned near harmonics are dominated (or "captured") by a single component; a response to a single component has consistent peak amplitudes over time and relatively small fluctuations at f0. In contrast, responses of fibers tuned between harmonics reflect beating of multiple components that pass through realistically broad physiological filters over a wide range of levels. Beating of multiple components occurs even at low characteristic frequencies (CFs), where relatively sharp filtering near threshold has motivated models based on resolution of harmonics. Across the population of AN fibers, the amplitude of f0 fluctuations in responses varies in a manner that encodes pitch. We refer to this pattern of f0-related fluctuation amplitudes as the neural fluctuation-place (NFP) model. The pattern of NFs across CFs is transformed into a rate-place representation at the level of the midbrain because inferior colliculus neurons (IC) are sensitive to fluctuations of their ascending inputs. Here, we tested the ability of the NFP model to encode pitch over wide ranges of sound level and f0. The NFP model extends naturally to phenomena such as pitch-shifts perceived in response to inharmonic tone complexes or other paradigms, such as pitch carried by high-frequency harmonics in background noise.

Methods: The NFP profiles were studied using models for AN fibers and IC neurons with band-suppressed (ICBS) tuning to amplitude-modulated tones. ICBS neurons are excited by unmodulated stimuli and suppressed by modulations. Thus a population of ICBS neurons has increased rates for CFs near harmonic frequencies (where inputs are 'captured' and have relatively flat, or unmodulated responses) and decreased rates for CFs between harmonics (where the inputs fluctuate at f0, or in response to background noise). An estimate of f0 was derived from the pattern of average rates across the ICBS population using a harmonic pattern recognizer (Goldstein et al., 1978, JASA 63:486).

Results: Estimates of f0 based on average-rate profiles across a population of model ICBS neurons were accurate across a wide range of f0s and levels. The harmonic pattern recognizer strategy was originally developed to analyze AN average-rate profiles, but AN rates saturate at moderate to high SPLs. In contrast, the pattern of AN fluctuations, and thus the rates at the level of the ICBS cells, is robust over a wide range of sound levels. Interestingly, background noise enhances NF profiles by increasing fluctuations in the responses of fibers tuned between harmonics.

Conclusions: The NFP model for pitch is based on realistic tuning and nonlinear properties of AN fibers and IC neurons. This model overcomes limitations of classical models for pitch based on rate-place or temporal fine structure representations. Comparison to other models for pitch will be discussed.

2. Psychometric and Subcortical Neurometric Measures of Temporal Discrimination in Rhesus Macaques
Category: Other

Chase Mackey*[1], Samantha Hauser[1], Namrata Temghare[2], Ramnarayan Ramachandran[2]
[1]*Vanderbilt University,* [2]*Vanderbilt University Medical Center*

Background: Temporal envelope fluctuations are ubiquitous in nature and serve as critical cues for perception of complex sounds. While psychophysical sinusoidal amplitude modulation (SAM) processing tasks have evaluated the perceptual salience of SAM features, and neurophysiological studies have described the transformation of neural representations from early temporal to later rate-based code, few studies have used the same metric in each domain to establish explicit neuron-behavior links. Further, studies typically use rodents, which exhibit lower perceptual and neuronal AM sensitivity than primates, and are often are limited to detection paradigms that do not provide a complete picture of AM sensitivity. To establish psychometric-neurometric relationships in a primate model, we recorded responses to SAM noise and compared derived neurometric measures in the cochlear nucleus (CN) and inferior colliculus (IC) to psychometric measures of modulation frequency (MF) discrimination of nonhuman primates (NHPs).

Methods: Two NHPs (Macaca mulatta) performed a reaction-time Go/No-Go lever-release MF discrimination task. Standard signals were 500 ms, 76 dB SPL, 20 Hz SAM broadband noise (BBN) bursts presented diotically in free-field. Probe stimuli were same as standard or differed pseudorandomly from the standard in MF by 2-64 Hz. Psychometric functions were fit with Weibull cumulative distribution functions (cdfs) to extract psychometric threshold and slope. Neurophysiological responses were obtained to unmodulated noise and SAM BBN (MF = 2-1024 Hz in 1/3 octave steps) in the CN and IC of two awake NHPs. MTFs were constructed from firing rate (FR) and vector strength (VS) for each neuron. Receiver operating characteristic analysis was used to calculate the discriminability of MF along sloping portions of the FR-based and VS-based MTFs near the best MF of each neuron, construct neurometric functions, and to extract neurometric threshold and slope.

Results: Psychometric thresholds to discriminate modulation frequencies (∂MF) were 5-7 Hz, consistent with data in humans and NHPs of the same species. IC and CN neurons in our sample often exhibited tuned responses to SAM BBN. In ~30% of CN neurons, FR-based thresholds could not be extracted; both FR- and VS-based thresholds could be extracted in most neurons. Neurometric thresholds in the CN and IC spanned a large range (2-200 Hz ∂MF). FR- and VS-based neurometric thresholds in the CN were comparable, and were comparable with FR-based thresholds in IC. VS-based neurometric thresholds in the IC most closely matched psychometric thresholds. The most sensitive half of IC neurons had thresholds similar to psychometric thresholds.

Conclusions: The present findings provide the first direct comparison of subcortical neurometric and psychometric measures of temporal discrimination in NHPs, corroborate evidence of transformations in SAM processing, and extend theories of the subcortical neural basis of SAM perception to primates. These data can inform future NHP studies of noise-induced hearing loss.

3. Beyond Encoding Space – Neuronal Spatial Tuning in the Auditory Cortex
Category: Primary Auditory Cortex

Yi Zhou*[1], Vergil Haynes[1], Sharon Crook[1]
[1]*Arizona State University*

Background: Understanding the organizing principles of sensory cortices is fundamental to a theory of how coordinated neural activities support the analysis of complex environments, (e.g., listening to individual voices in a noisy restaurant, identifying a familiar face in a crowd). Spatial hearing – the ability to localize a source based on the sounds it emits – is critical to navigating complex auditory scenes. In the auditory cortex (ACx), location-sensitive neurons have been widely reported, but their spatial selectivity is not organized across cortical areas in

any way we can currently identify. Intracellular measurements reveal that both excitatory and inhibitory synaptic inputs to the cortex exhibit spatial tuning (Kyweriga et al. 2014). This suggests that refinement of input selectivity contributes to heterogeneous location selectivity in auditory cortex – but for what computational purposes? We investigated the mechanisms of spatial tuning of single neurons in ACx for sound source encoding when the acoustic environment contained multiple sounds presented from different locations.

Methods: We recorded neural activity (single-unit spikes and local field potentials) in the auditory cortex of two adult marmoset monkeys. We investigated the spatial-location selectivity of neurons over the full 360o horizontal plane. The spatial functions of a neuron were measured based on its responses to best-frequency tones and broadband noises (100-500 ms duration). The changes in neurons' spatial tuning were then evaluated when additional broadband noise stimuli was presented, following the Target (random location) +Masker (fixed location) paradigm. Two temporal arrangements of the additional noise stimuli (Masker) were considered: (1) M simultaneously presented with T and (2) M preceding T by 200-ms.

Results: We found that cortical neurons across cortical layers show diverse spatial selectivity with respect to the peak direction and width of their spatial tuning. While the spiking activities of many cortical neurons showed hemifield preference (front, back, left, and right), the non-responsive regions were actively inhibited. When additional sound was presented, spatial tuning was less affected by maskers that preceded the target than those that were presented simultaneously. However, the extent of spatial release from masking was affected by the onset/sustained patterns of the masker responses and the extent of cortical inhibition/suppression in both target and masker responses. In a subset of neurons, spatial tuning could change its peak direction and tuning width, maintaining the heterogeneous nature of spatial selectivity at the population level.

Conclusions: The observed results suggest that neuronal spatial tuning in the auditory cortex is an important response feature, playing a fundamental role in segregating multiple sound sources.

4. Location-Specific Facilitation in Marmoset Auditory Cortex
Category: Binaural Hearing and Sound Localization

Chenggang Chen*[1], Xiaoqin Wang[1]
[1]*Johns Hopkins University*

Background: It has been well established that responses of neurons in auditory cortex are influenced by stimulus context. Contextual modulations can occur in spectral, temporal or spatial domain. However, comparing to spectral and temporal contextual effects, much less is known on spatial contextual effects in auditory cortex.
Methods: In this study, we explored how spatial contextual modulations evolve over time by stimulating neurons in awake marmoset auditory cortex with sequences of sounds either randomly from various spatial locations (equal probability mode) or repeatedly from a single location (high probability mode).
Results: To our surprise, instead of inducing adaptation as expected from well documented stimulus-specific adaptation (SSA) literature, repetitive stimulation in the high probability mode from spatial locations away from the center of a neuron's spatial receptive field evoked lasting facilitation observed by both extracellular and intracellular recordings from single neurons in auditory cortex. Nearly half of the sampled neuronal population exhibited this spatial facilitation, irrespective of stimuli type and visibility of the test speaker. Facilitation with longer duration occurred when the repetitive stimulation was delivered from speakers with firing rates ranked lower than the best speaker's firing rate under the equal probability mode. The extent of the facilitation decreased with decreasing presentation probability of the test speaker. Interestingly, the induced facilitation did not change spatial tuning selectivity, tuning preference and spontaneous firing rate of the tested neurons.
Conclusions: Taken together, our findings revealed a location-specific facilitation (LSF) instead of SSA to repetitively presented sound stimuli which has not been observed in auditory cortex. This form of spatial contextual modulation may play an important role in supporting such functions as auditory streaming and segregation.

5. Decoding the Location of Attended Talker in a Spatial Multi-Talker Setting From Invasive Neural Signals
Category: Binaural Hearing and Sound Localization

Vishal Choudhari*[1], Prachi Patel[1], Stephan Bickel[2], Ashesh D. Mehta[2], Nima Mesgarani[1]
[1]*Columbia University,* [2]*Hofstra Northwell School of Medicine*

Background: The cocktail party is an environment consisting of simultaneous talkers. In such scenarios, hearing-impaired listeners can experience difficulty in attending to a specific talker in the presence of other interfering

talkers. Most cognitively-controlled hearing aids try to address this problem by decoding the attended talker from neural signals using auditory attention decoding (AAD) algorithms, separating the speech mixture into clean streams and selectively amplifying the attended speech stream. In most auditory attention decoding (AAD) algorithms, a representation (envelope or spectrogram^) of the attended speech is reconstructed from neural signals and compared with the speech representations of the talkers in an acousitc scene. The decoded attended talker is chosen as one whose speech representation yields the highest correlation with the reconstructed speech representation.

As talkers in a cocktail party environment are often spatially separated, a supporting approach for an AAD algorithm could be decoding the location where attention is directed, i.e., the location of the attended talker. Recent deep learning-based multi-channel speech separation algorithms preserve location information (interaural cues) in the separated binaural speech streams. The attended location decoded from neural signals can then be compared with the locations of talkers (estimated from their binaural speech streams) to improve the attended talker decoding accuracy.

Methods: We recorded from seven epilepsy patients using depth electrodes (sEEG) as they listened to spatial multi-talker speech stimuli generated using head-related transfer functions (HRTFs). Two parallel speech streams (one male, one female) arrived at the subject from two locations at different azimuthal angles: -45 degrees (front-left) and +45 degrees (front-right). For every block of trials, subjects were asked to attend to a pre-specified talker (male or female) while the locations of the speakers swapped pseudorandomly between trials.

Results: We find electrodes whose steady-state high-gamma mean response levels (MRLs) are modulated by attention to a talker location. Such electrodes have significantly higher steady-state high-gamma MRLs when attending to a talker on the contralateral side than the ipsilateral side. Attended location can be decoded on a trial-by-trial basis significantly above chance levels. Attended location can also be decoded on a window-by-window basis significantly above chance levels, with a window duration as small as 0.5 seconds. Decoding accuracies improve with an increase in window durations.

Conclusions: The ability to reliably decode attended location suggests a potential for improving the performance of existing AAD algorithms by incorporating spatial information. It also encourages investigation of AAD algorithms that are computationally simple, consume less power and work in real-time for deployment on battery-operated hearing aids.

6. Exploring the Use of Auditory Nerve and Brainstem Electrophysiology to Improve Spatial Hearing in Children Using Bilateral Cochlear Implants
Category: Binaural Hearing and Sound Localization
Angela Fung*[1], Jaina Negandhi[2], Alan Blakeman[2], Robel Alemu[1], Sharon Cushing[1], Blake Papsin[1], Karen Gordon[1]
[1]*University of Toronto, Hospital for Sick Children,* [2]*Hospital for Sick Children*

Background: The study aims to improve spatial hearing in children using bilateral cochlear implants (CIs) by: 1) defining levels at which left and right CI input are best matched in the auditory system, and 2) assessing if matched levels will then promote binaural integration. If asymmetric sound input can be identified and corrected using objective measures, particularly for children who cannot provide feedback about their hearing, this may improve access to spatial/binaural cues early in development. We hypothesized that: 1) levels where electrically-evoked auditory nerve responses (ECAPs) from each CI are amplitude-matched indicate matched bilateral input, and 2) these levels predict a higher degree of binaural integration, resulting in decreased bilaterally-evoked auditory brainstem responses (BLeABRs) and a perception of balanced input.

Methods: ECAPs and BLeABRs were collected in 9 children (mean(SD) = 12.7(2.9) years of age, with 4.6(5.3) years of bilateral use). Matched input levels were first chosen by comparing the ECAPs measured on each side during implant surgery. BLeABRs were recorded across inter-implant Level Differences (ILDs) from these "centre levels" at 0, ±4, ±8, and ±12 clinical units (CU). This process was conducted for 3 different electrode pairs along the CI array (3, 9, and 18). Participants completed a behavioural lateralization task using the same ILD stimuli, where they indicated if they perceived the sound on the left or right of their head. Similar responses collected in a previous cohort (n=9, mean(SD)= 5.3(3.5) years of age) were also analyzed. In this cohort, ILDs were larger (±10, ±20 CU) and only collected at apical CI electrodes.

Results: Levels evoking equal unilateral ECAP responses predicted decreases in BLeABR amplitude at the apical electrodes when including large ILDs (±20 CU) ($R^2 = 0.098$, p= 0.0012). This correlation was not significant for responses evoked by mid-array or basal electrode pairs. Bilateral EABR amplitudes did not change significantly across the narrower ILD range (up to ±12 CU). Lateralization of bilateral input within the ±12 CU ILD range was

highly variable, and balanced perception did not consistently occur at ILD=0 as defined by matched ECAP amplitude, nor when compared to matched ECAP thresholds.

Conclusions: In this study, children with bilateral CIs showed poor sensitivity to narrow ranges of ILDs as measured at the auditory nerve, brainstem and with behavioural lateralization. Better correspondence between matched ECAP levels and binaural EABR at wider ILDs, and at apical electrodes only, suggest a limit of sensitivity to ILD in the auditory brainstem of children with bilateral CIs and/or a limited sensitivity of the electrophysiological measures, particularly at pathways stimulated by more basal cochlear areas. The high variability in behavioural measures of ILD also indicates an overall impaired binaural processing of ILDs in these children, which cannot be ruled out.

7. The Dynamics of Head Movement During a Digit Detection and Localization Task
Category: Binaural Hearing and Sound Localization
Nathan Higgins*[1], Erol Ozmeral[1], Angkana Lertpoompunya[1], David Eddins[1]
[1]*University of South Florida*

Background: In difficult listening environments the auditory system relies on variety of tools to separate competing sound sources. Binaural spatial cues and monaural spectral cues based on head-related transfer functions play critical roles in this process. For those to be useful and reliable cues for segregating sound sources, they must be integrated with a larger proprioceptive system that incorporates head position and head movements to maximize the chance of separating different auditory objects.

Methods: Head tracking data were collected while participants detected digits embedded in a stream of non-digit words presented from one of thirteen possible loudspeaker locations. Participants fixed their head location at 0° to begin each trial but were free to move their head upon sound onset, and were tasked with pressing a button upon detection of a digit. Additional variables included four different signal-to-noise ratios (SNRs) of speech stream to babble background. Data were collected from normal-hearing (NH) and hearing-impaired (HI) individuals, with and without hearing aids for both groups. The maximum head-movement velocity was calculated for each trial for trajectories corresponding to yaw, roll, and pitch.

Results: In easy listening environments (i.e., larger SNRs), participants-initiated yaw- head-movements towards the source-location approximately 500 to 1000 ms before the button press that indicated a digit was detected. In difficult listening conditions (i.e., poorer SNRs), yaw- head-movements were initiated 500 to 1000 ms after the button press. This pattern was observed in both normal hearing- and hearing-impaired listeners, with and without hearing aids. In a separate analysis, the 30 participants were split into three even groups corresponding to small-intermediate- and high-pitch movers (based on average pitch velocity). The group of high-pitch movers was a mix of NH and HI participants. In difficult SNR conditions, high pitch-movers performed significantly better on the digit detection task than intermediate or small pitch-movers. The difference in performance between groups on the digit detection task was not observed in the aided condition.

Conclusions: For easy versus difficult SNRs, the timing difference in yaw head-movement initiation relative to time-to-button-press might indicate a behavioral pattern characterized as a "wait for more information" strategy rather than an "active searching pattern," potentially interpreted as a need for head-stability for accurate collection of spatial information. In the pitch dimension, high-pitch movers gained a performance advantage for digit detection compared to low-pitch movers. Disruption of this advantage due to the presence of hearing aids might indicate that these listeners potentially 1) have heightened sensitivity to binaural or monaural cues compared to low-pitch movers or 2) have developed a strategy to maximize one or more spatial cue, evidenced by this pitch movement (e.g. head tilting).

8. Relationship Between Persistence and Listening Effort in Younger and Older Listeners
Category: Hearing Loss: Consequences and Adaptation
Sridhar Krishnamurti*[1], Susan Teubner-Rhodes Earp[1]
[1]*Auburn University*

Background: Older adults report greater listening effort than younger adults, especially for degraded speech [1]. Individuals facing effortful listening during time-compressed (i.e., rapid) speech may resort to persistence, the ability to exert effort to overcome difficulty [2], as a top-down processing strategy. Indeed, persistence predicts neural activity in the dorsal anterior cingulate cortex for errors when recognizing speech in background noise [2]. Pupil dilation is an objective measure that can track the listening effort associated with speech intelligibility for young, middle-aged, and older adults [3-4]. Because the pupil response reflects cortical inputs to the autonomic

nervous system, increases in pupil dilation indirectly measure the attention system's response to increasing task demands [5]. Our study investigated effects of age and persistence ability on listening effort during pupillometry for TCS.

Methods: The present study examined how age and persistence affect accuracy and pupil dilation (i.e., listening effort) during recognition of Time Compressed speech (TCS) in healthy younger and older adults. We report results from 13 younger adults aged 18-35 years and 6 older participants aged 60-80 years. Participants completed background questionnaires to obtain demographic information and were screened for mild cognitive impairment. They completed verbal working memory and processing speed assessments, and the Wisconsin Card Sorting Task-64 [6] to assess individual differences in shifting and persistence. Participants underwent a vision screening and a standard audiological assessment. Then, they completed a speech recognition task. On each of 36 trials, participants listened to and repeated 4 words presented at time-compression rates of 0% (normal speech), 30% (rapid speech), and 60% (very rapid speech). Pupil diameter was measured during listening using Micromedical Video Nystagmography goggles, in an illumination-controlled environment.

Results: There were no statistically significant effects of age group (young versus old) for 0% TCS {$F_{(1, 15)}$= 3.28; p=0.09); 30% TCS {$F_{(1, 7)}$= 0.026; p=0.87); 60% TCS {$F_{(1, 15)}$= 3.15; p=0.09). Accuracy on the speech recognition task was used as a covariate to account for possible effects of aging. We found no significant interaction effects for 60% TCS but time*accuracy interactions for 30% TCS and time*age-group interactions for 0% TCS.

Time Compression significantly affected pupil dilation ($F_{(1.03, 8.22)}$ = 6.86, p = .03) and this effect interacted significantly with persistence ($F_{(1.03, 8.22)}$ = 8.18, p = .02) and time window ($F_{(1.44, 11.50)}$ = 5.47, p = .03).

Conclusions: In individuals with higher persistence, pupil dilation increased linearly from 0% to 30% to 60% compression, with peak dilation occurring 2-3 seconds after trial onset. In contrast, individuals with lower persistence exhibited maximal pupil dilation in the 30% condition, peaking 4-5 seconds after trial onset. Younger and older individuals with lower persistence exhibited a drop-off in a physiological index of listening effort as speech rate rises.

Wednesday, February 9, 2022

12:00 p.m. – 2:00 p.m.
Glenis Long Memorial Symposium

Celebrating Glenis Long: Linking Acoustics, Psychoacoustics, and Otoacoustic Emissions
Pim Van Dijk, *University Medical Center Groningen*

Glenis R. Long (1943-2021) dedicated her career to the study of auditory function in a variety of species using psychoacoustic and otoacoustic tools. Over her career, Long also trained many students and built several productive research collaborations. This symposium will highlight the work of Long's various trainees and collaborators in an homage to the friend and mentor that Long was. One underlying theme of Long's work was the meticulous examination of the intricacies of cochlear function in general and mechanics in particular from various points of view. Specializations of the auditory system that allow precise echolocation in bats will discussed to represent work conducted by Long earlier in her career. In the domain of human hearing, the use of classic psychophysics to discern the properties of the cochlear amplifier and nonlinearity will be featured in other talks. The discovery and exploration of the microstructure in hearing thresholds will be presented as a pivot to the use of otoacoustic emissions to study cochlear function. The groundbreaking work done by Long and colleagues to understand otoacoustic emission generation and propagation will be connected to other presentations in which models of cochlear mechanics are leveraged to design and interpret phenomenological studies on the influence of various stimuli, age, and pathologies on otoacoustic emissions. In a final category of presentations efforts to understand the development and maturation of the auditory system using otoacoustic emissions will be discussed. Taken together the symposium will highlight the benefits of examining the cochlea using a variety of tools from a variety of points of view, an approach that Glenis Long used extremely profitably throughout her career. This collection of talks will celebrate the work started by and with Long while forecasting the next generation of questions to be asked and investigations to be performed.
Linda Hood, *Vanderbilt University Medical Center*

Psychoacoustics of Horseshoe Bat Frequency Hyperacuity and Its Extraordinary Window Into Cochlear Mechanics
James Simmons, *Brown University*

Echolocating horseshoe bats (Rhinolophus) make fine frequency adjustments of their tonal ultrasonic broadcasts to compensate for upward Doppler shifts in biosonar echoes by locking them onto an internal reference frequency. Behavioral tests done by Glenis Long established the reality of correspondingly acute frequency discrimination and sharp auditory tuning at the frequency of echoes. The cochlear mechanisms that create exceptionally narrow tuning reveal a degree of evolutionary ingenuity that is yet another example of how biosonar pushes the limits of auditory science.

Synchronization as a Key Mechanism Underlying Auditory Processing?
Bastian Epp, *Technical University of Denmark*

There is broad agreement that the auditory systems is a highly efficient, non-linear and active system that enables communication also in noisy environments. Active hair cells in the inner ear of amphibians show complex dynamic behaviour, including effects of clustering and entrainment to external stimuli. Despite potential differences in biophysical properties can various phenomena connected to mammalian hearing be connected to entrainment effects in the inner ear and beyond. Following Glenis' footsteps, I will collect findings from modelling, psychoacoustics and physiology that indicate the relevance of synchronization in auditory information processing.

Swept a Long: Measuring Otoacoustic Emissions Using Continuously Varying Stimuli
Christopher Shera, *University of Southern California*

At the 2004 Midwinter Meeting of the ARO, Glenis Long and colleagues introduced a method for measuring distortion-product otoacoustic emissions (DPOAEs) using primary-tone stimuli whose instantaneous frequencies vary continuously with time. (At the same meeting, Stephen Neely described a method for continuously varying the primary levels.) In contrast to standard OAE measurement methods, in which emissions are measured in the sinusoidal steady state using discrete tones of well-defined frequency, the swept-tone method sweeps rapidly across frequency, often at rates exceeding 1 oct/s. The resulting response waveforms are then analyzed using an appropriate filter (e.g., by least-squares fitting or heterodyning). Although introduced as a convenient way of evaluating DPOAE fine structure by separating the total OAE into "generator" and "reflection" components, the swept-tone method has since been extended to other emission types in a diverse population of subjects and has proved an efficient and valuable tool for probing cochlear mechanics. One day---a long time coming---swept tones may even find their way into the audiology clinic.

Long Tones From the Ear: Spontaneous Otoacoustic Emissions and Their Perception
Pim Van Dijk, *University Medical Center Groningen*

Spontaneous otoacoustic emissions are faint tones generated in the ear. Their frequency coincides with local minima in the audiogram, as extensively studied by Glenis Long. A near threshold tone becomes audible over the intensity range for which it also starts to synchronize the spontaneous otoacoustic emission. This suggests that the inner ear vibration produced by the SOAE generator provides the actual stimulus to the hair cells and subsequent stages of the auditory system. This talk will highlight Glenis's work regarding the perceptual effects associated with spontaneous emissions, and will outline recent work showing the relation between tone detection and emission synchronization.

Otoacoustic Emissions and the Medial Olivocochlear Reflex
Linda Hood, *Vanderbilt University Medical Center*

Otoacoustic emissions, measured in the presence of an additional stimulus, provide an objective, non-invasive window into the auditory efferent system. The medial olivocochlear reflex (MOCR) allows insight into normal properties and characteristics of disorders that involve auditory neural pathway function. Study of the MOCR presents challenges, requiring the thorough and meticulous approaches like those consistently applied by Glenis Long in her research. It is hoped that it is not too "Long" into the future before MOCR assays have broad

application in the evaluation of auditory efferent function in persons across the lifespan in both clinical and research settings.

Glenis Long as the "Impedance Matcher": Translating Basic Science to Clinical Applications
Beth Prieve, *Syracuse University*

Glenis Long's unbounded curiosity about auditory science fueled her enthusiasm for collaboration with basic scientists across disciplines, as well as clinical researchers. Glenis operated as an "impedance matcher", translating the language of physicists and mathematicians to that familiar to clinical scientists and clinicians. Glenis was eager to extend the use of her laboratory's invention of measuring DPOAEs using primaries swept in frequency over time for clinical uses. This presentation focuses on data using the sweeping technique in adult ears with hearing loss and infants.

Friday, February 11, 2022

8:00 a.m. - 10:00 a.m.
Workshop - Translational Delivery Approaches for Inner Ear Therapies

Translational Delivery Approaches for Inner Ear Therapies
Chair: Peter Steyger, *Creighton University*

Recent advances in understanding the mechanisms involved in inner ear diseases have accelerated the development of therapeutic approaches for hearing loss, tinnitus and other hearing disorders. Despite these remarkable advances, progress towards new treatments remains limited, in large measure because of the physiological barriers hindering delivery of drugs, gene and cells to the inner ear. Local delivery methods have been developed with the goal of avoiding systemic side effects and low dosage in the targeted organ. Intratympanic injection is currently the main translational delivery technique. With the rapidly growing field of gene- and cell-therapy for hearing disorders, other innovative solutions for direct delivery to the inner ear are required, while still being efficient and fully translational.
This workshop will provide an interactive opportunity to discuss the challenges and opportunities of inner ear delivery technology, and emerging approaches toward safe and efficacious dosing of emerging therapeutic compounds. We will cover the different aspects of inner ear delivery, from promising therapeutic strategies to small molecules and cell therapy. It will aim to clarify the translation of delivery approaches used in preclinical animal models to clinical trials in patients. Discussion will focus on practical aspects, including the experience of local delivery in the middle and inner ear in preclinical animal models, with a focus on preclinical delivery case studies and challenges for delivery in humans.
The workshop will be chaired by Professor Peter Steyger, Creighton University, and co-chaired by Dr. Rami Tzafriri, CBSET. Speakers will be scientific experts from companies working to develop hearing therapies including Otonomy, Sensorion, and CILcare, with an ENT Surgeon-scientist from Academia.
This 60-minute workshop is open to all. We encourage open participation and questions from attendees. Please feel free to submit your questions or research examples in advance. To do so please contact: gaelle.naert@cilcare.com

Delivery Approaches to the Inner Ear Used in Animal Models
Gaëlle Naert, *CILcare*

Intratympanic delivery has become a common method used in animal models and in human clinical trials. To overcome physical limitations of the inner ear, innovative approaches have been developed to enable the repeated or long-lasting delivery of drugs in the middle ear using different formulations, while trying to minimize injury to the eardrum. In addition, a direct delivery in the inner fluid is necessary for some therapies, requiring surgical procedures and specific devices.
This presentation will provide an overview of the delivery methods used in animal models and innovative strategies developed to counteract the complex nature of the inner ear.

Factors in Intratympanic Drug Delivery for the Inner Ear
Bonnie Jacques, *Otonomy Inc.*

Intratympanic injection is an effective and minimally invasive means to deliver therapeutics ranging from small molecules to complex biologics to treat inner ear disease. Several factors should be considered in developing an optimized approach for intratympanic administration where the goal is to provide a suitable pharmacokinetic profile enabling appropriate drug action on the target cells. Tailoring the formulation characteristics to the unique properties of the therapeutic can optimize the delivery for therapeutic benefit as well as ensure safe delivery via the middle ear. These issues will be discussed using examples from Otonomy's programs for the treatment of inner ear disorders.

Local Delivery of Inner Ear Gene Therapies
Laurent DESIRE, *Sensorion*

Gene therapy holds great promise for the treatment of congenital or later-onset hearing loss and for restoring audition in monogenic disorders. The number of preclinical successes and clinical trials is increasing. However, the field is facing translational challenges towards clinical practice implementation, for which safe and efficient delivery of gene therapy products are needed and hampered by the inner ear's unique anatomy and sensitivity. Adeno-associated virus (AAV) is a frequently used gene-delivery vehicle, owing to its lack of pathogenicity, persistence and variety of useful serotypes. Here, we will discuss current approaches for optimized local delivery of inner-ear AAV gene therapies in preclinical studies.

Challenges in Delivering Novel Therapeutics to the Inner Ear in Humans
Ronald Pennings, *Radboud University Medical Center*

Inner ear therapy is an exciting new field of research and innovative treatments for sensorineural hearing loss are rapidly progressing along the translational pathway towards clinical testing for safety and efficacy in humans. Despite the current progress, still limited experience exists on how to best deliver these novel therapeutics to the inner ear. This presentation focuses on the clinical aspects of drug delivery in humans to treat sensorineural hearing loss. An overview of current approaches for delivering novel inner ear therapeutics to the human inner ear are presented, along with the challenges that may occur during clinical trials that evaluate safety and efficacy.
Rami Tzafriri

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

gEAR Workshop - Gene Expression Analysis Resource
Chair: Ronna Hertzano, *University of Maryland School of Medicine*
Co-Chair: Joshua Orvis, *Institute for Genome Sciences*

The gEAR, gene Expression Analysis Resource (umgear.org), is a cloud-based 'one-stop-shop' for viewing and analyzing inner ear-related multi-omic data, without requiring programming skills. With numerous multi-omic datasets organized in thematic profiles, it's easier than ever to learn about inner ear-related gene expression. Whether you are a long-term gEAR user or completely new to the gEAR, you'll have the opportunity to learn everything from the gEAR basics to the newest features. Based on last year's success, this year we will again feature multiple breakout rooms hosted by gEAR experts, allowing users to choose which skills to learn based on their individual needs and familiarity with the gEAR. Topics covered will include: basic gEAR skills, Analysis - basic skills, Analysis - advanced, Dataset curation and upload, and visualization of epigenetic data.

gEAR Workshop - Gene Expression Analysis Resource
Yang Song, *Institute for Genome Sciences*

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gEAR Workshop - Gene Expression Analysis Resource
Beatrice Milon*, University of Maryland Baltimore*

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gEAR Workshop - Gene Expression Analysis Resource
Kevin Rose*, University of Maryland, Baltimore*

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gEAR Workshop - Gene Expression Analysis Resource
Kathleen Gwilliam*, University of Maryland School of Medicine*

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11:00 a.m. – 12:30 p.m.
gEAR Advanced

gEAR Workshop – Gene Expression Analysis Resource
Chair: Ronna Hertzano*, University of Maryland School of Medicine*
Co-Chair: Joshua Orvis*, Institute for Genome Sciences*

The gEAR, gene Expression Analysis Resource (umgear.org), is a cloud-based 'one-stop-shop' for viewing and analyzing inner ear-related multi-omic data, without requiring programming skills. With numerous multi-omic datasets organized in thematic profiles, it's easier than ever to learn about inner ear-related gene expression. Whether you are a long-term gEAR user or completely new to the gEAR, you'll have the opportunity to learn everything from the gEAR basics to the newest features. Based on last year's success, this year we will again

feature multiple breakout rooms hosted by gEAR experts, allowing users to choose which skills to learn based on their individual needs and familiarity with the gEAR. Topics covered will include: basic gEAR skills, Analysis – basic skills, Analysis – advanced, Dataset curation and upload, and visualization of epigenetic data.

Yang Song, *Institute for Genome Sciences*
Beatrice Milon, *University of Maryland, Baltimore*
Kevin Rose, *University of Maryland, Baltimore*
Kathleen Gwilliam, *University of Maryland School of Medicine*

POSTER PRESENTATIONS

Genes Related to SNPs Identified by Genome-Wide Association Studies of Age-Related Hearing Loss Show Restriction to Specific Cell Types in the Adult Mouse Cochlea

Na Xue*[1], Lei Song[2], Qiang Song[3], Joseph Santos-Sacchi[4], Hao Wu[2], Dhasakumar Navaratnam[3]
[1]*Shanghai Ninth People's hospital,* [2]*Department of Otolaryngology-Head and Neck Surgery, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China,* [3]*Yale University School of Medicine,* [4]*Yale University School of Medicine, Surgery, Neuroscience, Cellular and Molecular Physiology*

Category: Aging
Background: ARHL has been thought to result from disordered hair cell function and their loss. ARHL has a significant genetic component. In this paper, we sought to explore the expression in the cochlea of genes that were associated with single nuclear polymorphisms (SNPs) implicated in ARHL.
Methods: The adult wild-type mice cochlea were dissected to perform single-cell RNA sequencing. By performing principal components analysis and clustering, 18 clusters were separated and using specific cell type markers, one cluster of hair cells two clusters of supporting cells, three clusters of stria vascularis cells, six clusters of fibrocytes, two clusters of erythrocytes, two clusters of macrophages/ monocytes, and two clusters of B-lymphocytes were identified. Then the SNPs related to age-related hearing loss that were identified by three GWAS papers were used and their expression patterns in cochlear cell types were ascertained.
Results: 1. Genes linked with common variant polymorphisms associated with ARHL are widely expressed cell types of the cochlea isolated by single-cell RNA sequencing. 2. There is variable gene expression in cochlea cell types that stratifies with ARHL risk, with genes associated with SNPs with low p values being preferentially expressed in hair cells. 3. GSEA identified shared genesets between Alzheimer's disease and ARHL suggesting common mechanisms. 4. Other genesets identified by GSEA include vesicle movement and apical polarity in hair cells and supporting cells suggesting mechanisms of ARHL.
Conclusions: Genes associated with SNPs with the highest significance were preferentially expressed highly in hair cells while genes associated with SNPs with a lower significance were expressed more universally. In addition, we find significant overlap with genesets associated with Alzheimer's disease suggesting shared mechanisms, and genesets enriched for apical cell polarity and vesicle recycling suggesting mechanisms of cell death/ dysfunction with aging.

The Effects of Aging on 2-Tone Tuning Curve Diversity in L2/3 of Mouse Primary Auditory Cortex

Katherine Maximov*[1], Jonah Mittelstadt[2], Ji Liu[2], Kanold Patrick O.[1]
[1]*Johns Hopkins University,* [2]*University of Maryland*

Category: Aging
Background: Presbycusis, or age-related hearing loss, is caused by changes in both the inner ear (periphery) and the central auditory system. Many of the peripheral structures that degrade with age have been identified and characterized, but there is still a dearth of information pertaining to what changes occur in the aging central auditory pathway.
Methods: To gain a better understanding of how basic features of the auditory cortex change with age, we performed in vivo 2-photon Ca2+ imaging on L2/3 of the auditory cortex of both young (n=6, 11-24 weeks old) and aged (n=6, 12-17 months old) mice that retain peripheral hearing well into old age (CBA/CaJ). We played two sets of auditory stimuli to characterize both frequency response areas (FRAs) and inhibitory sidebands. The first stimulus consisted of 16 pure tone frequencies between 4 kHz and 53.8 kHz evenly spaced on a logarithmic scale and allowed us to obtain FRAs. The second stimulus consisted of playing two tones, which when played together, allow us to characterize inhibitory sidebands which might sharpen neuronal tuning. We next performed PCA and k-means clustering to classify tuning curves based on their shapes (i.e. what frequencies and sound levels produced significant responses).
Results: In young animals, we found distinct classes of tuning curves, suggesting a variety in circuit makeup of excitatory and inhibitory inputs to different excitatory cells. In aged animals, we found decreased diversity and changes in shape of the classes of tuning curves.
Conclusions: These results suggest that aging causes changes in circuit organization of the central auditory pathway including A1. This work may help elucidate the central factors that contribute to behavioral deficits in presbycusis.

Ribbon Synapse Volume Gradients in Quiet-Aged Gerbils

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Category: Aging
Background: The Mongolian gerbil (Meriones unguiculatus) is a popular model for age-related hearing loss since they have a relatively short lifespan and their hearing range includes lower frequencies and is, therefore, similar to humans. Type I afferent auditory-nerve fibers are connected to inner hair cells via specialized ribbon synapses. These synapses comprise a presynaptic ribbon and postsynaptic glutamate receptor patch. In this study, we investigate whether the synapse volume gradients in quiet-aged gerbils differ from those found in young-adult gerbils.
Methods: Cochleae from quiet-aged gerbils between 34 and 41 months of age were collected and immunostained using a label for hair cells (anti-MyoVIIa), presynaptic ribbons (anti-CtBP2), and postsynaptic glutamate receptor patches (anti-GluA2). Whole mounts were examined at cochlear locations corresponding to 2, 16, and 32 kHz using confocal microscopy. Analysis is ongoing to include additional locations. Custom-written image analysis software was used to determine the location, number, and volume of pre- and postsynaptic elements. Functional synapses were defined by the colocalization of the pre- and postsynaptic immunolabels. Pre- and postsynaptic volumes were normalized to their respective medians per image stack.
Results: The number of functional synapses was significantly reduced in aged compared to young-adult gerbils. Most functional synapses were located in the bottom half of the inner hair cells. A volume gradient in pre- and postsynaptic elements along the modiolar-pillar axis was found. There was also a positive correlation between the volume of pre- and postsynaptic elements, with larger synapses being located on the modiolar side.
Conclusions: The reduction in the number of functional synapses in old compared to young-adult gerbils is consistent with previous findings (Gleich et al., Exp Gerontol. 2016; Steenken et al., Neurobiol Aging. 2021). The location of ribbon synapses within inner hair cells as well as volume gradients of synaptic elements matched those reported previously in young-adult gerbils (Zhang et al., Hear Res. 2018), indicating that either the volumes of synaptic elements do not change with age or that the volumes change uniformly (since absolute volumes cannot be reliably compared). In aging mice, an increase in the size of both modiolar and pillar ribbon synapses was found, potentially compensating for the decrease in synapse numbers that was also observed (Jeng et al., J Physiol. 2020). Our results in aging gerbils, together with previous results showing variations in these gradients across mouse strains (Reijntjes et al., Hear Res. 2020), suggest that these gradients do not contribute to either hearing in younger animals or age-related hearing loss.

Psychophysical and Electrophysiological Measures of Frequency Modulation Acuity: Effects of Age and Modulator Phase
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Category: Aging
Background: Aging, independent of hearing loss, is associated with degradations in temporal fine structure (TFS) processing. Importantly, TFS processing plays a role in recognition of speech in noise and spatial release from masking. One approach to studying TFS processing is via binaural measures. Prior work demonstrated that psychophysical thresholds for detecting low-rate frequency modulation (FM) on a low-frequency carrier depends on the interaural phase of FM; thresholds are lower when FM is out-of-phase across ears compared to in-phase. This binaural advantage is due to the added cue of dynamic binaural beats that are present in the out-of-phase condition, a cue that relies on TFS processing. To study the impact of aging on TFS processing, we measured the range of modulation rates over which the binaural benefit in FM detection is evident in order to determine its age-dependency. In addition, an electrophysiological analog of the binaural advantage was implemented to provide an objective measure against which to reference the psychophysical performance. The hypothesis was that aging is associated with a restricted frequency range of binaural benefit in both behavioral and electrophysiological measures.
Methods: Participants included young (18-30 yrs), middle-aged (40-55 yrs), and older (65-80 yrs) adults with normal or near-normal audiometric thresholds. FM detection thresholds were measured for modulation rates ranging from 4-32 Hz carried by a nominally 500-Hz, 65-dB SPL tone. Modulators were either in-phase or out-of-phase across ears. The electrophysiological acoustic change complex (ACC) was also measured for in-phase and out-of-phase frequency modulators carried by a continuous 65-dB SPL, 500-Hz tone for FM rates of 4-32 Hz.

Results: For all age groups, behavioral thresholds were relatively constant across FM rate for in-phase modulators. In contrast, FM detection thresholds were markedly lower for out-of-phase FM at low rates but rose with increasing FM rate to converge with in-phase thresholds at higher rates. In both in- and out-of-phase conditions, older adults exhibited higher thresholds than young adults. Electrophysiological results were broadly consistent with these patterns: For out-of-phase modulators, the amplitude of the response (N1-P2) diminished and the N1 latency increased as the rate of FM increased. ACC responses from older adults exhibited increased latency and decreased amplitude and dissipated at lower FM rates than in young adults.

Conclusions: All age groups received a binaural benefit in the psychophysical task for out-of-phase modulators at lower rates of FM, eventually converging with the in-phase conditions by 32 Hz. Similarly, the electrophysiological data mirrored the behavioral data in that ACC responses diminished as rates of FM increased. The increased thresholds in both in- and out-of-phase conditions in older adults, as well as the diminished ACC responses, suggests reduced binaural processing capacity associated with aging. In summary, the results are consistent with age-related degradations in temporal processing.

Acute Nicotine Administration Has No Effect on ABR Thresholds and Wave 1 Amplitudes in Young or Old Mice

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Category: Aging

Background: Aging causes a deterioration of auditory processing leading to impaired speech processing, social isolation, and cognitive decline. The age-related deficits can occur with minimal hearing loss, indicating central changes. Such central changes are not easily modifiable with hearing aids, leaving many elderly humans with limited options to improve auditory function. Age-related processing deficits may be tied to the reduction of high affinity nicotinic acetylcholine receptors (nAChRs). Therefore, nAChRs agonists, such as nicotine, may enhance sensory and cognitive processing by acting on the remaining receptors. Our previous studies with acute nicotine injection in aging FVB mice (model with minimal presbycusis) and EEG recordings showed considerable improvement with old mice responses becoming more similar to young mice responses. Previous research also found that non-smoking humans given nicotine showed significant changes in the auditory brainstem response (ABR) including reduced wave 1 amplitude, and slower latencies. Therefore, our previous results of nicotine's effect on EEGs in old mice may include a peripheral/early brainstem component. Therefore, here we tested if acute nicotine had an effect on ABR thresholds, amplitudes, and latencies in young and old mice.

Methods: The current study recorded click ABRs at sound levels between 20-90 dB SPL in both male and female young adult (Y) (1-3 months) and older adult (O) (13-19 months) FVB mice. Recordings were taken from anesthetized mice (ketamine/xylazine/acepromazine (80/10/1 mg/kg, i.p.) ~9-10 minutes after nicotine administration, well within the timeframe of nicotine metabolism. The controls for each group received a saline injection (Y: N = 7, O: N = 5) and the experimental groups (Y and O: N = 8) received an i.p. injection of nicotine. Directly after injection, ABRs were recorded. Hearing thresholds, wave 1 amplitudes and latency were analyzed for all groups.

Results: The threshold, wave 1 amplitude and latency of ABR recordings were analyzed using two-way ANOVA. Older FVB mice had ~10-12 dB threshold elevation ($F(1,50) =7.4$, $p=0.009$), consistent with previous studies. Nicotine had no effect on ABR threshold in either the Y or the O group of mice ($F(2,50)=0.22$, $p=0.805$). Old mice also had a lower mean wave 1 amplitude at suprathreshold levels in comparison to Y mice (65dB SPL $F(1,24)=9.55$, $p=0.005$ and 80dB SPL $F(1,24)=15.33$, $p=0.001$). However, nicotine had no significant effect on wave 1 amplitude ($F(1,24)=0.00$, $p=1.00$). The analysis of wave 1 latency showed no main effect of age or nicotine treatment, or interactions.

Conclusions: In contrast to data in humans, nicotine did not affect wave 1 amplitude and latency or ABR hearing threshold in young or old mice. These findings suggest that nicotine's influence on improved auditory responses as measured with EEGs may be related to effects on the central auditory system rather than the auditory periphery and early brainstem.

The Changes in Ion Channels of Age-Related Hearing Loss by Intermittent Hypoxia and D-Galactose Injection

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Category: Aging
Background: Age-related hearing loss (ARHL), also known as presbycusis, is an emerging complication in the aging population worldwide. A gradual decrease of hearing function with increasing age is often perceived as an inevitable part of the human aging process. The overall contribution of ARHL to hearing impairment and decreased quality of life is underestimated. Since the average life expectancy of the population is increasing, hearing loss has significant implications on general health and quality of life.
Methods: We used a method based on the induction model of obstructive sleep apnoea syndrome (OSAS), wherein cell aging is promoted by temporarily blocking the supply of oxygen. This model was designed so that the increase in ROS in the blood rapidly damage the auditory organs. Due to this mitochondrial damage has been considered responsible for the death of auditory hair cells due to aging.
D-galaactose (D-gal) injection animal models, established by administering successive subcutaneous D-gal injections to animals for 8weeks, have been frequently used in aging studies. Accelerated aging of the brain, kidney, liver, and blood cells has been proven in animal models using the D-galactose injection.
Results: Continuous oxidative stress and D-gal injection accelerated cellular aging. Expression of ARHL and ROS-associated factors UCP2, Cdh23, Myo7a and Myo6 was altered by oxidative stress in auditory organ cells. In addition, the expression of ion channels Kir4.1, NKCC1, KCNQ4, and Pendrin were also changed in ARHL models.
Conclusions: We found that intermittent hypoxia and D-galactose injection accelerated cellular senescence in a short-term. This has been demonstrated through changes in age-related factors and ion channels in an age-related hearing loss models. This study provides a realistic and accurate animal model that can be used for ARHL studies and suggest a relation between aging and ion channels.

Category-Level Encoding of Human Voice in Superior Temporal Sulcus Revealed by Direct Electrophysiological Recordings
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Category: Auditory Cortex and Thalamus: Human Studies
Background: Recognizing a voice is one of the most complex, yet socially important, feats of human auditory perception. In a rapid and near-automatic fashion, listeners can extract mood, gender, age, and identity. Voice perception depends on the brain's ability to seamlessly extract abstract acoustic cues from a complex auditory signal. Specialized regions of auditory cortex in superior temporal gyrus (STG) and superior temporal sulcus (STS), termed temporal voice areas (TVAs), play a crucial role in this process. However, the nature of voice representation by TVAs, and whether it depends on linguistic cues, remains intensely debated.
Methods: We performed intracerebral recordings in 8 patient-participants undergoing epilepsy surgery evaluation while they listened to natural sounds stimuli (duration of 2 seconds each) and performed a one-back task.
Results: Human vocal sounds (e.g., laughter, humming, crying) could be decoded from non-vocal sounds using neural activity in auditory cortex, completely independent of speech. Via the simultaneous recording of multiple regions in auditory cortex, we show that voice selectivity increases along the auditory hierarchy from primary auditory cortex to the STG and STS. Our results show an early, less-selective temporal window of neural activity in the STG and STS followed by a sustained, strongly voice-selective window. Encoding models demonstrate that STG/STS voice selectivity is best explained by voice category-level information compared to acoustic features. This is in stark contrast to neural activity recorded from primary auditory cortex, which could be explained predominately by acoustic features.
Conclusions: These findings are consistent with models positing categorical encoding of voice in TVAs and further elaborate the temporal dynamics of these representations.

Comparing the Timescale of Analysis for Speech and Music Selective Cortex
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Category: Auditory Cortex and Thalamus: Human Studies

Background: Recent work suggests that human non-primary auditory cortex contains distinct neural populations that respond selectively to speech and music, respectively. However, little is known about what these neural populations compute or how they integrate information over time. In this study, we used "quilting" – a technique for scrambling sound waveforms while minimizing artifacts – to investigate how the temporal structure of complex auditory stimuli is encoded in high-level, category-selective regions of human non-primary auditory cortex.

Methods: We used fMRI voxel decomposition techniques to isolate speech and music-selective components of auditory cortical responses in a set of fifteen participants. We then measured the response of these components to speech and music stimuli that varied in the extent of naturalistic temporal structure. Specifically, we created quilted versions of German speech (which was unintelligible to our participants) and instrumental music using segment durations ranging from 30ms to 2000ms, and then measured fMRI responses to these sounds. By combining participants' voxel responses to these quilted stimuli with their previously measured component weights, we were able to estimate the components' responses to the quilted stimuli and thus their dependence on temporal structure.

Results: Replicating prior findings, the response of the speech-selective component increased with segment duration up to about half a second, after which the response began to plateau. We found that the response of the music-selective component was qualitatively similar, suggesting a similar timescale of analysis. Importantly, neither neural population showed a significant effect of quilting for the non-preferred stimulus type. We applied the same analysis to four other auditory cortical response components that reflect spectral and modulation features, and none of them showed an effect of quilting comparable to what we observed for the speech and music-selective components.

Conclusions: Our findings suggest that speech and music-selective responses in human nonprimary auditory cortex are specialized for processing the naturalistic temporal structure of their preferred sound category, but analyze information on similar timescales. Further, this kind of sensitivity to temporal structure in speech and music appears to be limited to higher-order processing in category-selective regions of non-primary auditory cortex.

Responses to Complex Sounds in Core, Belt and Parabelt Areas Reveal General Aspects of the Physiological Organization of Primate Auditory Cortex

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Category: Auditory Cortex and Thalamus: Structure and Function

Background: In prevailing models of primate auditory cortex (ACtx), 3 hierarchically connected areas – the core, belt and parabelt – are believed to serially process auditory inputs to produce selective representations of complex and/or behaviorally-relevant sounds. Although many studies have established the anatomical properties that delineate the auditory cortical fields in primates, relatively few physiological studies exist to test hypotheses regarding functional models of auditory cortical organization.

Methods: We used multielectrode arrays (16-64 channels) to record from single neurons across core, lateral belt, and parabelt areas of the awake/passive squirrel monkey (SQM) ACtx in 4 hemispheres (3 right and 1 left) of 3 animals (all female). Our recordings span multiple fields within each area: primary auditory cortex (A1) and the rostral field (R) in the core; caudal (CL), middle (ML) and anterior (AL) fields in the lateral belt; and caudal (CPB) and rostral (RPB) fields in the parabelt. We presented 3 types of complex sounds: amplitude-modulated broadband noise (AM); frequency-modulated tone sweeps (FM); and a spectrotemporally-modulated broadband noise ("dynamic moving ripple" [DMR]). We analyzed a diverse range of neural activity features in response to these sounds (e.g., response latency, phase-locking precision, firing rate, stimulus decoding) and mapped these response features across multiple cortical fields.

Results: We found two distinct topographic gradients: (1) A caudal-rostral gradient corresponding to differences in temporal response features such as response latency and phase-locking precision; and (2) A medial-lateral gradient corresponding to topographic differences in multiple response features such as firing rate and stimulus decoding accuracy. Factor analysis of the topographic organization of all tested response features revealed multiple gradients underlying the physiological organization of auditory cortex. Based on these analyses, the caudal fields within each area (A1, CL and CPB) are functionally distinct from the corresponding rostral fields (R,

AL and RPB) based on their different temporal precision. On the other hand, the medial-lateral gradient captures functional differences between putative core, belt and parabelt areas.

Conclusions: These results provide a novel description of the physiological organization of primate auditory cortex for multiple sound stimuli and multiple response features. The apparent organization of the primate ACtx depends critically on the response features being measured. Namely, physiological differences in firing rate and stimulus decoding between core, belt and parabelt fields support the prevailing model of a hierarchical, serial transformation of sound representations, while striking similarities in temporal response features between fields across different areas support parallel processing. These data suggest a refinement of the prevailing model such that the traditional core-belt-parabelt hierarchy exists within a rostral-caudal gradient primarily defined by the temporal precision of responses to sounds. Thus, serial and parallel streams in ACtx give rise to a diverse range of topographically-organized sound representations.

Identifying Neural Circuits That Engage Effortful Listening in Gerbils

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Category: Auditory Cortex and Thalamus: Structure and Function

Background: The deficits associated with developmental hearing loss (HL) can often be attributed to degraded sensory processing. However, cognitive skills that are engaged during auditory task performance can be overburdened in those with HL. For instance, individuals with HL may exert additional listening effort, defined as the allocation of cognitive resources during auditory tasks (Pichora-Fuller et al., 2016). This added mental effort can influence the ability to perceive sounds accurately, despite audiometric correction (i.e., hearing devices). Evidence from human studies suggest that the cingulate cortex, within the limbic lobe, is engaged during difficult listening conditions and can exert top-down modulation of the auditory cortex (AC). Here, we asked whether the cingulate cortex (Cg) sends anatomical projections to the AC, and whether it mediates effortful listening in gerbils.

Methods: Retrograde and anterograde virus tracers were injected into AC and Cg, respectively, to determine anatomical connectivity in gerbils. To assess effortful listening, 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, gerbils were trained to discriminate between "Go" stimuli consisting of a range of AM rates (4.5 to 12 Hz, broadband noise carrier, 100% depth modulation) 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. Performance was deemed to be asymptotic when animals reached 3 sessions in which false alarm (FA) rates were <30%, the average d' for the three easiest GO values (8, 10, 12 Hz) was >2, and the number of trials per AM rate was >15. When animals reached asymptotic performance, bilateral cannulae were implanted in Cg. To determine whether Cg mediates effortful listening, muscimol was infused bilaterally approximately 30 minutes before testing to pharmacologically inactivate Cg during task performance. Behavioral performance was collected across 7 testing days, where infusions of muscimol and saline were interleaved such that within-subject comparisons could be made.

Results: Viral tracing experiments revealed a strong projection from Cg to the AC, including both core and dorsal regions. Next, we asked whether locally inactivating Cg impairs perceptual performance. We found that Cg inactivation disrupted performance only for difficult listening conditions: thresholds for the 1s blocks (i.e., 'easy' blocks) remained the same across saline and muscimol conditions (~5 Hz AM in both cases), whereas thresholds for 0.25s blocks were elevated only for muscimol conditions (saline: ~5.5Hz AM; muscimol: ~7Hz AM).

Conclusions: Taken together, the results reveal a descending cortical pathway from Cg to AC that mediates perceptual performance during difficult stimulus conditions. This pathway is a plausible circuit that may be undermined by developmental HL.

Cortical Encoding of Long-Term Temporal Dependencies in Music Perception

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Category: Auditory Cortex and Thalamus: Structure and Function

Background: The human brain is shown to estimate temporal contexts to process time-series signals such as speech and music. Recent deep learning models are able to capture both short and long temporal dependencies

using mechanisms such as attention, more accurately than previous models such as hidden Markov models. To shed light on how the human auditory cortex encodes temporal dependencies at various scales, we used a transformer neural network model to predict the neural responses in the primary and nonprimary auditory cortex of neurosurgical subjects as listened to Bach compositions.

Methods: The model used is a 12-layer sequence-to-sequence multi-tasking transformer model trained on music MIDI data with both an encoder and a decoder. The encoder is trained to predict the masked input values, and the decoder is trained to predict the next music note given all previous music notes. In addition, the model is trained for both chord-to-melody and melody-to-chord generation in a sequence-to-sequence manner.

We apply a 2D t-SNE transformation on features from each layer of the encoder. The resultant 2d representation is used to predict the EEG and ECoG recordings with Temporal Response Function (TRF) from subjects listening to the same 8 pieces of music composed by Bach. We use leave-one-out cross-validation to select the best models for each piece of music and report the correlations between the recording and the predicted results. We have 20 EEG subjects and 3 ECoG subjects in total.

We also perform the clustering analysis on the t-SNE results to show the contextualization of the transformer features.

Results: The correlations of predictions are monotonically increasing over layers for both ECoG and EEG data. The correlation with ECoG data ranges from 0.21 to 0.31 for different channels averaged across subjects, whereas acoustic features have correlations ranging from 0.33 to 0.42 for different channels. The highest correlations across all 64 channels on EEG data are on average 0.043 across subjects using features from the last layer.

From clustering analysis, we have found that music notes from the same piece of music are gradually clustered together over layers, suggesting contextualization happens over layers. The clusters are not obvious from the first few layers. Music notes with the same pitch and duration do not get clustered together over layers.

Conclusions: We present a new method for studying music processing in the human auditory cortex by analyzing learned representations from deep learning models. In particular, features from the encoder of the transformer can be used to predict both EEG and ECoG recordings with high correlations. The correlations increase monotonically over layers, which is correlated to the contextualization of the transformer indicated by the clustering of t-SNE representations over the same piece of music.

Spectral and Contextual Coding of Tone-Varying Sequences is Hierarchically Organized From the Midbrain to the Prefrontal Cortex

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Category: Auditory Cortex and Thalamus: Structure and Function

Background: One essential function of the auditory system is detecting unpredicted sounds, as revealed by differential neural activity to predictable and unpredictable sounds. According to the predictive coding framework, this effect can be explained by the interplay of repetition suppression and prediction error signaling. This work investigates the functional specialization of the rat auditory system by combining a frequency oddball paradigm and two complementary tone-varying control sequences: the many-standards and the cascade.

Methods: The cascade sequence is generated by a constant and predictable transition probability of successive tones, presumably undergoing stronger repetition suppression than the many-standards control, where the transition between consecutive tones is fully random. Nevertheless, responses between both sequences were not significantly different on average. However, because the frequency steps among successive tones were also different, we tested whether control responses were affected by the step frequency from the previous tone in the sequence.

Results: The data revealed a processing hierarchy in which the inferior colliculus and the medial geniculate body were sensitive to any frequency-step difference regardless of the control type. In the AC, the lemniscal fields still preserve some involvement in spectral coding. Although the suprarhinal auditory field is influenced by the largest frequency steps, it encodes all other frequency differences equally, showing some contextual processing. Importantly, neurons in the posterior auditory field can predict all the frequency variations demonstrating a robust contextual or global processing. Similarly, the medial prefrontal cortex also proves these integrative functions at larger spectral and temporal scales. Altogether, these results demonstrate a hierarchical processing, in which less spectral features are progressively encoded giving rise to contextual processing downstream.

Conclusions: Furthermore, we explored the functional specialization of the AC fields. Although tones in the control sequences underwent neural adaptation comparable to deviant events they did not violate a regularity as the deviant does. Thus, a difference in the neural activity between deviant and control tones indicates the emergence of prediction error signaling, whereas a difference between control and standard tones indicates its repetition suppression effect. Single unit recordings demonstrated by far the largest prediction error signals in the posterior auditory field, while the lemniscal fields (primary auditory cortex, the anterior auditory field, the ventral auditory field) and the nonlemniscal suprarhinal auditory field were dominated by repetition suppression effects. The nonlemniscal posterior auditory field is mostly engaged in context-dependent processing underlying deviance detection, as opposed to the other AC fields which are sensitive to stimulus-dependent effects underlying differential degrees of neural adaptation.

GluA3 Subunits Are Required for the Appropriate Assembly of AMPA Receptors at Cochlear Afferent Synapses and for Presynaptic Modiolar-Pillar Features

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Category: Auditory Nerve

Background: Hearing depends on activation of AMPA-type glutamate receptors (AMPARs) on the post-synaptic terminals of auditory nerve fibers. Noise-induced cochlear synaptopathy is caused by excitotoxic overactivation of those receptors; antagonizing the calcium-permeable subset of AMPARs (CP-AMPARs) pharmacologically can prevent this synaptopathy. Cochlear AMPARs are tetrameric heteromers comprised of the pore forming subunits GluA2, 3, and 4 where absence of GluA2 results in a CP-AMPAR channel with increased permeability to $Ca2+$ and $Na+$. AMPAR tetramers form as dimers of dimers, with the GluA2/3 dimer being energetically favored and prominent. In the absence of GluA3, GluA2/4 would be the only heterodimer. Homodimers and homomeric tetramers may exist but are less stable energetically.

Methods: We used global Gria3KO mice to ask what happens to cochlear ribbon synapses in the absence of subunit GluA3. With immunohistofluorescence and confocal microscopy we studied molecular anatomy with antibodies to GluA2, GluA3, GluA4, and the presynaptic ribbon protein CtBP2/Ribeye. With transmission electron microscopy and serial section reconstruction we studied pre- and post-synaptic ultrastructure. With ABR, we tested cochlear output. Male WT and KO mice were compared at 5 weeks of age following normal rearing in an animal facility with ambient noise of 55-70 dB SPL.

Results: ABR thresholds and wave-I amplitudes of 5-week-old male Gria3KO mice were similar to male WT littermates, as were the numbers of paired and unpaired synaptic puncta. However, synaptic molecular anatomy and ultrastructure were altered. In addition to absence of GluA3 immunoreactivity, analysis of confocal images showed that ribbon synapses of Gria3KO mice had smaller AMPAR arrays that contained less GluA2 and more GluA4 relative to WT. Changes to the GluA4:GluA2 ratio in Gria3KO were greater for synapses on the pillar side of the inner hair cell (IHC), resulting in the emergence of modiolar-pillar gradients in GluA expression. Ultrastructurally, the IHC modiolar-pillar differences in presynaptic ribbon size, ribbon shape, and vesicle size seen in WT was diminished or reversed in Gria3KO. Postsynaptic terminals of KO animals had large vacuoles not seen in WT.

Conclusions: Loss of GluA3 alters GluA2 and GluA4 subunit relative abundance, increasing the GluA4:GluA2 ratio, which may increase the number of GluA2-lacking CP-AMPARs at cochlear ribbon synapses. Additionally, changes to the presynaptic ribbon suggest transsynaptic developmental effects, reminiscent of previous reports on synapse ultrastructure in the cochlear nucleus of Gria3KO mice. Although young male Gria3KO mice have ABR and synapse numbers similar to WT, we hypothesize these molecular-anatomical alterations to AMPAR subunits result in synapses with increased vulnerability to AMPAR-mediated excitotoxicity that may lead to synapse loss and hearing loss as the mice age. We propose the increase in GluA4:GluA2 ratio (i.e., $Ca2+$-permeability index) is causally related to the appearance of large vacuoles in terminals of Gria3KO mice.

Altered Morphological Properties of Neonatal Spiral Ganglion Neurons Cultured In-Vitro on Graphene- And Hexagonal Boron-Nitride-Based Substrates

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Category: Auditory Nerve
Background: Poor pitch perception and significant variability in efficacy still limit the use of current cochlear implant technology. These limitations are likely caused by low spatial resolution and broad neuronal stimulation, and researchers have recently addressed them by introducing novel graphene-based materials.
Graphene and other two-dimensional materials possess various properties, making them a desirable candidate for future cochlear implants. Graphene might support neural growth due to its unique electric properties, biocompatibility with neuronal cells, and ability to interface with neuronal tissue effectively. Moreover, recent studies of hexagonal boron nitride (hBN) showed promising applications in neuroengineering. How graphene and hBN interact with spiral ganglion neurons (SGN) morphology is still not known.
Methods: This study used three graphene-based substrates (single layer graphene - SLG, hydrogenated graphene - HG, and fluorinated graphene - FG) and single-layer hexagonal boron nitride – SLhBN, as surfaces for in-vitro culturing of neonatal SGN extracted from P5 rat pups. The cultures were immunocytochemically stained either at four days in vitro (4DIV) or eight days in vitro (8DIV) with beta-III-tubulin and S-100, identifying neural and glial cells on fluorescence images, respectively. We then used the custom-made machine learning-based image processing to segment identified neurons in images. We compared morphological properties of neuronal cultures grown on graphene-based and SLhBN surfaces with those grown on control glass coverslips.
Results: Our in-vitro cultures from six experiments yielded 33107 single individual neurons and 16043 neurons forming clusters of various sizes. We found that all types of graphene-based surfaces and SLhBN significantly altered morphological properties compared to standard glass coverslips. The mixed-effect model of different morphological measures such as soma size, neuronal area, perimeter, elongation, number of branching, and the total length of all branches revealed that HG, SLG, and SLhBN substrates represented a more favorable environment for spiral ganglion neuron growth than those grown on control coverslips.
Conclusions: These findings reveal substantial interaction between material properties of graphene- and hBN-based substrates on in-vitro SGN cell cultures, which may affect neurophysiological properties of spiral ganglion neurons.

Comparing the Biophysical Properties of Spiral and Vestibular Ganglion Neurons From Neonatal Rats
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Category: Auditory Nerve
Background: The membranes of auditory and vestibular afferent neurons each contain diverse groups of ion channels that lead to heterogeneity in their intrinsic biophysical properties. Previous in vitro work in both auditory- and vestibular-ganglion have individually examined this remarkable diversity, but there are few direct comparisons between the two ganglia.
Methods: This work compares the firing patterns of neonatal vestibular- (postnatal day, P3 through P22) and spiral ganglion neurons (P3 through P16) in Long-Evans rats. The data were recorded by whole-cell patch-clamping in previous studies from our laboratory. Recordings in vestibular ganglion were from disassociated and cultured neurons, whereas recordings in spiral ganglion neurons were from acute semi-intact preparations.
Results: Indicative of an overall heterogeneity in ion channel composition, both ganglia exhibit qualitatively similar firing patterns ranging from sustained-spiking to transient-spiking in response to current injection. The range of resting potentials, voltage thresholds, current thresholds, input resistances, and first-spike latencies are similarly broad in both ganglion groups. The covariance between several biophysical properties (e.g., resting

potential to voltage threshold and their dependence on postnatal age) was similar between the two ganglia. Cell sizes were on average larger and more variable in VGN than in SGN. One subgroup of VGN stood out as having extra-large somata with transient-firing patterns, very low-input resistance, fast first-spike latencies, and required large current amplitudes to induce spiking. Despite these differences, the input resistance per unit area of the large-bodied transient neurons was like that of smaller-bodied transient-firing neurons in both VGN and SGN, thus appearing to be size-scaled versions of other transient-firing neurons.

Conclusions: Although auditory and vestibular afferents serve very different functions in distinct sensory modalities, their biophysical properties are more closely related by firing pattern and cell size than sensory modality.

Disentangling Peripheral Versus Brainstem Components of the Frequency Following Response

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Category: Auditory Nerve

Background: Frequency-following responses (FFR) are evoked potentials resulting from populations of neurons responding phase-locked to the carrier frequency of a pure tone. Previous studies have shown that FFR amplitudes decline with increasing age, even in listeners with clinically-normal audiometric thresholds. This has been attributed to an age-dependent decline of neural synchrony in the central auditory system, but our recent work suggests that an age-dependent degeneration of auditory-nerve fibres in the periphery could also contribute to this FFR reduction. Here, we present a method to disentangle peripheral versus brainstem components of the FFR. This method could later be used to investigate the dominant source of such age-related reduction in the FFR.

Methods: We developed a method to disentangle peripheral versus brainstem contributions to the FFR in humans. This was achieved by placing one EEG electrode on the vertex, one on the ipsilateral mastoid, and a third tympanic membrane (TM) electrode on the eardrum. FFRs to 10-ms pure-tone bursts were recorded at frequencies of 516 and 1086 Hz, presented at 80 and 105 ppSPL. Stimuli were presented 6000 times at a presentation rate of 9 Hz. Auditory brainstem responses (ABR) with the same electrode montages were recorded using 100-μs clicks presented at 115.5 ppSPL, repeated 6000 times at a 9 Hz presentation rate.

Results: FFRs were recorded with a traditional vertical montage (mastoid versus vertex). This classical montage emphasized brainstem components, revealed as well-defined waves-III, -IV and -V morphologies, and diminished wave-I amplitudes in the click-ABR. The FFR showed a change in amplitude and phase at about 5-6-ms, followed by a steady-state response until 16-ms. This indicates the presence of a brainstem contribution. Replacing the vertex electrode with the TM electrode (i.e., TM versus mastoid montage) isolated peripheral FFR components. Only the summating potential and waves-I and -II were present in the click-ABR. The FFR latency was about 1 ms and the response lasted until 11 ms (for the 10-ms long stimulus). A frequency dependency was observed. The peripheral FFR component showed a response to both 500-Hz and 1-kHz tones, but the 500-Hz response was only represented in the brainstem-enhanced component. By utilizing the distinctive phase relationship between the peripheral and central responses, it was possible to disentangle the response amplitudes from two separate sources and examine their interaction in the central response.

Conclusions: Different electrode montages and stimulus frequencies enhance peripheral versus brainstem components of the FFR. This was confirmed using ABR recordings, in which different montages enhanced or attenuated different waves. We aim at using this method to study age-related effects in peripheral versus brainstem components of the FFR.

Round Window Kainate Application Increases the Fraction of High-Spontaneous Rate Fibers in Gerbil Auditory Nerve

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Category: Auditory Nerve

Background: Glutamate excitotoxicity may play an instigating role in the de-afferentation observed after noise overexposure. Previous work has shown that the glutamate agonist kainic acid (KA) applied to the round window niche in gerbils, induced a cochlear synaptopathy (~50% loss of synapses) but little is known about the phenotype of remaining auditory nerve fibers (ANFs). To investigate the functional properties of remaining ANFs, single-fiber recordings were performed two weeks after a KA application.

Methods: Artificial perilymph containing 25 mM kainic acid was infused into the round window niche for 1 hour in young adult female Mongolian gerbils. High-impedance (50-100 MΩ) glass microelectrodes were inserted in the auditory nerve to assess the functional properties of remaining ANFs.

Results: KA application did not modify the thresholds and CF distributions of remaining fibers compared to control animals. However, the spontaneous rate (SR) distribution of the remaining fibers was strongly compressed toward high-SR values with almost no low-SR fibers and the emergence of a very high-SR pool of fibers (SR>80 spikes).

Conclusions: Application of KA in the round window niche of gerbils depresses the pool of low-SR ANFs, while increasing the SR of surviving fibers.

Cx30 (GJB6) Plays a Critical Role in Neural Development and Distribution in the Cochlea

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Category: Auditory Nerve

Background: Gap junction (GJ) connexin gene mutations induce high incidence of hearing loss, indicating that connexin GJs play an important role in hearing function. However, connexin GJ functions in the cochlea and hearing are not fully understood. Cx26 and Cx30 are predominant isoforms in the cochlea. Both Cx26 mutations and Cx30 mutations can cause hearing loss. However, there are no Cx26 and Cx30 expressions in the hair cells and spiral ganglion neurons in the inner ear. Our previous studies reveal that Cx26 deficiency can cause cochlear developmental disorders. In this study, we found that Cx30 deficiency can cause spiral neuron development and distribution disorders.

Methods: Cx30 KO mice and littermate wild-type (WT) mice were used. ABR threshold, DPOAE, and cochlear microphonics (CM) were recorded to assess cochlear and hearing function. The spiral neuron development and distribution were examined by immunofluorescent staining microscopy. The ribbon synapses were also examined.

Results: Cx30 KO mice showed hearing loss in comparison with WT mice. In comparison with WT mice, innervations of auditory nerves with inner hair cells (IHCs) in Cx30 KO mice were significantly reduced. Ribbon synapses in Cx30 KO mice were also reduced and demonstrated a "deer-hoof-print" like distribution under IHCs. However, deletion of Cx26, which also caused hearing loss, had no such effect.

Conclusions: These data indicate that gap junction gene Cx30 can modify cochlear neural development and distribution, even though there is no Cx30 expression in the spiral ganglion neurons. These results also reveal that connexin GJs play a critical role not only in the cochlear development but also in the neural development in the inner ear.

Using Musical Pitch Interval Comparisons to Assess Cochlear Implant Frequency-To-Place Maps

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Category: Auditory Prostheses

Background: Music perception remains challenging for many cochlear implant (CI) recipients, due perhaps in part to the frequency mismatch that occurs between the electrode-neural interface and the frequencies allocated by the programming. Individual differences in ear anatomy, electrode array length, and surgical insertion can lead to great variability in the positions of electrodes within the cochlea, but these differences are not typically accounted for by current CI programming techniques. Flat panel computed tomography (FPCT) can be used to visualize the location of the electrodes and calculate the corresponding spiral ganglion characteristic frequencies. Such FPCT-based CI frequency mapping may improve pitch perception accuracy, and thus music appreciation, as well as speech perception. The present study seeks to develop a behavioral assessment metric for how well place-based pitch is represented across the frequency spectrum.

Methods: Listeners were asked to match the pitch interval created by a three-tone sequence (low-high-low) across different frequency ranges to estimate the extent to which the pitch map is evenly distributed across the CI array. This test was piloted with pure tones in normal-hearing listeners, using both unprocessed and vocoder-processed sounds to simulate both even and warped frequency-to-place maps. We hypothesized that the vocoded stimuli

would be more difficult to match in terms of pitch intervals than unprocessed stimuli, and that a warped map (as may occur with current clinical maps) would produce poorer matches than a veridical and even map (as may be achieved using FPCT-based frequency allocation).

Results: Preliminary results suggest that the task can reveal differences between veridical and warped maps in normal-hearing listeners under vocoded conditions.

Conclusions: Next steps will be to test this procedure in CI users and compare results with traditional clinical maps and FPCT-based frequency allocation to determine whether the new FPCT-based maps result in improved pitch-interval perception.

Towards Optogenetic Cortical Implants for Hearing Impaired
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Category: Auditory Prostheses

Background: Cochlear implants are among the most successful sensory restoration devices, which in many cases, drastically improve hearing in impaired patients by directly stimulating the auditory nerve. However, they still suffer from limitations and cannot be applied to patients with auditory nerve loss. To explore news ways of solving these limits, we are currently developing methodologies to demonstrate in mice, the feasibility of auditory rehabilitation using a cortical optogenetic implant. Being the most downstream information processing structure, the cortex allows a larger interface and interpretation of more complex stimuli.

Methods: We first established benchmarks of mouse hearing using a Go/NoGo discrimination for 3 important features of auditory perceptions. Frequency discrimination, amplitude modulation and, robustness to noise. Two other benchmarks for frequency modulation and discrimination of harmonics are in preparation. These benchmarks are aimed to be compared to perception obtained with the implant. A pilot implantable optogenetic stimulator was manufactured by the Mathieson lab (Uni. of Strathclyde) to project 2D light patterns on the auditory cortex. We are currently calibrating this device to estimate the spatio-temporal precision of the activity patterns induced. In parallel, we implemented an algorithm to convert sounds to tonotopic activation patterns, and we explore other methods based on AI tools.

Results: Behavioral experiments allowed us to measure frequency perception accuracy over a range of 16 stimuli of different tone frequencies (6000Hz to 16000Hz). Amplitude modulation for 16 AM frequencies (20Hz to 200Hz). Results show that background noise of increasing levels contributes to diminish the discrimination capacities of mice.

For the encoding model, we could use tonotopic maps of the auditory cortex obtained through intrinsic imaging, to construct a linear model which computes projections of the sound spectral content onto the tonotopic axis at the surface of the brain. As an alternative model, we also developed a deep autoencoder network that heavily compresses the sound representation while keeping nearly all spectro-temporal information intact.

Conclusions: These results pave the way for generating artificial auditory perception via cortical stimulation and benchmark them against auditory discrimination performance of normally hearing mice.

What Cues Are Children With Cochlear Implants Using to Understand Speech?
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Category: Auditory Prostheses

Background: Cochlear implants (CIs) are largely programmed using a one-size-fits-all approach for both post-lingually deafened adults and congenitally deafened children. For adults with CIs and children with normal hearing (NH), there is a clear and well-recognized relationship between spectral resolution and speech recognition. However, for children with CIs, the results have been mixed with some demonstrating a significant relationship (Horn et al., 2017) and other studies showing no relationship (e.g., Jung et al., 2012; Gifford et al., 2018). Despite this, children with CIs regularly achieve high levels of open-set speech recognition; thus it may be the case that children with CIs place greater perceptual weight on temporal versus spectral cues for speech recognition (Landsberger et al., 2018; 2019)—but additional research is needed. Thus the purpose of this study was to investigate the relationship between spectral and temporal resolution and speech recognition in children with CIs. We hypothesized that 1) there would be a relationship between spectral and/or temporal resolution and speech

recognition, and 2) improvements in spectral and/or temporal resolution over time would correspond to improvements observed for speech recognition.

Methods: Participants included 20 children (6-12 years) with at least one CI. Performance was compared between a baseline assessment and 12-months later. Ear-specific, psychoacoustic measures included sinusoidal amplitude modulation detection (4, 32, 128 Hz) and spectral modulation detection (0.5 and 1.0 cyc/oct) both using 3-interval forced choice with 2-down, 1-up tracking with stimuli presented in the free field at 65 dB SPL. Speech recognition in quiet and noise was also obtained in the free field for each ear individually using CNC words, BabyBio sentences in noise (+5 and 0 dBB), BKB-SIN, and adaptive HINT in noise.

Results: At both the baseline and 1-year time points, there was no noted relationship between either spectral or temporal resolution and all measures of speech recognition (uncorrected $p > 0.05$ for all correlations). However, improvements observed for temporal modulation detection thresholds for a 32-Hz rate were significantly correlated with improvements observed for adaptive HINT in noise ($r = -0.90$, $p = 0.01$). No other relationships between change in spectral and/or temporal resolution and speech recognition over time were significant (uncorrected $p > 0.05$ for all correlations).

Conclusions: Unlike their peers with NH and adults with CIs, children with CIs may place greater perceptual weight on temporal versus spectral cues as evidenced by a significant relationship between changes in temporal modulation detection and speech recognition over a 1-year period of experimental observation. As such, it is possible that children with CIs may benefit from specialized speech coding strategies accentuating temporal cues, though substantial additional research is needed. This longitudinal study is ongoing and will follow up to 60 children for a 2-year period.

Electrically-Evoked Auditory Brainstem Responses Produced via a Novel Auditory Nerve Implant

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Category: Auditory Prostheses

Background: The cochlear implant (CI) is an extremely successful neural prosthetic capable of restoring hearing abilities in those with severe to profound hearing loss. The CI has clear limitations however, as CI users have reduced performance in noisy environments and music perception. The main cause of these shortcomings is that the CI electrodes do not directly contact the auditory nerve fibers; current must first travel across the bony wall of the cochlea. This poor electrode interface causes increased current spread and reduces the resolution to which specific frequency fiber groups can be activated. Recent studies have demonstrated the benefits of direct auditory nerve stimulation, showing lower activation thresholds and more confined current spread. These advantages could help overcome limitations of the CI and activate a greater number of independent frequency channels of information. To develop and commercialize an auditory nerve implant (ANI), our group is currently performing electrophysiological studies in animal models.

Methods: Acute, non-survival experiments were performed in the cat (n=4) and rhesus monkey (n=2) using a 3x5 Utah Slanted Electrode Array (USEA) developed by Blackrock Neurotech. The electrically-evoked auditory brainstem response (eABR) was recorded through percutaneous needle electrodes at the scalp and mastoid. A translabyrinthine surgical approach was employed to expose a distal section of the auditory nerve immediately exiting the cochlea. All animals remained fully anesthetized throughout the surgical procedure and for the duration of the electrophysiological experiments. Charge-balanced biphasic pulses were presented, with stimulation polarity altered after each trial. All trials for a given current level were averaged and subjected to a 4th order Butterworth bandpass filter with a passband of 300-3000 Hz. Several current levels were presented to demonstrate different levels of recruitment of auditory nerve fibers.

Results: Multiple component waves of the eABR were observable in the cat and rhesus monkey, showing strong activation of auditory processing centers in the brainstem. Upon changing the active stimulation site on the electrode array, different ABR waveforms were observed, due to activation of different nerve fiber groups. As the current level was increased, stronger recruitment of nerve fibers was evidenced through decreases in latency and increases in amplitude of eABR waves.

Conclusions: eABRs collected in acute animal experiments have verified that our ANI device can robustly activate the auditory system at various levels of intensity. Upcoming chronic experiments in monkeys will demonstrate long-term stability and safety of our device, ultimately paving the way for clinical translation into human patients. We seek to introduce a new type of auditory prosthetic that can serve patients who may not be suitable candidates for a cochlear implant.

A Novel Viral Vector, AAV-CCP16 Results in High Viral Transfection in the Mouse Cochlear Nucleus (CN)

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Category: Auditory Prostheses

Background: The auditory brainstem implant (ABI) is a neural prosthesis that provides sound sensations by electrically stimulating the cochlear nucleus (CN). It is hypothesized that ABI performance is limited by channel crosstalk and current spread to non-auditory neural structures. A possible technology to overcome these challenges is a light-based ABI. Light can be highly focused and targeted to specific cell types that are modified to express optically sensitive ion channels (opsins). Adenovirus associated vectors (AAVs) such as Anc.80 and AAV-9 have been used to deliver opsins to spiral ganglion neurons and CN neurons. A novel AAV, AAV.CPP.16, has demonstrated more efficient transfection in the brain as well as greater blood brain barrier (BBB) penetration. Here, we use AAV.CPP.16 to compare its neural transfection capacity with that of Anc.80 in the CN.

Methods: CBA/Caj mice underwent suboccipital craniotomy and exposure of left CN. AAV Anc.80 with Chronos or AAV.CPP.16 (2.76*109 genome copies) was injected into the CN using direct micro-syringe injection over 5 minutes. One month following viral transduction, Anc.80 mice underwent revision craniotomy and re-exposure of CN. A 16-channel NeuroNexus probe was placed into the right inferior colliculus (IC) to record evoked multiunit activity during radiant exposure with a pulsed 488 nm laser via optical fiber. AAV.CPP.16 were not tested because their construct lacked an opsin. Following electrophysiologic recordings, mice were sacrificed and transcardially perfused. Brainstem tissue was dissected, transverse sections were collected and stained. Neural viral transfection was assayed by confocal analysis of fluorescence levels in CN and other brainstem areas.

Results: The percentage of the total CN neurons transduced by AAV Anc.80 (4 mice) was 38.18% (range: 14.55-63.14 %). AAV Anc.80+ neurons were distributed throughout CN in the ventral and dorsal subdivisions and were associated with robust IC neural responses during optical stimulation of CN. High IC spike rates were correlated with larger numbers of transfected CN neurons. AAV.CPP.16 injections (4 mice) resulted in higher numbers of transfected neurons (79.56%, range: 69.35 87.14%) and greater fluorescence in CN compared to Anc.80 ($p<0.05$). The cochlear nucleus area measured was consistent between both virus. Some AAV.CPP.16 injected mice had fluorescence just ventral to the injected CN and possibly in the ventral acoustic stria (VAS), suggesting a greater spread of virus from the dorsal to the ventral cochlear nucleus. No expression was seen in the contralateral CN.

Conclusions: AAV.CPP.16 was twice as efficient compared to Anc.80 in transfecting neurons in mouse CN. This study elucidates the potential of AAV.CPP16 to efficiently transduce neurons of the cochlear nucleus. Future studies will use AAVCPP.16 with opsins to photosensitize central auditory neurons through surface or systemic intravenous virus delivery and decrease injury to CN microstructure.

Comparison of Simple Impedance and Modeled-Impedance Components Over Time After Cochlear Implant Insertion Suggests a Dominant Role of the Electrode-Tissue Interface

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Category: Auditory Prostheses

Background: Following cochlear implantation, it is common for intrascalar tissue to form, ultimately leading to bone in some cases. Biological material adhering to the electrode surface has also been observed. Previous work has linked this tissue growth to changes in electrical impedance, a measure which is readily recorded in all commercial cochlear implant clinical software. Despite the prevalence, a clear physical link between impedance and intrascalar tissue has not been established. Furthermore, there is no accepted way to noninvasively monitor this tissue formation.

Methods: To further investigate the relationship between intrascalar tissue and impedance, we employed a multifrequency, electrochemical circuit model of impedance enabling biologically relevant explanatory power to the measure. Twenty-six chronically implanted guinea pigs had impedance data regularly collected in three ways: 1) a multifrequency "complex" impedance measures, 2) a "simple" impedance magnitude for a 1 kHz sinusoid, and 3) an impedance value derived for trains of 30 μs/ph 2.1 μs IPG biphasic pulses of varying amplitude. The complex impedance data were fit to a circuit model and the resulting four elements were each compared to the simple measures of impedance.

Results: The temporal pattern (changes over time after implantation) of the simple impedance for 1 kHz sinusoids and combined complex impedance measures closely agreed for most animals regardless of electrode type, while impedance derived from the pulse train had similar patterns but with less dramatic temporal changes. When decomposed via the equivalent circuit model, the impedance patterns over time agreed well with only one of four elements, the constant phase element (CPE), an element which is believed to track the quality of the electrode interface.

Conclusions: As preliminary analysis does not suggest a significant relationship between extent of intrascalar tissue and impedance, another phenomenon may be driving impedance change. The fact that the pattern and level of the CPE is matched closely by the 1 kHz simple impedance suggests the simple impedance measure is reflective of conditions on or very near the electrode surface rather than the presence of fibrous and bone tissue growth in the rest of the scala tympani. Impedance changes over time suggest a complex and, in some cases, changing relationship between the implant and the scala tympani environment after implantation.

Variability of Cochlear Implant Outcomes in a Large German Cohort With a Genetic Hearing Loss Etiology

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Category: Auditory Prostheses

Background: The cochlear implant is an established and successful treatment for severe to profound hearing loss, yet outcomes after surgery are variable and largely unexplained. To some extent variability is attributed to hearing loss etiology, more specifically genetic factors have been proposed to impact cochlear implant outcome. The clinical and genetic heterogeneity of hereditary hearing loss complicates preoperative counseling on cochlear implant outcome prediction based on genetic diagnosis. The "spiral ganglion hypothesis" suggests that genetic factors that negatively affect cochlear implant outcome are expressed in the relevant neuronal compartment of the cochlea and the auditory pathway that are targeted by the electric stimulation of the cochlear implant. We sought to find corroborating evidence supporting this hypothesis and to identify further factors that might influence cochlear implant outcome.

Methods: A large German cohort of cochlear implant recipients (n=76) with a confirmed genetic diagnosis and documented postoperative CI outcome for 123 implanted ears were included in the study. Preoperatively all patients underwent clinical, radiologic and audiological examinations to evaluate their cochlear implant candidacy. For adult recipients the outcome measures were based on at least one year of postoperative audiological follow-up and for children with congenital or pre-/perilingual hearing loss onset on five years follow-up, respectively.

Results: The nine most frequent causal genes were GJB2 (13%), TMPRSS3 (8%), MYO15A (7%), and COL4A3, LOXHD1, MYO7A, SLC26A, USH2A, WFS1 (each 4%).

Patients with causal genetic variants affecting the neural targets of the cochlear implant showed a significantly worse outcome, therefore corroborating the "spiral ganglion hypothesis". The only other factor that demonstrated to be a significant predictor for less favorable hearing outcome was a long time interval between the onset of hearing loss and cochlear implant surgery.

Conclusions: We provide corroborating evidence for the "spiral ganglion hypothesis" by demonstrating that genetic neuronal causes of hearing loss negatively influence speech recognition in cochlear implant recipients in a large German cohort.

Toward Behavioral Assessment of Acoustic Frequency and Cochlear Implant Channel Discrimination Performance in Rodents Combined With Chronic Midbrain Recordings

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Category: Auditory Prostheses

Background: Contemporary cochlear implants (eCI) directly stimulate spiral ganglion neurons (SGNs) using electric current to partially restore auditory function in patients diagnosed with profound sensorineural hearing loss or deafness. However, patients report poor speech understanding in noise, which can be explained by the limited number of independent stimulation channels. Multiunit recordings from the inferior colliculus (IC) of anaesthetized animals further demonstrated the limited spatial resolution from eCI stimulation, which could be fundamentally improved by spatially more selective optogenetic CIs (oCI). Here, we aim to combine chronic IC recordings together with acoustic/channel discrimination behavioral experiments for comprehensive assessments of channel discrimination in eCI and oCI.

Methods: In a first group of experiments, Mongolian gerbils (n=10) were pre-trained using the ShuttleBox (SB), a negative reinforcement paradigm encouraging avoidance behavior, to discriminate deviant pure tones from background pure tones. Animals were subsequently chronically implanted with an eCI (MED-EL electrode arrays with up to 5 electrodes, n=3) and subjected to CI channel discrimination tasks in the SB. In a second group of experiments, non-trained Mongolian gerbils (n=4) were chronically implanted in the IC with a multiunit neural probe (Neuronexus, A1x32-6mm-50-177-A32) and tonotopy was derived over time using 100-ms pure tones spanning 0.5 to 32 kHz in awake as well as anaesthetized states.

Results: Animals were successfully trained on an acoustic discrimination task, psychometric curves were fitted to data collected and frequency difference limits using our setup were estimated (Weber fraction of 6.4+/-1.95%, R2 of 93.65+/-6.05%, n=6). Data collected from eCI-implanted animals show increased false alarm rate compared to prior implantation. Tonotopy of the IC was successfully derived in animals prior to chronic implantation of multiunit neural probe (4.33 octaves/mm, n=3), however chronic recordings showed degrading signal levels over time - mean absolute deviation (MAD) levels decreased from 4.48µV to 2.34 µV.

Conclusions: We established acoustic discrimination experiments using the ShuttleBox and tested frequency difference limits. Now we move on to test channel discrimination performance in CI implanted animals. Chronic neural recording of the IC demonstrated reduction of signal to noise levels over time, which may indicate scarring tissue and limits the timeline of the experiments. Alternative recording electrodes are being investigated. Nonetheless, combining chronic IC recording with behavioral experiments seems feasible. This is a promising step towards comparison of state-of-the-art CI channel discrimination performances with next-generation CI technologies using optogenetic stimulation.

Evaluation of Cochlear Health in DTR Mice With Chronic Cochlear Implants

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Category: Auditory Prostheses

Background: Cochlear implant (CI) animal models provide an important opportunity to improve outcomes for CI users. It is assumed that CIs function by electrically stimulating healthy spiral ganglion neurons (SGNs), however in many deafened animal models, inner hair cell (IHC) ablation is tied to poor SGN survival. In contrast, the deafened diphtheria toxin receptor (DTR) mouse allows for IHC elimination while preserving SGN density. By implanting DTR mice, we aim to understand the contributions of SGN density and IHCs to CI function.

Methods: Implantation was performed with a single platinum/iridium ball electrode in the scala tympani at age 14 weeks on average in hearing wild type (WT) mice (n=3), hearing DTR mice (n=7), and DT-deafened DTR mice (n=13). Electrically-evoked auditory brainstem responses (EABRs) were recorded weekly under anesthesia. EABR amplitude growth function (AGF) slopes were calculated objectively using methodology previously described (Skidmore et al. 2021). To avoid the possibility of SGN loss in long-term deafened DTR-mice, maximum survival time was fixed at 2.5-3 months post-deafening. Hearing mice were allowed to survive until a

complication occurred (e.g. implant failure, anesthesia stress, or vestibular overstimulation). Cochlear sections were obtained, and SGN density and IHCs were assessed.

Results: Histology confirmed IHC loss in the DT-deafened mice. In most mice, SGN density was preserved throughout the cochlea, however in a subset of deafened and implanted-hearing mice, SGN loss was observed at the base in the region of the implant. In most mice, implants remained functional for at least 1 month post-implantation. High EABR AGF slopes were only seen in mice with high SGN density, consistent with findings in guinea pigs and mice with stable long-term multichannel implants. Regarding the effect of IHC preservation, data were variable, and more animals need to be tested to establish clear trends.

Conclusions: The chronically implanted DTR mouse is useful for studies of cochlear health. Further studies are needed to better quantify the relative contributions of SGN density and IHC presence to CI function, particularly with larger numbers of mice and other mouse models of hereditary deafness. This will allow the design of appropriate interventions to improve clinical outcomes in CI users.

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Monopolar, Bipolar and Four-Point Impedance Measurements for Predicting Cochlear Implant Electrode Placement

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Category: Auditory Prostheses

Background: Positioning of cochlear implant (CI) electrode arrays close to the spiral ganglion cell bodies within the modiolus increases frequency selectivity and thereby improves hearing outcomes. Measurements of electrical impedance recorded at the CI's electrode contacts could potentially be used to assess electrode positioning in real-time during electrode array insertion. In this study, we characterized the relationship between three different electrical impedance measurements and electrode-modiolar distance.

Methods: Monopolar impedances, bipolar impedances in response to monopolar stimulation, and four-point impedances are made during step-wise CI insertions in human cadaveric temporal bones. The distance between the electrodes and the modiolus is assessed at each step using cone beam computed tomography. Linear mixed regression is used to assess the relationship between the impedances and electrode-modiolar distances. The experimental results are compared with an existing lumped-element model of an implanted CI and with clinical impedance data.

Results: Bipolar and four-point impedance strongly correlate with electrode-modiolar distance, while monopolar impedances are only minimally affected by changes in electrode positioning with respect to the modiolus. An overall model specificity of 62% was achieved when incorporating all impedance parameters. This specificity could be increased beyond 73% when prior expectations of electrode array positioning are incorporated in the model.

Conclusions: Bipolar and four-point impedances are promising measures to predict electrode-modiolar distance in real-time during CI electrode array insertion.

Cochlear Implantation Facilitates the Use of Spatial Cues to Segregate Competing Speech in Mandarin-Speaking Unilaterally Deaf Listeners

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Category: Auditory Prostheses

Background: Binaural hearing plays an important role in segregating competing speech when spatial cues are available. However, bilateral and bimodal cochlear implant (CI) patients often do not benefit from spatial cues to segregate competing speech. The aim of this study was to investigate whether CI patients with unilateral hearing loss can utilize spatial cues for recognition of target speech in the presence of symmetrically placed masker speech.

Methods: Speech recognition thresholds (SRTs) for competing speech were measured in 12 adults with normal hearing (NH) and 12 unilaterally deaf CI patients. Among the 12 CI patients, five had unrestricted acoustic hearing in the non-implanted ear (single-sided deafness, SSD) and the remaining seven had restricted acoustic hearing in the non-implanted ear (asymmetric hearing loss, AHL). For unilaterally deaf patients, SRTs were measured with the CI on or off. SRTs were measured for target sentences produced by a male talker in the presence of two masker talkers. The target sentence was always presented via loudspeaker directly in front of the listener (0°), and the maskers were either co-located with the target (0°) or spatially separated from the target and symmetrically placed at ±90°. Three segregation cue conditions were tested to measure masking release (MR) relative to the baseline condition (where no talker sex or spatial cues were available): 1) Talker sex, 2) Spatial, and 3) Talker sex+spatial.

Results: With the CI on, overall SRTs were significantly lower (better) for NH listeners than for SSD CI or AHL CI listeners, and significantly lower for SSD CI than for AHL CI listeners. For NH listeners, MR was largest for the Talker sex+spatial condition, followed by the Spatial and Talker sex conditions. The pattern was different for SSD CI listeners, where MR was largest for the Talker sex+spatial condition, followed by the Talker sex and spatial conditions. For AHL CI listeners, MR was largest for the Talker sex+spatial condition, with no significant difference between the Talker sex and Spatial conditions. Across all conditions, MR was significantly larger with the CI on than off. Across all CI patients, MR was highly correlated with pure tone average (PTA) thresholds in the non-implanted ear for the Talker sex and Talker sex+spatial conditions, but not for the Spatial condition.

Conclusions: Different from bilateral or bimodal CI patients, cochlear implantation may benefit unilaterally deaf patients' utilization of spatial cues to segregate competing speech. The amount of MR due to spatial cues did not depend on the extent of acoustic hearing in the non-implanted ear. The results suggest that with sufficient acoustic hearing in the non-implanted ear, unilaterally deaf CI patients may effectively combine contralateral acoustic and electric hearing to segregate competing speech.

Temporal Processing at Different Levels of the Auditory Pathway in Cochlear Implant Recipients
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Category: Auditory Prostheses

Background: The benefits of cochlear implantation for postlingually deafened individuals vary widely across patients, especially for speech perception performance in noise (Robert et al., 2013; Budenz et al., 2011). Several lines of research suggest that temporal processing is crucial for understanding speech in noise and accounts for substantial variations in patient speech perception outcomes (Sagi et al., 2009; Fu, 2002). Psychophysical and electrophysiological measures of temporal processing (e.g., gap detection) have been studied in both acoustic (e.g., Hoppe et al., 2013; He et al., 2012; Lister et al., 2007; Martin and Boothroyd, 2000) and electrical hearing (e.g., Zhang et al., 2013; Kirby and Middlebrooks, 2012). It has been suggested that the physiological status of the auditory nerve (AN) may be important for auditory sensitivity to temporal gaps (Zhang et al., 2013; Kirby and Middlebrooks, 2012). To date, the relative contributions of the AN and the auditory cortex to the perception of temporal gap cues in cochlear implant (CI) users remains unknown. This study aimed to investigate the association among the temporal response properties of the AN, the cortical encoding of temporal gaps and the perceptual sensitivity to temporal gap cues in postlingually deafened adult CI users.

Methods: To date, nine subjects have been recruited and tested for this study. All subjects were implanted with a Cochlear™ Nucleus® device in the test ear(s) with full electrode insertions. Two electrodes with the largest differences in the speed of recovery from neural adaptation at the AN were selected for each subject. Within-channel gap detection thresholds (GDTs) were measured using pulse trains with a pulse rate of 900 pulses per second (pps) per channel presented directly to individual electrodes using both psychophysical procedures and electrophysiological measures of the auditory change complex (ACC).

Results: Our preliminary results showed that GDTs measured using psychophysical and electrophysiological approaches were significantly correlated. ACC thresholds were comparable to psychophysical thresholds for gap detection. No significant correlation was observed between adaptation recovery of the AN and the ACC GDT thresholds across subjects. However, within individual CI users, electrodes showing slower recovery from neural adaptation at the AN had a larger ACC GDT.

Conclusions: These preliminary results suggest that temporal gap sensitivity is affected by adaptation recovery of the AN within individual CI users. ACC could serve as a reliable indicator for auditory gap detection.

What Builds the Precedence Effect?
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Category: Binaural Hearing and Sound Localization
Background: Most of what is known about how listeners localize in reverberant environments has been for single sound presentations over headphones of a simulated direct sound (lead) followed milliseconds later by a simulated reflection (lag), each presented with different interaural disparities. Within some range of lead/lag delays, listeners report hearing a single sound source located near the position of the leading sound source – these effects of precedence collectively called the precedence effect. In everyday environments, sound sources often emit relatively continuous sounds or repeat them often in succession, for example speech. Also, listeners often move. Previous studies have shown that repetition of the same stimulus under simulated reverberant conditions can alter listeners' spatial perception of the stimulus so that, for the same lead/lag delay where a direct sound and its echo would be reported with one stimulus presentation, a fused stimulus perceived near the lead is reported after multiple presentations, the so-called "buildup of the precedence effect." Explanations for this effect have often centered on the idea that listeners "learn" the spatial acoustics of the environment. An alternative explanation is that listeners simply learn the temporal order and timing of reflections and are relatively unaffected by the spatial cues contained in stimuli that are presented after the lead.
Methods: We sought to disambiguate these two theories by comparing listener behavior under conditions where listeners were (1) presented a single stimulus (2) presented with multiple stimuli (3) presented with multiple stimuli and rotated their heads, and (4) presented with multiple stimuli and the location of the lag sound source changed for each location while the lead remained at a single location. Stimuli were presented over loudspeakers placed 15 degrees apart that surrounded the listener. Listeners' perceived sound-source localization and the rate of fusion were measured.
Results: Initial results suggest that movement of the listener and/or simulated reflection (lag) did not disrupt the buildup of the precedence effect.
Conclusions: These results offer support for the notion that the buildup of the precedence effect may be more associated with learning of the temporal aspects of reverberation rather than the spatial characteristics of the room that produced the reverberation.

The Complex-Valued Correlation Coefficient Across Frequency Channels Accounts for Binaural Detection
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Category: Binaural Hearing and Sound Localization
Background: Differences in interaural phase configuration between a target and a masker can lead to substantial binaural unmasking. This effect is decreased for masking noises having an interaural time difference (ITD). Combining two noise tokens with opposite ITDs (double-delayed noise) in most cases further reduces binaural unmasking. Thus far, modeling of these detection thresholds assumed both a mechanism for internal ITD compensation and a larger binaural than monaural bandwidth. However, neither has yet been demonstrated in mammals. This study introduces an alternative mechanism to account for a large extent of binaural unmasking involving monaurally derived peripheral filter bandwidths and no internal delay compensation.
Methods: A quantitative multi-channel model is proposed which involves monaurally derived peripheral filter bandwidths (ERB = 79 Hz at 500 Hz center frequency). Instead of the so far required cross-correlation function, the complex correlation coefficient is evaluated, i.e. only one complex number at lag zero. To account for the reduced unmasking in double-delayed noise, an alternative explanation is suggested: Unmasking is assumed to be impaired by a lower interaural coherence in off-frequency regions (Marquardt and McAlpine 2009, JASA pp. EL177 – EL182), implemented as an across-channel incoherence interference mechanism. This mechanism differs from wider filters since it has no effect when the masker coherence is constant across frequency bands. The model further contains a monaural energy discrimination pathway to account to conditions where no interaural cue is provided.
Results: The model predicts a large range of binaural unmasking data with high accuracy. This includes the different thresholds between single- and double-delayed noise. Furthermore, it accounts for data sets that thus far were associated with a wider binaural than monaural bandwidth.

Conclusions: A quantitative binaural model was presented that does neither require internal delay compensation nor wider filters and appears to be well compatible with the physiology of the mammalian binaural system. The model can account for binaural unmasking including complex maskers like double-delayed noise. This helps resolving the inconsistency that simulation of some data sets requires wide filters while others require narrow filters.

Comparison of Click Vs. Chirp Stimuli in Evoking the Binaural Interaction Component (BIC) of the Auditory Brainstem Response (ABR)

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Category: Binaural Hearing and Sound Localization
Background: Chirp stimuli are specifically designed to enhance temporal synchrony in the responses of the auditory nerve and brainstem by accounting and compensating for the frequency- and level-dependent delays accrued by the traveling wave in the cochlea. Chirps have been shown to produce larger amplitude responses in specific waves/peaks of the auditory brainstem response (ABR). Here we test the hypothesis that chirp stimuli can increase the amplitude of the binaural interaction component (BIC) of the ABR. The BIC of the ABR is a potential biomarker for binaural hearing. However, clinical use of the BIC has been limited because it is unreliably measured using traditional click or tone stimuli in humans. Here we generate and compare different chirp stimuli methods to maximize the BIC.
Methods: ABR peak latencies were measured in response to tone bursts over a range of stimulus frequencies and intensity levels in seven chinchillas. Stimuli consisted of 5-ms tone bursts (1, 2, 4, 8, 16, 24 kHz) presented via sealed and calibrated insert earphones from ~10 dB below to 50 dB above ABR detection threshold. Latencies of ABR waves I-IV and the BIC DN1 wave (the largest amplitude peak in the BIC) were plotted against frequency and then fit to a power function: $Tau = k*f^{\wedge}(-d)$, where tau is latency in seconds, f is frequency, and k and d are constants. The values of k and d were then used to construct a series of sound level-specific chirps for three above-threshold stimulus intensity levels based on 1) monaural ABR wave I 2) monaural ABR Wave IV and 3) BIC DN1.
Results: Monaural ABR peak latencies decreased systematically with increasing sound frequency as expected from the corresponding latencies due to cochlear delay. Consistent with other studies, our results show that monaural ABR peak amplitudes were enhanced by chirps which compensate for that latency relative to those measured with clicks. Surprisingly though, BIC DN1 peak latencies did not show a similar frequency-dependent latency shift. Rather BIC DN1 latencies appeared to be relatively constant despite increases in stimulus frequency.
Conclusions: Traditional chirp stimuli designed to optimize monaural ABR peak amplitudes by accounting for frequency-dependent cochlear delays appear ill-suited for eliciting optimal BIC peak amplitudes. Traditional broadband clicks or other non-traditional chirp stimuli may be preferable for eliciting an optimal BIC.

Tonotopic Distribution and Projection Pattern to Inferior Colliculus of Inhibitory and Excitatory Cell Types in the Lateral Superior Olive of Mongolian Gerbils and Mice: Cellular Specializations for Low-Frequency Hearing

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Category: Binaural Hearing and Sound Localization
Background: Principal neurons (PNs) of the lateral superior olive (LSO) nucleus in the brainstem compare inputs from the two ears. They receive excitatory inputs driven by the ipsilateral ear and inhibitory inputs driven by the contralateral ear. These cells are important for sound localization on the horizontal plane and are sensitive to intensity and timing differences between the ears. There is cellular diversity within the LSO that is not well understood that may underlie its processing of multiple sound localization cues.
LSO PNs project to upstream auditory processing centers and consist of glycinergic inhibitory and glutamatergic excitatory cells. There is some disagreement on their relative numbers, distribution, and projection pattern perhaps due to differences between animal models and cell identification methods. Additionally, these parameters have not been determined in two important animal models, Mongolian gerbils and mice.

Gerbils are an important rodent model system as they have low-frequency (LF) hearing. This is significant because so much of human speech is LF and our selective attention relies on sound localization. Mice lack LF hearing but have the advantage of availability of many genetic manipulations. Additionally, the comparison between rodents with and without LF hearing may provide insights into the cellular organization and evolution of this ability.

Methods: We used in situ hybridization to label inhibitory and excitatory LSO neurons combined with retrograde tracer injections into the inferior colliculus (IC) to examine cell type-specific projection patterns. To identify inhibitory LSO PNs, we targeted transcripts of sodium- and chloride-dependent glycine transporter 2 (GlyT2, Slc6a5). To identify excitatory LSO PNs we targeted vesicular glutamate transporter 2 (vGlut2, Slc17a6) transcripts as it is the dominant vGlut in the LSO. FluoroRuby (10%, 150 nL total) was pressure injected into the central IC unilaterally.

Results: We processed tissue from 10 injected gerbils (P32-35). There was no overlap between cell types and excitatory ones were more numerous (76%). Inhibitory LSO neurons almost exclusively projected ipsilaterally making up 41% of the ipsilateral projection and exhibited a moderate low frequency bias (10% difference High-Low). Two thirds of excitatory neurons projected contralaterally and had a slight high frequency bias (4%). One third of excitatory LSO neurons projected ipsilaterally and were strongly biased toward the LF limb (37%).

We also made injections in 9 C57Bl6/J mice (P38-48). Preliminary assessment of ongoing analysis suggests that there is little to no overlap between GlyT2 and vGlut2 positive cells and contrary to our gerbil findings, most ipsilaterally projecting cells are inhibitory.

Conclusions: The excitatory ipsilateral projection from LSO to IC in gerbils is also found in cats, but not rats and likely not in mice. The association with LF-hearing species and the LF bias we discovered in gerbils, suggest this pathway may be a specialization for LF ability.

A Conceptual Framework for Understanding Interaural Asymmetry With Bilateral Cochlear Implants

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Category: Binaural Hearing and Sound Localization

Background: Bilateral cochlear implants (BiCIs) have been provided to patients with severe to profound hearing loss more frequently in recent time. BiCIs can result in several benefits, including improvements in speech understanding in noise and sound source localization. However, the amount by which each patient benefits varies considerably. One reason for this variability is difference between the two ears' hearing function, i.e., interaural asymmetry.

Methods: Thus far, investigations of interaural asymmetry have been highly specialized within various areas. The goal of this presentation is to provide a new framework under which these studies can be integrated synergistically. This framework begins with a binaural signal, ends with a behavioral response, and consists of two interim stages: encoding and decoding of binaural information.

Results: Encoding of binaural cues is represented using excitation-inhibition (EI) of signals from the left ear and right ear according to where the signal originated in space. Encoding is limited by peripheral changes associated with hearing loss and implantation for listeners with BiCIs. To test the impacts of poor encoding, model EI cells were presented with stimuli containing binaural cues. These model EI cells were highly predictive of the perceived location for listeners with BiCIs and in simulations of normal hearing (NH), both with and without interaural asymmetry. This demonstrates, via one highly relevant mechanism of binaural processing, that poorer encoding resulting from interaural asymmetry is associated with poorer binaural outcomes.

Decoding of binaural cues is represented using predictive coding. Predictive coding assumes that expectations based on prior sensory experience and transmitted via top-down connections are compared against sensory input (i.e., bottom-up connections). When listening bilaterally in the presence of interaural asymmetry, the predictions for each ear may be considerably different from one another. Speech perception experiments from listeners with BiCIs and simulations in NH were evaluated. For listeners with BiCIs, speech perception was evaluated independently in each ear and again for bilateral presentations. For listeners with NH, temporal fidelity was manipulated independently in both ears from good to poor. Results showed that there was a disproportionate effect of the poorer ear on divided attention and speech segregation, limiting speech understanding.

Conclusions: This new framework can be used to integrate physiological, behavioral, and modeling studies to gain a clearer understanding of the implications of interaural asymmetry and devise strategies for optimal patient interventions.

Cortical Mechanisms of Across-Ear Speech Integration Investigated Using Functional Near-Infrared Spectroscopy

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Category: Binaural Hearing and Sound Localization

Background: Cochlear implants (CIs) can rehabilitate bilateral hearing in listeners with single-sided deafness (SSD-CI), and in listeners with bilateral deafness (BiCI). Behavioral studies suggest that patients in both groups benefit from bilateral hearing in tasks involving sound localization or speech understanding in noise; however, they perform worse than do typical-hearing (TH) listeners. This study examined how TH listeners presented with SSD-CI and BiCI simulations integrate speech that is streamed to both ears in an alternating manner, to assess auditory attention and across-ear integration. We further explored whether neural underpinnings of these effects are revealed in cortical activity patterns measured using functional near-infrared spectroscopy (fNIRS).

Methods: We tested 20 NH listeners with sequential segments of spoken sentences alternating between ears at rates of 2, 4, 8, and 32 Hz. We simulated SSD-CI and BiCI hearing in this group by presenting vocoded speech in the right ear or in both ears, respectively. We expected to observe a V-shaped speech intelligibility function across rates: At low rates listeners might perform well by switching attention between ears, whereas at high rates listeners might perform well by bridging the shorter silent gaps in either ear. At intermediate alternating rates (around 4 Hz), if neither strategy is effective, speech intelligibility should decrease. We obtained speech intelligibility scores using this stimulus, and fNIRS measures in two brain regions: the bilateral auditory cortices (AC), which are sensitive to intelligible speech, and the bilateral dorsolateral prefrontal cortices (DLPFC), which play a role in auditory attention.

Results: Speech intelligibility results in TH and SSD-CI simulated conditions demonstrated high scores across all alternating rates. A V-shaped intelligibility function was not observed in either condition, and TH scores were marginally higher than SSD-CI scores at 2 and 4 Hz. In the BiCI simulated condition, a V-shaped speech intelligibility function with was observed with lowest scores at 4 Hz. BiCI scores were significantly lower than both SSD-CI and TH scores at all alternating rates ($p < 0.001$). For fNIRS data, we analyzed the relationship of fNIRS response amplitudes across rates, listening conditions and brain regions. The left AC (LAC) and right DLPFC exhibit differing patterns of activation in response to alternating speech stimuli. Importantly, in conditions with high speech intelligibility scores, cortical activity indicated different listening strategies across the four alternating rates. Addition of degraded speech in SSD-CI and BiCI conditions altered patterns of LAC and right DLPFC activity compared to the TH speech condition.

Conclusions: Our findings suggest that degraded speech inputs in one or two ears impacts across-ear integration of speech stimuli, and that fNIRS measures might reveal differences across conditions not revealed by speech intelligibility data alone.

Binaural (pre)processing for Contralateral Sound Field Attenuation and Improved Speech-In-Noise Recognition

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Category: Binaural Hearing and Sound Localization

Background: Understanding speech presented in competition with other sounds is still one of the main challenges faced by people with hearing loss. Here, we present and evaluate a binaural sound (pre)processing algorithm aimed at improving speech intelligibility in multi-talker, free-field listening scenarios.

Methods: The algorithm enhances the signal-to-noise ratio in the ear ipsilateral to the target sound by attenuating the contralateral sound field. This is achieved by linear, weighted subtraction of the contralateral stimulus, with a weight equal to the ratio of ipsilateral to contralateral head-related transfer functions (HRTF) averaged over an appropriate azimuth range (regarded as a parameter). We assess the benefits provided by the algorithm alone and in combination with two independently functioning hearing aids (one per ear). Assessments include simulations of

intelligibility as well as experimental evaluations in monaural and binaural listening, for several target-masker spatial configurations and for listeners with normal hearing and hearing loss.

Results: We found good agreement between simulated and measured benefits, with speech reception thresholds in noise improving up to 15 dB in some conditions. We also found that the algorithm (1) does not significantly alter the localization of sound sources in the horizontal plane, and that (2) it qualitatively accounts for binaural unmasking for speech in competition with multiple maskers and for multiple target-masker spatial arrangements.

Conclusions: Altogether, findings show that the algorithm can improve the intelligibility of speech in free-field multi-talker listening scenarios without the user's intervention and without making any assumption about the characteristics of the sound sources or their location. [Work supported by the Spanish Ministry of Science and Innovation (grant PID2019-108985GB-I00) and by MED-EL GmbH].

Brain-Derived Neurotrophic Factor is Required for Activity-Dependent Tonotopic Refinement of Mntb Neurons

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Category: Brainstem: Structure and Function

Background: In the mammalian brain, auditory brainstem nuclei are arranged topographically according to acoustic frequency responsiveness. During postnatal development, the axon initial segment (AIS) of principal neurons undergoes structural refinement depending on its location along the tonotopic axis within the medial nucleus of the trapezoid body (MNTB). However, the molecular mechanisms underlying the structural refinement of the AIS along the tonotopic axis in the auditory brainstem have not been explored. We tested the hypothesis that brain-derived neurotrophic factor (BDNF) is a molecular mediator of the structural development of the MNTB in an activity-dependent manner.

Methods: Using BDNF heterozygous mutant (BDNF+/−) mice, we examined the impact of global BDNF reduction on structural and functional development of MNTB neurons by assessing AIS structure and associated intrinsic neuronal properties.

Results: BDNF reduction inhibits the structural and functional differentiation of principal neurons along the tonotopic axis in the MNTB. Augmented sound input during the critical period of development has been shown to enhance the structural refinement of the AIS of MNTB neurons. However, in BDNF+/− mice, MNTB neurons did not show this activity-dependent structural modification of the AIS.

Conclusions: Taken together, structural development and functional refinement of auditory brainstem neurons require physiological levels of BDNF to establish a proper tonotopic organization.

Arousal States Modulate Frequency Following Responses Across Humans and Guinea Pigs

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Category: Brainstem: Structure and Function

Background: Frequency following responses (FFRs) are scalp-recorded electrophysiological signals that reflect ensemble neural activity phase-locked to stimulus periodicity. FFRs are used to assay fine-grained auditory processing in healthy as well as clinical populations. Emerging evidence in the past decade has shown that top-down modulatory factors such as attention and stimulus expectation modulate FFRs. Arousal states are reflective of locus coeruleus-norepinephrine networks and have been shown to modulate the activity of auditory neural circuits that contribute to the FFRs. However, little is known about the influence of arousal states on the FFRs and its significance in understanding auditory processing through the lens of FFRs. We leveraged a multimodal neuroimaging approach combining pupillometry with electroencephalography to assay the modulatory effects of arousal states on the FFRs. We used a cross-species approach (human and guinea pig) to provide a scaffolding for future studies geared towards understanding the precise neural underpinnings of such top-down influences on the FFRs. Stimulus expectation has been shown to significantly modulate arousal states. Thus, we also assessed if stimulus expectation mediated changes in arousal state differentially modulated the FFRs.

Methods: We performed simultaneous scalp electroencephalography and pupillometry while humans or guinea pigs listened to repetitively presented pitch-varying stimuli. Peak pupillary dilation (PPD), a proxy for arousal level, was measured for each trial, and FFRs were sorted based on a median split of the PPD (high PPD – high arousal and low PPD – low arousal).   FFR pitch strength was quantified using sliding-window autocorrelation analysis in each group. To assess stimulus expectation mediated arousal effects on the FFRs, an oddball paradigm was adopted and the FFRs to the rare stimulus in the oddball were compared between high- and low-arousal states.

Results: FFR pitch tracking accuracy differed across arousal states, with higher pitch tracking accuracy in low arousal states and lower pitch tracking in high arousal states. This trend was statistically significant at individual subject-levels in both model species ($p<0.001$, paired t-tests on bootstrapped FFR trials). On the other hand, stimulus expectation effects showed an opposite trend, where FFRs to rare stimuli showed higher pitch strength in high arousal states.

Conclusions: Our results show that FFRs are significantly modulated by arousal states in both model species. Our results also show a dissociation between FFRs in exploratory arousal states and stimulus expectation driven arousal states. Our results lay the groundwork for future research that will elucidate the precise neural mechanisms linking arousal states with FFRs.

The Auditory Brainstem Response in Hatchling Chicken

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Category: Brainstem: Structure and Function

Background: The auditory brainstem response (ABR) is an invaluable assay in clinical audiology as well as animal research. The embryonic chicken is an extensively studied model for auditory development, form, and function, especially brainstem microcircuitry. However, there exists minimal modern research methods for recording ABRs in hatchling chickens. While there are modern ABR studies in avian species like the finch, budgerigar, and owl, these models are altricial species that undergo further development after hatch. The chicken is a precocious animal with near mature auditory function during late embryonic and early hatchling stages. Therefore, the ABR in hatchling chickens is not only an objective measure of auditory neural synchrony and hearing sensitivity, but an invaluable in-vivo methodology to compare to in-vitro molecular and developmental research.

The auditory brainstem of birds is well correlated to that of mammals. Both species have an auditory nerve that provides excitatory, glutamatergic input to distinct cochlear nucleus structures. The avian cochlear nucleus magnocellularis (NM) is considered analogous to the mammalian anterior ventral cochlear nucleus (AVCN). NM sends an excitatory projection to nucleus laminaris (NL), which is analogous to the mammalian medial superior olive (MSO). This auditory microcircuitry is critical for sound localization and early auditory temporal processing. Therefore, the composition of the ABR up to auditory midbrain is likely comparable across species.

Methods: Recordings from 43 wildtype hatchling chickens (post-hatch age P1-P2) always presented with 3 positive going peaks within 6 ms of a suprathreshold click stimulus. Peak-to-trough amplitudes ranged from 2-11 µV at high intensity levels, exhibiting appropriate latency-intensity functions.

Results: The timing of peaks was consistent across animals, allowing for identification of waves I, II, and III, with an average click threshold of 40 dBSPL. A subset of hatchlings had additional microstructure within wave II, or additional positive peaks after 6 ms, posing the possibility of four and five ABR peak waveforms. While exhibiting at least three positive peaks within 6 ms is consistent across avian species, mapping peaks to specific brainstem structures requires further study.

Tone burst ABRs were acquired at nine frequencies ranging from 100 Hz to 4000 Hz. The range of best sensitivity was found between 750-2000 Hz, with an average threshold of 30 dBSPL at 1000 Hz. Not only were click and tone burst ABRs characterized, but important variations in methodology were explored. Hatchling body temperature, age in hours post hatch, and alternative positioning of the reference electrode all resulted in important changes in ABR morphology.

Conclusions: Overall, the ABR methods outlined here permit accurate and reproducible recording of in vivo auditory function in hatchling chicken that could be applied to different stages of embryonic development. Such findings are easily compared to human and mammalian models of hearing loss, aging, or other auditory-related manipulations.

Functional and Molecular Profiling of Cell Diversity and Identity in the Auditory Brainstem: Patch-Seq in the Lateral Superior Olive (LSO)

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Category: Brainstem: Structure and Function

Background: The LSO is a prominent integration hub in the auditory brainstem which is involved in sound localization. Towards this task, it processes information in a fast and temporally precise manner. The LSO comprises highly heterogeneous cell types. The nature, extent, and origin of the heterogeneity are unclear. Investigation of the molecular determinants of cellular heterogeneity has become possible through next-generation RNA-sequencing which has revolutionized the entire field of biology. One powerful multi-modal single-cell RNA-sequencing approach is patch-seq, which combines electrophysiological characterization with global transcriptomic analysis, thus allowing to include functional and anatomical properties (e.g. tonotopic location) into the analysis.

Methods: We here employed patch-seq in adolescent mice to decipher the biophysical, genetic, and anatomical determinants of LSO neurons. Furthermore, we used immunohistochemistry for validation, and electrophysiology to investigate promising candidates obtained by patch-seq.

Results: Two major neuron types were identified, corresponding to LSO principal neurons and lateral olivocochlear (LOC) neurons. We found novel marker transcripts specific to both neuron types and validated some findings via immunohistochemistry. Differentially expressed genes analysis revealed 348 genes with higher expression in principal neurons. Among these, enriched transcripts were annotated to several gene ontology terms, e.g. "ion channel activity", "calcium binding", and "protein kinase activity". Transcripts coding for potassium channels and modulators were especially prominent in principal neurons, namely Kcnh7, Kcnab3, and Kcnip1. Transcripts with higher expression in principal neurons also included Cacng5 and Ryr3, coding for calcium binding proteins, as well as Fgfr2, Wnk1, and Sgk3 coding for protein kinases which play a role in ion channel modulation. We reason that such molecules play essential roles in fast and temporally precise information processing underlying sound localization. We found 78 genes expressed higher in LOC neurons for which details will be presented on the poster. The expression level of 38 genes correlated with biophysical properties and the tonotopic location of principal neurons, but not of LOC neurons. Kcnh7, which codes for ether-à-go-go-related gene potassium channel 3 (ERG3), was one of the genes with the highest correlation coefficient. We therefore analyzed ERG channels electrophysiologically and found that they regulate the excitability of principal neurons. Furthermore, we detected two distinct subtypes of principal neurons, namely onset and sustained firing neurons. They differed mainly in transcripts coding for axon guidance and cell adhesion molecules. Because cell adhesion molecules participate in target finding and forming functional synapses, we assume that onset and sustained firing principal neurons project to different brain regions.

Conclusions: Collectively, our results significantly expand the knowledge on the biophysical, genetic, and anatomical determinants of neuronal heterogeneity in the LSO and provides a screening tool for candidate transcripts and proteins for future projects.

Regulation of Synaptic Inhibition and Ca2+ Signaling by mGluR5 in the Developing Sound Localization Circuit in the Mouse Brainstem

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Category: Brainstem: Structure and Function

Background: In the lateral superior olive (LSO), activation of metabotropic glutamate receptors (mGluRs) with generic (non-subtype-specific) agonists modulates neural properties, causing membrane depolarization, inhibiting neurotransmission, and increasing intracellular Ca2+ concentration. It is unknown whether mGluR5 is involved in any of these modulatory functions, especially during early development. Here, we combine whole-cell patch recording and Ca2+ imaging to examine the modulatory effects of mGluR5 on both electrical and Ca2+ responses of LSO neurons during development.

Methods: Brainstem slices were prepared from C57/B6 mice of either sex, and whole-cell current-clamp recordings and Ca2+ imaging experiments were performed at 35 °C. Cells were loaded with a Ca2+ indicator dye Fura2-Na (1 mM) and biocytin (0.5%). mGluR5 is one of the two members of group I mGluRs (the other member is mGluR1). There is no specific antagonist for mGluR5. To activate mGluR5, we bath-applied a group I mGluR

agonist 3,5-DHPG (200 μM) in the presence of mGluR1 antagonist JNJ16259685 (10 nM). Cell morphology was revealed with biocytin staining after physiology recordings.

Results: To confirm the feasibility of Ca2+ imaging using the patch-filled approach, we applied 60 mM KCl-containing ACSF, which depolarized and excited LSO neurons, and produced large Ca2+ increases. To further test the sensitivity of the Ca2+ imaging method, we injected suprathreshold current pulses to make the cell fire action potentials while performing Ca2+ imaging. A 100 Hz pulse train stimulation delivered at the rate of 1 train/s triggered physiological Ca2+ responses. Pharmacological activation of mGluR5 enhanced spontaneous inhibitory transmission and depolarized LSO neurons, resulting in bursting sIPSPs, with minimal Ca2+ responses. Biocytin staining revealed bipolar dendritic arborization, typical of principal cells in the LSO in coronal sections.

Conclusions: These data demonstrated the feasibility of combining whole-cell recording and Ca2+ imaging in LSO neurons, and revealed a novel phenomenon that mGluR5 enhanced spontaneous inhibitory transmission at the MNTB-LSO synapse.

Inhibitory Inputs Onto Spherical Bushy Cells Have Distinct Roles in Shaping Neuronal Responses

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Category: Brainstem: Structure and Function

Background: Neuronal inhibition is crucial for temporally precise and reproducible signal transmission in the auditory brainstem. In the cochlear nucleus (CN), inhibition has been shown to modulate responses of spherical bushy cells (SBCs) which are integral to the sound localization circuit. The cellular mechanisms underlying this inhibition have been described both in vitro and in vivo, including their functional consequences. Inhibition in the CN is dominated by two cell types, the tuberculoventral (TV) and D-stellate (DS) cells. However, the dense architecture of the cochlear nucleus has so far precluded linking the physiological properties of inhibition to the underlying anatomical structures.

Methods: To dissect the influences of TV and DS cells on the activity of SBCs we combined experimental recordings from SBCs obtained under natural acoustic stimulation with a recently published biophysical model of CN neurons (Manis and Campagnola 2018). We constrained the model using single-neuron specific spike trains and previously estimated neuronal and circuit properties to approximate the physiology of the gerbil auditory brainstem. Synaptic strength of the excitatory endbulb input was adjusted to match the experimental data.

Results: In this configuration, the model cells in the CN created responses resembling the experimental data. In particular, inhibition mediated by TV and DS cells enhanced sparsity and reproducibility in SBC compared to their respective auditory nerve input. We showed that this improvement was indeed caused by TV and DS cells, since removing both cell types from the model rendered the SBC responses similar to their auditory nerve inputs. Including only DS inhibition rescued the increase in both sparsity and reproducibility, while TV inhibition alone showed little effect.

Conclusions: In summary, we predict that DS inputs to SBC cells dominate the improvements in neuronal coding observed in vivo and therefore play a central role in gain control of SBC responses in adverse acoustic environments, such as broadband background noise.

The Ultrasonic Hearing Abilities of the Domestic Cat as Assessed With Auditory Brainstem Responses

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Category: Brainstem: Structure and Function

Background: The upper hearing limit of humans is 20kHz, and sounds having frequencies above 20kHz are considered ultrasonic. Ultrasonic frequencies are important to a variety of species, many of which have a well-defined and much higher hearing limit compared to humans. A common model used for auditory research is the domestic cat (Felis catus). Even though it is generally accepted that cats can hear ultrasonic frequencies, there are notable discrepancies in the literature regarding the full extent of the cat's hearing abilities. Prior work involving the ultrasonic hearing abilities of cats mostly come from psychophysical methods. However, these methods can be very time-consuming, and results are often based on a small number of animals. To overcome these issues, we used auditory brainstem responses (ABRs) to evaluate the sensitivity of the cat's auditory system to ultrasonic

frequencies. Both the amplitudes and latencies of the ABR waves were evaluated and used to determine the ABR threshold, thereby evaluating the cat's hearing sensitivity in response to different stimuli.

Methods: Wideband stimuli (clicks) and ultrasonic pure tones between 8kHz and 80kHz were presented to each cat (n=6) at intensities ranging between 80dB and 20dB. Each stimulus produces electrical responses that reflect the neurophysiological activity in the auditory nerve and the auditory nuclei in the brainstem. These responses were recorded via subdermal electrodes. Afterwards, the amplitude and latencies of each of the waves were evaluated.

Results: The ABR waveforms change in response to the different stimuli presented. For all stimuli tested, the amplitudes of each of the waveform peaks decrease with decreasing intensity levels. The intensity levels at which each of the peaks in the waveform remains present, differ when the frequency of the pure tone stimulus is varied. These results were used to determine the ABR threshold for each of the frequencies presented.

Conclusions: Since cats are a common animal model in hearing research, it is crucial to validate and expand our current understanding of their full hearing abilities. Knowing how the ABR waveforms change when ultrasonic stimuli with frequencies in the cat's upper hearing range are presented to cats is useful for determining the ABR threshold at those frequencies. The information about the cat's sensitivity to ultrasonic sounds as determined from the ABR data, will also be useful in conjunction with data from psychoacoustic experiments to more conclusively establish the upper hearing limit of cats. Further, it could also provide insight into the neural mechanisms that might be involved when cats are perceiving these high frequency signals, and ultimately better our understanding of their hearing abilities.

In Vivo Multiwavelength Fluorescence Imaging of Cholesteatoma for Surgical Guidance
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Category: Clinical Otolaryngology and Pathology
Background: Cholesteatoma is a common and difficult to treat middle ear condition, so the only method of management is to surgically remove the diseased tissue. However, the difficulty of identifying the entirety of the cholesteatoma during the procedure is a major restraint, leading to a high percentage of relapse and causing severe hearing loss, balance issues, and even brain infection (meningitis).

Methods: We propose a multiwavelength fluorescence-based imaging strategy to assist during surgery and reduce cholesteatoma relapses. The study was conducted on Mongolian gerbils, induced with cholesteatoma via eustachian tube ligation, with a sample size of n = 5. Auditory brainstem response (ABR) measurements were performed before and after inducing cholesteatoma. After 6 weeks of inducing the cholesteatoma, the diseased tissue was surgically removed from the gerbil following either typical surgical procedure or using surgical guidance in the form of autofluorescence excited by multiple wavelengths at 405 nm, 450 nm, and 520 nm that specifically targeted collagen, NADH, and keratin found in cholesteatoma tissue. This was done with an otoendoscope connected to a CMOS monochrome camera containing multiple long pass filters at 425nm, 475nm, and 600nm.

Results: Intensity of fluorescence was calculated for each of the excitation wavelengths. Histology confirmation was performed using autofluorescence microscopy which corroborated the in vivo findings and presence of multiple fluorophores in cholesteatoma tissue.

Conclusions: Overall, the results indicate that by taking advantage of the autofluorescence of components specific to cholesteatoma, such as keratin, collagen, and NADH we can utilize its contrast for real-time surgical guidance. By doing so, we can better remove the diseased tissue, prevent more relapses and thus surgical procedures, and ultimately reduce the high risks and costs for both healthcare providers and patients.

Facial Nerve Abnormalities in Temporal Bones With Inner Ear Malformations
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Category: Clinical Otolaryngology and Pathology
Background: Inner ear malformations (IEMFs) are responsible for 20% of all cases of congenital sensorineural hearing loss, frequently requiring cochlear implants or auditory brainstem implants for hearing rehabilitation. To allow optimal cochlear implantation results with minimum risk of complications, the most frequent anatomical

features of these IEMFs need to be understood in depth. The objective of this study was to perform an otopathologic analysis of temporal bones (TBs) with inner ear malformations to determine the most frequent abnormalities affecting the facial nerve (FN).

Methods: From the Otopathology Laboratory at the University of Minnesota we selected 38 TBs from donors with IEMFs. These TBs were serially sectioned at a thickness of 20 μm, and every 10th section was stained with hematoxylin and eosin. We excluded 10 temporal bones who had severe processing artifacts that prevented a full analysis of the FN. The cochlear and vestibular malformations were classified as per Sennaroğlu and Bajin. We performed otopathologic analyses of the internal auditory canal (IAC) diameter, the angle of the first genu, the relationship of the FN with the oval window (OW), the facial recess (FR) at the level of round window (RW), and overall development of the FN. The gathered data were statistically analyzed.

Results: Our final study group included 28 TBs. From those TBs, 5 (17.8%) were classified as cochlear hypoplasia-II, 20 (71.4%) as cochlear hypoplasia-III, 2 (7.1%) as incomplete partition-II (Mondini deformity), and 1 (3.5%) was classified as an isolated vestibular malformation. We found abnormalities with FN in 24 TBs (85.7%) associated with IEMFs. The diameter of IAC was observed as hypoplastic in 2 TBs (7.1%). The angle of the first genu was obtuse in 11 TBs (39.2%). FN was abnormally located in 11 TBs (39.2%) compared to expected relationship of the FN with the OW (i.e., superior, and lateral to the OW). The FR was narrow (<2.5 mm) in 6 TBs (21%). Finally, the FN was hypoplastic in 18 TBs (64.2%). We did not find a significant correlation between the presence of abnormalities in the FN course and the type of IEMF or development of the IAC.

Conclusions: Our study demonstrated a high prevalence of FN abnormalities in patients with IEMFs. Some of the abnormalities we found would constitute significant challenges for the traditional facial recess approach for cochlear implantation. Our results suggest that the facial nerve course should be carefully evaluated preoperatively to reduce the risks of surgical complications.

Association Between Osteoporosis and Audio-Vestibular symptoms: A Systematic Review and Meta-Analysis

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Category: Clinical Otolaryngology and Pathology

Background: Osteoporosis is a chronic systemic skeletal disease, characterized by low bone mass, progressive microarchitectural deterioration and increased bone fragility. A bone mineral density (BMD) T-score of -2.5 or below is a diagnosis of osteoporosis. Usually aging has been associated with increased risk of osteoporosis among other factors. Nonetheless, osteoporosis could also be a risk factor for hearing loss due to demineralization of the temporal bone, ossicles and cochlear capsule. Most studies have focused on older subjects but gene interactions during adulthood could explain early hearing loss findings in this population. However, there is scarce evidence about audio vestibular outcomes in patients with osteoporosis under 65 years of age. Therefore, we conducted a systematic review (SR) and meta-analysis (MA) to determine if there was an association of audio vestibular symptoms in patients with early-onset osteoporosis.

Methods: A systematic search was designed according to the PRISMA guidelines and was registered in PROSPERO. Search was conducted in PubMed (1946-), Embase (1947-), and Web of Science Core Collection (1900) using terms related to early-onset low BMD and hearing loss. Mean age (+/- SD), the proportion (%) of patients with low BMD, hearing loss, benign paroxysmal positional vertigo (BPPV) or vestibular disorders were calculated in the SR. Pooled odds ratio (OR) with their corresponding 95% confidence intervals (CI) were calculated for the MA. Heterogeneity was assessed and quantified using Cochrane Q statistics and I2 statistics

Results: : A total of 26 articles underwent full text review for the SR. Data was extracted for 213.890 subjects with osteoporosis representing 65% of patients with hearing loss and 35% of patients with BPPV. The mean age was 58-years-old (SD, 7.76) with 78% of women. From these studies, only 12 met inclusion criteria for the MA. Six were assessed pursuing the association between osteoporosis and hearing loss. A random effect model was used due to high heterogeneity (87.92%). An increased risk for developing hearing loss was observed in patients with osteoporosis (OR = 1.52, 95% CI 1.06-2.19; p<0.02) compared to controls. Six studies reported the association between osteoporosis and BPPV, high heterogeneity (88.61%) was found across studies. A significant increased odds for developing BPPV was observed in individuals with low BMD and/or osteoporosis (OR= 1.58 ,95% CI 1.02-2.4; p < 0.04) compared to controls. Potential publication bias was examined using funnel plots,

including the effect size and standard error from all studies. There was a no significant (p <0.05) small study effect.

Conclusions: Individuals with osteoporosis demonstrated a positive association for hearing loss and BPPV compared to healthy controls. Particularly younger patients with osteoporosis who are not at risk for presbycusis could have inner or middle ear disorders. Future studies should assess the histological changes in the inner ear during early-onset osteoporosis.

Questionnaires in Otology

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Category: Clinical Otolaryngology and Pathology
Background: Patient-reported outcome measures (PROMs) are valuable tools in assessing the quality of health care from a patient perspective and are increasingly used by otologists. However, selecting the right questionnaire has proven to be a difficult and time-consuming task. To facilitate this process, we will provide a comprehensive overview of existing questionnaires and assess the questionnaires with multiple ear complaints.
Methods: A systematic literature search was conducted using the EMBASE and PubMed medical databases. 13,345 unique records were extracted. Questionnaires addressing any otologic complaint (tinnitus, hearing loss, earache, otorrhoea, and ear-related pressure sensation, vertigo, itch, or dysgeusia) were identified. All questionnaires were evaluated for eligibility by two independent researchers. COSMIN checklists were used to describe the characteristics of the questionnaire, and a rating list was used to evaluate the clinometric aspects of the multiple complaint questionnaires.
Results: A total of 155 unique questionnaires were selected: 33 tinnitus questionnaires, 23 vertigo questionnaires, 84 hearing loss questionnaires, and 15 multiple complaint questionnaires. The majority of the questionnaires were symptom specific. We found that multiple complaint questionnaires often lack a good design with concept elicitation and patient involvement, resulting in accurate validation of questionnaires with a mediocre design.
Conclusions: We present a comprehensive overview of the existing 155 unique questionnaires in otology. This overview enhances questionnaire selection. When focusing on questionnaires on multiple ear complaints the design often lacks patient involvement. More attention on quality of the design and patient input should be given when selecting a questionnaire for use in research and clinical practice.

Cochlear Implant Outcomes and Tumor Characteristics in Patients With Neurofibromatosis Type 2 and Bilateral Vestibular Schwannoma

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Category: Clinical Otolaryngology and Pathology
Background: Bilateral vestibular schwannomas (VS) seen in most patients affected by neurofibromatosis type 2 (NF2) present a challenge with regards to disease management. The natural history of bilateral VS in NF2 and potential treatments carry significant risk of profound bilateral hearing loss, and treatment planning must incorporate auditory rehabilitation strategies. Traditionally, the only treatment available for an ear with profound hearing loss in NF2 patients was an auditory brainstem implant (ABI). Recent literature suggests that cochlear implants (CI) are a viable alternative to ABI in patients whose cochlear nerve is anatomically preserved. CI carries several advantages over ABI, offering more aggressive management options for bilateral VS and reducing surgical complexity and intraoperative risks. This study seeks to further explore the viability of CI for hearing rehabilitation in patients with NF2, to aid in clinical decision making and counseling for these patients.
Methods: This study is a retrospective case series of 5 patients with NF2 and bilateral VS who underwent cochlear implantation to treat bilateral profound sensorineural hearing loss. Specific variables reviewed included patient demographics, CI electrode type, VS dimensions, duration of hearing loss, treatment modality for VS ipsilateral and contralateral to CI, pre-operative and post-operative pure-tone averages (PTA) and speech reception scores (sentence and word scores). Daily CI use, telephone use, and subjective comments from the patient were also reviewed.

Results: Five patients (3 female, 2 male) underwent unilateral cochlear implantation over the last nine years. The mean age at implantation was 54 years (range 36 – 78 years). Two patients did not receive tumor treatment in the implanted ear, and three were treated with tumor resection and/or radiation therapy in the implanted ear. The mean ipsilateral duration of hearing loss was 103 months and all patients had profound hearing loss bilaterally at time of implantation. The mean ipsilateral VS dimensions at time of implantation were 14 mm x 7.2 mm x 6.1 mm. All patients experienced improved PTA following implantation (average of 63 dB), and achieved enhanced open-set speech recognition in quiet (mean of 10% pre-activation and 57% post-activation). All patients reported improved lip-reading skills and increased environmental sound awareness. Four out of five patients continue to use their CI daily, demonstrating improved open-set speech recognition at the time of their last evaluation.

Conclusions: In patients with NF2 and bilateral VS in which the cochlear nerve is anatomically preserved, cochlear implantation can be a viable treatment option for hearing rehabilitation. This study revealed that all patients benefitted from their CI, in both objective and subjective audiometric performance measures. PTA and sentence scores improved, and all patients reported enhanced lip reading and environmental sound awareness. Research funded by the American Otological Society Fellowship Grant and the UW-Madison Department of Surgery / Division of Otolaryngology.

Chromatin Remodelers and Lineage Specific Factors Interact to Target Enhancers to Establish Pro-Neurosensory Fate within Otic Ectoderm

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Category: Development: Cellular/Systems
Background: Specification of Sox2+ pro-neurosensory progenitors within otic ectoderm is a prerequisite for the production of sensory cells and neurons for hearing. However, the underlying molecular mechanisms driving this lineage specification remain unknown.
Methods: We performed conditional deletion of the ATPase subunit Brg1 or inactivation of both Eya1/Six1 to demonstrate a critical role of Brg1 or both Eya1/Six1 in pro-neurosensory fate induction within the otic ectoderm. Co-immunoprecipitation (co-IP), chromatin immunoprecipitation followed by deep sequencing (ChIP-seq), ChIP-qPCR, and RNA-seq were performed to identify a cooperative interaction between Eya1/Six1 and Brg1 in regulating Sox2 expression through co-binding to distal 3' Sox2 cis-regulatory elements (CREs)/enhancers. Transgenic and mutagenesis analyses were used to examine each enhancer activity in vivo.
Results: We show that the Brg1-based SWI/SNF chromatin-remodeling complex interacts with the neurosensory-specific transcriptional regulators Eya1/Six1 to induce Sox2 expression and promote pro-neurosensory-lineage specification. Ablation of the ATPase-subunit Brg1 or both Eya1/Six1 results in loss of Sox2 expression and lack of neurosensory identity, leading to abnormal apoptosis within the otic ectoderm. Brg1 binds to two of three distal 3' Sox2 enhancers occupied by Six1, and Brg1-binding to these regions depends on Eya1-Six1 activity. We demonstrate that the activity of these Sox2 enhancers in otic neurosensory cells depends explicitly on binding to Six1. Furthermore, genome-wide and transcriptome profiling indicate that Brg1 may suppress apoptotic factor Map3k5 to inhibit apoptosis.
Conclusions: Together, our findings reveal an essential role for Brg1, its downstream pathways, and their interactions with Six1/Eya1, in promoting pro-neurosensory fate induction in the otic ectoderm and subsequent neuronal lineage commitment and survival of otic cells.

Transcriptional Dynamics of Delaminating Neuroblasts in the E10.5 Otic Vesicle

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Category: Development: Cellular/Systems
Background: The inner ear derives from a thickening of the ectoderm adjacent to the developing hindbrain called the otic placode. Between embryonic days (E) 8.5 and 10.5 in mice, the otic placode invaginates to form the otic pit and then the otic vesicle, a fully enclosed structure from which both sensory and non-sensory tissues of the inner ear originate. The developing otic vesicle can be roughly divided into two segments: the dorsal region, which gives rise to mainly non-sensory structures, and the ventral region, which houses a neurosensory competent domain that produces the hair cells and supporting cells of the cochlear and vestibular systems, as well as the neurons of the cochleovestibular ganglion (CVG). An abundance of research has recently highlighted the

susceptibility of CVG neurons to noise damage and aging in the adult cochlea, resulting in hearing deficits. Therefore, furthering our understanding of the transcriptional cascades that lead to the development of neurosensory precursor cells may provide invaluable insight into how these cells can be regenerated to treat inner ear dysfunction.

Methods: We utilized single-cell RNA-sequencing (scRNA-seq) to assess the cellular heterogeneity of the developing otic vesicle and delaminating neuroblasts. Tissues from E10.5 Pax2-Cre;ROSA26CAG-tdTomato animals were micro-dissected, and single-cell suspensions were subjected to fluorescence activated cell sorting (FACS) to purify target cells. RNA reverse transcription, cDNA amplification, and sequencing library construction were performed using the Clonetech v3 SMARTER kit and Illumina's Nextera platform. In total, 491 high depth single-cell transcriptomes were acquired, with an average sequencing depth of 1.5x106 reads and between 4,000-7,000 unique transcripts per cell.

Results: Clustering analysis of the 491 cells using CellTrails reveals otic vesicle cells and delaminating neuroblasts. Several contaminating cell types were also identified, including epidermal cells and cells from the adjacent developing hindbrain. The computational alignment of otic vesicle and neuroblast cells along a pseudotime developmental trajectory reveals the cascading changes in gene expression that occur when neuroblasts are generated and develop into nascent neurons.

Conclusions: Here we present a high-depth scRNA-seq dataset that assesses the transcriptomes of E10.5 mouse otic vesicle and neuroblast cells. This dataset represents a valuable resource to the hearing community to characterize gene expression in otic vesicle cells, as well as along the developmental time course of delaminating neuroblasts into CVG neurons.

Physiological Properties of Utricular Hair Cells and Afferents in Gpr156-/- Mice Lacking a Mirror-Image Hair Cell Organization
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Category: Development: Cellular/Systems
Background: In otolith organs (utricle and saccule), hair bundles of hair cells have varying orientations that reverse along a line of polarity reversal (LPR) located within or at the edge of the central striolar zone. Recently, the EMX2-GPR156-Gαi cascade was shown to be important for establishment of LPR during development (Kindt et al., Nat Commun 12:2861, 2021). Gpr156-/- as well as Emx2-/- otolith organs lose the LPR without any other clear anatomical defects. In this study, we examined whether the loss of LPR in Gpr156-/- mice affects physiological properties of hair cells and primary afferent neurons.

Methods: Whole-cell patch clamp recordings were made from hair cells or afferent calyceal endings in excised utricles from post-natal (P12-100) Gpr15+/- and Gpr156-/- mice. Voltage-gated currents were measured in voltage clamp mode and current-step evoked voltages were recorded in current clamp mode. Mechanoelectrical transduction (MET) currents were evoked by step and sinusoidal hair bundle deflections applied with a rigid probe. Similar recordings were made from calyceal terminals of afferents, and the receptive fields of individual afferents in the utricular nerve were visualized by diffusion of Alexa594 included in the normal KCl-based internal solution. All data were collected from the lateral extrastriola (LES), where hair bundle orientation fails to reverse by deletion of Gpr156.

Results: We found that dominant voltage-gated potassium conductances did not differ significantly between hair cells of Gpr156+/- and Gpr156-/- utricles (n = 13-20 for each combination of cell type (I and II) and genotype). The transducer currents and receptor potentials of type II hair cells in null and het animals had comparable properties (operating range, sensitivity, adaptation time course and extent).

We also determined whether zone-specific firing pattern is affected by loss of LPR. In wildtype animals, afferent neurons innervating the striola tend to fire transiently in response to depolarizing current steps, whereas those innervating the extrastriola, including LES, fire in a more sustained fashion. Current steps applied to LES calyces of both Gpr156+/- and Gpr156-/- utricles elicited multiple spikes (sustained responses).

In normal utricles, each afferent innervates hair cells only on one side of the LPR. We are investigating whether this strict innervation pattern is altered in utricles of Gpr156-/- mice. Although the LPR is missing, the location of the striolar/LES boundary does not appear to be disrupted, according to calbindin immunoreactivity. Our preliminary results have found no aberrant dendritic arbors that innervate hair cells across the striolar/LES boundary.

Conclusions: Some physiological properties are conserved in hair cells and afferent terminals of the LES in utricles without bundle orientation reversal as a result of deletion of Gpr156.

A First Upstream Regulator of Gαi-GPSM2 During Hair Bundle Morphogenesis

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Category: Development: Cellular/Systems

Background: Sensory cells in the inner ear use their hair bundle to capture mechanical stimuli, including sound. The hair bundle consists of actin-based membrane protrusions, or stereocilia, forming rows of graded height. This staircase-like architecture is key for mechano-electrical transduction. We and others previously showed that inhibitory G- protein alpha subunits (GNAI) in complex with the scaffold G-protein signaling modulator 2 (GPSM2) are critical for stereocilia placement, elongation, and row identity. In these roles, GNAI are dissociated from the heterotrimeric G-protein complex, and maintained in a GDP-bound state by GPSM2. How GNAI(GDP)-GPSM2 is produced is unknown, and it also remains unclear how GNAI(GDP)-GPSM2 is first addressed to the bare zone, the lateral hair cell surface devoid of microvilli, and then enriched at the tip of abutting stereocilia in the tallest row. We previously identified a Guanine nucleotide Exchange Factor (GEF), DAPLE, as essential to pattern and orient the hair bundle by coupling hair cell intrinsic (GNAI-GPSM2) and tissue level polarity. In this new study, we identify a protein with complementary GTPase-accelerating (GAP) activity, RGS12, as critical to produce the GNAI(GDP)-GPSM2 complex polarized by DAPLE.

Methods: We used constitutive Rgs12, Daple and Gpsm2 mouse knock-outs, immunolabelling and confocal microscopy to detect and quantify protein amounts in distinct hair cell sub-compartments. We used Auditory Brainstem Response tests to assess hearing. A new Gnai3-Egfp mouse reporter was used to revisit precise GNAI localization. Interactions between DAPLE, RGS12, GNAI, and GPSM2 were tested by co-immunoprecipitation in lysates from transfected HEK-293T cells.

Results: Rgs12 mutants are severely deaf, and F-actin labeling at various stages reveal that hair bundles have supernumerary rows and variably stunted stereocilia. The GNAI-GPSM2 complex is absent at the bare zone and inconsistently enriched at stereocilia tips, and is instead ectopically detected at stereocilia rootlets. Interestingly, RGS12 is enriched and polarized at the apico-lateral junction, colocalizing there with DAPLE. GNAI is transiently present at the junction as a substrate for the DAPLE/RGS12 GEF/GAP complex, but only immunodetected at the adjacent bare zone once stabilized by GPSM2. RGS12 and GPSM2 both have GoLoco domains to bind GNAI(GDP), and co-immunoprecipitation and immunolabeling in mutants suggest that RGS12 and GPSM2 can compete for binding GNAI in a dose-dependent manner.

Conclusions: We identify a new protein, RGS12, as essential for hair bundle morphogenesis and auditory function. Our results suggest a model where DAPLE at the hair cell junction dissociates the heterotrimeric G-protein complex, allowing RGS12 to produce GNAI(GDP) and retain it via its GoLoco domain. GNAI(GDP) could then be captured and transferred to the adjacent apical membrane (bare zone) by GPSM2. Together, our results suggest that free GNAI(GDP) is produced at the lateral hair cell junction before being deployed at the apical membrane to sculpt the hair bundle with GPSM2.

Role of Ednrb in the Development of the Stria Vascularis

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Category: Development: Cellular/Systems

Background: The sensory cells of the inner ear that are responsible for the conversion of sound vibrations into nerve impulses require a specific environment, which is rich in potassium and with an electric potential. This specific medium, called endolymph, is generated by the stria vascularis which is a specialized epithelial structure located in the lateral wall of the cochlea. The stria vascularis is composed of three different cell types: marginal cells, intermediate cells, and basal cells. Intermediate cells, like melanocytes, originate from neural crest cells and synthesize melanin. In a previous study, we found that the expression of endothelin receptor B (EdnrB), which is essential for melanocyte development, persists in intermediate cells long after birth. This is in contrast to skin melanocytes that rapidly downregulate the expression of EdnrB after birth.

Methods: To analyze the role of EdnrB in the migration and differentiation of the intermediate cells of the stria vascularis, we used a cell-specific inducible mouse model in which we specifically delete EdnrB expression at

different key stages of the intermediate cells' development. The proper development and function of the stria vascularis were then assessed by auditory brainstem response, endocochlear potential, and histology.
Results: Our preliminary results indicate that postnatal expression of EDNRB in the intermediate cells is not essential for hearing but its absence could lead to an increase of the hearing threshold.
Conclusions: This study will allow us to understand the different roles that EdnrB play in the intermediate cell and stria vascularis development.

JAG1 is Necessary for Inner Hair Cell Stereocilia Development During Cochlear Maturation

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Category: Development: Cellular/Systems
Background: The Notch signaling pathway plays multiple essential roles in the embryonic development of inner ear sensory regions, including the establishment of the sensory progenitors as well as the decision to become either a hair cell or a supporting cell. Despite these important early roles, there is a limited understanding of Notch function postnatally, when the sensory regions are still immature. Uniquely, the Notch ligand JAG1, becomes localized to supporting cells during cell fate acquisition and continues to be expressed in supporting cells postnatally and into adulthood, suggesting a role in cochlear maturation or maintenance. Previously we demonstrated that conditional deletion of Jag1 in maturing supporting cells (Sox2CreER/+Jag1fl/fl) causes auditory neuropathy and inner hair cell stereocilia defects at 6-weeks of age. Here, we further characterize the role of JAG1-Notch signaling in stereocilia morphogenesis and/or maintenance throughout postnatal cochlear development.
Methods: We utilize a Cre/loxP recombination system to conditionally delete JAG1 in supporting cells (Sox2CreER/+Jag1fl/fl) and assess for effects on hair cell function and stereocilia morphology. To evaluate the role of Jag1 in cochlear maturation, pups were given a single intraperitoneal injection (I.P.) of tamoxifen (75µg/g body weight) on postnatal day (P)0 and P1. To evaluate the role of Jag1 in later cochlear maintenance, mice were given a single I.P. tamoxifen injection (75µg/g body weight) for 5 consecutive days starting at one month of age. Auditory function was assessed over time with Auditory Brainstem Responses (ABR) and Distortion Product Otoacoustic Emissions (DPOAE). Ultrastructure analyses were conducted using scanning electron microscopy (SEM) of cochlear sensory regions at different time points throughout postnatal development. FM1-43 functional assays of hair cell mechanotransduction were performed to determine how the onset of the inner hair cell stereocilia defects correlate with dysfunctional hair cell mechanotransduction.
Results: JAG1 deletion in supporting cells at P0/P1 (during cochlear maturation) resulted in inner hair cell stereocilia fusion that begins before the onset of hearing. This fusion appears between P8-P10 in ~10% of inner hair cells and progresses to ~70% at 6-weeks when we have previously observed hearing loss. Surprisingly, JAG1 deletion at 1 month of age had no effect on hearing when assessed at 4 months, suggesting that JAG1 does not function later in cochlear maintenance.
Conclusions: We conclude that JAG1 is required for maturation of the inner hair cells during cochlear development.

Cell Type-Specific Release Modes of Alpha-Tectorin (TECTA) Regulate the Domain-Specific Organization Patterns of the Tectorial Membrane

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Category: Development: Cellular/Systems
Background: The tectorial membrane (TM) is an apical extracellular matrix (ECM) produced by cochlear supporting cells and plays a critical role in auditory transduction. The TM displays domain-specific architectures along its radial axis: dense limbal domain attached to the spiral limbus and the extended body domain hovering over the organ of Corti. In particular, alpha-tectorin (TECTA) forms dense non-collagenous fibers in the limbal domain and short crosslinking fibers associated with parallel collagen fibrils in the body domain. Our recent finding showed that both surface-tethering and the release of TECTA play critical roles in TM morphogenesis. The surface-tethering of TECTA prevents the diffusion of secreted TM components, while its release mediates the matrix growth. However, the release mechanism for the GPI-anchored TECTA is unknown. Noting that the C-terminus of TECTA contains external hydrophobic patch (EHP) sequences that block the polymerization of the

released TECTA, we hypothesized that distinct release mechanisms of TECTA might mediate its domain-specific organization. The removal of the EHP by proteolytic shedding may induce the formation of dense fibers in the limbal domain, while full-length TECTA released by GPI-anchor lipase preserves EHP and forms crosslinking fibers in the body domain.

Methods: To identify the releasing enzymes of TECTA, we overexpressed TECTA in HEK293T cells with various cell-surface sheddases and monitored the release of TECTA into the culture medium by western blots. To examine the role of each cleavage in the domain-specific organization of the TM, we used animal models in which either the releasing enzyme is knocked out, or each release mode of TECTA is specifically blocked and studied how these mutations impact the organization of the TM by immunohistochemistry and Transmission electron microscopy (TEM).

Results: Our HEK293T cell release assay showed that TECTA could be released from the producing cells by two distinct releasing mechanisms: proteolytic cleavage via transmembrane serine protease (TMPRSS) and GPI-anchor cleavage via GDE3, a GPI-anchor lipase. TECTA fragment released by TMPRSS forms homo-multimers in the culture medium, while full-length protein released by GDE3 does not. Our in-situ hybridization showed that the Gde3 is predominantly expressed in the greater epithelial ridge (GER) that forms the body domain, while TMPRSS1 and 2 show a broader expression pattern. TEM of GDE3 knockout mice showed an irregular organization pattern for the collagen bundles, with a significant increase in their average curvature in the body domain. Furthermore, we identified TECTA mutations that specifically block either proteolytic shedding or GPI-lipase cleavage and generated corresponding mouse models.

Conclusions: Our findings indicate that the distinct release modes of TECTA mediate the domain-specific organization of the TM and that the GPI-anchor cleavage of TECTA plays a critical role in the organization of parallel collagen fibrils in the body domain.

Live Imaging of Opposite Hair Bundle Orientation Establishment in the Mouse Utricle as Mediated by the Transcription Factor Emx2

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Category: Development: Cellular/Systems

Background: The mechanotransduction apparatus of sensory hair cells (HCs), known as the stereociliary bundle or hair bundle, is thought to form by the mother centriole (MC) first docking at the apical HC surface, which allows the MC to form the base of the kinocilium. This kinocilium then moves from the center of the HC to a destinated location at the periphery, where the stereociliary staircase is subsequently built adjacent to the kinocilium and collectively, they form the hair bundle. The orientation of the hair bundle on the surface of a HC dictates the cell's directional selectivity. Notably, the macula of the utricle and saccule exhibit a line of polarity reversal (LPR), across which hair bundle orientations are in mirror images. The transcription factor Emx2, expressed only on one side of the LPR, serves as the master regulator that mediates hair bundle reversal within its expression domain. To understand how Emx2 mediates this effect, we investigated when Emx2 is required to mediate hair bundle reversal by comparing hair bundle establishment in wildtype HCs across the LPR, based on their centriole migration. We asked whether the centrioles in the Emx2-positive HCs first move to the default position similar to Emx2-negative HCs before reversing their course to the opposite side of the HC. Alternatively, if Emx2-positive HCs are intrinsically different from their counterparts, the centrioles may move directly from the center to their destinated positions in the periphery. Additionally, we investigated the time required for ectopic Emx2 to reverse the course of centriole trajectory in HCs.

Methods: Embryonic day 13.5 Atoh1-Cre; Rosa-tdT; Gfp-Centrin utricles, in which HCs are tdTomato-positive and the centrioles are Gfp-positive were live imaged for 48 hours. For analyses of centriole migration in ectopic Emx2 HCs, Gfp-Centrin utricles infected with AAV-Emx2 virus or Atoh1-Cre; Rosa-Emx2 and Gfi1-Cre; Rosa-Emx2 utricles in Rosa-tdTomato and Centrin-Gfp background were used.

Results: In Emx2-positive HCs, centriole migration is fundamentally similar to that of Emx2-negative HCs, starting in the apical center and moving to the periphery, except the centrioles migrate to the opposite end of the cell comparing to Emx2-negative HCs. Ectopic Emx2 reversed centriole trajectory within 10 to12 hours after gene activation. Furthermore, our live-imaging results revealed a potential involvement of the daughter centriole in coordinating the migration of the MC/kinocilium towards the periphery

Conclusions: The direct trajectory of centrioles toward their destinated locations in the Emx2-positive HCs suggest that cells are already pre-staged by Emx2 prior to hair bundle establishment. Secondly, the short lapse in time between the putative onset of ectopic Emx2 activation and the consequential change in centriole trajectory suggests that the hair bundle reversal effect of Emx2 does not require a cascade of multiple transcriptional activation events.

Macrophages Are Recruited Into the GER Region of the Developing Cochlea, but Are Not Essential for GER Regression

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Category: Development: Cellular/Systems

Background: Programmed cell death (PCD) plays a critical role in the development and maturation of the cochlea. Significant remodeling occurs among columnar cells of the greater epithelial ridge (GER), leading to tissue regression and formation of the inner sulcus. In mice, this event occurs between postnatal days 5-15 (P5-15) and is regulated by thyroid hormone (T3). During this developmental time, the cochlea also contains a large population of macrophages. Macrophages are frequently involved in the phagocytic clearance of dead cells, both during development and after injury. However, the role of macrophages in the developing cochlea is unclear. This study examined the link between developmental cell death in the GER and the recruitment of macrophages into this region.

Methods: Studies used CX3CR1-GFP and CX3CR1-DTR mice which express EGFP and humanized diphtheria toxin receptor (DTR) under control of the endogenous CX3CR1 promoter, respectively. To eliminate macrophages from the developing cochlea, CX3CR1-GFP and CX3CR1-DTR mice were treated with the CSF1R antagonist BLZ945 and diphtheria toxin (DT), respectively. In addition, CX3CR1-GFP mice received thyroid hormone (T3) at P0 and P1, to promote premature cell death in the developing cochlea. Cochleae were fixed and examined between P3-30. Specimens were labeled with combinations of anti-GFP (GFP+ macrophages), anti-CD45 (macrophages), anti-cleaved caspase 3 (apoptosis marker) antibodies and DAPI. All cochleae were imaged as whole mounts or frozen sections using confocal microscopy. Hearing was assessed using auditory brainstem response (ABR).

Results: Cell death in the basal GER begins at P5 and enhanced numbers of macrophages were observed at P7. This pattern of macrophage recruitment was unchanged in mice that were genetically deficient for CX3CR1, the sole receptor for fractalkine (a known macrophage chemoattractant). Earlier onset of developmental cell death in the GER was induced by T3. We found that injection of T3 caused GER cell death to begin at P3, and this premature PCD was accompanied by earlier recruitment of macrophages. We further found that depletion of macrophages from the developing cochlea (using CX3CR1DTR/+ mice and CSFR1 antagonist BLZ945 treatment) had no effect on the pattern of GER regression.

Conclusions: Our results suggest that macrophages are recruited into the GER region after initiation of developmental PCD, but that they are not essential for GER regression and cochlear remodeling.

Defining Inner Ear Organoid Cell Type Specification at Single Cell Resolution

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Category: Development: Cellular/Systems

Background: Inner ear development requires the complex interaction between tissues and cells derived from multiple embryologic origins. Current knowledge of the complexities of inner ear organogenesis is limited primarily to animal models. Although similarities between animal models and human exist, the uniquely human aspects of inner ear development remain largely unknown. Using a recently developed organoid model of human inner ear development starting with pluripotent stem cells, we constructed a time-based map of in vitro inner ear organoid generation.

Methods: Inner ear organoid derivation promotes the generation of an entire sensorineural including hair cells, neurons, and Schwann cells. Although we have characterized many of these cell types, we have yet to fully define this developmental process. Our goal here was to construct a time-based map of cell diversity during inner ear organoid differentiation. We sampled sequential timepoints using single cell transcriptomics and immunohistochemistry.

Results: We identified the expression pattern of inner ear organoid generation as it progresses from pluripotent stem cells to surface ectoderm then to otic tissue types following treatment with FGF, BMP, and Wnt signaling modulators. In addition to these 'on-target' cell populations which directly contribute to otic development, we also identified other tissue types developing in this culture system, such as neural crest, epidermis, and mesenchyme. Additionally, we characterized developing neuronal populations arising from multiple sources.

Conclusions: We traced with transcriptomics and immunohistochemistry the generation of surface ectoderm from pluripotent stem cells. The surface ectoderm then gives rise to cranial placode tissue, which ultimately generates otic placodes and otic vesicles. The otic vesicles contain sensory epithelium with hair cells. This work provides the foundation upon which to further optimize elements of this inner ear organoid culture system as well as model genetic inner ear disease when paired with gene editing.

Identification of GJB2's Upstream Regulatory Elements Facilitates Design of Safe, Precision AAVs and Recovery of Hearing in a GJB2-Deficient Mouse Model

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Category: Genetics A: Genomics and Gene Regulation

Background: Mutations in Gap junction beta protein 2 (GJB2) are the leading cause of non-syndromic, prelingual deafness (DFNB1), with an estimated US prevalence of 180,000 cases. Most of these deafness-causing mutations are recessively inherited, loss-of-function alleles, making them attractive targets for an AAV-based gene replacement therapy. One challenge in developing such a therapy lies in the ability to achieve the proper expression pattern in the cochlea. Specifically, the risk/benefit ratio must be optimized by targeting a sufficient fraction of normally GJB2-expressing cells to restore function while excluding expression from other critical sensory cells to avoid toxicity.

Methods: Adult or neonatal mice (wildtype or GJB2 deficient mutants) received inner ear injections of AAVs driving Gjb2 or nuclear GFP expression from a ubiquitous promoter or a proprietary regulatory element. AAVs were administered locally via the posterior semi-circular canal. Hearing function was assessed using auditory brainstem responses and distortion product otoacoustic emissions. After animal takedown, immunohistochemistry labeling was performed to assess cochlear morphology and transgene expression.

Results: We first looked to evaluate whether ubiquitous AAV-mediated Gjb2 expression in the inner ear could lead to detrimental effects. Wildtype adult mice were injected with AAV CMV Gjb2 and taken down one to two weeks later. A large fraction of the animals exhibited elevated hearing thresholds 2 weeks post-treatment. Histological analysis showed accumulation of GJB2 protein in inner hair cells after just 1 week, followed by almost complete loss of these cells by 2 weeks, suggesting that ectopic Gjb2 expression in hair cells can be toxic and lead to hair cell death. In order to address this, we next sought to identify a combination of GJB2 proximal and distal regulatory regions which would allow us to more closely mirror the endogenous GJB2 expression pattern. Bioinformatic analyses and a neonatal cochlear explant screen identified a lead proximal promoter/enhancer combination which successfully drove expression in GJB2-expressing cells while excluding expression from hair cells and neurons. In vivo experiments further confirmed the observed expression pattern in the mouse cochlea. Lastly, we evaluated whether this proprietary promoter driving Gjb2 expression could enable hearing recovery in a GJB2 deficient mouse model. We found that neonatal delivery of our AAV gene therapy in mutant mice deficient for GJB2 led to outer hair cell preservation and reduced hearing thresholds in injected ears compared to contralateral and naïve controls.

Conclusions: Our results underscore the importance of using a GJB2 specific promoter to avoid toxicity in AAV-based gene therapy for DFNB1. Here, we show preliminary data demonstrating the identification of a promoter/enhancer combination which can mediate hearing recovery in a GJB2 deficiency mouse model. These findings represent significant progress towards the development of a gene therapy to restore natural hearing to DFNB1 patients.

Unmasking the Mesenchyme: Cellular Diversity During Cochlear Development

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Category: Genetics A: Genomics and Gene Regulation
Background: The cochlea consists of a variety of specialized cells, all of which are essential to normal hearing. However, certain cell types, such as hair cells and supporting cells, are studied more comprehensively than others. An often-overlooked cell population is the otic mesenchyme cells, which are the most numerous cells in the cochlea during development. Several human deafness genes are specifically expressed in otic mesenchyme cells including Tbx18, Coch, Otos, Col2a1, Slc26a4, and Pou3f4. Additionally, cochlear mesenchyme cells play a role in generating the endocochlear potential, modiolus formation, spiral ganglion neuron survival and axonal guidance, and are essential for the maturation of normal hearing in both humans and mice. Based on the diverse roles of otic mesenchyme cells, we hypothesize that these cells can be clustered into groups that are spatially and transcriptionally distinct. In this study, we characterized the transcriptome of otic mesenchyme cells, at the single cell level, at multiple timepoints during cochlear development.
Methods: Whole cochlea scRNA-seq was performed at embryonic (E) day 15.5, and postnatal (P) days 2 and 7. Analyses were performed using Seurat, DEsingle, and Monocle. Otic mesenchyme cells were identified and subclustered based on expression of Pou3f4 and Tbx18. All datasets are available for visualization and analysis via the gEAR portal (gene Expression Analysis Resource | umgear.org/mesenchyme). Validation of scRNA-seq results was completed using immunohistochemistry and RNAscope™.
Results: Clustering of P7 otic mesenchyme cells revealed four transcriptionally distinct populations, each expressing unique marker genes. These markers include Emilin2 (Type I Mesenchyme – Basilar Membrane), Tgfbi (Type II Mesenchyme – Spiral Limbus), Runx2 (Type III Mesenchyme – Modiolar Bone), and Car3 (Type IV Mesenchyme – Lateral Wall). Furthermore, scRNA-seq and immunohistochemistry indicate that otic mesenchyme cell populations diversify throughout cochlear development. Pseudotime analysis of the E15.5, P2, and P7 scRNA-seq datasets elucidate key transcriptional regulators in each otic mesenchyme population during early cochlear development.
Conclusions: We show that otic mesenchyme cells are transcriptionally and spatially distinct, and while derived from a relatively homogenous cellular population, can be grouped into four major populations at P7, which correspond to later distinct mesenchyme-derived cellular populations in the mature inner ear.

Generating Whole-Body and Mosaic Mutant Mice Using Improved Gene-Editing via Oviductal Nucleic Acid Delivery

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Category: Genetics B: General
Background: Creating genetically engineered animal models is key to discovering protein function. Recently, a new technique called iGONAD (improved Gene-Editing via Oviductal Nucleic Acid Delivery) was described for quickly and easily generating mouse mutants by electroporating CRISPR/Cas9 into embryos that remain in the oviducts (Gurumurthy et al., Nature Protocol, Vol.14, p.2452-2458, 2019). We have successfully used this approach to create several knockout mouse lines and have also characterized mosaic founders directly.
Methods: Oviducts were accessed via a dorsal incision between E0.7 and E1.5 and a solution containing commercially available CRISPR guide RNA complexed with Cas9 protein was injected before electroporation with tweezer-type electrodes and a square- wave pulse. Electroporation drives the gene-editing mixture into embryos, which remain in the oviducts throughout the procedure. In some instances, the tyrosinase gene was simultaneously targeted along with the gene of interest. After pups were born, genomic DNA was isolated from tail snips and the targeted region was amplified by PCR. Then reannealed products were screened by T7 endonuclease digestion to identify insertion and deletion mutations. Subcloned PCR products were subsequently analyzed by Sanger sequencing to characterize the mutation. Founders were either bred to create a new line or sacrificed to analyze stereocilia morphology.
Results: We produced deletions of 2 nucleotides in Eps8, 346 nucleotides in Tmie, and 20 nucleotides in ESPNL, all of which resulted in frameshift mutations. Founders were backcrossed to B6 before intercrossing heterozygous mice to generate homozygous mutants. Corresponding to predicted loss of function from the frameshift mutations, the phenotypes of these new lines are similar to those of published knockouts, including hearing loss and stereocilia dysmorphology. We also found that some founders were essentially whole-body mutants. Others were mosaic, even within the cochlea, with some hair cells showing knockout phenotypes while others were apparently unaffected.

Conclusions: CRISPR/Cas9, delivered via iGONAD, is an accessible approach for mutating genes in mice. Founders can be used to establish new lines or can be directly analyzed.

AAV-Mediated Gene Therapy With OTO-825 Rescues Hearing Loss and Cochlear Degeneration in a Clinically Relevant Tissue Specific Mouse Model of GJB2 Congenital Hearing Loss

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Category: Genetics B: General

Background: GJB2 mutations represent the most common cause of genetic hearing loss in humans. GJB2 encodes for connexin 26 (CX26) a gap junction protein that is natively expressed in fibrocytes of the spiral limbus and spiral ligament as well as supporting cells within the organ of Corti. Prior results from mouse and human studies have shown that GJB2 mutations lead to elevated auditory brain response (ABR) thresholds and degeneration of supporting and hair cells. To rescue this GJB2 deficient phenotype we sought to deliver functional copies of GJB2 via intracochlear administration of AAV. We first identified a novel class of AAV capsids that efficiently transduce cochlear cell types that natively express CX26. We then further optimized the AAV construct by packaging the novel capsid, promoter, and human GJB2 gene elements with and without a Flag tag (OTO-825-Flag and OTO-825, respectively). Here, we have utilized a constitutive Cre mouse model of GJB2 hearing loss to evaluate the therapeutic potential of OTO-825.

Methods: To assess in vivo rescue of CX26 deficiency we generated a mouse model with inner ear deletion of GJB2 by crossing Cx26loxp/loxp mice with mice expressing Cre driven by the inner ear specific promoter P0 (P0-Cre). The onset of P0-Cre occurs embryonically and previous studies have reported disrupted plaque formation as early as E14.5 in this model. Postnatal mice were injected with 1μL OTO-825, OTO-825-FLAG, or vehicle via the posterior semicircular canal and later assessed for various efficacy endpoints as early as P30. Auditory sensitivity was measured by ABR after which cochleae were collected and immunohistochemically processed with anti-CX26, anti-FLAG, and phalloidin to assess for tropism and cochlear morphology. For evaluation of tropism, cochlear and lateral wall whole mounts were imaged on a Zeiss LSM880 confocal microscope and FLAG or CX26 coverage was quantified.

Results: An intracochlear injection of OTO-825-FLAG into wildtype mice during the postnatal period provides extensive cochlear coverage including all cell types that natively express CX26. P0-Cre mice exhibit a substantial reduction in CX26 expression and the presence of a flat epithelium phenotype where there is a complete loss of hair cells and supporting cells and severe to profound hearing loss. Intracochlear administration of OTO-825 to P0-Cre mice substantially restored CX26 expression and greatly reduced the occurrence of a flat epithelium phenotype, and more importantly demonstrated functional improvement in hearing across multiple frequencies as measured using ABRs.

Conclusions: We demonstrate that a single intracochlear injection of OTO-825 is capable of rescuing CX26 deficient hearing loss and cochlear pathologies. These encouraging pre-clinical results support the further development of OTO-825 as a clinical candidate for the treatment of congenital hearing loss caused by GJB2 mutations.

OSBPL2 Mutations Cause Hearing Loss via Defective Autophagy

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Category: Genetics B: General

Background: Hearing loss is the most common sensorial disorder, affecting approximately 1-2 in 1000 individual worldwide. Mutations in OSBPL2 has been reported cause an autosomal dominant progressive nonsyndromic hearing loss 67 (DFNA67). Up to now, four different frameshit mutations have been identified.

Methods: western blot, immunohistochemistry, immunofluorescence, proteomics, auditory brainstem response (ABR), Distortion product otoacoustic emissions (DPOAE), scanning electron microscopy, transmission electron microscopy

Results: We found that OSBPL2 mutant protein formed cytoplasmic aggregates in vitro, bound to autophagy-related proteins and reduced autophagic flux suggesting that the accumulation of mutant OSBPL2 abrogated cellular proteostasis and autophagy. We also generated transgenic mouse model overexpressing OSBPL2-p.Q53Rfs*100(hQ53R-TG) and Osbpl2 knockout mice(Osbpl2 KO). hQ53R-TG mice showed hearing loss and abnormal cochlear morphology, but Osbpl2 KO mice did not, suggesting that the presence of OSBPL2 mutant protein is important for the pathogenesis of DFNA67. Injection of rapamycin, an activator of autophagy, decreased the cellular aggregates in cells and partially prevented hearing loss in hQ53R-TG mice.

Conclusions: Our findings suggest that frameshift mutations in OSBPL2 lead to hearing loss due to defective autophagy by accumulation of mutant protein and rapamycin can partially rescue DFNA67.

Single-Cell RNA-Sequencing for Juvenile and Adult Mouse Cochlear Sensory Epithelia

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Category: Genetics B: General

Background: Single-cell RNA-sequencing (scRNA-seq) has played an integral role in scientific advancements in biomedical research. scRNA-seq allows scientists to study gene expression at the single-cell level, answering critical biological questions about cellular heterogeneity and development, providing insight on transcriptional dynamics and regulatory networks. Large quantities of cells are necessary for accurate reflection of biological states and meaningful conclusions to be drawn from high-throughput scRNA-seq. The adult cochlea has proved difficult to access because cells are embedded in a dense bone, limiting the study of heterogeneous molecular and biological processes at the adult stage. Our group has successfully performed scRNA-seq and bioinformatic analysis on the adult mammalian cochlea sensory epithelial cells.

Methods: Basilar membranes were collected, and cells were dissociated from mice at P14 and P28 from C57BL/6 mice (n=8 and 12) and P70 from CBA mice (n=12). All steps for cDNA library were performed following the 10x Genomics protocol. Chromium Next GEM Single Cell 3' Reagents Kit v3.1 was used. Libraries were sequenced on an Illumina Nextseq and raw sequencing counts were processed using CellRanger (v6.0.1). Single-cell data was analyzed and clustered using Seurat (R package v4.0.4).

Results: P14 was composed of one biological replicate but two technical 10x runs. P28 and P70 were composed of two biological replicate 10x runs. Results are the average of the two runs merged. The sensitivity of gene detection was evaluated using total genes detected, median genes per cell, and valid UMI's, revealing the overall efficiency of the experiment. Total genes detected: P14 (19,370 genes), P28 (20,755 genes), P70 (20,419 genes); median genes per cell: P14 (760.5 genes/cell), P28 (720 genes/cell), P70 (474 genes/cell); all time points presented valid UMI's > 99.9%. Mean reads per cell were evaluated to determine the resolution for making biological discoveries: P14 (91,329 reads/cell), P28 (13,111.5 reads/cell), P70 (15,551 reads/cell. Manual filtering of raw counts based on nCount, nFeature, and mitochondrial percentage left, P14: 2,926 cells, P28: 19,277 cells and P70: 26,465 cells. Clustering analysis workflow and t-distributed stochastic neighbor embedding (tSNE) analysis revealed various sensory epithelial cell populations for each time point, such as hair cell populations and supporting cell populations. Inner and outer hair cells (IHC and OHC) for each time point. IHC: P14 (45), P28 (166), P70 (259); OHC: P14 (441), P28 (1,030), P70 (585).

Conclusions: We are among the first groups to successfully sequence adult mouse cochlear sensory epithelial cells at multiple time points for two distinct mouse strains. All three time points show high sensitivity, revealing overall efficiency for capturing mRNA molecule, reverse transcription, second strand synthesis and pre-amplification. Finally, optimal cell counts were collected to perform clustering analysis, identify cell types, specifically mechanosensory cells, and draw biological conclusions from analysis.

Evidence for Variants in TMTC4, a Novel Deafness Gene, in Human Hearing Loss and Unfolded Protein Response Activation

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Category: Genetics B: General

Background: We have recently identified Transmembrane and tetratricopeptide repeat 4 (Tmtc4) as a novel deafness gene in mice. Mice lacking Tmtc4 have normal onset of ABR thresholds at postnatal-day 13 (P13), with rapid progression to complete deafness by P26. Tmtc4 deficiency is also associated with dysregulation of Ca2+ flux between the cytoplasm and endoplasmic reticulum (ER), ER stress, and the unfolded protein response (UPR).
Methods: One proband (M/25) presented with bilateral profound sensorineural hearing loss (SNHL), while his older sister (F/28) had bilateral moderate mid-frequency SNHL. Exome sequencing was performed from blood from one proband (M/25) and subsequent bioinformatics analysis was done to narrow down the candidate variants. Potential candidate variants were Sanger-sequenced and segregated with hearing loss in affected and unaffected family members to determine the causative variants. Lymphoblastoid cells were derived from these individuals. 1 μM thapsigargin was applied to cells for 6 hours to induce ER calcium depletion and UPR activation. Expression levels of three UPR markers (BiP, indicative of UPR activation; CHOP, correlated with pro-apoptotic activity of the UPR; and S-XBP1, associated with pro-homeostatic activity of the UPR) were measured by qPCR and quantified against GAPDH and unexposed controls using the 2deltadeltaCT method. The ratio of CHOP over S-XBP1 was used as a marker of the pro-apoptotic state of the UPR. One-way ANOVA followed by Tukey's multiple comparison test was performed, with pairwise comparisons for each proband relative to their normal-hearing relatives.
Results: Two individuals in a Korean family, each with progressive SNHL, were found to be compound heterozygous for two candidate pathogenic missense variants in TMTC4 (c.547 G>A: p.Glu183Lys (CADD phred 27.00) and c.575 C>T: p.Ala192Val (CADD phred 31.00)), while the parents and the unaffected sibling each only carried one of these variants. Lymphoblastoid cells were derived from each proband, the unaffected sibling, and one unaffected parent. qPCR analysis revealed that cells from the affected probands had a significantly increased ratio of CHOP to S-XBP1 expression, both at baseline and upon UPR-induction with thapsigargin ($p < 0.001$). The thapsigargin-induced increase in CHOP/S-XBP1 ratio correlated strongly with pure-tone average in the four subjects ($p < 0.05$, $R2 = 0.94$).
Conclusions: Deficiency of Tmtc4 has been shown to cause rapidly progressive hearing loss in mice due to sensitization of cochlear cells to pro-apoptotic UPR activation. Our findings here are the first evidence that missense variants of TMTC4 in humans are associated with progressive hearing loss. Functional analysis of cells from affected probands and unaffected family members are consistent with these TMTC4 missense variants affecting UPR sensitivity. These results together suggest that TMTC4 can cause progressive hearing loss due to UPR dysregulation in humans, findings that will be strengthened by identifying additional similarly affected families.

Exploring the Missing Heritability in Subjects With Hearing Loss, Enlarged Vestibular Aqueducts, and a Single or No Pathogenic SLC26A4 Variant

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Category: Genetics B: General
Background: Pathogenic variants in SLC26A4 have been associated with autosomal recessive hearing loss (arHL) and a unilateral or bilateral enlarged vestibular aqueduct (EVA). SLC26A4 is the second most frequently mutated gene in arHL. Despite the strong genotype-phenotype correlation, a significant part of cases remains genetically unresolved. Recently, an EVA-associated haplotype (Caucasian EVA (CEVA)), characterized by 12 single nucleotide variants located upstream of SLC26A4 was found to be enriched in cases with an EVA and a single (M1) or without (M0) a (likely) pathogenic variant in SLC26A4 (Chattaraj et al. J Med Genet 2017;54:665-673). The genetic defect of this CEVA haplotype remained elusive. Digenic inheritance of variants in SLC26A4 and FOXI1 was also reported but later debated. In this study, we addressed the missing heritability of EVA-associated hearing loss.
Methods: A patient cohort was investigated, consisting of 28 M0 and M1 index cases of Dutch origin and diagnosed with HL in combination with a unilateral or bilateral EVA. The presence of the CEVA haplotype was determined by Sanger sequencing. To identify genomic variation including structural variants, we performed whole exome sequencing (Illumina HiSeq), short- and long-read (PacBio) whole genome sequencing and optical genome mapping (Bionano).

Results: We found the CEVA haplotype and a delimited V1-CEVA haplotype to be significantly enriched in our M1 patient cohort (10/16 cases). The CEVA haplotype was also present in two M0 cases (2/12). Short- and long-read whole genome sequencing and optical genome mapping could not prioritize any of the variants present within the CEVA haplotype as the likely pathogenic defect. Short-read whole genome sequencing of the six M1 cases without this haplotype and the two M0/CEVA cases only revealed previously overlooked or misinterpreted splice-altering SLC26A4 variants in two cases, who are now genetically explained. No deep-intronic or structural variants were identified in any of the M1 subjects. We addressed potential digenic inheritance of variants in SLC26A4 and FOXI1 but did not obtain indications for this. However, we found the previously reported c.677C>T FOXI1 variant to be enriched in the studied cases which suggests that the c.677C>T FOXI1 variant might contribute to the etiology of HL and EVA.

Conclusions: With this study we confirmed the enrichment of the CEVA haplotype in M1 subjects with HL and an EVA. Also, we have provided important insights that will pave the way for elucidating the missing heritability in M0 and M1 SLC26A4 cases. For pinpointing the pathogenic effect of the CEVA haplotype, additional analyses are required addressing defect(s) at the RNA, protein, or epigenetic level. Pilot experiments using patient-derived induced pluripotent stem cells are ongoing.

Investigating Activity-Dependent Synaptic Plasticity at Cochlear Ribbon Synapses

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Category: Hair Cells: Anatomy and Physiology

Background: In the mammalian cochlea, hearing relies on highly specialized ribbon-type synapses between sensory inner hair cells (IHCs) and postsynaptic spiral ganglion neurons. Prior to hearing onset, developing IHC physiology is characterized by spontaneous burst firing patterns that are exclusive to a defined early-postnatal period, during which a range of dynamic structural and functional refinements shape the presynaptic morphology. To date, despite numerous evidences indicating the indispensability of this Ca2+-based spontaneous firing for the functional maturation of the auditory pathway, the impact of this intrinsically-generated activity on IHC presynaptic structural development remains uncertain. Moreover, while previous work on other ribbon-bearing systems – such as zebrafish lateral line neuromast hair cells – indicates homeostatic modes of structural plasticity upon genetic or pharmacological activity manipulation, the degree to which mammalian ribbon synapses can adapt to alterations in presynaptic activity levels remains to be determined. Therefore, we set out to investigate this phenomenon at cochlear ribbon synapses using genetic, pharmacological, and optogenetic approaches.

Methods: To analyze the effects of activity modulation on ribbon synapse morphology we prepared short-term explant cultures of the organ of Corti from postnatal day (P)5 mice. First, to investigate the impact of presynaptic disruption of IHC exocytosis during early development, we employed immunohistochemistry to inspect ribbon synapse morphology of Otof-KO mice and wild-type control cultures. Second, to test if acute modulation of spontaneous activity could trigger homeostatic structural plasticity of IHC ribbon synapses, we treated organ of Corti explant cultures of C57Bl6J mice with either (i) the Ca2+-channel inhibitor isradipine, or the Ca2+-channel agonist (RS)-BayK8644. Lastly, using a combination of patch-clamp electrophysiology and immunohistochemistry, we devised and validated a novel tissue culture-based model system to conduct cell type-specific, patterned, long-term optogenetic stimulation of channelrhodopsin-2 (ChR)-expressing IHCs within a standard tissue culture incubator.

Results: Genetic loss of Otoferlin resulted in lower numbers of synaptically-engaged ribbons, but increased volumes and integrated as well as maximal fluorescence intensity values of IHC ribbons as well as their corresponding postsynaptic densities (PSD). Acute pharmacological inhibition of presynaptic L-type voltage-gated Ca2+-channels (CaV1.3) using isradipine lead to a mild increase of PSD fluorescence intensity, while CaV1.3 agonism via BayK8644 induced the opposing effect on pre- and postsynaptic level. Finally, long-term patterned optogenetic stimulation of ChR2-expressing IHCs resulted in decreased ribbon and PSD fluorescence intensities – a finding consistent with BayK8644-mediated CaV1.3 agonism.

Conclusions: Positive as well as negative modulation of IHC presynaptic activity exerts opposing effects on ribbon synapse morphology – both, on the pre- as well as the postsynaptic side, thus indicating efficient homeostatic plasticity mechanisms taking place during developmental maturation.

Transcriptional Regulation of MYO7A Isoforms

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Category: Hair Cells: Anatomy and Physiology
Background: Unconventional myosin VIIA (MYO7A) is important for hair cell development, and our previous study provided evidence that MYO7A is the molecular motor that tensions the MET complex. We also showed that MYO7A isoforms are differentially expressed in the inner and outer hair cells (IHCs and OHCs). Interestingly, these isoforms are have distinct transcription start sites. In this study, we investigated the enhancer regions and transcription factors regulating Myo7a isoform expression and their functional roles in the inner ear.
Methods: Published ATAC-seq databases were consulted to predict novel cis-regulatory units of the mouse Myo7a gene. The function of regulatory unit candidates was evaluated by luciferase assay in RPE cells. Enhancer-specific deletion mouse lines were generated using CRISPR/Cas9. Myo7a expression levels were measured by qPCR and immunofluorescence microscopy. The UniBind2021 track in the UCSC genome browser indicating robust direct transcription factor (TF)-DNA interactions was used for identifying TF candidates. Six2fl/fl mouse line was crossed with Atoh1-Cre mouse line to generate homozygous Six2 conditional deletion mice.
Results: By analyzing published ChIP-seq and ATAC-seq databases, we identified multiple novel Myo7a gene enhancer candidates in the mouse genome. We screened these candidates using luciferase assays, and selected the most robust candidate to generate a enhancer-specific deletion mouse. The deletion of this enhancer reduced MYO7A in both IHCs and OHCs in a tonotopic manner, with a ~80% MYO7A signal reduction at basal turns and ~50% reduction at apical turns.
The center of this enhancer harbors a binding motif for the transcription factor SIX homeobox 2 (SIX2). This binding site is highly conserved in mammals and shows robust interaction with SIX2 in mouse and human kidney tissue. Immunofluorescence imaging showed that SIX2 is differentially expressed in the cochlea, with predominant expression in OHCs and a lower expression in IHCs. SIX2 expression displayed a tonotopic gradient in OHCs, increasing in its expression from the apex towards the base. This expression pattern correlated well with the expression gradient of a MYO7A isoform (MYO7A-N) in the cochlea.
To directly investigate whether SIX2 contributes to the formation of the MYO7A gradient in cochlear hair cells, we deleted SIX2 specifically in hair cells. The conditional deletion of SIX2 resulted in severe developmental defects in cochlear hair cells. This suggests that in addition to the potential role in regulating MYO7A, SIX2 has additional and fundamental roles in hair cell development. In future studies, we will identify SIX2 target genes in the inner ear by applying single-cell RNA-seq, and investigate its global chromatin interaction using ChIP-seq.
Conclusions: Our previous study revealed an unexpected complexity of Myo7a isoforms in auditory hair cells. Here we identified potential cis and trans-regulatory factors of Myo7a expression, potentially suggesting a role of transcriptional regulation of MYO7A isoforms in tuning hair cell function.

Taperin is Not Essential for Mechanotransduction but Contributes to the Linearity of Hair Bundle Motion
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Category: Hair Cells: Anatomy and Physiology
Background: Taperin is localized at the base of hair cell stereocilia and essential for their proper formation (Rehman et al., 2010; Chen et al., 2016; Men et al., 2019). Pathogenic variants of human TPRN are associated with nonsyndromic deafness DFNB79 (Rehman et al., 2010). Although the involvement of taperin in the macromolecular complex stabilizing stereocilia base is well established (Salles et al. 2014; Zhao et al., 2016; Liu et al., 2018), its exact role in the mechanosensory function of hair cells is yet unknown. The goal of this study was to explore the mechano-electrical transduction (MET) and mechanical properties of the stereocilia bundles in the auditory hair cells of a mouse homozygous for a genomic deletion of Tprn starting after codon 259 and ending with codon 749 (Tprntm1(komp)Vclg).
Methods: Using whole cell patch clamp recordings of young postnatal auditory hair cells, we have investigated MET currents evoked by deflection with a fluid-jet of stereocilia bundles. Simultaneously with patch clamp recordings, the movements of the hair bundles were monitored by a high-speed camera at 3,000 fps. We also examined stereocilia ultrastructure with transmission electron microscopy (TEM) and serial sectioning with a FIB-SEM.

Results: In both inner (IHCs) and outer (OHCs) hair cells of homozygous Tprntm1(komp)Vclg mice, we recorded MET responses with apparently normal amplitude, suggesting that taperin deficiency does not disrupt hair cell mechanotransduction. In the control wild type and heterozygous mice, fluid-jet stimulation produced slightly asymmetrical movements of hair bundles in positive and negative directions due to the contribution of tip links and MET apparatus when the bundle is deflected toward kinocilium. Besides this slight directional asymmetry, the mechanical responses of both IHCs and OHCs were relatively linear. In contrast, homozygous Tprntm1(komp)Vclg hair cells often exhibited substantial nonlinearity of the hair bundle deflections. They have abnormally low stiffness at resting position and increasingly higher stiffness when the bundle was deflected by more than 50-100 nm or 100-200 nm in OHCs or IHCs, respectively. Ultrastructural observations revealed abnormalities of the homozygous Tprntm1(komp)Vclg stereocilia taper, which may underlie these physiological observations.

Conclusions: We concluded that taperin is not essential for mechanotransduction but is involved in shaping the mechanical properties of stereocilia at their pivot point.

Ribbon Precursor Dynamics During Synapse Assembly in Cochlear Inner Hair Cells

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Category: Hair Cells: Anatomy and Physiology

Background: Sensory inner hair cells (IHC) in the mammalian cochlea harbor highly specialized presynaptic structures, so-called 'synaptic ribbons'. At the presynaptic membrane, ribbons constitute the main scaffold of the presynaptic active zone (AZ), and enable the indefatigable and temporally accurate encoding of auditory stimuli. The presynaptogenesis of IHC is characterized by the gradual increase in ribbon size and the tethered synaptic vesicle pool – mediated by the translocation of floating cytosolic ribbon precursors towards developing AZs, where they likely fuse with already membrane-anchored ribbons. Although assumed a dynamic process, the molecular pathways underlying the developmental assembly of the IHC ribbon-type AZs has remained largely elusive thus far. Interestingly, synaptically-engaged ribbons in other ribbon-bearing systems – such as the retina and lateral line neuromasts hair cells in zebrafish – have been shown to display activity-dependent structural plasticity, thereby arguing for an adaptive scaffold. Whereas such plasticity remains to be confirmed for IHC ribbons, positional and structural adaptation of mature IHC ribbon size has recently been reported in response to inner ear injury. Yet, real-time in situ analysis of such events is still lacking to date. To now address this shortcoming, we devised an organotypic culture-based model system to perform live-cell imaging experiments and monitored ribbon synapse assembly and activity-dependent structural plasticity at the AZ membrane of early postnatal mice.

Methods: We optimized an organ of Corti organotypic culture approach to conduct adeno-associated virus (AAV)- mediated labeling of synaptic ribbons in situ in transgenic mice that express different fluorescent reporters in pre- and postsynaptic structures to establish tissue context. Transduced cultures were then used for multi-color live-cell imaging experiments to assess the morphology and trajectories of ribbon precursors in respect to the IHC plasma membrane and the extracellular context of postsynaptic SGN boutons. In this study, we employed a comprehensive methodological approach combining pharmacological manipulation with confocal and volumetric multi-color widefield microscopy that enables close to real time acquisition speed. Using this approach, we investigated the mobility of membrane-anchored and membrane-proximal ribbons over various timespans and extracted information on precursor size, velocity, etc. Moreover, we used super-resolution microscopy to assess the molecular composition of free-floating ribbon precursors.

Results: In developing IHCs, ribbon precursors at the AZ display a high degree of movement and a highly adaptable morphology. Tracing of high-speed dynamics allows for the characterization of remittent periods of diffusive motion and putative active transport at the AZ membrane. Moreover, a bidirectional exchange of vastly mobile ribbon material seemed to occur between membrane-bound, as well as cytosolic ribbon precursors.

Conclusions: Synaptic ribbons are highly dynamic during developmental assembly of the IHC presynapse. The morphological plasticity of ribbons does not follow a unidirectional trajectory,

but appears reversible and highly adaptable.

Generation of a Complete Loss-Of-Function Mouse Model to Assess the Biological Function of Loxhd1

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Category: Hair Cells: Anatomy and Physiology
Background: Loxhd1 is a deafness gene with poorly understood function that causes recessive age-related and congenital hearing loss in humans (DFNB77), dogs, and mice. Loxhd1 is a large gene (160 kb) composed of 41 exons encoding a suite of 15 repeats called PLAT (Polycystin/Lipoxygenase/Alpha-Toxin) domains, which in other proteins can bind proteins and lipids. Hearing loss mutations have been found in the exons that encode for nearly all of Loxhd1's PLAT domains. Our lab previously reported that the hearing loss caused by missense (Loxhd1Sba/Sba) and nonsense (Loxhd1T1308X/T1308X) mutations in the 10th PLAT repeat results from a hair cell mechanotrasduction defect with an onset later than postnatal day (P)7 (Trouillet A., J Neurosci, 2021). However, as 1) Loxhd1 can produce splice isoforms that terminate before the 10th PLAT domain, 2) Loxhd1 can produce new in-frame splicing events that skip mutated exons in a nonsense mutant (Loxhd1T1308X/T1308X), and 3) PLAT repeats share considerable similarities with each other, functional compensation may exist in our current mouse models. To reveal the entire function of Loxhd1, a complete loss-of-function mouse model is needed.
Methods: CRISPR/Cas9 technology was used to generate the Loxhd1 complete loss-of-function mouse model. We analyzed the auditory phenotype with auditory brain stem responses (ABRs) and distortion products of otoacoustic emissions (DPOAEs). Scanning electron microscopy (SEM) was used to assess the morphological phenotype of hair cells.
Results: After trying different inactivation strategies, we successfully produced a novel allele where the entire Loxhd1 coding sequence downstream of exon 1 was deleted (Loxhd1Delta). The Loxhd1Delta/Delta mice are viable, and we have confirmed a hearing loss phenotype at P21 by measuring ABRs and DPOAEs. Morphological assessment by SEM showed that Loxhd1Delta/Delta mice have cochlear hair cells with hair bundles until at least two months old. However, we observed the degeneration of the third stereociliary row as early as P7 in OHCs – which was not seen in either of our PLAT 10 mutant mouse models. By P60, all IHC third-row stereocilia disappear while all second-row stereocilia become thinner and lose their beveled tips.
Conclusions: We successfully generated a Loxhd1 complete loss-of-function mouse model, confirmed its hearing loss phenotype, and found that Loxhd1 is required for maintenance of the stereocilia in the shorter rows. We plan to assess the requirement of Loxhd1 for mechanotransduction before P7. Likewise, the molecular mechanisms by which Loxhd1 participates in hair cell mechanotransduction and shorter stereocilia maintenance require further investigation.

A Deep Learning Approach to Quantify Auditory Hair Cells in Organ of Corti Explants

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Category: Hair Cells: Anatomy and Physiology
Background: The neonatal organ of Corti explant culture is a frequently used experimental system for the investigation of auditory hair cells. These in vitro cultures allow the testing of protective substances or the study of structural components and molecular mechanisms. Hair cell survival is frequently assessed in these cultures, which is often performed by manual counting due to the complex morphology of the cells. However, manual counting is a time-consuming process in which inter-rater reliability is also an important concern.
Methods: In this study, we tested a deep learning approach for the quantification of auditory hair cells in organ of Corti explants cultured in vitro. By using StarDist, a publicly available platform and plugin for Fiji (Fiji is just ImageJ), we trained a custom deep learning model. We further validated this model in untreated and damaged (gentamicin, cisplatin) organ of Corti explants.
Results: The trained custom deep learning StarDist model reliably detected the hair cells in both control, gentamicin and cisplatin treated explants. Most importantly, there were no significant differences when compared to the manual counts of two different observers in any condition.

Conclusions: We show as a proof-of-concept, that deep learning is a valuable approach for the quantification of hair cells. Our trained StarDist model produces reliable hair cell counts. Therefore, it is a useful approach to facilitate the analysis of hair cells in organ of Corti explants and increasing reproducibility. The described approach can be implemented easily using the open-source StarDist plugin for Fiji.

The Potassium Channel Subunit Kv1.8 (Kcna10) Differentially Shapes Tuning, Gain, and Response Latency of Type I and II Vestibular Hair Cells

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Category: Hair Cells: Anatomy and Physiology

Background: Vestibular hair cells (HCs) convert head motions into receptor potentials that drive downstream gaze and postural reflexes. In amniotes, type I and II HCs (HCI, HCII) express large K+ conductances that differentially shape receptor potential (RP) gain, tuning, and timing. We investigated the roles in HCIs and HCIIs of Kv1.8, an understudied channel encoded by the Kcna10 gene. Kv1.8-null animals lack vestibular-evoked potentials normally evoked by transient head motions (Lee et al., Hearing Res 300 (2013),1). Kv1.8 forms conductive ion channels when heterologously expressed (Dierich et al., Cell Reports 32 (2020),1).

Methods: We stimulated HCs with voltages, currents, or hair bundle deflections and recorded HC whole-cell currents and voltages in intact utricles from Kv1.8-null mice and wildtype and heterozygous littermates (postnatal days 5-370). Sectioned utricular epithelia were stained with anti-Kv1.8 (Alomone).

Results: Kv1.8 immunoreactivity localized to the basolateral membranes of HCIs and, to a lesser extent, HCIIs. Kv1.8-null HCIs lacked the low-voltage-activated K+ conductance of mature control HCI (gK,L, V-half –84±1 (±SE) mV, n=88 wildtype, heterozygous). Kv1.8-null HCIs expressed a much smaller (by 95%) and less negatively activating K+ conductance (V-half –41±1 mV, n=51). As a result, input resistance was 20-fold greater in Kv1.8-null HCIs than wildtype HCIs, such that sinusoidal hair bundle deflections evoked larger and slower RPs. The low-pass corner frequency of RP re: transduction current (I-MET) fell from ~400 Hz (control) to ~25 Hz (Kv1.8-null), and phase lag at 20 Hz increased by ~25-30 degrees (3.7 ms).

Wildtype HCIIs typically express a fast-inactivating A-type conductance (g-A, V-half –27±1 mV, n=31). In Kv1.8-null HCIIs, g-A was reduced by ~92% and the steady-state Kv conductance by ~60%. Consequently, in Kv1.8-null HCIIs, voltage responses to step currents were slower to rise, did not rebound, and, in some cells, showed electrical resonance. Consistent with these effects, the low-pass corner frequency of RP re: I-MET fell from ~70 Hz (control) to ~17 Hz (Kv1.8-null), and phase lag at 20 Hz increased by ~30-35 degrees (4.6 ms).

In Kv1.8-null HCIs and HCIIs, the residual K+ conductance lacked rapid inactivation and activated positive to resting potential (V-half range –30 to –40 mV). Block by XE991 (IC50 ~10 μM, n=12) suggests that it includes Kv7 subunits.

Conclusions: We propose that Kv1.8 is a pore-forming subunit of K+ conductances modified by cell type-specific factors to have distinct voltage dependence and inactivation, producing g-K,L in HCIs and g-A in HCIIs. These Kv1.8-dependent conductances strongly affect response gain, tuning, and timing. Both g-K,L and g-A increase the low-pass corner frequency and reduce phase lag at stimulus frequencies >5 Hz.

Disparities in Hearing Healthcare: Barriers to and Predictors of Hearing Aid Acquisition

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Category: Hearing Loss: Consequences and Adaptation

Background: Amplification of sound through hearing aids, implantable technology, and other assistive devices can ameliorate hearing deficits and improve quality of life. For some patients, the pursuit of hearing aids is disproportionately restricted by financial factors and lack of insurance coverage. Though previous research into hearing healthcare disparities has examined race/ethnicity and socioeconomic factors, investigations are traditionally limited by small sample sizes of minority adults. Additional research is needed to fully characterize the extent to which race, ethnicity, and socioeconomic status impact hearing aid acquisition.

Methods: A retrospective review of over 2600 charts was performed for patients who underwent audiologic evaluation at a large public hospital between 01/01/2016 and 12/31/2020. Patients were included if diagnosed with

hearing loss for which treatment with amplification was warranted. Patients with history of prior hearing aid use were excluded. Preliminary analyses were performed on a sample of 100 patients. Differences in demographic characteristics, socioeconomic status, and hearing health status were assessed for hearing aid recipients and non-recipients using independent t-tests. For zip code analyses, Median Family Income was recorded from the Missouri Census Data Center 2019 Geographic Application. Predictors of hearing aid acquisition were identified using logistic regression modeling. All analyses were performed in R.

Results: Most patients were African American females in their early 60s. Approximately 20% of patients endorsed a history of noise exposure, with half attributing exposure to occupational sources. Most reported perceived hearing loss for less than 3 years at time of presentation. Approximately half of all eligible patients ultimately acquired hearing aids. Hearing aid recipients and non-recipients differed in sex (p = .015) but not age, race, or English as a first language. No significant differences were observed between the two groups with respect to Median Family Income (MFI), however both groups had a significantly larger proportion of patients residing in zip codes whereby the MFI falls below the United States average. Logistic regression modeling revealed self-reported financial difficulty negatively predicted hearing aid acquisition over and above age, sex, and English as a first language (estimate = -3.38, SE = 0.80, p<0.001).

Conclusions: Hearing aid recipients were more likely to be females, however our findings indicate age, race, and language do not predict whether a patient obtains amplification. Self-reported financial difficulty was a strong determining factor of whether a patient decided to pursue hearing aids even after accounting for other factors such as age or sex. These findings corroborate previously demonstrated research yet extend them to a diverse patient population ranging in racial, ethnic, and financial backgrounds.

Subclinical Hearing and Balance Deficits in Noise-Exposed Firefighters

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Category: Hearing Loss: Consequences and Adaptation

Background: Noise-induced hearing loss is the most prevalent occupational disease in the world. There is mounting evidence that early exposures to hazardous noise result in structural and functional changes in the inner ear, leading to hearing and balance impairment. Hazardous noise exposure increases risk for rapid onset and progression of noise-induced hearing loss. There is also increasing evidence that chronic or repeated exposure to noise results in long-term and irreversible damage to the vestibular system. Damage to the inner ear arising from noise is insidious and delayed in clinical presentation. Firefighters are at increased risk for noise-induced damage to the ear due to frequent exposure to hazardous levels of noise. The purpose of the study was to assess the prevalence of auditory and vestibular symptoms in firefighters with hazardous nois exposure.

Methods: Hearing, oculomotor, and balance function assessments were collected in 176 firefighters during annual physical assessments for the City of Miami Fire Service Members. Firefighters had a mean age of 38 years and years of service ranged from 1 – 31 years. Puretone thresholds were obtained from 250 – 8,000 Hz, distortion product otoacoustic emissions were obtained from 1,500 – 10,000 Hz. We administered a 10-minute Oculomotor, Vestibular, and Reaction Time test battery using a head-mounted display with two integrated high-speed 100 Hz infrared eye-tracking cameras (I-PAS™, Neurolign, Inc.)

Results: Results reveal significant deficits in cochlear outer hair cell function in the presence of normal audiograms. There was a significant decrease in DPOAE amplitude with increasing years of service (p<.01). Functional balance outcomes indicated that 15% of the study population had increased fall risk, while decrease in oculomotor function was associated with impairments in functional gait. Additionally, 26% of firefighters self-reported concerns about their balance. Ocular vergence tasks demonstrated significant disruption of symmetry with increasing years of service (p<.05), and there was a significant decrease in pupil constriction with increasing years of service (p<.01). Auditory reaction times also decreased with increasing years of service despite visual reaction times remaining unchanged.

Conclusions: Early changes in auditory, oculomotor, functional balance, and reaction times are worrisome in fire fighters and may be an unrecognized contributor to age-related balance impairment later in life Observed deficits are more prevalent with years in the fire service, but not associated with age. Collectively, the results demonstrate a high incidence of subclinical hearing and balance deficits in a cohort of otherwise healthy firefighters. Early detection of changes in the auditory and vestibular systems arising from noise is an important topic of research with major public health implications.

Development of Noise-Induced Hearing Loss (NIHL) Rat Models for Preclinical Efficacy Assessment

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Category: Hearing Loss: Consequences and Adaptation

Background: Occupational and recreational noise-induced hearing loss is a complex disease with an increasing global prevalence. Exposure to intense or prolonged sounds has a damaging effect on cochlear structures which are irreversible. Acoustic trauma can lead to permanent or transitory threshold shift (PTS and TTS) with different pathological features, including hair cell loss, degeneration of the auditory nerve, and damages to ribbon synapses, also known as synaptopathy. Preclinical models have been developed in rats to model different patterns of NIHL and give insights for future pharmacological treatments.

Methods: Five noise exposures were performed. Animals received a bilateral noise exposure at a noise band of 8-16 kHz at 105 dB for 2 hours (A), 110 dB for 1 hour (B), 110 dB for 2 hours (C), 115 dB for 2 hours (D), and 120 dB for 2 hours (E). DPOAE amplitudes and ABR thresholds were measured at baseline, T+1DAY, T+7DAYS, T+14DAYS and T+21DAYS for A, B, C groups, and at baseline, T+1DAY, T+7DAYS, T+14DAYS, T+21DAYS and T+35DAYS for groups D and E. Histological analyses were performed at T+21DAYS to count outer and inner hair cells and synaptic ribbons for groups A, B, and C. For groups D and E, histological analyses were performed at T+35DAYS to count outer and inner hair cells, fibers and SGN.

Results: A-105 dB 2h: TTS – ABR thresholds and DPOAE amplitudes return to BL – No hair cell loss – loss of synaptic ribbons at 25 and 32 kHz.

B-110 dB 1h: PTS – permanent increase of ABR thresholds and decrease of DPOAE amplitudes – No hair cell loss – no synaptic ribbon loss.

C-110 dB 2h: PTS - permanent increase of ABR thresholds and decrease of DPOAE amplitudes – No IHC loss, slight loss of OHC, loss of synaptic ribbons at 25 and 32 kHz.

D-115 dB 2h: PTS - permanent increase of ABR thresholds and decrease of DPOAE amplitudes - No IHC loss, significant loss of OHC at 16 and 32 kHz - no synaptic ribbons immunostaining – slight loss of fibers – no SGN loss.

E-120 dB 2h: PTS - permanent increase of ABR thresholds and decrease of DPOAE amplitudes – significant IHC loss from 16 kHz, complete OHC loss from 16 kHz - no synaptic ribbon immunostaining – loss of fibers – loss of SGN.

Conclusions: By varying the intensity of traumas by 5 dB steps and modifying the duration, we obtained different phenotypes of hearing loss, from simple functional impairment without cell loss to the activation of cell death pathways and thus degeneration of cochlear structures. These models allow to mimic different aspects of NIHL and to test efficacy of drugs targeting synaptogenesis, cell death (apoptosis or necrosis), oxidative stress, inflammatory responses, or cell regeneration.

3D Finite Element Modeling of Occlusion Effect With Hearing Protection Devices

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Category: Hearing Loss: Consequences and Adaptation

Background: Hearing protection devices (HPDs) like earplugs are used to protect military personnel from hearing loss resulting from blast exposure. A critical issue with earplugs is their effect on bone conduction (BC) hearing in the ear canal. The obstruction of the ear canal amplifies the wearer's own physiological noises in the air between the earplug and tympanic membrane (TM), a phenomenon known as the occlusion effect. This can cause serious discomfort in wearers. There is an urgent need to understand and limit the occlusion effect for earplug wearers. In this study, we used our 3D finite element (FE) model of the human ear to simulate the sound or blast overpressure transmission from the ear canal entrance to stapes footplate in an open or occluded ear canal, with BC vibrations applied to the canal wall. Our goal is to simulate the occlusion effect in the FE model for future study on improving earplug designs to reduce the occlusion effect.

Methods: The human ear FE model consists of the ear canal, TM, middle ear cavity, ossicular chain, and suspensory ligaments. First, sound pressure of 90 dB was applied at the ear canal entrance over the frequency range of 100 to 10,000 Hz in ANSYS Mechanical. Then, blast overpressure of 30 kPa was applied at the canal entrance for transient analysis over the time domain. BC stimulation was represented as harmonic vibrations of 10-5 m/s applied normal to the ear canal wall. In addition to the open ear, three earplug designs were tested and compared: foam, Combat Arms, and aerogel earplugs. The outputs include the pressure near the TM in the canal (P1) and stapes footplate displacement (dFP).

Results: The P1 pressure and footplate displacement dFP derived from the base model without earplug under 90 dB input were consistent with the published data. For all tested HPDs, P1 pressures had peaks at frequencies below 1000 Hz. The foam, Combat Arms, and aerogel earplugs displayed peak pressures of 8-10 Pa at 150, 550, and 800 Hz. The dFP peaks at similar frequencies were observed with a maximum value of 0.01-0.05 μm. These variations in P1 and dFP indicated that the occlusion effect with earplugs is sensitive to HPD design.

Conclusions: Our FE model has demonstrated the occlusion effect with HPDs caused by insertion of earplugs into the ear. The severity of the occlusion effect is related to the shape and material properties of the HPD. The limitations of the occlusion effect will be further investigated on design optimization of earplug geometries and materials using the FE model of the human ear.

Temporal Masking Underlies Reduced Speech Discrimination in Noise at High Sound Intensities
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Category: Hearing Loss: Consequences and Adaptation

Background: Natural sound processing used in everyday situations is fundamental to how humans interact with each other and with the external world. These natural sounds, such as speech, are complex time-varying waveforms containing rich spatiotemporal information critical to communication. It is known that sensory disorders such as hearing loss results in a breakdown of this information and causes distortions in the neural code, which subsequently leads to misinterpreted neural representations of complex sounds as information ascends the auditory pathway. As a result, perception of complex sounds such as speech is compromised. This problem is further complicated by the fact that sound intensity level varies in natural settings, both in quiet and in noisy backgrounds, and plays a critical role in speech perception. Somewhat paradoxically, despite increased audibility at high sound intensities, that perception and discrimination of speech is actually diminished, especially in the presence of background noise and also observed in hearing-impaired individuals. This is known as rollover of speech and is poorly understood in general.

Methods: To investigate this problem, we used a combination of awake-behaving in-vivo electrophysiology in Mongolian gerbils (Meriones Unguiculatus) to investigate how hearing loss affects the neural encoding of speech. We presented 22 Vowel-Consonant-Vowel (VCV) syllables to the gerbil and recording neural responses from the inferior colliculus (IC). We used a K-nearest neighbor (KNN) neural classifier to investigate whether IC neurons could discriminate between different consonants in normal hearing (NH) and noise-exposed hearing-loss (HL) animals. We hypothesized that there will be strong effects of temporal masking in the boundaries between consonant and vowels in syllables, and that these effects will be most pronounced at higher intensity levels and in HL conditions. We tested the effects of temporal masking by presenting the consonants isolated from the vowels and reclassifying to observe whether there was an improvement in discrimination.

Results: We found that rollover was already present in the IC and that performance in discrimination decreased when VCVs were presented in -2 dB SNR noise when compared to in quiet. The rollover was a more prominent dropoff in the NH animals whereas the dropoff plateaued in the HL animals. When the consonants were presented in isolation, the HL animals benefitted from the lack of temporal masking and performance in discrimination improved relative to the VCV presentations.

Conclusions: These results demonstrate that the failure of temporal processing, caused by temporal masking in speech syllable elements, is detrimental to speech perception, Therefore, temporal masking underlies the neural mechanism in rollover of speech. This advancement in knowledge could improve current hearing aid designs by mitigating the temporal masking from incoming sound features in speech. This will also pave the way for better diagnoses and personalized medicine treatments in the future.

Cessation of Developmental Spontaneous Activity Elicits Rapid Changes in Neuronal Excitability in a Mouse Model of Gjb2-Mediated Deafness

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Category: Inner Ear: Anatomy and Physiology
Background: Supporting cells within the organ of Corti modulate cochlear output by providing both structural and functional support. In both the developing and mature cochlea, these non-sensory cells are extensively coupled through gap junctions, coordinating intercellular signaling and ion and metabolite homeostasis. Mutations in Gjb2, which encodes the gap junction protein Connexin 26 (Cx26), are the most prevalent cause of congenital hearing loss, emphasizing the importance of cochlear gap junctions in auditory processing. However, the consequences of gap junction disruption on early pre-hearing spontaneous activity patterns and development of central auditory pathways are unknown.
Methods: To explore whether supporting cell expression of Cx26 in the organ of Corti is critical for proper maturation of central auditory circuits, we used a knock-in mouse line (Tecta-Cre) to restrict deletion of Cx26 to supporting cells within the developing cochlea (Cx26 cKO).
Results: Unexpectedly, inner supporting cells, hair cells, and spiral ganglion neurons still exhibited robust spontaneous activity in cochleae isolated from postnatal day 7 (P7) Cx26 cKO mice, exhibiting only modest differences from controls. Consistent with these observations, in vivo imaging of spontaneous neuronal Ca2+ transients in the inferior colliculus (IC) of P7 Cx26 cKO mice revealed that neurons continued to exhibit temporally and spatially correlated bands of activity within future isofrequency domains. These results indicate that in the absence of Cx26, robust spontaneous activity continues to pass through developing auditory circuits prior to hearing onset. Despite the persistence of pre-hearing neural activity and preservation of hair cells and SGNs, Cx26 cKO mice at hearing onset (~P12) exhibited significantly higher auditory brainstem response (ABR) thresholds than controls (>60dB), indicating that acoustic sensitivity is impaired. In vivo imaging revealed that neurons in IC and auditory cortex (AC) of awake, unanesthetized Cx26 cKO mice at this age had a much higher threshold, but could be activated by loud sounds. Moreover, suprathreshold pure tone acoustic stimuli elicited spatially restricted activation of auditory neurons within isofrequency domains, demonstrating that early tonotopic organization of the auditory system remains largely intact in these mice. With increasing age, Cx26 cKO mice retained responses to pure tones above 80 dB SPL; however, neuronal excitability in the auditory cortex rapidly increased, leading to enhanced Ca2+ response amplitudes, greater spatial activation, and degradation of precise neural tuning.
Conclusions: Together, these results suggest that preservation of spontaneous activity in the absence of Cx26 enables proper maturation of central auditory circuits, and that the transition to deficient cochlear output once spontaneous activity ceases triggers a rapid increase in central acoustic sensitivity. Early intervention to restore neural activity in the auditory pathway in patients with Cx26 mutations may reduce the probability of experiencing the maladaptive consequences of rapid gain control in auditory circuits, such as hyperacusis and tinnitus.

Characterizing Coturnix Japonica as a Model for Studying the Avian Inner Ear

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Category: Inner Ear: Anatomy and Physiology
Background: The senses of hearing and balance rely on mechanosensory hair cells. These cells are responsible for transducing mechanical stimulation into an electrical signal interpreted by the brain as sound or linear/rotational acceleration. In mammals, hair cells that are lost due to noise or ototoxic drug exposure do not regenerate, resulting in permanent inner ear dysfunction.
Unlike their mammalian counterparts, the inner ears of birds exhibit a remarkable regenerative response after hair cell death. Our lab has demonstrated that the hair cells of the P7 chicken cochlea (basilar papilla) are extruded from the sensory epithelium within 24 hours after an infusion of the ototoxic drug sisomicin into the inner ear via the posterior semicircular canal. Over the course of an additional 72 hours, a portion of the supporting cells re-enter the cell cycle, divide, and begin to express markers of new hair cells. Characterizing this regenerative response could lead to the translation of discovered pathways into the mammalian system. However, the chicken model does not lend itself well to detailed characterization of the outcome of the regenerative response: i.e., functional maturation of regenerated hair cells, including stereocilia bundle reformation, reinnervation, etc.

Therefore, we have aimed to characterize Coturnix japonica, or the Japanese quail, as a model for inner ear research in the context of our recent discoveries.

Methods: Inner ears of Japanese quails were dissected at several embryonic and postnatal ages to characterize the development of cochlear hair cells using immunohistochemistry and hybridization chain reaction (HCR).

Results: Immunohistochemical analysis with antibodies known to work in the chicken cochlea, such as MYO7A, SOX2, and TUBB3, shows that the overall morphology of the quail cochlea is very similar to that of the chicken. Additionally, HCR for marker genes of tall and short hair cells in the chicken cochlea demonstrates the existence of hair cell subtypes also in the quail cochlea.

Conclusions: The Japanese quail represents a suitable replacement model to chicken for studying the long-term hair cell regenerative response. The quail inner ear's overall structure and morphology are persevered compared to chicken, suggesting that discoveries made in the chicken can be directly translated. Additionally, fertilized quail eggs are generally available from local vendors. Finally, their small size, high fecundity, and a short time to sexual maturity (5-6 weeks) make the Japanese quail a good candidate for breeding colony establishment within institutional animal facilities.

Three-Dimensional Visualization of the Membranous Labyrinth: The Ductus Reuniens

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Category: Inner Ear: Anatomy and Physiology

Background: The labyrinth of the inner ear is a complex three-dimensional structure comprised of a membranous otic labyrinth (derived from ectoderm) and various soft tissues of mesodermal origin occupying the space between the otic labyrinth and bony otic capsule. The ductus reuniens (DR) joins the inferior portion of the saccule to the scala media providing the only endolymphatic connection between the vestibular and auditory end organs of the ear. The DR, however, remains poorly understood in both its morphology and spatial relationships to surrounding anatomy. In turn, its (patho)physiological role in the healthy and the diseased ear remains unclear. Recent studies are beginning to address this deficiency. For example, recent evidence from a large case series in 27 patients shows that vestibular receptor function can be preserved after surgical ablation of the cochlea highlighting that endolymph flow from the cochlea to the vestibular labyrinth via the DR is not necessary for normal function of the human peripheral vestibular system (Plontke et al 2021).

Methods: This study serves to elucidate the 3D morphology of the DR and its relationship to surrounding bony and membranous anatomy, providing a detailed, quantitative description of its morphology. Temporal bones from three humans and two guinea pigs were fixed using Karnovsky's fixative and then soaked in 2 % Osmium tetroxide. This process allowed better visualization of the membranous labyrinth. The samples were then scanned by micro-CT followed by 3D reconstruction of the saccule, DR, and initial portion of the scala media. Finally, univariate measurements of these structures were taken using the software 3D Slicer.

Results: The DR takes the form of a narrow tube which has a subtle hourglass-like shape expanding slightly on both ends contiguous with the saccule and scala media. The DR is curved concavely when viewed anteroposteriorly, adhering to the inferior bony wall of the vestibule just anterior to the posterior ampulla. 3D visualizations of the DR illustrate its small intralumenal width (<0.2mm) and its proximity to the round window with the lateralmost end of the DR situated 0.25mm (on average) superior to the posteromedial corner of the round window in humans.

Conclusions: Our results provide new visualizations of the DR, demonstrating its relationship to surrounding structures. Of particular importance is its proximity to the round window. Our observations indicate that care should be taken for surgical procedures involving the round window so as not to disturb the DR situated immediately superior to it. Our results also support the possibility of dislodged otoconia blocking the duct itself, which may impede endolymphatic flow and play a role in endolymphatic hydrops and Meniere's Disease. Plontke SK, et al. A case series shows independent vestibular labyrinthine function after major surgical trauma to the human cochlea. Communications Medicine 2021, in press.

Towards Identifying Force Relaying Elastic Elements in Drosophila Melanogaster NOMPC

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Category: Inner Ear: Anatomy and Physiology

Background: Sensitive hearing in Drosophila melanogaster requires the bona fide mechano-electrical transduction (MET) channel NOMPC (TRPN1) [1]. NOMPC possesses an ankyrin repeat (AR) domain consisting of 29 ARs at its amino terminus. These 29 ARs assemble into a helical structure [2], tether the channel intracellularly to microtubules [3], and are essential for mechano-gating [3]. Because of these findings it was hypothesized that the AR domain might function as the gating spring, an elastic element relaying forces to the channel gate.

Methods: We used Laser-Doppler-Vibrometry and simultaneous compound action potential recordings to assess the hearing performance and correlates of MET channel gating of adult flies in vivo. For the in vitro experiments, we recorded spontaneous single channel currents as well as stimulated channel activity in outside-out patches of NOMPC29+29AR and NOMPC expressing S2 cells. The different NOMPC constructs were additionally labelled with a GFP to allow for targeting patch clamp experiments.

Results: Consistent with previous reports [3], NOMPC29+29AR enabled mechano-activated currents in heterologous expression systems (S2 cells). The mechanosensitivity of stimulus evoked MET currents in NOMPC29+29AR positive S2 cells closely resembled those of native NOMPC, as did its adaptation properties. In addition, sensitive hearing remained unaltered when replacing NOMPC in the adult fly with NOMPC29+29Ar, as did the NOMPC-dependent nonlinear gating compliance of the fly's auditory receiver [1]. Hence, duplicating the NOMPC AR domain neither affects NOMPC mechanosensitivity, adaptation, and channel gating in vitro nor in vivo.

Conclusions: We report here that the 29 ARs of NOMPC do not function as an elastic element (gating spring) for the channel. What forms the spring will be the topic of the presentation.

Immunohistochemical Localization of Glucocorticoid and Mineralocorticoid Receptors in the Human Inner Ear

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Category: Inner Ear: Anatomy and Physiology

Background: In the present study we investigated the localization of glucocorticoid receptors (GCR) and mineralocorticoid receptors (MCR) in the human inner ear using immunohistochemistry. Corticosteroids are widely used to alleviate inner ear disorders among them Meniere's disease (MD). GCR and glucocorticoids have been studied in the mouse, rat, guinea pig and human inner ear.

Methods: Cryostat sections of the macula utricle obtained from patients who required trans mastoid labyrinthectomy for intractable vertigo due to MD (n=4), acoustic neurinoma (n=3), celloidin-embedded sections of human temporal bones from patients diagnosed with MD (n=3) and specimens with normal hearing and balance (n=3) were immunofluorescence (IF) stained using GCR rabbit affinity-purified polyclonal antibodies, glial fibrillary acidic protein (GFAP) or MCR mouse monoclonal antibodies. Digital fluorescent images were acquired using a high-resolution laser confocal microscope.

Results: In the macula utricle cryostat sections obtained from surgery (MD and acoustic neurinoma), GCR-IF was present in the nuclei of vestibular hair cells and supporting cells. GFAP-IF allowed the identification supporting cells. In celloidin-embedded sections, GCR-IF was present in the nuclei of hair cells and supporting cells of the organ of Corti. MCR-IF was present in the nucleus of supporting cells. GCR-IF was detected in cells nuclei of the Reissner's membrane, whereas MCR-IF was in cytoplasm. GCR-IF and MCR-IF were seen in cells nuclei of the spiral ligament. GCR-IF was seen in cells nuclei of the stria vascularis, whereas MCR-IF was in cytoplasm. GCR-IF was found in the nucleus of ganglion cells, whereas MCR-IF was present mainly in ganglion cell cytoplasm. In addition, GCR-IF was found in the nucleus of vascular endothelial cells and pericytes. In the saccule, GCR-IF was localized well in the nuclei of vestibular hair cells, supporting cells nuclei and stroma cells.

Conclusions: GCR and MRC distribution was ubiquitous in the cochlea and vestibular endorgans. obtained from ablative surgery and the inner ear archival celloidin sections. The differential expression of both receptors found in the human inner ear would help to design different therapeutic approaches of glucocorticoids administration.

Microarray Analysis of Lipopolysaccharide-Induced Endotoxemia in the Cochlea

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Category: Inner Ear: Cochlear Mechanics
Background: Lipopolysaccharide (LPS)-induced endotoxemia alters intracochlear homeostasis and potentiates aminoglycoside-induced ototoxicity. However, the pathophysiological mechanisms in the cochlea following systemic LPS-induced inflammation remain elusive.
Methods: In this study, three groups of mice received intraperitoneal injections [group A, saline control (n=10); group B, 1 mg/kg LPS (n=10); group C, 10 mg/kg LPS (n=10)].
After 24 h, gene expression in cochlea samples was analyzed using DNA microarrays covering 28,853 genes. A total of 505 differentially expressed genes (DEGs) ($\geq$ 2.0-fold change; $p<0.05$) were identified.
Results: Interferon- and chemotaxis-related genes, including gbp2, gbp5, cxcl10, and Rnf125, were dose-dependently upregulated by LPS-induced endotoxemia. These results were verified by RT-qPCR. Upregulated DEGs were associated with inflammation, positive regulation of immune responses, and regulation of cell adhesion, while downregulated ones were associated with chemical synaptic transmission and synaptic vesicle cycle.
Protein-protein interaction included four functional clusters associated with interleukin-4, -10, and -13 and G protein-coupled receptor (GPCR) ligand binding; activation of matrix metalloproteinases and collagen degradation; recruitment of amyloid A proteins; and neutrophil degranulation.
Conclusions: We provide a fundamental data on changes in the expression of genes in the cochlea in response to LPS-induced endotoxemia. These results provide mechanistic insight into the risk of aminoglycoside-induced ototoxicity under systemic inflammation.

Noise-Induced Changes in Reticular Lamina and Basilar Membrane Vibrations in Gerbil Cochleae

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Category: Inner Ear: Cochlear Mechanics
Background: Noise-induced alterations in cochlear mechanics have been studied by measuring the basilar membrane vibration using heterodyne laser interferometers. It has been demonstrated that acoustic overexposure decreases basilar membrane responses to low-level tones, mainly at frequencies close to the best frequency. There is no significant change at low frequencies and at high sound pressure levels. These changes have been interpreted as results of damaged outer hair cell functions and decreased gain of the cochlear amplification. To test this hypothesis, we measured intense sound-induced alterations in the reticular lamina and basilar membrane vibrations.
Methods: Young Mongolian gerbils of either sex with normal hearing at the age of 4 to 8 weeks were used in this experiment. The bulla on the left side was opened through a ventrolateral surgical approach, and the round window membrane was partially removed. The opening in the round window membrane was covered using a glass coverslip with a tilted angle to deflect the object beam of a heterodyne low-coherence interferometer to the cochlear partition. When the object beam was focused on the reticular lamina or the basilar membrane, the vibration magnitude and phase were measured as a function of frequency at different sound pressure levels before and after the noise exposure. The stapes vibration was also measured for determining the transfer function of the reticular lamina and basilar membrane.
Results: Before the noise exposure, the reticular lamina and basilar membrane vibrations show high sensitivity, sharp tuning, and nonlinear compression. The reticular lamina vibration is significantly larger than basilar membrane vibration at all frequencies and sound pressure levels. The reticular lamina and basilar membrane vibrations are approximately in phase at the best frequency and out of phase at low frequencies. After noise exposure, the reticular lamina vibration decreases significantly more than the basilar membrane vibration. While the decrease of the basilar membrane vibration occurs mainly at frequencies close to the best frequency at low sound pressure levels, the reduction of the reticular lamina vibration extends from the best frequency to low frequencies. The phase difference between the reticular lamina and basilar membrane vibration becomes smaller

or disappears. Moreover, the baseline shift of the reticular lamina vibration decreases and ultimately changes its direction.

Conclusions: Since the reticular lamina is measured from the apical ends of outer hair cells, the noise-induced changes in the reticular lamina vibrations reflect the decreased mechanical activity of outer hair cells. These micromechanical changes likely contribute to the noise-induced temporary threshold shift.

Do Organ of Corti Vibrations Change With Spontaneous Changes in Brain State in Awake Mice?

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Category: Inner Ear: Cochlear Mechanics

Background: While the presence of cortical and other high-level projections to the medial olivocochlear (MOC) efferent neurons have been identified and studied over the last several decades, their functional significance in top-down modulation of auditory processing remains poorly understood. More specifically, it is not yet clear whether the brain actively modulates cochlear amplification through auditory efferent neurons, and if so, for what purpose. Recently, studies have shown pupil size to be a reliable indicator of changes in brain state. Using pupillometry and optical coherence tomography (OCT), we non-invasively measured organ of Corti (OoC) vibrations and pupil diameter in awake mice. Our hypothesis was that spontaneous natural changes in brain state would correlate with the amount of cochlear amplification.

Methods: Wild-type CBA/CaJ and mutant alpha9 knockout (KO) mice of both sexes between 5 and 8 weeks old were used. Alpha9-KO mice lack α9-nicotinic acetylcholine receptor subunits at the basolateral surface of their outer hair cells where medial olivocochlear (MOC) efferent neurons terminate, effectively terminating higher-level neuronal signaling to the cochlea. Mice were headposted and trained to calmly rest while clamped atop a wheel in a dimly-lit soundproof booth. Sound stimuli were presented using a Fostex tweeter. OoC vibration measurements were taken non-invasively using a custom-built adjustable OCT device designed to be positioned directly outside the ear canal. Concurrently, an infrared pupillometry camera continuously imaged the pupil, and pupil diameters were measured from these images during data post-processing. Vibrometry and pupillometry data were then matched by timestamp.

Results: We imaged through the tympanic membrane and otic capsule bone to visualize the OoC within the apical turn at a radial orientation. When the mice were not moving, repeatable vibratory tuning curves could be measured from the area of the outer hair cells and Deiter's cells. The characteristic frequency of this location was about 9 to 10 kHz. Gain and tuning curve sharpness appear similar to what we have previously reported in anesthetized mice. Pupil diameter varied significantly throughout the 5-minute time frame of these experiments, with oscillating diameters from 0.29 mm to 1.64 mm. In both wild-type and alpha9-KO mice, we did not see any gross changes in vibratory magnitude or phase with changes in pupil diameter. However, a detailed statistical analysis is still in progress.

Conclusions: Our experimental setup permits the direct assessment of cochlear amplification and mouse brain state simultaneously, and ultimately may be used in behavioral experiments. While gross changes in cochlear physiology do not occur during spontaneous oscillations in brain state, subtle effects cannot be ruled out.

Deeper Investigation Into the Effect of Furosemide on Cochlear Vibratory Responses and Stereocilia Morphology

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Category: Inner Ear: Cochlear Mechanics

Background: The endocochlear potential, EP, the ~+ 80 mV potential in scala media is essential for normal cochlear amplification. In two separate previous studies, we reversibly eliminated the EP by an intravenous (IV) injection of furosemide in gerbil and measured the basal local cochlear microphonic (LCM) (Wang et al., 2019),

the vibrations of the OCC (Strimbu et al., 2020), and distortion product otacoustic emissions (DPOAEs). The EP, vibrations, evoked potentials, and DPOAEs recovered in ~ 2 hours post injection.

Velez-Ortega et al., 2019 have also shown that normal transduction currents are necessary to maintain stereocilia morphology. We hypothesize that the loss of EP will thus damage hair cell stereocilia and may, in part, explain the reversible loss of cochlear amplification.

In this study, we further investigate the effect of IV injection of furosemide to the morphology of the OHC bundles and the vibrations of the OCC by 1) ex-vivo imaging of the OHC bundles using scanning electron microscopy (SEM) and 2) In-vivo two-dimensional mapping of the axial vibration of the OCC using optical coherence microscopy (OCT) in gerbil.

Methods: For SEM experiments, young gerbils were anesthetized and DPOAEs were measured before and after furosemide injection (100 mg/kg IV) to assess the loss of the EP. Cochleae were extracted, fixed in 2.5% glutaraldehyde, dehydrated in ethanol, critical point dried, sputter coated, and imaged with a ZEISS VP SEM. Cochlear vibrations were measured with a ThorLabs Telesto III OCT system. By taking sequential recordings with a narrow, 10 um, spacing we construct two-dimensional maps of the axial displacements. Vibrations were measured before, after the furosemide injection, and in ~20-minute intervals for several hours post injection. DPOAEs were measured at the same time points and used to assess the efficacy of the injection and to provide a real time measurement of cochlear condition.

Results: Preliminary results from the SEM images of the OHC bundles after furosemide showed shortening at the first (shortest) row of the OHC stereocilia in the base. A morphological change of the OHC bundles in the middle and apical regions was not detectable.   We hypothesize that the first row of the OHC bundles is shortened or lose rigidity due to transducer current reduction with furosemide.

Post furosemide, BM vibrations resembled a passive cochlea while those in the OHC-region showed a loss of amplitude but retained the broadband nonlinearity. Vibrations were less tightly focused on the OHC-region. Preliminary data suggest that DPOAEs and vibration patterns could recover to baseline conditions over the subsequent 2 – 3 hours.

Conclusions: The EP is necessary for normal cochlear amplification and mechanoelectrical transduction. The loss of the EP in-vivo may affect bundle morphology and partially explain the loss of amplification.

An Improved Model of the Cochlear Microphonic and Auditory Nerve Neurophonic
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Category: Inner Ear: Cochlear Mechanics
Background: To low frequencies (below ~1500 Hz), the ongoing portion of electrocochleography (ECochG) is comprised of the cochlear microphonic (CM) and auditory nerve neurophonic (ANN). It is of interest to quantitatively determine the contributions of the CM and ANN in order to assess cochlear health. We have previously used a mathematical model to estimate the contributions of the CM and ANN to the ECochG response (Fontenot et al. 2017, Front. Neurosci. 11:592). Here, we present an updated model which provides good estimates while being more biophysically rooted.

Methods: The input is an 'average cycle' which is the average of all cycles in the steady-state part of the response. It then adjusts parameters to equations for the CM and ANN that provide the best fit to the average cycle and reports the amounts of CM and ANN that produced the best fit.

The CM is described by a sinusoid that may be symmetrically or asymmetrically saturated. In the previous version, this saturation was implemented by setting separate cut-off parameters for the peak or trough. In the current version, we use a Boltzmann function to describe the cochlear transducer with parameters for saturation and operating point.

The ANN is expressed by a convolution of the unit potential with a cycle histogram and multiplied by the number of responding fibers. In the previous version, the unit potential a single cycle of a sine wave with a frequency of 1100 Hz. In the current version, it is based on measured unit potentials from the literatures. In the previous model, the cycle histogram was modeled as a lognormal distribution with a spread of excitation parameter. In the new model, it is described as a gaussian distribution with the mean in the middle of the cycle, and a spread of excitation parameter influencing the standard deviation.

Results: To compare the models, we created a metric to independently determine the relative amount of ANN present. For each average cycle, we performed a cross-correlation with all the average cycles to high frequencies, which are CM-only, and measured the root-mean square values of the residuals in the case with the closest fit. The

smaller the residuals, the less ANN should be present because it is a closer match to a CM-only curve. The new modeling approach gives similar results to the previous version, with a stronger basis in biophysical properties. **Conclusions:** Separating the CM and ANN has historically used spectral or masking methods which are non-quantitative. Here we describe an improvement to a modeling technique that provides quantitative estimates of the amounts of CM and ANN. We show that the numbers scale well with an estimate of ANN proportion that does not make assumptions about what causes the shape of an average cycle.

Preliminary Investigation of the Relationship Between Temporal Bone 3D Motion and Intracochlear Pressure

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Category: Inner Ear: Cochlear Mechanics
Background: The temporal bone, including the otic capsule, undergoes a complex 3D motion pattern that depends on frequency of the BC stimulation. The correlation between the 3D motion of the surrounding bone and the intracochlear pressure difference across the cochlear partition is not yet known and is to be investigated.
Methods: Preliminary measurements were conducted in a single fresh frozen cadaver head, where the both medial and lateral bone surfaces of the temporal bone have been exposed. The skull bone was mechanically excited in the frequency range of 0.1- 10 kHz via the actuator of a bone conduction hearing aid (BCHA). Stimulation was applied to the ipsilateral mastoid via a conventional transcutaneous (5-N steel headband) and percutaneous coupling, sequentially. Three-dimensional motions were monitored, across the lateral and medial (intracranial) sides of the skull at the ipsilateral temporal bone, via a 3D laser Doppler vibrometer (LDV) moved by a customized robotic positioner. A total of 50-70 measurement points (~5 mm pitch) were distributed across each side of the bony surface of the temporal bone. The motion of the ipsilateral promontory and stapes were also recorded, sequentially. Additionally, intracochlear pressure in the scala tympani and scala vestibuli was measured via a custom-made intracochlear acoustic receiver (ICAR).
Results: The temporal bone surface, surrounding the otic capsule, remains rigid-like up to 5 kHz, in contrast to the parietal plate, which deforms above 1 kHz, with an onset of deformation near the stimulation already at 0.5 kHz. The magnitude of the complex ratio of the differential intracochlear pressure and the promontory motion increases with frequency, up until 5 kHz, beyond which it has a trend to decrease with frequency.
Conclusions: The area around the otic capsule appears rigid up to significantly higher frequencies than the rest of the skull surface, resulting in primarily inertial loading of the cochlear fluid.

Macrophage Differentiation and Cytokine Expression in the Vestibular Schwannoma Microenvironment

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Category: Inner Ear: Damage and Protection
Background: M2 macrophages and inflammatory cytokines within the vestibular schwannoma (VS) microenvironment have been linked to tumor growth and hearing loss. Our previous work found that VS from patients with non-serviceable hearing expressed more M2 macrophages than those with serviceable hearing. In this study, we seek to analyze: (1) the secreted cytokine profile of cultured VS, (2) how secreted cytokines changes when VS are co-cultured with monocytes, (3) how cytokine profile affects M2 macrophage subtypes and morphology, and (4) how macrophage subtype, macrophage morphology, and cytokine profile is correlated with hearing status.
Methods: VS tumors were harvested prospectively from patients undergoing microsurgical resection from 2019 to 2021. Six tumors from patients with serviceable hearing were matched for age, gender, and tumor size with six tumors from patients with non-serviceable hearing. Tumors were cultured for 14 days on 16 well slides both alone and in co-culture with human monocytes. Immunohistochemistry for S100B (Schwann cell marker), CD163 (M2 macrophage marker), and additional markers for M2 macrophage subtypes was performed, and representative confocal images were obtained. Tumor conditioned media was collected from each VS on day 14 and analyzed using an antibody array of 88 human cytokines. Cytokine expression levels were then digitally normalized and

quantified. A retrospective chart review was performed for those patients to obtain demographic information as well as clinical and radiographic features.

Results: Patients were stratified based on the AAO-HNS Hearing Classification Scale: A (n=5, 41.7%), B (n=1, 8.3%), C (n=0, 0%) and D (n=6, 50%). Immunohistochemistry shows monocyte differentiation into M2 macrophages in varying degrees across the patients. Preliminary cytokine array results show that tumor conditioned media in co-cultures of VS cells and monocytes express more interleukin (IL)-6, IL-8, macrophage chemoattractant protein-1 (MCP-1), and tissue inhibitor of metalloproteinases 2 (TIMP-2), when compared to VS cultures alone. We show the distribution of cytokines, M2 macrophage morphologies, and macrophage subtypes between VS from patients with and without serviceable hearing.

Conclusions: VS cells secrete cytokines that initiate monocyte polarization into M2 macrophages of various morphologies and subtypes. We describe the cytokine and macrophage profiles of VS cells from patients with unserviceable and serviceable hearing. Understanding the tumor microenvironment in VS may lead to novel targets and therapies for hearing loss related to VS.

Activation of ULK1 Dependent Autophagy by Nicotinamide Protects from Noise Induced Hearing Loss

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Category: Inner Ear: Damage and Protection
Background: Noise-induced hearing loss (NIHL) has the highest incidence in environmental related hearing loss, however there is no effective therapeutics for patients. NAD+ performed therapeutic potential in NIHL, but if NAD+ precursor Nicotinamide (NAM) protect NIHL is unclear.
Methods: C57BL/6 mice aged 4 weeks with NAM local supplementation were exposed to an 8-16kHz octave-band noise at 104 dB SPL for 2 hrs. ABR test and immunofluorescence were conducted to mice at 3, 7 and 14 days post noise. Cochleae of the mice were collected 1 day post noise for NAD+ level, mtDNA copy number, mitochondrial function measurement and TEM observation. The number of mitochondria in HCs and SGNs were counted at 3 days post noise. Autophagic flux in the SGNs was observed and demonstrated in the cochleae of CAG-RFP-EGFP-LC3B mice, and mRNA expressions of autophagy-related genes were analyzed through RT-PCR.
Results: NAD+ level reduced in cochlea after noise and noise exposure results in mitochondrial dysfunction in auditory neuron rather than hair cells. Supplementation of NAM maintain mitochondria homeostasis in cochlea and protect auditory neuron from neuroexcitatory toxic injury in vitro and in vivo. Pretreated NAM 2 days before noise injury through round window sustained NAD+ level, ROS level as well as OXPHOS gene expression levels. NAM treatment increased the number of mitochondria in the cochleae within the noise condition, especially in the IHCs and SGNs. Auditory Brainstem Response (ABR) threshold shifts significantly decreased by NAM treatment in comparison to the opposite ears with nearly 30 dB difference at the frequencies of 16 and 32 kHz after noise trauma, with synapse preservation in the IHCs. Gelatin-methacryloyl (GelMA) hydrogel encapsulated NAM improved delivery efficiency therefore protected hearing comprehensively from noise exposure. Autophagy was enhanced in spiral ganglion neuron (SGN) after NAM treatment, mainly through upregulating autophagy-initializing kinase ULK1. Local administration of ULK1 activator also protected from NIHL in mice.
Conclusions: Noise induced mitochondrial dysfunction and NAD+ depletion in the cochlea, could be protected by NAM through mitochondrial biogenesis and quality control. NAM protected NIHL in mice by local administration, through maintaining synapses and mitochondria function. GelMA improved medication delivery in inner ear, which facilitated small molecular drug treatment. NAM enhanced SGNs autophagy after noise trauma, by upregulation of ULK1. ULK1 activation protected from NIHL, suggesting ULK1 dependent autophagy enhancement as a potential intervention to NIHL.

Macrophage Infiltration in the Cochlea of Control and Kanamycin-Deafened Rats

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Category: Inner Ear: Damage and Protection
Background: Cochlear hair cells transmit sound information to spiral ganglion neurons (SGNs). SGNs gradually degenerate after hair cell loss and is associated with an immune/inflammatory response in the spiral ganglion. One characteristic of the immune response is an infiltration of immune cells with an increase in macrophage number and activation being prominent components. In neonatal rodents, macrophage number and activation may change in response to developmental loss of SGNs. To further investigate developmental changes and the role of the immune response in the spiral ganglion after deafening, we quantified macrophages (Iba1 immunoreactive cells), macrophage activation (CD68+), and neuronal density (Tuj1+) in neonatally deafened rats and age-matched hearing controls. Rats were deafened by daily kanamycin injection from postnatal day 8 (P8)-P16 to kill hair cells. We have previously shown that inner hair cells are not completely gone until P18-P19, and SGN death is not detectable until about P28.
Methods: Sprague-Dawley rats were intraperitoneally injected with kanamycin once daily from P8-P16 to kill hair cells. Cochlea from hearing and kanamycin-deafened rats were collected between P5 and P32, fixed with 4% paraformaldehyde, and prepared for cryosectioning. Cochlea were sectioned parallel to the mid-modiolar plane at 25 μm thick, then labeled with antibodies to identify neurons (Tuj1), macrophages (Iba1), and activated macrophages (anti-CD68). Sections were then imaged using confocal microscopy and the number of cells was counted at several locations along the basal-apical axis in the ganglion using FIJI/ImageJ.
Results: Previous studies have shown an increase in macrophages in the apical half of the cochlea at P70 in deafened rats. Here we show increased macrophage density in the apical half of the cochlea in kanamycin-injected rats as early as P16, prior to SGN loss. Macrophage density at the basal half of the cochlea, however, was not different between groups at any time point. We observe an increase in CD68 immunoreactivity in injected rats throughout the cochlea as early as P21. A similar pattern of developmental changes in macrophage density was seen in both injected and non-injected rats from P5-P12. From P5 to P8, injected and non-injected rats show a developmental decrease in neuron density in the apical half of the cochlea.
Conclusions: Changes in macrophage and neuron density occurring between P5-P12 are likely developmental changes of the cochlea rather than a consequence of kanamycin injection. The density of macrophages in the apical half of the cochlea is significantly higher in injected rats by P16, and the proportion of CD68+ macrophages is increased by P21. These changes occur prior to detectable SGN degeneration in the cochlea, suggesting that the increase in macrophage number and activity is not for the purpose of phagocytosis of dying neurons.

Long-Term Electrophysiological Correlates of Noise-Induced Temporary Threshold Elevations in Guinea Pigs
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Category: Inner Ear: Damage and Protection
Background: Auditory pathology resulting from noise-induced loss of synapses between sensory cells and auditory nerve fibers has been inferred from non-invasive measurements after noise exposures in a number of species but remains dependent on histological verification. Although the degree of noise-induced pathology and the extent of subsequent natural recovery of lost function vary among species thus far considered, the condition appears ubiquitous among mammals. Here, we report preliminary electrophysiological and otoacoustic emission findings from a study designed to develop a multivariate statistical model that confidently predicts the disorder in humans. An ancillary goal of the overall project is to determine the relative position of humans on the auditory synaptopathy continuum.
Methods: In this report, we summarize preliminary findings from a battery of electrophysiological and otoacoustic emission tests, prominently including Auditory Brainstem Responses (ABR), Envelope Following Responses (EFR), and Distortion Product Otoacoustic Emissions (DPOAE) following temporary threshold elevations. The goal is to identify those outcomes that best reflect sustainable differences between noise-exposed and control guinea pigs, an animal model selected for its similarity with human auditory performance. Although early physiological findings are presented here, ultimately, abnormalities in inner ear morphology, prominently including synaptopathy, will serve to validate the diagnostic power of the fully developed statistical model. That model will subsequently be evaluated against similar data collected in human subjects, with the goal of predicting the presence and degree of auditory pathology in humans.

Results: Immediately following exposure to an octave-wide band of noise centered on 4 kHz and delivered at 106 dB SPL for two hours, ABR thresholds were significantly elevated for frequencies at and greater than 2.8 kHz, as were click and DPOAE thresholds. Consistent with the loss of sensitivity, diminished wave I amplitudes and DPOAE amplitudes were observed immediately following exposure in all noise-exposed animals studied thus far regardless of stimulus level for frequencies above approximately 2.8 kHz, as well as for clicks. Both DPOAE and ABR thresholds recovered in all noise-exposed animals within 14 days following exposure. However, growth of wave I amplitudes with increasing stimulus level (i.e., the slope of amplitude-level curves) remained notably reduced in noise-exposed animals relative to control conditions. Differences in temporal processing capabilities were also observed, particularly with regard to the system's capacity to track repetitive stimuli with increasing presentation rates (e.g., reduced interstimulus intervals) and the extent to which frequency following (EFR) to the envelopes of sinusoidally amplitude-modulated carriers was maintained.

Conclusions: Our tentative assertion that long-lasting abnormalities remain following temporary noise-induced threshold elevation, most likely resulting from loss of inner hair cell synapses with high-threshold auditory nerve fibers, appears affirmed by outcomes of early electrophysiological and otoacoustic emission findings.

The Effect of Selective Inner Hair Cell Loss on Intensity Difference Detection Thresholds in the Chinchilla Animal Model

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Category: Inner Ear: Damage and Protection

Background: Intensity coding of acoustic stimuli plays a critical role in the perception of complex auditory stimuli such as speech. In previous studies, we showed that carboplatin-induced selective inner hair cell (IHC) loss in chinchillas had little effect on pure tone thresholds, but significantly affected pure tone thresholds in noise and detection of gaps in continuous noise. In a more recent study, we also showed that selective IHC loss had little effect on temporal summation as measured by detection of short duration pure tones. To assess whether the deficits seen in gap detection could be the result of poorer intensity coding, we devised a task to measure intensity difference detection (IDD). This psychophysical task was then used to evaluate chinchilla sensitivity to intensity changes before and after carboplatin, a treatment that reliably and selectively destroys IHC in this species. We hypothesized that IDD thresholds would increase following carboplatin treatment, indicating that loss of IHC produces deficits in intensity coding. If this hypothesis is supported, IDD tasks could be used as a suprathreshold assay of selective IHC loss.

Methods: Free feeding, young adult chinchillas were used for this study. Distortion product otoacoustic emissions (DPOAEs) and pure tone thresholds in quiet were evaluated to assay the status of normal cochlear nonlinearity and as a measure of overall hearing sensitivity. Chinchillas were conditioned to respond to changes in intensity compared to a continuous reference tone. IDD performance was assessed at 1, 2, 4, 8, and 12 kHz and at two continuous reference tone levels, moderate (50 dB SPL) and high (70 dB SPL). The moderate-level tones initially increased by 15 dB SPL (65 dB SPL) and the high-level tones initially increased by 10 dB SPL (80 dB SPL). An automated stair step procedure was used to determine IDD threshold as intensity decreased by 0.5 dB SPL for correct responses and increased by 2 dB SPL for incorrect responses until the lowest level at which the animal achieved 66% correct was obtained. Final IDD thresholds were determined by averaging thresholds from three test sessions. Following baseline testing, chinchillas received a single dose of 75 mg/kg of carboplatin. DPOAEs, pure tone thresholds, and IDD thresholds were re-assessed after a three week recovery period.

Results: Following carboplatin treatment there was no significant elevation of pure tone thresholds and no significant changes to DPOAE; results suggesting that hearing sensitivity had not changed. In contrast, chinchilla IDD increased following carboplatin treatment, suggesting that loss of IHC directly impacted sensitivity to intensity changes.

Conclusions: Our preliminary data supports the hypothesis that chinchilla IDD resembles that of humans and that this model could be used to study the effects of cochlear pathologies involving IHC.

Optimization of a Cisplatin-Induced Rat Ototoxicity Model: Comparison of Various Repeated Slow Intravenous Infusion Regimens Associated With Supplemental Rehydration Protocols for Optimized Long-Term Hearing Loss and Survival Rates

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Category: Inner Ear: Damage and Protection
Background: Cisplatin is used to treat both adult and pediatric tumors and routinely forms the backbone of chemotherapy regimens. Cisplatin-induced ototoxicity (CIO) is an unmet medical need for both these populations, potentially resulting in multifaceted decrease in quality of life and language acquisition, social and academic development in the pediatric population (Gurney et al., 2007).
Several CIO paradigms have been developed in animal models using various dosing schedules and routes of administration to improve hearing loss while reducing nephrotoxicity consistency. Harrison et al. (2016) showed that one key factor of ototoxicity was the cumulative dose of cisplatin when administered with repeated infusions at lower doses. We have previously presented that the 2x5mg/kg cisplatin regimen induced strong and reproducible hearing loss 4 weeks after the last injection. However, this model highlighted that global survival and nephrotoxicity remain difficult to control. Consequently, we decided to continue to optimize our model stability by using lower cisplatin dosing regimen associated with supplemental rehydration to sustain renal function and animals' physiological state.
Methods: Following baseline ABR and DPOAE audiometry recordings, female 7-weeks old Wistar rats received multiple intravenous infusion of cisplatin during 30 minutes under isoflurane anesthesia, of either 2x5mg/kg once/7 days, 4x2.5mg/kg once/7 days or 4x2.5mg/kg once/3 days. Two rehydration protocols were performed for 35 days and compared, consisting in oral saline administration once or twice daily +/- saline/5% glucose i.p. +/- 48h twice daily oral saline prehydration before first infusion + single saline intraperitoneal bolus administered immediately after each infusion. For each treatment group, T+35 days final audiometry was performed for hearing loss characterization, overall physiological state was monitored bi-daily and survival rate was determined as the percentage of remaining animals vs the number of originally included animals per group.
Results: Our results supported the importance of the maximal cumulative dose for optimal ototoxicity but also highlighted the beneficial impact of rehydration and of the infusion window timing to obtain optimized correlation between hearing loss and survival. Animals receiving 4x2.5mg/kg cisplatin all showed 100% survival, but animals infused once/7 days showed no significant T+35d ABR threshold shifts, whereas animals infused once/3 days showed average ABR threshold shift of 19.6dB across all frequencies with a frequency-dependent increase from 8kHz to 32kHz from respectively 7 to 32dB. These values are comparable to our previous data obtained with the 2x5mg/kg treatment modality, while allowing a more consistent survival rate.
Conclusions: These data highlight the fact that for the same cumulative dose, the schedule of infusion has major incidence on cisplatin's ototoxicity and associated mortality. We demonstrate here that shortening the 4x2.5mg/kg cisplatin regimen to one infusion each 3 days represents the best balanced model to obtain hearing loss suitable to test otoprotective therapies associated with ethically acceptable high survival rates.

Reversible Impulse Noise Induced Hidden Hearing loss, Metabolic Disturbances and Hair Cell Ciliary Changes in Mice
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Category: Inner Ear: Damage and Protection
Background: Recent studies demonstrated that reversible continuous noise exposure may induce a temporary threshold shifts (TTS) with a permanent degeneration of auditory nerve fibers, although hair cells remain intact. In this study, we investigate the impact of impulse noise induced TTS exposure on cochlear function, morphology and metabolic state
Methods: CBA/J Mice were exposed to an impulse noise (peak pressure: 146 dB SPL, 1 pulse/second, for 700 seconds) to provoke a maximum elevation of ABR thresholds 30 minutes following noise exposure, and returning to nearly normal 30 days after exposure. The effects of noise exposure were assessed using complementary approaches combining morpho-physiology, biochemistry and molecular biology
Results: Our results showed that the exposures caused a mean elevation of ABR thresholds ~ 30 dB and a reduction in DPOAE amplitude 30 minutes after exposure. 4 weeks later, ABR thresholds and DPOAE amplitude were back to normal at the higher frequency region (8-32kHz). A small level of PTS remained in the lower

frequencies. Morphological evaluations revealed a disturbance of the stereociliary bundle of outer hair cells, mainly in the outer hair cells located in the regions coding the frequencies from 4 to 20 kHz. On the other hand, the reduced suprathreshold ABR amplitudes remained until 4 weeks later. Loss of synapse numbers was observed 24h after exposure, with full recovery two weeks later. Transmission electron microscopy revealed morphological changes at the ribbon synapses by two weeks after exposure. Molecular investigations revealed increased levels of the oxidative stress immediately after and maintained for 2 weeks after exposure.

Conclusions: These results clarify the pathology underlying impulse noise-induced sensory dysfunction, and suggest possible links between impulse noise injury, cochlear cell morphology and metabolic changes and hidden hearing loss.

Identification of an FDA-Approved Drug Combination for the Prevention of Cisplatin-Induced Ototoxicity

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Category: Inner Ear: Damage and Protection

Background: Cisplatin is a first-line chemotherapy prescribed to 20% of all cancer patients. However, more than 80% patients receiving cisplatin treatment developed permanent hearing loss. Despite this debilitating side effect, there are no FDA-approved drugs to prevent cisplatin ototoxicity.

Methods: A combination of FDA-approved drugs was identified through our computational drug screening using retrospective clinical data and gene expression data. The drug combination was validated in zebrafish lateral line neuromast assay. Cell assays were also performed to further validate the protective effect and to examine whether the drug combination affects cisplatin anti-tumor effect.

Results: Our computational and experimental results show that the drug combination exhibited higher otoprotective effect than the drugs alone. The drug combination did not attenuate cisplatin anti-tumor effect in vitro.

Conclusions: The drug combination demonstrates promising potential for the prevention of cisplatin-induced hearing loss. It will be further validated in vivo.

Continuous Monitoring of Occupational Noise Exposure in Firefighters Using Apple Watch

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Category: Inner Ear: Damage and Protection

Background: According to The National Institute for Occupational Safety and Health (NIOSH), each year, about 22 million American workers are exposed to hazardous noises. Additionally, about 34% of the workers are in environments where hearing protection devices (HPD) are not used. As a result, each year about $242 million is spent on compensation of hearing loss disabilities. Occupational noise exposure and hearing loss are prominent in the fire service, as firefighters are exposed to many loud noise sources during their service. The present study utilized the Apple Watch to continuously measure environmental noise levels in on-duty firefighters.

Methods: Participants included sixteen firefighters from the Coral Gables Fire Department, and sixteen adult non-firefighter control subjects. Firefighters were recruited from a variety of roles to ensure noise exposure profiles were appropriately representative of exposures in the fire service. All participants wore an Apple Watch for 3 separate 24-hour shifts and completed a post-shift survey self-reporting on perceived exposures over the 24-hour study period. Cumulative exposures were calculated for each shift using a custom MATLAB script and noise dose was calculated relative to the NIOSH Recommended Exposure Limit of 85 dBA as an 8-hour time-weighted average.

Results: Equivalent exposure over an 8-hour duration in firefighters was 81.97 ± 2.9 dB compared to 72.35 ± 5.53 dB among control subjects. On average, firefighters were exposed to 85dB or higher levels of noise for 5 ± 3.06 hours compared to 1 ± 1.52 hours in controls subjects. Among firefighter participants, 15% of the work shifts exceeded the NIOSH 85-dBA standard. Members of the rescue team had greater average exposure than members of the engine team. The averaged self-reported exposure time among firefighters was 18.5 minutes. Only 2 of 16 firefighters reported use of HPDs.

Conclusions: Among the noise sources related to fire rescue, the air horns and sirens are ~115 dB while air chisels and gasoline-powered circular saws generate noise between 110-120 dB. Thus, even less than a minute of exposure may be hazardous as per NIOSH standards. Our study shows that firefighters have significant hazardous noise exposure and may be at an increased risk of noise-induced hearing loss.

Influence of Fractalkine Receptor CX3CR1 Deletion on Cochlear Hair Cell Survival and Macrophage Expression in Chronic Suppurative Otitis Media
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Category: Inner Ear: Damage and Protection
Background: Chronic Suppurative Otitis Media (CSOM) is a neglected disease that afflicts 330 million people worldwide and is the most common cause of permanent hearing loss among children in the developing world. It is characterized by a chronically discharging infected middle ear. We have demonstrated that CSOM causes macrophage associated sensory hearing loss.
Methods: In this report, we examined the influence of fractalkine receptor (CX3CR1) deletion (CX3CR1GFP/GFP) in CSOM. We investigated in our novel pseudomonas aeruginosa PA CSOM animal model, previously validated to mimic the human disease.
Results: We observed partial outer hair cell (OHC) loss in the cochlear basal turn, no OHC loss in the middle and apical turns in both CX3CR1GFP/GFP and wild type (WT) mice at 14 days after bacterial inoculation. The number of OHCs in the base remained as 26.6/100 µm of the basilar membrane in CX3CR1GFP/GFP mouse and 27.0/100 µm of the basilar membrane in WT mouse. There was no significant difference (p = 0.95). In contrast to OHC loss, no IHC loss was found in all cochlear turns. We also counted F4/80 macrophages in hair cells area and outer sulcus region with Z-stack images in whole mount samples. Macrophages have 6.0/100 µm of the basilar membrane in CX3CR1GFP/GFP mice and 5.6/100 µm of the basilar membrane in WT mouse. There was also no significant difference (p = 0.68).
Conclusions: Together, the data did not support the correlation in HC loss and macrophage numbers between fractalkine receptor deletion and WT CSOM. We will further investigate the immune responses in the whole cochlea in CX3CR1GFP/GFP comparing with WT mouse.

Differential Gene Expression in the Developing Mouse Cochlea Caused by Congenital Cytomegalovirus Infection
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Category: Inner Ear: Damage and Protection
Background: Congenital cytomegalovirus (cCMV) infection is a major source of sensorineural hearing loss in infants and children worldwide, and the mechanism of hearing loss is unknown. We have used a murine model of congenital CMV, where newborn mice are peripherally inoculated with mouse CMV on the first day of life, to investigate genes expressed during congenital CMV infection with the intent to focus on gene expression that is significantly altered during viral infection that may contribute to hearing loss.
Methods: Bulk RNA-sequencing was performed to evaluate differences in gene expression between normal and infected mouse cochleae at P7 and P14. Cochlear tissue was harvested from anesthetized mice and separated into two specimens labeled: lateral wall (LW), containing the stria vascularis and spiral ligament and modiolus (MO) which included the sensory epithelium, the osseus spiral lamina, and spiral ganglion neurons. Total RNA was extracted using the Arcturus PicoPure RNA Isolation Kit (Applied Biosystems). Samples were sequenced by the Genome Technology Access Center (GTAC) at Washington University School of Medicine using the Takara Clontech SMARTer technique.
Results: Principal Component Analysis (PCA) confirmed four distinct gene expression profiles corresponding to the four experimental groups (control LW and MO, CMV-infected LW and MO). Subsequent differential expression analysis supported several important findings. In CMV infected cochleae, inflammatory markers were markedly elevated as expected, and there were some that were more highly expressed in the lateral cochlear compartment versus the medial compartment. Expression of TNF and interferon gamma were greater than ten times higher than in control ears. Interferons and interferon stimulating genes, cytokines, and chemokines were

significantly upregulated. Genes that are classically known to be important for hair cell and spiral ganglion development were not significantly changed in CMV infected ears.

Conclusions: Bulk RNA-sequencing confirms host interferon gamma signaling plays a major role in CMV infection, and other immune signaling pathways are activated in the cochlea during congenital CMV infection. Some pathways may prove effective targets for treatments and therapies to mitigate the risk of permanent sensorineural hearing loss.

Synaptic Loss and Functional Deficits in Guinea Pigs After Exposure to Noise That is Likely Experienced in Human Daily Life

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Category: Inner Ear: Damage and Protection
Background: Significant loss of ribbon synapses in cochleae was seen after exposure of brief noise exposure at the highest level without permanent threshold shift (PTS) in animals. Such data is difficult to be translated into human cases where noise exposure is usually at much lower level. Based upon the animal data, coding-in-noise deficits (CIND) is speculated as the major problem associated with the selective loss of auditory nerve fibers (ANF) with low spontaneous rate (LSR). However, data from human subjects with long history of noise exposure cannot confirm such deficits. Moreover, the exclusive role of LSR ANFs in signal coding at high sound level has been challenged by temporal fluctuation profile hypothesis (Carney, 2018, doi: 10.1007/s10162-018-0669-5). Based upon this hypothesis the fluctuation of ANF responses play a critical role in efferent feedback to regulate the gain of outer hair cells (OHCs). However, such gain control has not been verified.
Methods: Guinea pigs were exposed to fluctuated noise with Leq below 90 dB SPL, which was given several hours a day over a month. Synaptic loss was quantified by immunohistology against the presynaptic ribbons and postsynaptic terminals. Coding of signal at high sound level against background noise was examined by the masking on envelope following responses (EFR) in far field and near field (nfEFR recorded from round window). The role of temporal fluctuation on efferent feedback was examined by the contralateral suppression (CS) on transient CAP at 16 kHz.
Results: (1) The repeated noise exposure at the low level over the long period was applied to have accumulated total dose more than the brief noise exposure at high level (2 h at 106 dB SPL). However, amount of the synaptic loss was much smaller. (2) The masking effect on both EFR and nfEFR is much larger when a stationary masker is used as compared with the effect of masker that is temporally fluctuating, suggesting that auditory system can code signal in the dips of noise, probably relying upon the temporal processing. (3) Between the control and noise groups, the effect of masking is largely different when fluctuating masker is used, while the difference is not significant for stationary masker. (4) Now evidence is seen for the larger effect of CS by using temporarily fluctuated signals as compared with the stational signal.
Conclusions: Temporarily fluctuated noise at lower level produces much less damage to ribbon synapses at equal energy basis. CIND should be observed by using fluctuated masker, which mimics the masker in real life and allow the auditory system to use the cue in dips. CIND is likely real but is due to the deterioration of temporal processing in ANFs with the defeated synapses.

Effects of Mitochondria Dynamics Disruption on The Functioning of Mouse Inner Ear

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Category: Inner Ear: Damage and Protection
Background: Mitochondria are highly dynamic organelles that play a critical role in cell homeostasis and energy balance. They can undergo continuous fission/fusion as well as turnover through mitochondrial biogenesis and mitophagy to maintain optimal cellular function. Disruption of this dynamic balance under bioenergetic or oxidative challenges could lead to age-related disorders through cells senescence or death. The protein phosphoglycerate mutase 5 (PGAM5) is a component of the inner mitochondrial membrane that regulates mitochondria dynamics by acting as a mitochondrial stress sensor to multiple cellular pathways. Here, we aimed to evaluate the effect of PGAM5 deficiency on hearing function through aging and in response to noise exposure.

Methods: PGAM5 constitutive knockout mice (PGAM5-/-) were bred and aged to P60, P120, and P180. Additionally, a separate cohort of P60 mice were exposed to a moderate noise insult of 100 dB octave band noise for 2 hours. Suprathreshold auditory brainstem response (ABR) testing and whole mount immunofluorescence staining was performed for each group of mice and wild-type littermate mice.

Results: PGAM5-/- mice exhibited progressive middle to high frequency (12, 16, 24 and 32 kHz) hearing loss compared to their littermate wildtype mice. Whole mount immunofluorescence staining indicated abnormal intracellular distribution of the PGAM5-/- mice mitochondria, more shrunken cytoplasm volume, and greater hair cell death in basal regions of cochlea. Noise-exposed P60 PGAM5-/- mice demonstrated a larger middle and high frequency permanent threshold shift; additionally, outer hair cell death in knockout mice was 25% greater in the region encoding for 32 kHz frequency.

Conclusions: Our results support the hypothesis that PGAM5-regulated mitochondrial dynamics is essential for optimal functioning in the inner ear under normal and stress response conditions. The PGAM5 knockout mice model can be useful to understand this specific damage mechanism in the various cell types of the inner ear which may assist with future insight in inner ear disease pathophysiology.

Roles of Sirtuins in the Protection Against Cisplatin Ototoxicity

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Category: Inner Ear: Damage and Protection

Background: Cisplatin ototoxicity (CO) in chemotherapy affects an estimated 100-300 thousand cancer patients in the USA annually, causing symptoms including severe hearing loss, tinnitus, dizziness, etc. CO is related to the accumulation of reactive oxygen species (ROS) in the cytoplasm and mitochondria of the cochlear cells, inducing damage associated mainly with outer hair cell (OHC) loss. Sirtuins, a family of NAD+-dependent deacetylase, are critical enzymes for stress resistance pathways and regulators of the intrinsic anti-ROS systems. Sirtuin family contains seven members (SIRT1-7), which differ in catalytic activities and subcellular locations (SIRT1 and 2: cytoplasm, SIRT3-5: mitochondria, SIRT1, 2, 6, 7: nucleus). Through the regulation of ROS, sirtuins govern cellular processes, including homeostasis, responses to stress, DNA damage repairing, inflammation, and apoptosis. However, the role of sirtuins in hearing protection against CO has not been systematically studied. We have shown that SIRT3 is involved in the hearing protective effect of honokiol (HNK) and can also increase animal survival in cisplatin treatment without compromising its antitumor effects. In this study, we further investigated the protective effects of HNK against CO on ribbon synapses in the inner hair cells and the potential roles of sirtuins in the cytoplasm and mitochondria.

Methods: A transgenic mouse model from Jackson Laboratory expressing mouse mammary tumor virus (MMTV) polyomavirus middle T agent (PyMT) oncogene (Stock #: 002374) was used in this study. The female carriers of the MMTV-PyMT oncogene develop palpable mammary tumors at the age of ~2 months. When the tumor size reached 500 mm3, the mice were treated with a chemotherapy regimen consisting of 3 cycles. In each cycle, 4 doses of HNK (10 mg/kg/day) and/or cisplatin (4 mg/kg/day) were administered across 4 days, followed by a 10-day recovery interval. The entire chemotherapy regimen lasted for 42 days, or the animals reached the humane study endpoint due to weight loss ($\geq$25%) or tumor growth ($\geq$1500 mm3). Auditory brainstem responses (ABRs) were recorded before the treatment and after each cycle (days 14, 28, and 42) to assess the cochlear function. After the treatment, the cochleae were harvested to observe the changes in synaptic components and the expression of SIRT1, 2, 3, 5 through immunostaining.

Results: HNK treatment decreased ABR threshold elevation and prevented the loss of pre-and post-synaptic components in cisplatin chemotherapy. Meanwhile, HNK further suppressed tumor growth and improved the survival of the animals. The expression of sirtuins in the cytoplasm (SIRT1 and 2) and mitochondria (SIRT3 and 5) increases with HNK treatment.

Conclusions: HNK potentiates the antitumor effect of cisplatin and protects hearing in chemotherapy. This protective effect of HNK against CO is associated with the upregulation of sirtuins in the cytoplasm and mitochondria.

Fate Mapping Reveals Heterogeneity in Cochlear Macrophages in Steady-State and After Acoustic Trauma

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Category: Inner Ear: Damage and Protection

Background: The mammalian cochlea contains resident macrophages. Sterile injury to the cochlea is associated with activation of resident macrophages and recruitment of predominantly monocytes from circulation, which differentiate into macrophages (monocyte-derived macrophages). The precise roles of resident and recruited macrophages in hearing loss and cochlear pathology are unclear. We have reported that macrophages promote the survival of spiral ganglion neurons (SGN) via fractalkine (CX3CL1-CX3CR1) signaling after cochlear injury (Kaur et al., 2015, 2018, 2019). However, it remains elusive whether CX3CR1-expressing resident and recruited macrophages are functionally distinct and differentially promote SGN survival. To define the mediators of macrophage-induced neuroprotection and to harness the protective capacity of these cells clinically, it is necessary to delineate the specific cell type (i.e., CX3CR1-expressing resident or recruited macrophages) that promote SGN survival after injury. We used a robust fate mapping technique wherein CX3CR1-expressing resident and recruited macrophages are endogenously labeled with different fluorescent reporters to define heterogeneity in cochlear macrophages in terms of origin, spatial and temporal distribution, morphology, fate, and function after acoustic trauma.

Methods: Tamoxifen inducible CX3CR1YFP−CreER/YFP−CreER mouse line was crossed with Rosa-lsl-tdTomato (R26RFP) reporter mouse line. The progeny CX3CR1YFP−CreER/wt:R26RFP were injected with tamoxifen and euthanized at various time points post injection to determine Cre recombination efficiency in CX3CR1 lineage and their turnover rate in blood and cochlea. A cohort of tamoxifen-injected CX3CR1YFP−CreER/wt:R26RFP mice were allowed to recover for 60 days ("wash out" period) followed by noise exposure to distinguish and define heterogeneity in CX3CR1-expressing resident and recruited macrophages in the injured cochlea. Tissue was analyzed by flow cytometry, fluorescent immunohistochemistry, and confocal imaging.

Results: By 60 days post tamoxifen administration, CX3CR1-expressing cochlear resident macrophages (98 +/- 1.7% recombination efficiency) and blood circulating CX3CR1 lineage (2.5 +/- 1.1% recombination efficiency) displayed distinct YFP+ RFP+ and YFP+ RFP− phenotype, respectively. Examination of recombined cochlear resident macrophages over a period of one year indicate that their turnover rate is considerably slower than circulating monocytes/macrophages (1-3 days) and is like the long-lived brain microglia. Acoustic trauma in "washed out" mice show that spiral ganglion and spiral lamina contains both resident macrophages (YFP+ RFP+) and recruited monocyte-derived macrophages (YFP+ RFP−), compared to sham exposed mice that only possessed resident macrophages. Such increase in macrophage numbers is partly due to infiltration of circulating monocyte-derived macrophages whose numbers increases at 1-day and peak at 1 week after trauma and partly due to in situ proliferation and migration of resident macrophages. Macrophage morphometric analysis indicate that morphology is not a good indicator to distinguish CX3CR1-expressing resident and recruited macrophages in the noise-injured cochlea.

Conclusions: These data establish the use of genetic fate mapping to definitively distinguish CX3CR1-expressing resident macrophages from recruited monocyte-derived macrophages in both naive and injured cochlea.

In Vivo Gene Editing Using CRISPR/Cas9 System Partially Improves Hearing Loss in a Dominant-Negative KCNQ4 Mouse Model

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Category: Inner Ear: Damage and Protection

Background: Hearing loss is a common sensory disorder that affects 466 million people or approximately 6.1% of the world population (World Health Organization, 2018; https://www.who.int/pbd/deafness/estimates). Among the many cases of hearing loss, DFNA2 non-syndromic hearing loss is characterized by symmetric, predominantly high-frequency sensorineural hearing loss (SNHL) that is progressive across all frequencies, which is related to a pathogenic variant in the Potassium voltage-gated channel subfamily q member 4 (Kcnq4). Kcnq4 expressing in outer hair cells (OHCs) plays an important role in potassium recycling in the inner ear. However, mutation of KCNQ4 exerts a strong-dominant negative effect to the channel, causing degeneration of OHCs, leading to adult-onset hearing loss. Among several mutations in Kcnq4, p.W276S is located in the pore region and

directly affects potassium ion selectivity. Despite its high prevalence and wide therapeutic window, targeted therapy is currently not available for AHL.

Recently, many research groups have been conducting research to improve hearing through gene therapy using the CRISPR/Cas9 system to improve hearing in the hearing loss mouse model.

Here, we generated a mouse model harboring Kcnq4W276S/+ and investigated the hearing correction effect through inner ear gene therapy using CRISPR/Cas9 system in Kcnq4W276S/+ mouse.

Methods: sgRNAs were generated to target c.830G> C, a missense mutation region in exon5 of the Kcnq4 gene. Two anc80L65 vectors were packaged with N-term of SpCas9, sgRNA sequence and C-term of SpCas9, respectively. Post-auricular incision was performed in mice between postnatal day one (P1) and P3 for inner ear injection through round window membrane. At 7 weeks, auditory brainstem response (ABR) measurements were performed to determine whether hearing was improved. In vivo Indel rate was measured through deep sequencing method. Immunohistochemistry was performed to compare the number of live hair cells in the injected and non-injected sides. We developed a new technique using live-cell imaging, Tl+ flux assay, to measure the membrane potential of the OHCs ex vivo.

Results: In the 7weeks ABR results, the hearing of the AAV-injected ear was improved by 20dB on average compared to the non-injected ear, and the whole cochlea of the injected ear with improved hearing showed 0.6% indel's. Also, through live cell imaging, it was confirmed that the OHCs membrane potential of the AAV-injected ear was more hyperpolarized, suggesting KCNQ4 activity was improved, but no difference was confirmed in cochlear histology.

Conclusions: In vivo gene editing with Anc80L65-CRISPR/Cas9 in Kcnq4W276S/+ mouse was successfully performed. However, low correction rate should be more improved and the underlying mechanism needs to be further elucidated.

Oncomodulin-Deficiency Increases Sensitivity to Inflammation

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Category: Inner Ear: Damage and Protection

Background: Approximately 15% of American adults between the ages of 20 and 69 have high frequency hearing loss due to exposure to loud sounds, and 50% of Americans over 75 years old are affected by presbycusis (https://www.nidcd.nih.gov/health/statistics). Although substantial progress has been made in determining the genetic and cellular functions disrupted by acquired hearing loss, comparatively little is known about its underlying causes. Chronic noise leads to excesses in cytosolic calcium levels in outer hair cells (OHCs). Since calcium regulation acts antagonistically with inflammatory pathways, we focused on the role of oncomodulin (OCM), a mobile calcium-binding protein preferentially expressed in OHCs, in regulating inflammatory mechanisms that lead to hearing loss. Chronic noise and inflammation can directly activate toll-like receptor 4 (TLR4) pathways to stimulate transcription of pro-inflammatory and pro-oxidant signals. The activation of TLR4 signaling is central to inflammation arising from acoustic insult as well as infection. We propose that OCM plays a role in suppressing common pro-inflammatory pathways upregulated by cochlear injury. We hypothesize that aging and noise act through inflammatory pathways to induce outer hair cell (OHC) dysfunction and loss.

Methods: WT and KO mice were pre-treated with TAK-242 or vehicle 24 hours prior to lipopolysaccharide (LPS). LPS was administer at 5mg/kg 2 days in a row. Hearing thresholds were collected before pre-treatment and 48 hours after LPS. Cochlea were collected at 48 hours to determine cell stress, neuronal and OHC numbers, and inflammatory response.

Results: We found that Ocm KO mice were more sensitive to LPS-induced inflammation, had elevated DPOAE thresholds, and greater morphological damage than similarly treated WT mice. There was no increase in DPOAE thresholds under any condition (LPS only, TAK-242 only, etc.,) in WT mice. Our LPS only injections increased DPOAE thresholds 30-40 dB in KO mice but showed no effect on DPOAE thresholds in WT mice. The KO mice also showed decreased co-expression of afferent synaptic proteins (CtBP2 and GluR2) and increased activation of stress markers to a greater extent compared with WT mice and controls. TAK-242 is a specific inhibitor of TLR4 receptors and did not interfere with hearing. Importantly, TAK-242 pretreatment plus LPS showed only a mild increase in DPOAE thresholds. The average DPOAE threshold shifts across all frequencies for TAK-242 pre-treatment plus LPS was +15 dB, compared to +35 dB for LPS alone. These results suggest calcium signaling and TLR4 are major factors in LPS-induced hearing loss. They also show that TAK-242 may correct dysregulated

calcium signaling by blocking TLR4 response to cochlear insult. At least qualitatively, KO mice treated with TAK-242 did not show any morphological damage.

Conclusions: Using a mouse model with defective calcium regulation within cochlear OHCs, we show that inhibition of TLR4 signaling reduces hair cell loss and synaptopathy.

Protocol Optimization for Aminoglycoside-Induced Ototoxicity in the C57BL/6 Cdh23 Ahl+ Mouse Line and the Identification of Natural Compounds as Otoprotectants

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Category: Inner Ear: Damage and Protection
Background: Aminoglycosides (AGs) are broad-spectrum antibiotics widely used to treat serious Gram-negative bacterial infections such as septicemia and meningitis. Although AGs are extremely effective, they carry the risk of adverse side effects including hearing and balance problems (ototoxicity) and kidney damage (nephrotoxicity). To date, no drugs have been approved by the Food and Drug Administration for protection against AG-induced hearing loss (AHL) which emphasizes the immediate medical need for the search of therapies.
Initial screening of a small library of natural and synthetic compounds in zebrafish identified piperlongumine a putative otoprotectant against AHL. Piperlongumine is a natural alkaloid isolated from the long pepper (Piper Longum L.) known to have many beneficial activities. Moreover, piperlongumine can suppress tumor growth and metastasis in bladder, breast, and lung tissues without affecting normal tissues. Piperlongumine can also act as a medicinal adjuvant improving the efficacy of antibiotics in in vitro assays
Methods: 7-8 weeks C57BL/6 animals carrying the corrected Cdh23 gene (C57Bl6 Cdh23 Ahl+, Jackson's Lab: 018399) were used. Animals were given kanamycin (50mg/mL, Sigma-Aldrich, K0254) at 500, 600, or 700 (mg/kg b.w.) s.q. for 14 days twice a day with a gap of at least 5 hours between the two daily doses. Piperlongumine (Cayman Chemicals, 11006) was dissolved in corn oil (5mg/mL) and injected IP once a day for 17 days at 10, 20. or 40 (mg/kg b.w.) Auditory brainstem responses and distortion product otoacoustic emissions were performed pre- and post-treatment. After the last hearing test, the organ of Corti was isolated, immunostained for Myosin VIIa, and hair cells quantified. HPLC-MS/MS was performed in whole cochlea homogenates for piperlongumine's detection.
Results: Administration of piperlongumine alone during the 17 days did not show any signs of general toxicity, monitored by body weight and animal behavior. In the case of kanamycin alone, animal general toxicity was relatively low with no death or body weight loss. For 700mg/kg b.w., of kanamycin, post-hearing tests showed these animals have a severe hearing deficit at frequencies 16kHz and above, with very few hair cells left at those frequencies. Conversely, animals receiving 500mg/kg b.w. did not show any significant hearing deficits compared to vehicle-injected animals, while animals receiving 600mg/kg b.w.. showed moderate hearing deficits at frequencies 22.6kHz and above. Distribution analysis of piperlongumine in the cochlea demonstrated that piperlongumine can cross the blood labyrinth barrier after 2 hours of IP administration. More importantly, animals co-treated with piperlongumine and kanamycin showed protection at frequencies 22.5kHz and above (immunohistochemistry assessment is underway).
Conclusions: We found that a protocol of 600mg/kg b.w. for 14 days twice a day is optimal for the induction of AG-ototoxicity in the C57Bl6 Cdh23 Ahl/+ mouse line. Piperlongumine showed no general toxicity and was protective against AHL at 40mg/kg b.w.

Knockout of KSR1 Gene and BRAF Inhibitor Dabrafenib Protect Against Noise Induced Hearing Loss

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Category: Inner Ear: Damage and Protection
Background: Hearing loss is a major unmet medical need in our society, affecting approximately 500 million individuals worldwide. One of the most common causes of adult-onset hearing loss is noise induced hearing loss (NIHL). Currently, there are no Food and Drug Administration (FDA)-approved drugs to protect from NIHL. Oral delivery of the FDA-approved BRAF kinase inhibitor, dabrafenib, was previously shown to reduce noise induced hair cell death in adult mice as well as noise induced phosphorylation of extracellular signal–regulated kinase (ERK) in cochlear cells.

The Kinase Suppressor of Ras 1 (KSR1) scaffold protein assembles the RAF/MEK functional pair in the mitogen-activated protein kinase (MAPK)/ERK signal transduction pathway, which is important in cell proliferation and cell survival.

Methods: KSR1 wild type (WT) and knockout (KO) C57BL/6 mice aged 6-8 weeks of age were used in our experiments. Auditory brainstem response (ABR) of the mice was measured 7 days prior to noise insult as a baseline hearing test. Adult mice were exposed to 100 dB sound pressure level (SPL) at 8-16 kHz for 2 hours. Dabrafenib was given orally twice daily to adult WT and KO mice at 12 mg/kg for 3 days, and a post-ABR was recorded 14 days after the last dabrafenib dosage. Mice were then euthanized and cochleae immediately isolated. Age- and genotype-matched sham exposed mice served as experimental controls to examine the expression of synaptic ribbon scaffolding protein Ctbp-2 and phosphorylated extracellular signal-related kinase (pERK) in the normal cochlea. Cochleae were processed for fixation, decalcification, and sectioning. The immunolabeled sections were imaged on a fluorescent laser scanning confocal microscope (Zeiss LSM 700) and captured images were processed on Zen Blue (Zeiss) software. Ctbp-2 and pERK fluorescent intensities were compared between sham- or noise-exposed WT and KO mice after noise trauma.

Results: KO sham control KSR1 mice showed significant resistance to noise-induced hearing loss compared to WT mice (20-30 dB at 8, 16, and 32 kHz). Dabrafenib significantly protected against noise-induced hearing loss in WT KSR1 C57BL/6 mouse models (20-30 dB protection at 8,16 and 32 kHz frequencies) compared to the WT sham controls. KO mice treated with dabrafenib showed a similar level of protection (20-30 dB at 8, 16, and 32 kHz frequencies) compared to the WT sham controls. Synaptic ribbon Ctbp-2 and pERK staining of the cochleae of the different mouse experimental groups is currently quantified.

Conclusions: These combined results confirm dabrafenib's mechanism of protection through inhibition of the MAPK pathway and the importance of the MAPK pathway in the mechanism of NIHL.

Effects of Intracochlear Injection via the Round Window on Auditory Brainstem Response and Histopathology in the Non-Human Primate

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Category: Inner Ear: Drug Delivery

Background: Viral vectors show promise in the treatment of disorders in the otic and vestibular systems. To evaluate transduction efficacy and safety of potential drugs, animal models are used with the goal of delivering vectors into the cochlea, close to the target hair cells. The cynomolgus monkey is a common animal model used for this purpose due to anatomical and physiological similarities with humans. A surgical approach has been used to gain access to the cochlea, via round window membrane (RWM). The goal was to determine the effect of the surgical procedure on the main endpoints (ABR and histopathology) used to evaluate safety.

Methods: Animals either underwent a surgical procedure (transmastoid approach to expose the middle ear and inject 10 – 30 uL phosphate buffered saline (PBS) via the RMW), a sham surgery (full surgery but no piercing of the RWM), or no surgical procedure. Auditory brainstem responses (ABR) at 1, 4, 8 and 16 kHz were measured prior to surgery and 6 weeks post. At necropsy, the ears were fixed and decalcified. The tissue was step-sectioned, processed to slide, and stained with H and E.

Results: ABR thresholds in untreated ears were within ±10 dB of the pretreatment baseline, and for sham injections within ±15 dB. In ears given PBS, thresholds were generally higher than baseline and showed a higher variability, particularly at 8 kHz and 16 kHz, ranging from + 5 to +40 dB. In PBS-injected inner ears, minimal to mild inflammation of the RWM was accompanied by focal fibrosis, small bone debris and/or phagocytized foreign material. In PBS and sham ears, findings related to the surgical procedures were present, consisting of inflammation, fibrosis and new bone formation. No abnormalities in untreated ears were present.

Conclusions: In conclusion, effects of the surgical procedure remained present after 6 weeks, with additional minimal to mild inflammation in ears where PBS was administered via the RWM. ABR thresholds were slightly increased 6 weeks after sham surgery, but were much more variable with potential for moderate threshold elevation after PBS injection, mostly at the higher frequencies evaluated. This may be due to the hair cells responsible for higher frequency response being closer to the site of injection.

Use of the Herpes Latency Promoter to Control Neurotrophin Delivery to the Inner Ear

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Category: Inner Ear: Drug Delivery

Background: Numerous studies have demonstrated that supplementation of the neurotrophic factors brain BDNF and NT-3/Ntf3 can support spiral ganglion survival using delivery of supraphysiologic levels of growth factor from a pump or using a variety of gene therapy strategies. Survival and integrity of the spiral ganglion are vital for hearing in background noise and for optimal functioning of cochlear implants. The actual physiological levels of growth factors within the inner ear have been difficult to determine but are likely present at extremely low levels within the normal inner ear

Methods: We evaluated the use of the herpes latency promoter, a weak, long expressing promoter system, to deliver BDNF or NT 3 Using an adenovirus serotype 28 derived adenovector delivery system and compared to vectors using a hcmv promoter. Adult macular organ explants were treated with neomycin followed by neurotrophin expressing vectors. Neurotrophin concentrations were assayed in conditioned medium. Adult mice were then treated with neomycin followed by vector treatment. Controls consisted of non-ototoxin-treated mice exposed to vectors.

Results: Using an adenovirus serotype 28 derived adenovector delivery system, neomycin treated mice showed survival of spiral ganglion cells after delivery of either growth factor. Expression of BDNF and NT-3 could be demonstrated in the damaged organ after gene delivery for prolonged periods. Treatment of normal-hearing mice with vectors expressing high levels of growth factors resulted in hearing loss.

Conclusions: Delivery of neurotrophins using gene therapy may support spiral ganglion survival. Low strength promoter systems should be investigated to provide more physiologic levels of growth factor support

Non-Invasive Targeted Temperature Management of the Inner Ear in a Human Cadaver Model

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Category: Inner Ear: Drug Delivery

Background: Previous studies have demonstrated that acute application of mild hypothermia (a 4-6 °C decrease) has an otoprotective effect following cochlear trauma such as implantation, noise overexposure, and cisplatin ototoxicity. Mild hypothermia is known to regulate pro-inflammatory pathways, accumulation of reactive oxygen species, and apoptosis. A systemic cooling approach utilized thus far is not clinically feasible for application to the inner ear. The purpose of this study was to determine the feasibility of inducing mild hypothermia in the cochlea through a localized and noninvasive approach while avoiding damage to the surrounding structures.

Methods: Temperature recordings were gathered on four cadaver heads (two females, two males, cut mid-sagitally, twelve repetitions). A metal bead bath was used to pre-warm and maintain specimens at a temperature range that simulated average human conditions. A custom-designed cooling device was placed over the skin surface at the temporal bone area for either 30 or 60 minutes. Thermistors were placed at the middle ear, scalp, ear canal, skull, bead bath, and cooling pack. Computational Fluid Dynamics was used to analyze the heat transfer to the cochlea using anatomically accurate models of the same samples. Multiple transient analyses were performed to determine the time and extent of temperature drops

Results: The present study showed that the external cooling device successfully reduced the temperature at various cadaver locations. Thermistors placed near the inner ear displayed a mean temperature drop of 4.2°C within a 60-minute cooling period and 2.5°C within a 30 minute cooling period. The temperature at the inner ear structures continued to reduce for an average of 15 minutes once the device was removed. Female cadaver heads exhibited a higher temperature drop when compared to male heads at both 60 minute and 30-minute trials. Control thermistors placed at the scalp and away from the cooling device showed a mean drop of 2.5°C and 1.5°C for the 60- and 30-minute rounds respectively. A mesh-independent study confirmed the numerical model was stable and provided consistent results that matched the experimental observations.

Conclusions: Experimental observations in this study suggest that hypothermia can be efficaciously obtained through an external localized cooling system. Experimental observations in the human temporal bones combined with the numerical simulations informed device designs for the eventual human applications.

Effects of Several Corticosteroids on Spiral Ganglion Neuron Survival and Neurite Length

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Category: Inner Ear: Drug Delivery

Background: Corticosteroids were divided into glucocorticoids and mineralocorticoids and were already commonly used as treatment for Menière's Disease or sudden deafness in clinics as well as during cochlear implantation. The main beneficial effect of corticosteroids are anti-inflammatory and immunomodulatory effects. On the one hand, corticosteroids should protect cochlear neurons and, on the other hand, inflammation should be attenuated at the implantation side. In this study, we used four different clinically used corticosteroids (fludrocortisone acetate, TriamHEXAL® [triamcinolone acetonide], Fortecortin®Inject [dexamethasone 21-phosphat disodium salt] and Prednisolut® [prednisolone succinate]) and investigated their effect with respect to the clinically used concentrations on the survival rate and neurite length of spiral ganglion neurons (SGN) in vitro.

Methods: The SGN were isolated from neonatal (P3-5) Sprague Dawley rats and were enzymatically and mechanically dissociated. For all experiments, the SGN were pre-cultivated with 10% fetal calf serum (FCS) for 24 hours. After additional 48h (total 72h) of cultivation, the SGNs were fixed, stained and the neuronal survival rate and neurite length were determined. In the first experiments, after pre-cultivation the SGN were cultured in the presence of different concentrations of the selected corticosteroids to determine their respective dose-response curves. In the second experimental setting, we used two inhibitors (mifepriston as a glucocorticoid inhibitor and spironolactone as a mineralocorticoid inhibitor) to reverse the inhibitory effects of the corticosteroid treatment.

Results: We identified for each of the used corticosteroids a concentration for normal or high survival of SGN (related to the positive control: treatment with 10% FCS) and a concentration for less survival of SGN (related to the negative control). For fludrocortisone we identified 0.4 mg as concentration for normal or high survival of SGN and 4.0 mg as concentration for less SGN survival. For TriamHEXAL® 1.0 mg and 4.0 mg, for Fortecortin®Inject 0.005 µg and 0.010 µg and for Prednisolut® 0.25 mg and 1.5 mg, were identified respectively. Especially for increasing concentrations of Prednisolut®, the neurite length was subsequently reduced in a dose-dependent manner. The two inhibitors have not been able to reverse the reduced SGN survival after a treatment with high concentrations of the corticosteroids.

Conclusions: The determined concentrations of the in vitro tested corticosteroids showed that – depending on the substance – there is only a small therapeutic window for a harmless treatment. Thus, it should be careful decided which patients receive a corticosteroid treatment during cochlear implantation especially with respect to the protection of functional residual hearing.

Optical Coherence Tomography Imaging of Gold Nanoparticles After Posterior Semicircular Canal Injection in Mice

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Category: Inner Ear: Drug Delivery

Background: Delivering therapeutics into the cochlea while preserving hearing has been a challenge. Intratympanic injection requires the solution and therapeutic to cross the round window membrane to reach the perilymphatic space, resulting in variable concentrations reaching the perilymph often 100-1000x lower than the concentration injected into the middle ear space. Direct injection of therapeutic through the round window membrane can result in hearing loss. A hearing preservation technique to inject solutions into the posterior semicircular canal is commonly used for viral-mediated gene delivery in mice. We sought to assess the reliability of this technique to deliver material to the cochlear apex.

Methods: Gold nanoparticles were made using a 50nm spherical gold core and functionalized using AlexaFluor488-polyethylene glycol-OPSS and 5kDa methoxy-polyethylene glycol-SH. Artificial perilymph containing 140mM NaCl, 2mM KCl, 2mM MgCl2, 2mM CaCl2, and 20mM HEPES with pH 7.4 and 304-307mOsm/kg was used as a carrier for the gold nanoparticles. Wild type CBA/CaJ mice were anesthetized using a mixture of ketamine and xylazine, and surgery was performed to expose the cochlea and the posterior semicircular canal. Optical coherence tomography images of the cochlea and organ of Corti vibrometry measurements were

recorded to assess cochlear physiology at baseline, before and after a canalostomy, and during and after injection. All animal experiments were approved by the USC Institutional Animal Care and Use Committee.

Results: Gold nanoparticles with a size of 83.1 +/- 2.0 nm and zeta potential -12.46 +/- 1.27 mV that do not aggregate in physiologic salt conditions were synthesized. Gold nanoparticles in artificial perilymph were injected into the posterior semicircular canal. We found that 1 uL injected at 0.5 ul/min allowed the gold nanoparticles to reach the apical turn of the cochlea, without causing distortion of Reissner's membrane. Vibrometry measurements revealed mild changes in cochlear physiology, which are being characterized.

Conclusions: Posterior semicircular canal perfusion is a reliable and relatively safe way of delivering drugs throughout the cochlea.

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Coating of Cochlear Implant Electrode Arrays to Release Anti-inflammatory Substances
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Category: Inner Ear: Drug Delivery
Background: Cochlear implants (CI) are the most effective treatment option for hearing loss. Opening of the cochlea and insertion of the electrode promotes formation of fibrous tissue around the electrode array. Among current strategies to reduce this tissue formation are deposition of steroids in the cochlea during cochlea implantation and slow release of dexamethasone from the silicone body of the electrode array. The aim of the current study is to combine this slow release with a faster release of anti-inflammatory substances from a polymeric surface coating. To achieve this, otherwise approved drugs such as diclofenac were tested in vitro for application to cells from the inner ear, release characteristics and the influence of the coating on electrode contact impedances.

Methods: Diclofenac, dexamethasone (DMS) and enalapril were tested on freshly isolated SGN, cultured for 48 hours. Cells were stained immunocytochemically before evaluation of cell survival and neurite length. For drug release measurements, PLLA coated samples ($\varnothing$ = 6 mm) were placed in 1 ml fresh artificial perilymph and released drugs were quantified by HPLC. Impedance measurements of flat rectangular silicone samples coated with 10 µm PLLA and loaded with 10 % or 20 % diclofenac, were measured for 24 hours in 0.9 % NaCl solution without and for another 24 hours with pulsatile electrical stimulation.

Results: At concentrations of $2*10 4$ mol/l, surviving SGN were barely found with all three substances. Survival increased to about 100 % at a concentration of $8*10 6$ mol/l for DMS and diclofenac and remained stable for lower substance concentrations. Using enalapril, the highest survival of SGN with about 76.8 % was achieved at a concentration of $8*10 6$ mol/l. In contrast, neurite length was not affected for all substances. PLLA coating reduced the release of DMS from the silicone carrier whereas incorporation of diclofenac enhanced the DMS release slightly again. For the release of diclofenac, a significantly higher burst release was detected for samples containing 20 % diclofenac. Incorporation of 10 % diclofenac into a PLLA coating results in initial impedances of >10 MΩ whereas with 20 % diclofenac initial impedances were between 1 MΩ and 10 MΩ. For both concentrations, impedances could drop under electrical stimulation to below 10 kΩ or remain at >1 MΩ.

Conclusions: Diclofenac might be suitable for application in the cochlea but strategies to avoid coating of the contacts have to be developed.

Translational Development of ACOU085 for the Prevention of Cisplatin-Induced Hearing Loss (CIHL)
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Category: Inner Ear: Drug Delivery
Background: Ototoxicity from chemotherapeutic cancer treatment with cisplatin remains a significant, debilitating side-effect for survivors, with a direct impact on hearing, learning and social interactions, as well as long-term impact on cognitive abilities. Currently, no proven and safe treatment without risk of impacting event free survival exists to prevent cisplatin induced hearing loss (CIHL).

ACOU085 is a small molecule drug candidate demonstrated to significantly reduce hearing loss and cochlear hair cell death when administered locally in a guinea pig model of CIHL (Löwenheim, 2021). Here we present data supporting the translational development of ACOU085 for clinical testing, using a proprietary, sustained-release formulation for transtympanic administration.

Methods: A sustained-release formulation of 6% w/v ACOU085 was administered transtympanically to groups of SAMP8 mice (10 µL) and guinea pigs (100 µL) (n=3-7 per bioanalytical time-point). Drug loads relative to total inner ear volume (respectively 240 µg/µL and 245 µg/µL for mice and guinea pigs) were comparable between species. At fixed time-points, animals were euthanized by anaesthesia overdose, and samples of inner ear tissue prepared for bioanalysis using HPLC-MS/MS.

Results: In both species, transtympanic administration of the 6% w/v ACOU085 sustained-release formulation resulted in comparable mean peak inner ear tissue exposures of 215±165 µM (mice) and 189±89 µM (guinea pigs) at 6h post-administration and continued to rise to 341±58 µM in guinea pigs at 48h post-administration (time-point not tested in mice). Inner ear tissue exposures in mice fell below the bioanalytical lower limit of quantification (LLOQ) corrected for sample dilution for timepoints beyond 7 days, whereas significant inner ear tissue exposures in guinea pig samples were still detectable at 21 days post-administration (1154±1273 nM).

Conclusions: These data support the ability of the proprietary, sustained release formulation of ACOU085 to achieve significant, high micromolar levels of inner ear exposure in both mice and guinea pigs. As the drug load relative to inner ear volume was practically identical between the two species, along with the ratio of administration volume to inner ear volume (~4), the data suggests that differences in ACOUS085 exposure duration (and potentially also peak exposure) could be primarily driven by the absolute volume of formulation administered (100 µL vs 10 µL).

Efficacy studies in a guinea pig CIHL model suggest that clinical drug loads of ~35 µg/µL ACOU085 relative to inner ear volume delivered over the course of at least one week should provide a reasonable translational margin for achieving significant and clinically relevant hearing preservation. With the developed proprietary, sustained-release formulation, this is feasible for 2-3% w/v formulations administered at well-established volumes of 200-300 µL. Despite this resulting in a slightly lower ratio of administration volume to inner ear volume in humans (~1.2-1.6), the larger absolute volume is expected to drive sufficient peak and sustained exposures.

Energetic Depletion and IsK Mutations Destabilize Potassium Transport Across Marginal Cells and Vestibular Dark Cells

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Category: Inner Ear: Membranes and Fluids

Background: Our sense of hearing and balance relies on mechanotransduction by auditory and vestibular hair cells that drain potassium from the endolymphatic fluid in the inner ear. This potassium must be resupplied to the endolymph, a process that requires significant expenditure of energy by marginal cells in the cochlea and vestibular dark cells in the vestibule.

Methods: We constructed a biophysical model of ion transport across these epithelial layers by implementing mathematical expressions for known ion transporters expressed in the marginal/dark cells, including the Na/K-ATPase, NKCC and chloride channels at the basolateral face and an IsK conductance composed of KCNQ/KCNE1 subunits on the apical face. The transepithelial potassium current (IKte) can then be studied as a function of desired parameters of the system. Two parameters of interest are the potassium concentration in the apical fluid (endolymph, normally 150 mM) and basal fluid (normally ~ 4mM). The dependency of IKte on external potassium concentrations can be considered a phase diagram illustrating the stable states of the system and can be studied to gain insight into how potassium resupply to the endolymph is altered in conditions that alter potassium homeostasis.

Results: IKte was found to increase monotonically for basolateral potassium concentrations up to ~ 7 mM, and then approach saturation.   The system exhibits stable behavior below values of endolymph potassium of ~160 mM for basal potassium concentrations up to 15 mM. However, above 160 mM, IKte was found to reverse and multiple stable states are present based on numerical solutions of the differential equations at steady state. Energetic depletion was modelled by decreasing the ATP available for the Na/K pump. ATP levels below 1 mM caused marked shifts in the phase diagram, lowering the steady state IKte contours and causing reversal at normal values of endolymphatic potassium. Increasing the conductance of Isk also altered the phase states of the system.

In particular, when Isk exceeded ~15x its normal value, regions of hypersensitivity became apparent in the phase diagram at elevated levels of basolateral potassium at normal levels of ATP.

Conclusions: The model predicts that the combination of reduced ATP and increased IsK can have dramatic effects on the system even at normal basolateral levels of potassium. Increased IsK conductance is implicated in susceptibility to noise-induced hearing loss, and the model suggests that energetic depletion may further destabilize inner ear potassium transport. The results indicate the power of computational modeling in gaining understanding of instabilities in potassium recycling that lead to hearing loss and vestibular dysfunction.

Using Microprisms, the 2P Calcium Imaging Revealed a Functional Distinction Between the Dorsal and Lateral Cortices of the Mouse Inferior Colliculus.

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Category: Midbrain: Structure and Function
Background: The inferior colliculus (IC) is a critical midbrain structure for the processing of auditory stimuli. The IC is a hub that permits widespread convergence of both bottom-up and top-down projections involving auditory, somatosensory, visual, motor and arousal-related brain regions. Dorsal (DC) and lateral (LC) cortices form the non- lemniscal division of the IC and receive most of the descending projections directed to the IC. LC only contains periodic modules of GABAergic cells and terminals which stain for a range of metabolic markers. Interestingly, the auditory inputs from the auditory cortex or central nucleus of the IC strongly avoid these inhibitory modules and instead form dense projections to the matrix areas that surround the modules. This anatomic distinction between DC and LC could implicate a functional distinction between the two subdivisions.
Methods: Two-photon imaging was used here to characterize the functional activity of the GABAergic and non-GABAergic cells in the LC as well as in the DC.
Results: Consistent with a previous report (Wong and Borst 2019), imaging the surface of the IC showed that both GABAergic and non-GABAergic cells of the DC showed mirror-image tonotopic mapping of high to low and then from low to high frequency along the medial to lateral axis. In contrast, side view imaging of the LC using 450 microprisms showed that both GABAergic and non-GABAergic cells were less responsive to the pure tone sound, were not tonotopically organized, and were more responsive to amplitude-modulated broadband noise.
Conclusions: This finding implicates a functional distinction between DC and LC, which functions more to process complex sound.

Neural Population Activity in the Shell Inferior Colliculus Predicts Behavioral Outcomes

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Category: Midbrain: Structure and Function
Background: Active listening requires not only correctly identifying primary sound features, but also predicting their behavioral relevance. Behaviorally relevant representations are well documented in the auditory cortex, but whether similar activity arises earlier in the central hierarchy is hotly debated. The dorsal and external nuclei of the inferior colliculus (shell IC) are important midbrain circuits that receive a variety of acoustic, multi-sensory and neuromodulatory signals (Gruters and Groh 2012). We tested the hypothesis that behavior and/or outcome signals are present in the shell IC during an auditory task using Ca2+-imaging, machine learning, and a reward-based discrimination task in mice.
Methods: We expressed the Ca2+-indicator GCaMP6f in shell IC neurons of 4 CBA/C57 Bl-6J mice. Animals were subsequently trained to discriminate the presence or absence of amplitude modulation in a bandpass noise stimulus using a GO/NOGO paradigm. Following training, we used 2-photon microscopy to record neural activity from the same neurons across 7 consecutive sessions as mice performed the task; modulation depth was varied to obtain psychometric functions. We analyzed the population activity at various epochs during the trial, and used a support vector machine (SVM) classifier to predict the trial outcome (mice's behavioral responses) from neural population activity.
Results: Mice's modulation detection thresholds did not significantly change over the course of 7 days, indicating stable performance in our conditions. Likewise, the average neural trajectories and principal components did not change strongly over time, suggesting that population-level representations of task-related variables are largely stable in the shell IC. We used the fluorescence data from multiple shell IC neurons as training data for an SVM

classifier to predict the outcome of each trial (hit, miss, false alarm, correct rejection). As expected, classification accuracy was highest after sound offset (~ 85%, chance: 40%). However, significant classification was achieved even if activity was integrated over the first (60%) or second (70%) half of the sound stimulus only. Remarkably, a similar accuracy was achieved when the SVM was exclusively trained on neural activity occurring prior to mice's behavioral response (70%). We can further conclude from the confusion matrices that this result is unlikely to simply reflect motor- or motor preparatory activity, because hits and false alarms can be reliably distinguished. **Conclusions:** Collectively, our data argue that neural population activity in the auditory midbrain reflects a mixed selectivity of predictive- and feedback information about behavioral outcome in response to behaviorally relevant sound features.

Neurotransmitter Phenotype and Axonal Projection Patterns of VIP-Expressing Neurons in the Inferior Colliculus

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Category: Midbrain: Structure and Function
Background: We previously identified a class of neurons in the inferior colliculus (IC) labeled in the vasoactive intestinal polypeptide (VIP)-IRES-Cre mouse line. VIP neurons are physiologically and morphologically homogeneous, exhibiting stellate morphology and sustained firing patterns. They receive direct inputs from the cochlear nucleus and contralateral IC, suggesting that they integrate ascending and commissural information. Almost no VIP neurons immunostain for markers of GABAergic cells, suggesting that the VIP population is glutamatergic. Here, we continue characterizing VIP neurons, with an emphasis on neurotransmitter phenotype and axonal projections.
Methods: We used fluorescent in situ hybridization (FISH, with RNAScope) to identify cells that express VIP, VGLUT2 (vesicular glutamate transporter 2, a marker of glutamatergic cells), and tdTomato in VIP-IRES-Cre x Ai14 (tdTomato-reporter) mice. Tissue was collected from mice of both sexes, aged P45 – P58.
We injected AAV1.CAG.FLEX.eGFP.WPRE.bGH into VIP-IRES-Cre x Ai14 mice of either sex aged P21 – P35 and harvested tissue three weeks later.
Results: Our data show that 93.2% of tdTomato+ neurons were VIP+ and 99.2% were VGLUT2+, confirming that the IC neurons labeled in VIP-IRES-Cre mice are glutamatergic and almost always express VIP. Surprisingly, VIP expression was also observed in a sizeable population of glutamatergic cells that did not express tdTomato. Thus, VIP-expressing neurons in the IC are glutamatergic, and, due to under-labeling in the mouse line, the population of VIP neurons in the IC may be 5x larger than previously predicted.
Ascending projections terminated in the medial geniculate nucleus (all subdivisions) and in the nucleus of the brachium of the IC. Within the midbrain, VIP axons terminated within the injected and contralateral IC (all subdivisions), in the intercollicular tegmentum, in the superior colliculus and in the periaqueductal gray. Descending projections targeted numerous auditory nuclei, including the dorsal and ventral nuclei of the lateral lemniscus and the ventral nucleus of the trapezoid body. Sparse projections were present in the cochlear nucleus (primarily the dorsal subdivision). In each of the target areas, the majority of axons were thin (<0.75 um diameter). Boutons also ranged in size, with the largest boutons limited to the thalamus and midbrain.
Conclusions: VIP neurons form a sizeable population of glutamatergic neurons in the IC. As a population, VIP neurons contribute to practically all projections from the IC, including projections to higher and lower auditory centers as well as several multisensory areas. In addition, VIP neurons project to other cells within the ipsilateral IC and in the contralateral IC, contributing to the extensive local processing that occurs within the IC. These widespread excitatory projections are in a position to affect virtually every function associated with IC pathways, including auditory perception, feedback to lower auditory centers and a variety of brainstem driven behaviors.

Determining the Role of the Auditory Midbrain in Perceptual Learning

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Category: Midbrain: Structure and Function

Background: Sensory stimuli that are alike in nature, such as tones with similar frequencies or slightly different shades of the same color, can be difficult to differentiate at first. However, training can lead to improvement in one's ability to discriminate between the stimuli, a process called perceptual learning. Auditory perceptual learning is important for language learning, and it can also improve the use of assisted listening devices (Fu and Galvin, 2007). Auditory cortex is an important cortical hub for sensory information that also receives functional inputs from frontal regions associated with higher-order processing. Previous research has shown that perceptual learning strengthens the top-down modulation of auditory cortex. However, it is unclear whether these learning-related changes first emerge elsewhere in the ascending auditory processing pathway and are inherited by the auditory cortex, or arise in the cortex de novo. The inferior colliculus (IC) has been shown to display spectrotemporal task-related plasticity (Ryan and Miller, 1977; Slee and David, 2015), making it an attractive candidate region for the target of top-down projections that modulate neural improvement in perceptual learning.

Methods: To explore this possibility, single-unit recordings were obtained from the IC of awake, freely-moving Mongolian gerbils during perceptual learning on an amplitude modulation detection task.

Results: Our results will determine whether the gerbil IC displays task-related plasticity, and whether learning-related plasticity occurs in the IC during perceptual training

Conclusions: These findings will contribute to a deeper understanding of the circuits behind perceptual learning, which can aid translational research in hearing loss and cochlear implant use.

Testing of an Implantable Umbo Microphone in Temporal Bone

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Category: Middle and External Ear

Background: Beyond concerns with cosmesis, hearing-aid devices such as cochlear implants with external components are vulnerable to damage, rely on magnetic fixation (which can cause skin issues with pressure or have issues with poor attachment), often cannot be used during sleep, underwater, or during intense sports or with helmets. External microphones suffer from wind noise and do not take advantage of the gain and filtering of the outer ear. An implantable microphone is a key component in the creation of fully implantable hearing aid devices, which would overcome these problems.

We designed a microphone prototype that mounts between the cochlear promontory and umbo. It resembles a drum with piezoelectric polyvinylidene fluoride (PVDF) membrane to sense umbo motion. Previously we reported promising data from bench testing that was verified with analytical and finite-element models. Here, we provide updates from human cadaveric temporal bone experiments.

Methods: With cadaveric temporal bone experiments, we determined both how umbo velocity changed due to mechanical loading of the microphone and how the transfer function relating umbo and microphone displacement related to the microphone output voltage. A speaker applied pressure into the sealed ear canal with a calibrated probe tube microphone 1-2 mm from the tympanic membrane detecting the pressure. Umbo velocity was measured using laser Doppler vibrometry. The implantable microphone was placed between the umbo and the bony surface of the cochlea (the promontory). With spacers, we varied microphone positioning offset to vary how much the PVDF pushed against the umbo.

Results: Umbo velocity was found to be attenuated 5-15 dB due to the presence of the microphone depending on positioning offset. Varying offset height resulted in frequency-dependent performance changes. If the height was too low, poor coupling between the microphone and umbo occured. Increasing height (pushing up against the umbo) resulted in increased fluctuations with frequency due to rocking of the microphone base on the uneven surface of the promontory. Smoothing the promontory surface and firmly gluing the microphone base resulted in a smoother frequency response. This result agreed with our analytical model from 200 Hz to 6 kHz, but deviated with a peak in magnitude with associated phase transition around 10kHz.

Conclusions: Initial temporal bone testing shows promising results for this implantable umbo microphone. Importance of stable placement was revealed through this testing and will be improved in future design and implantation methods.

Effects of Head Tilting on Laser Measurements of Middle Ear Diffusion

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Category: Middle and External Ear
Background: Otitis media with effusion (OME), characterized by the presence of fluid in the middle ear cavity, affects approximately five out of six children by the time they turn three years old. To detect fluid buildup in the middle ear, doctors commonly use a pneumatic otoscope. However, the pneumatic otoscope's accuracy relies heavily on the severity of the buildup and leads to a subjective diagnosis. In an effort to create a less subjective diagnosis procedure, we developed an algorithm to process the full-field tympanic membrane surface motion data acquired by a scanning laser Doppler vibrometer (SLDV). We postulated that the MEE position would change when the head is tilted 30° to the side and this change could be detected by SLDV with analysis using new algorithms. To test the hypothesis, we proposed taking measurements in two human temporal bones and analyzing the data with algorithms developed in our lab.
Methods: Otitis Media was replicated by injecting a small amount (0.1ml) fluid into the middle ear of two human cadaver ears. Next, we placed an ear on the tilting milling table to achieve the desired tilt of 30° to the side. The SLDV was then adjusted to the same angle with laser beams in order to view the entire tympanic membrane. Then the full-field surface motion of the tympanic membrane in response to 90 dB sound pressure across the frequency range 200~8000 Hz was recorded by the SLDV. We developed an algorithm to process the raw data, including noise reduction, feature calculation, and analysis of variance (ANOVA).
Results: This preliminary study tested the effects that tilting the head had on detection of otitis media in the middle ear cavity. The results indicated that the average sound displacement across the bottom half of the TM between the frequency bands .8 kHz to 6.5 kHz of the tilted head were significantly lower than that of the control/liquid filled ear in the normal position.
Conclusions: This preliminary study proves a practical application of quantitative assessment in diagnosing OME. The new tilting approach amalgamated with the new algorithm offers a less subjective and more reliable process to detect small MEE.

An Automatic 3D Reconstruction of Middle-Ear Ossicles From Cone Beam Ct Images Using Machine Learning

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Category: Middle and External Ear
Background: In these days, a personalized-medical treatment becomes important due to the 4th industrial revolution. If a three-dimensional-personalized finite element (FE) model of auditory periphery can be quickly and correctly constructed from the CT images of a patient, it can be helpful for medical doctors to diagnose and prescribe to a specific disease. As the first step for development of the personalized FE model, we pursue to reconstruct middle-ear ossicles from cone beam CT images.
Methods: From the cone beam CT images containing whole head structure, middle-ear ossicles were detected by YOLOv5x which has benefits in fast object detection. The resolution of the detected ossicles was too low to reconstruct a 3D FE model. Therefore, the super-resolution was performed by Deep Back Projection Network to obtain the higher resolution of the detected ossicle images. In addition, since the gap between sliced CT images was too wide to build the FE model, we developed the house code to deduce the sliced image in the mid of two CT images.
Results: Based on the CT images and deduced images, the 3D FE model of middle-ear ossicles could be constructed. Using a CT image set of middle-ear ossicles produced by a 3D printer, all the process were validated. The accuracy of the reconstructed model was over 90%.
Conclusions: An automatic process to construct a 3D FE model of middle-ear ossicles has been established based on patients' cone beam CT image. The personalized FE model constructed by the machine learning process can be helpful for medical doctors not only to diagnose a disease but also to predict the result of an operation or treatment.

Conductive Hearing Loss Estimated From Acoustic Measurements in Ears With Otitis Media With Effusion

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Category: Middle and External Ear

Background: Wideband acoustic immittance (WAI) measurements have demonstrated clinical utility in the differential diagnosis of otitis media with effusion (OME; Merchant et al., 2021). Specifically, WAI has been shown to reliably differentiate not only the presence of an effusion, but also its volume. This is important because effusion volume has been shown to drive the amount of conductive hearing loss (CHL) that results from a given episode of OME (Al-Salim et al., 2021). The goal of this work was to determine how well CHL could be estimated directly from measured WAI in ears with OME.

Methods: Sixty-three ears from a previously published study on OME (Merchant et al., 2021) were included. Ears with OME (49 ears from 26 participants) were grouped based on effusion volume observed during tympanostomy tube placement surgery (full effusion, partial effusion, and ears clear of effusion but with a recent diagnosis of OME) and were compared with age-matched healthy normal control ears (14 ears from 9 participants). Audiometric thresholds were obtained for a subset of 34 ears distributed across the four effusion-volume groups. For ears with OME, thresholds were obtained no more than 72 hours prior to tube placement. Initial estimates of CHL were based on either average absorbance or the component of the response thought to represent damping in the ossicular chain, as determined by utilizing a computational modeling approach. Better CHL predictions were obtained by taking advantage of features discovered in the data.

Results: An electrical-analog model of ear-canal acoustics and middle-ear mechanics was fit to each WAI absorbance measurement. The model produced good fits to the measured absorbance for all groups. Both of the two initial CHL estimates were well correlated (87%) with pure-tone average (PTA, average at 0.5, 1, 2, and 4 kHz). However, the CHL estimates based on damping revealed an unexpected feature. In roughly a third of the ears (11/34), these estimates were too large by a factor of two. Adjusted estimates that accounted for this observation improved the PTA correlation to 97% and achieved a prediction error of less than 3 dB (mean absolute difference).

Conclusions: Overall, these results suggest that WAI absorbance, combined with a computational model, can estimate behavioral audiometric thresholds within a clinically meaningful margin of error. This is a significant finding given the challenges associated with behavioral audiometric testing in pediatric populations where OME is most common. This work also suggests the possible existence of two types of ears: pressure detectors and power detectors.

Plasma Activated Medium Induce Ferroptosis like Cell Death in Human Schwannoma Cell Line

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Category: Other, Tumor, Skullbase, Basic Research

Background: Until now, the workhorse treatment of vestibular schwannoma (VS) is surgical removal, but there is risk of cranial nerve damage during trying to remove completely to prevent recurrence. Therefore, the development of adjuvant antitumor treatment after or during surgery can solve this problem.

Plasma is an ionized gas, and research to be used for anti-aging or anti-cancer, etc. has been conducted in recent decades. In particular, since plasma can be directly applied to the affected area for anticancer purposes, it is being sought as an adjuvant therapy during or after surgery. Previously, we reported the result of effective elimination of remnant tumor cells after tumor removal using application of in situ cold atmospheric plasma. However, as a limitation in this study, it has been suggested that it may be difficult to control the area of plasma applied, and that the generation of plasma may vary depending on the surrounding environment. Recently, in order to solve these shortcomings, plasma activated medium (PAM) has been developed. PAM is obtained by discharging plasma in a liquid, and has the advantage of being relatively more stable and easy to control compared to plasma in gaseous state. Here, we investigated the feasibility of VS treatment using PAM.

Methods: Experiments were conducted using the human vestibular schwannoma cell line (HEI193), and all experiments were performed 24 hours after 5000 cells were plated. Cell death was quantified using MTT assay, and the mechanism of triggering cell death was explored using various ROS scavengers and cell death inhibitors. The mechanism was verified by Western blotting and immunofluorescences. In addition, all experiments were independently repeated at least 3 times.

Results: PAM effectively killed HEI193 cells. PAM induced half of cell death in HEI-193 within 6 hours after PAM treatment. As a result of confirming by western blot, DNA damage and apoptosis markers were observed after 4 hours. Moreover, it was confirmed through ROS scavengers and cell death inhibitors (z-VAD-fmk, Necrostatin-1 and Ferrostatin-1) that the cell death pathway is ferroptosis induced by ROS-induced lipid peroxidation, which was recently discovered.

Conclusions: It was confirmed that PAM induces ferroptosis, not necroptosis, which was previously reported by us, and killed HEI193 cells very effectively, which was irreversible.

Variability in the Manual Segmentation of Temporal Bone Structures

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Category: Other, Surgical Simulation

Background: Surgical simulation of the temporal bone (TB) has relevancy for both pre-operative preparation and surgical training, allowing users a means for comprehending patient specific anatomy and refining surgical techniques. Computer-based surgical simulation requires "segmentation" of image data to be used in the simulation for identifying individual structures and landmarks. There are two methods of segmentation: manual (MS) and automated (AS). Manual segmentation is often used as the "gold standard", however, there can be significant variability in manual segmentation between observers. MS has been used in many studies to validate AS, however many of the validation studies were performed using only one or two reviewers. The inherent variability of MS for segmenting TB structures has not been truly characterized. In this study, we propose to evaluate intra- and inter-rater variability of MS in temporal bone structures.

Methods: Ten pre-operative temporal bone CBCTs scans obtained from adult patients who had undergone cochlear implant (CI) surgery at The Ohio State University were used for this study. The cochlea, mid-modiolar axis, round window, facial nerve, and chorda tympani of each CBCT scan were manually segmented by the six reviewers. The reviewers were trained in manual segmentation technique via a one-on-one training session and an accompanying instructional document. Reviewers were evaluated on their tracing technique with a practice scan to ensure that adequate training in MS technique had been given. Each reviewer segmented individual CBCT scans two times with a 1-month interval. Inter-rater variability was assessed by evaluation of Quantitative metrics, namely Dice Coefficient and average Hausdorff distance.

Results: Agreement between reviewers was highest for the cochlea followed by the facial nerve with median DICE coefficient (MDC) scores ranging between 0.863-0.908 and 0.725-0.832 and median average Hausdorff distance (MAHD) ranging between 0.119-0.223 and 0.411-1.571 over ten images, respectively. Inter-rater agreement was lowest among reviewers for the chorda tympani (MDC range: 0.384-0.689; MAHD range: 0.462-4.086) and round window (MDC range: 0.129-0.441; MAHD range: 0.704-3.595). One image set had low MDC scores and high MAHD values across multiple structures, indicating a high degree of variability. After reviewing this image, the low resolution of the CT scan may have led to this outcome.

Conclusions: Preliminary statistical analysis indicates that substantial inter-rater variability can exist in the manual segmentation of TB structures. Tracings of the round window had consistently higher MAHD values and lower MDC scores than other structures, although the chorda tympani had the highest single MAHD. Agreement among reviewers was highest for the cochlea, which had the lowest MAHD values and highest MDC scores with the smallest ranges for both. This suggests that smaller and often less defined structures such as the round window and chorda tympani appear to have the greatest degree of variability among tracers.

Abnormal Eye Movement-Related Eardrum Oscillations (EMREOs) in Individuals With Auditory System Dysfunction

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Category: Other, Auditory-Visual Integration

Background: Our senses work together to help us understand and interact with the world around us. Eye movements are critical to linking spatial hearing and vision – every eye movement shifts the relative relationship

between the visual (eye-centered) and auditory (head-centered) frames of reference, thus requiring constant updating of incoming sensory information in order to integrate the two sensory inputs across space. Neurophysiological studies have previously shown evidence for eye movement-related modulation of auditory signals within the brain, and we recently discovered a unique type of low frequency otoacoustic emission that accompanies eye movements. These eye movement-related eardrum oscillations (EMREOs) occur in the absence of external sound and carry precise information about saccade magnitude, direction, and timing (Gruters et al 2018, Murphy et al 2020).

However, it is not well understood how these eye movement-related effects in the auditory periphery contribute mechanistically to hearing. Two auditory motor systems may be involved in generating EMREOs: the middle ear muscles and/or the cochlear outer hair cells. To gain insight into which systems are involved and how they contribute, we are presently investigating the EMREOs in human subjects with dysfunction involving these systems compared to a normal hearing population.

Methods: Subjects were seated in a sound booth, facing a monitor, and maintaining stable head position using a chinrest. Microphones recorded oscillations in both ear canals while eye movements were simultaneously tracked with a video eye tracker. The impact of hearing loss on the response is examined by comparing responses from individuals with different hearing losses to population data from normal hearing subjects.

Results: We find that EMREOs are abnormal in subjects with hearing impairment, most commonly with the EMREO being abnormally small in individuals who have impaired outer hair cell or stapedius function.

Conclusions: These findings tie the EMREO's properties to peripheral motor systems known to play a mechanistic role in auditory perception. Future work is needed to assess if patients with these types of hearing loss have specific impairments in the perceptual process of integrating visual and auditory spatial information.

Ototoxic Effects of Drugs Used in COVID-19 Therapies in Comparison to Well-Known Ototoxic Agent (Gentamicin) in Male Wistar Rats

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Category: Other, Ototoxicity

Background: Many medications are under investigation as novel therapies to treat COVID-19. Among them, Hydroxychloroquine, Azithromycine, and Colchicine have been identified as potentially being ototoxic. The aim of this study was to determine if these drugs, administered similarly to clinic protocol (route of administration, doses, and regimen schedule), have any effects on hearing. A reference compound, Gentamicin, well-known for having ototoxic effects, was used for comparative purposes.

Methods: Male wistar rats (Janvier labs) were randomly divided into seven groups : one sham group (no treatment)(A), five groups treated with either Hydroxychloroquine (62 mg/kg, per os once a day for five days)(B), Azithromycin (51.5 mg/kg, per os once a day for five days)(C), Colchicine (0.1 mg/kg, per os once a day for five days)(D), Lopinavir / Ritonavir (41.5 mg/kg / 10.5 mg/kg, per os twice a day for ten days)(E), Ivermectin (0.2 mg/kg, per os once a day for five days)(F) or one group treated with Gentamicin (160 mg/kg, ip injection once a day for five days).

DPOAE at 4 ,8, 16 ,24 and 32 kHz (F2/F1=1.2, intensity=63dB) and ABR at 4, 8, 16, 25 and 32 kHz (from 90 to 0 dB in 10 dB steps) were measured at baseline (T0), T+10DAYS, T+24DAYS and T+38DAYS.

Results: In the Hydroxychloroquine, Azithromycin and Colchicine treated groups, a significant increase of ABR thresholds was observed for at least one frequency from T+24DAYS compared to the Sham group. Conversely, in the Lopinavir / Ritonavir and Ivermectin treated groups, no significant increase of ABR thresholds was observed. In the Gentamicin treated group, a significant increase of ABR thresholds was observed at the high frequencies compared to the Sham group from T+24DAYS.

DPOAE amplitudes decreased in the Azithromycin treated group only at 32 kHz at T+38DAYS compared to the Sham group, but no difference was observed in the Hydroxychloroquine, Colchicine, Lopinavir / Ritonavir and Ivermectin treated groups throughout the study. In the Gentamicin treated group, a significant decrease of DPOAE amplitudes measured at 45 dB and 63 dB was observed at 32 kHz at T+38DAYS compared to the Sham group.

Conclusions: The hearing loss observed in the groups treated with Hydroxychloroquine and Azithromycin was progressive and more pronounced than in the other treated groups. The effects on hearing of Colchicine, Lopinavir / Ritonavir and Ivermectin in our experimental conditions were negligible (mean loss <10 dB) which suggests they are not ototoxic. The ototoxicity of these compounds remains to be studied in the long term. Gentamicin, known to

be an ototoxic compound, induced, at the tested dose and treatment regimen, a slight hearing loss at the high frequencies.

Priorities in Hearing - Towards a Common Goal

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Category: Other, Research Strategy

Background: In hearing, there is an intrinsic link between knowledge of the normal functioning system and clinical application. However, grant proposals do not always substantiate the links clearly with evidence, for grant panels and funders. Funders themselves also make strategic decisions about areas to fund and can benefit from expert consensus. A field can move forward more effectively if there is a consensus on the big problems, how they inter-relate and whether and how they can be solved. We believe that there is currently no contemporary source which describes the priorities in the field of hearing research. In this vacuum, funding of hearing research is likely to lose out to other areas. Our objective is to consult the worldwide field of hearing researchers about what they think the priorities in hearing research should be for the next 5-10 years.

Methods: We used a mixed methods approach to develop the priorities. To generate an initial set of priorities, an open response questionnaire was sent to 40 experts of international standing (determined mainly by Professorial status). There were 18 responses. These responses were subject to a thematic analysis. An initial analysis was generated by an experienced qualitative psychologist. The categorisation of themes was presented to a subject expert alongside quotes for each theme to ensure content validity, and the theme categorisation was refined. The refined analysis was then subject to blind review and comment by the remaining authors, and a consensus reached by deliberation. All authors all agreed on a final categorisation of themes, with care taken to minimise bias.

Results: The themes were used to generate closed questions for a questionnaire which is intended to canvas the views of the field of hearing research world-wide. The questionnaire allows for rapid data collection following the themes defined the process above, but also crucially allows for expression of supplementary and dissenting viewpoints. The questionnaire (to be shared in the presentation) asks respondents to rate the importance of different themes and sub-themes, the tractability of making significant progress in the themes and sub-themes, whether themes and sub-themes were currently receiving the appropriate amount of attention. These data will be analysed using principal component analysis, in order to establish underlying and inter-related themes.

Conclusions: The present work aims to benefit all hearing researchers by providing a large-scale canvas of opinion on the state of hearing research and current priorities. This aims to provide clarity and evidence of mandate to grant funding bodies, who are at best ambivalent towards funding the field of hearing research. Now more than ever the time is ripe for the field to pool its collective understanding of the field for mutual benefit and to re-invigorate funding research in our field.

Dual Effect: BRAF Inhibitor Dabrafenib Protects Against Cisplatin-Induced Kidney Injury and Hearing Loss

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Category: Other, Dual Protection for Hearing and Kidney

Background: Cisplatin is a chemotherapy agent used for cancers of the head and neck, reproductive system, lungs, and others. Its beneficial use, however, is limited by side effects including acute kidney injury (AKI) and hearing loss. There are currently no FDA-approved drugs to treat these significant side effects, which can also be dose-limiting. Recently, the oral drug dabrafenib was identified as protective against cisplatin-induced hearing loss in mice. Dabrafenib is a specific BRAF inhibitor shown to reduce activation of the MAPK pathway in inner ear cells after cisplatin treatment. BRAF is also expressed in kidney cells, and the MAPK pathway is upregulated in vivo after cisplatin treatment. Thus, a similar mechanism may contribute to damage in both tissues, making dabrafenib a potential therapeutic candidate for cisplatin-induced kidney injury.

Methods: Initial experiments in a human kidney proximal tubular cell line (HK2) were done to determine IC50 values and confirm the upregulation of the MAPK pathway in kidney cells. Then animal models were used to

further assess the nephroprotective effect. Dabrafenib (12 mg/kg body weight) was administered by oral gavage for three consecutive days, twice daily, to FVB mice receiving a single dose of cisplatin (30 mg/kg body weight). Following this model, nephroprotection was evaluated through BUN levels, histology, apoptosis, and mouse survival.

Results: The initial cell viability assay of HK2 cells showed a dose-dependent increase in cisplatin-induced cell death and an IC50 of 5 µM. Treatment with dabrafenib alone showed no toxicity and improved cell viability when given with cisplatin at an IC50 of 0.77 µM. Western blot analysis showed upregulation of the MAPK pathway in HK2 cells after cisplatin treatment and downregulation of the pathway when co-treated with dabrafenib and cisplatin.

In the mouse models, BUN, a kidney injury marker, was evaluated on day 3, which show increased levels in cisplatin-treated mice that are then reduced with administration of dabrafenib. On day 21, histological analysis by H and E and PAS staining show kidney injury in cisplatin-treated mice which is significantly reduced with dabrafenib treatment. Immunofluorescent staining demonstrates decreased expression of P-ERK, a downstream target in the MAPK pathway, in mice treated with both cisplatin and dabrafenib. Additionally, reduced levels of apoptosis were seen in the dabrafenib and cisplatin-treated mice via TUNEL assay. Importantly, there is a significant increase in survival of mice in the group treated with cisplatin and dabrafenib compared to cisplatin alone.

Conclusions: Overall, dabrafenib demonstrates reduction of cisplatin-induced kidney damage and improved survival in mice, in addition to protection against cisplatin-induced hearing loss. This provides promising results that dabrafenib could reduce both the hearing loss and AKI side effects commonly seen in those requiring cisplatin chemotherapy for treatment of various cancers, including those of the head and neck.

Limbic Stress Receptors: Key Signatures That Bridge Peripheral Hearing and Cognitive Function
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Category: Other, Top-down signaling
Background: Emerging evidence for associations between hearing impairment and cognitive decline implies that hearing is dependent on the formation and storage of auditory memories in the limbic system in a mood- and arousal-related manner.
Methods: Considering glucocorticoid release upon stressful and exciting situations that result in altered auditory perception, we were interested in the contribution of mineralocorticoid- (MR) and glucocorticoid receptors (GR) on hearing function, using a tamoxifen-inducible CreERT2/loxP system to generate single or double deletion of MR and GR in limbic brain regions of adult mice.
Results: While threshold sensitivity in MRGR conditional knockout (cKO) double mutants were unchanged, early and late ABR waves, CAP latencies, and ASSR, suggested a direct beneficial effect of limbic MR/GR function on auditory-nerve processing. Analysis of single MR or GR cKO revealed that the phenotype of MRGR cKO mice resulted from opposing influences on auditory fiber responses, i.e. stimulating and inhibiting action: Limbic MR deletion reduced IHC ribbon numbers and ABR wave I responses, leaving later waves, and synchronization to amplitude-modulated tones, unchanged. This indicates that limbic MR activation mayalter auditory nerve fiber discharge rates. In contrast, limbic GR deletion improved early and late ABR waves without reducing IHC ribbon numbers. CAP thresholds, latency, and synchronization to amplitude-modulated tones were improved. This suggests that limbic GR activation affects neural response synchrony, thus influencing temporal auditory processing.
Conclusions: Our findings suggest that MR/GR stress hormone receptors are candidate factors for positive- and negative cochlear pre-cognitive processing during auditory cue perception and auditory cognitive dysfunction.

The Molecular Basis of Outer Hair Cell Electromotility is Defined by Prestin's Conformational Cycle
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Category: Other, Structural Biology of Hearing

Background: In mammals, auditory sound detection relies on a mechanical amplification process to achieve its remarkable sensitivity and frequency selectivity. This "cochlear amplifier" depends on one of two types of sensory receptor cell in the cochlea, the outer hair cells (OHC). OHCs undergo voltage-dependent longitudinal contractions or elongations at their basolateral membrane, triggered by the concerted action of millions of fast "motor" proteins. It is now overwhelmingly agreed upon that this molecular motor is the protein Prestin (SLC26A5), the only piezoelectric member of the SLC26 family of anion transporters. Knock out or impairment of Prestin causes severe hearing loss. Despite Prestin's key role in hearing, the mechanism by which mammalian Prestin senses voltage and transduces it into cellular-scale movements (electromotility) is not fully understood.

Methods: Here, we have used single particle cryo-electron microscopy (cryo-EM) to determine the structure of Prestin under various ionic conditions and in complex with the reversible inhibitor salicylate. These structural and trajectories were further supported by site-directed mutagenesis, patch-clamp electrophysiology and electrostatic calculations.

Results: We modulated Prestin function by changing the anion type. We used the same principle to drive and capture Prestin structures at six distinct conformations, including two states in complex with the known Prestin-inhibitor compound, Salicylate. While Prestin has kept a "Transport-like-movements", these structural trajectories point to novel mechanisms of voltage-dependent area changes, highlighting the evolutionary distinction between electromotile Prestin and other SLC26 transporters. The periphery helix TM1 and TM6 accounts in large part for Prestin's area expansion and actively influences the physical state of the surrounding bilayer. Mutating the evolutionarily conserved residues on TM6 helix largely affected Prestin's electromechanical properties.

Conclusions: I) Our data suggests that the bound anion together with its coordinating charged residues and helical dipole act as a dynamic voltage sensor.

II) Analysis of all anion-dependent conformations reveals how structural rearrangements in the voltage sensor are coupled to conformational transitions at the protein-membrane interface, suggesting a novel mechanism of area expansion.

II) Prestin structure in complex with the reversible inhibitor salicylate directly delineates the anion-binding pocket and establishes the mechanism of inhibition as direct biding competition with physiological anions.

Analysis of the Natural and Synthetic CV Stimuli for Cortical Auditory Evoked Potential
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Category: Other, Central Auditory Evoked Potential

Background: Cortical auditory evoked potential (CAEP) is late response of auditory evoked potential. For the stimulation of CAEP, speech signals including natural and synthetic speeches were known to be effective. However, when compared to click, tones and synthesized speech sounds, the natural speech sounds were thought to be less reliable for recording CAEP due to their highly complex time-varying signals. Therefore, investigating and selecting the appropriate speech materials for CAEP response and identifying CAEP waveforms according to the selected speech materials depending on the gender of speaker (GS) and the gender of listener (GL) were aimed for this study.

Methods: There were two experiments. For the comparison of natural and synthetic stimuli, 21 young announcers (9 males, 12 females, 22 ±1.7 years) participated. In this experiment plosive /g/ and /b/ and aspirated plosive /k/ and /p/ combined with /a/ were selected. This /ga/-/ka/, /ga/-/ba/, /ga/-/pa/, /ka/-/ba/, /ka/-/pa/, and /ba/-/pa/ were formulated in forwarding and backwarding orders. These were recorded from Korean native professional male and female announcers. All the bisyllables were presented randomly to the participants and they were asked to pick one what they heard on response pad and identify whether the sound was natural or synthetic. For CAEP measurement, 40 young adults (20 males, 20 females, 23.5±2.04 years) participated. The CAEP waveforms were obtained with natural and synthetic /ka/ and /pa/ which were selected through first experiment.

Results: In comparison of natural and synthetic stimuli, only SM (stimulation mode) elicited the significant difference showing higher scores in the natural speech sound mode. Among all the stimuli, the correction rate difference was biggest (74%) at /ka/-/pa/ and /pa/-/ka/. As a result, they were selected as stimulation materials for the CAEP measurement. The SM showed shorter latency with P2 and N1-P2 complex with natural speech sound and N2 with synthetic speech sound. The amplitude of P2 was larger with natural speech sound. The SD (stimulus difference) showed larger amplitude of P2 and N1-P2 for /pa/. The GS showed shorter latency of P2, N2 and N1-

P2 complex and larger amplitude of N2 with female speakers. And the GL showed shorter latency for N2 and N1-P2 and larger amplitude for N2 with female listeners.

Conclusions: Overall, several variables affected N2, P2 and N1-P2 complex but the P1 and N1 were not affected. The results of the P2 and N1-P2 complex were significantly different according to the SM and SD showed the significant differences in the latencies of P2 and N1-P2 complex. The N2 latency and amplitude seemed to be affected by differences in perception, including attention and language perception in the cerebrum. Conclusively, in the view of endogenous and exogenous features, it was found that N2 and P2 were mainly affected by the endogenous factor.

Characterizing the Joint-OAE Profile in Individuals With Endolymphatic Hydrops

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Category: Otoacoustic Emissions

Background: Endolymphatic hydrops (EH), a hallmark of Ménière's disease, is caused by an increased volume of endolymph in the scala media of the cochlea, either from over-secretion or ineffective re-absorption. The ensuing alterations in cochlear morphology can cause disruptions in cochlear mechanics and the generation of otoacoustic emissions (OAEs). Hence, OAEs may be an ideal tool for diagnosing and monitoring EH in humans. Given recent advances in the measurement and understanding of OAEs a careful study of their utility in detecting EH is warranted. We are now able to rapidly measure the two distinct classes of emissions—OAEs arising from nonlinear distortion such as distortion product (DP) OAEs, and OAEs arising from coherent linear reflection such as stimulus-frequency (SF) OAEs — nearly simultaneously and in doing so, exploit information offered from two distinct OAE generation mechanisms. An earlier study from our lab has reported what appears to be a striking SFOAE-DPOAE profile in one subject with EH: reduced or non-measurable DPOAEs with near-normal SFOAEs, thus motivating this follow-up study to characterize the two classes of emissions in individuals with EH.

Methods: A joint-OAE profile is generated from the near-simultaneous measurement and relational analysis of DPOAEs and SFOAEs in the same ear. Here, this OAE profile was recorded in 10 ears with EH and in a group of roughly age-matched normal hearers. Both OAEs were evoked with rapidly sweeping tones at 10-12 stimulus levels (calibrated in forward pressure level) across five octaves.

Results: Results show greatly reduced or non-measurable DPOAEs in the region of EH involvement, most often the low-frequencies, combined with SFOAE levels that are near-normal. Most subjects with EH showed this pattern, but not all. Factors such as stage of disease and/or its current status (active versus quiescent) are likely to influence the outcome but were not controlled here. Although SFOAE levels are generally higher than DPOAE levels in normal hearers (and this control group was no exception), individuals with EH showed exaggerated differences between the two emission types, most of which could be attributed to decreased DPOAE levels.

Conclusions: The joint-OAE profile appears able to detect and characterize EH via a signature result observed in most of the diseased ears (and in no ears with normal hearing). Results suggest that the OAE generation processes are disrupted by the pathophysiology of EH, which may include a stiffening of the cochlear partition and damage to the outer hair cell bundle. We speculate that the intracochlear generation of nonlinear-distortion OAEs is more disrupted than that of coherent-reflection emissions in ears with EH. More work is warranted to control potentially confounding factors and to determine whether this joint-OAE profile observed in ears with EH provides specificity of diagnosis and sensitive monitoring of disease progression.

Synaptic Zinc Release at Specific Cortical Synapses Shapes Intracortical Synaptic Transmission in Mouse Auditory Cortex

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Category: Primary Auditory Cortex

Background: The primary auditory cortex is crucial for the perception of sounds. This cortical area is highly enriched in synaptically released zinc which acts as a potent modulator of cortical function and sound processing. Zinc (as Zn^{2+}) is loaded into synaptic vesicles by the zinc transporter protein ZnT3 where it is coreleased with glutamate during synaptic transmission. Synaptically released zinc shapes multiple aspects of synaptic signaling and can inhibit AMPA and NMDA glutamate receptor function.

The primary auditory cortex contains highly organized networks of neurons that form precise synaptic microcircuits and process auditory information. Despite the importance of synaptic microcircuit organization of the cortex and the different functional properties of these synapses for auditory function, synaptic mechanisms that support the diversity and specificity of these connections are less-well understood. Mounting evidence strongly suggests that synaptic zinc is only released from a subset of glutamatergic cortical neurons and in a cortical layer-specific manner. Since the principals governing cortical microcircuit organization relate to both cortical layer and neuronal type, this suggests that synaptic zinc may contribute to the specificity and diversity of intracortical synaptic microcircuits.

Methods: Here, to understand the role of synaptic zinc signaling at specific cortical synapses, we optogenetically stimulated different presynaptic neuronal populations and recorded the synaptic inputs they provide to the same class of postsynaptic cortical neuron. In acute brain slices of the auditory cortex, we performed whole-cell patch-clamp recordings from identified layer 5 corticocollicular neurons while optogenetically stimulating inputs from specific presynaptic neuronal populations of cortical Cre-expressing neurons in layer 2/3 and layer 5.

Results: Our results reveal that the effects of synaptic zinc release are cell-type specific and we also find a novel role for synaptic zinc in potentiating AMPA receptor function at specific intracortical synapses.

Conclusions: Together, these results reveal a novel role of synaptic zinc within the cortex and suggest that synaptic zinc signaling contributes to the diversity and specificity of intracortical synaptic connections that support normal cortical function and auditory processing.

Prenatal Opioid Exposure Results in Hypo-Connectivity of Excitatory and Inhibitory Intra-Cortical Circuits in Mouse Primary Auditory Cortex

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Category: Primary Auditory Cortex
Background: Opioid used by pregnant women results in neonatal opioid withdrawal syndrome (NOWS)and lifelong neurobehavioral deficits that result from perinatal opioid exposure. Animal models of NOWS show impaired performance in an auditory discrimination task suggesting abnormalities in sensory processing, especially in the auditory cortex.
Methods: To investigate the consequences of perinatal opioid exposure on auditory cortex circuits, we administered fentanyl to mouse dams in their drinking water throughout gestation and until litters were weaned at postnatal day (PD) 21.We then investigated how auditory cortical microcircuits in adult are altered by performing laser scanning photostimulation (LSPS) combined with whole-cell patch clamp recordings from Layer (L)2/3 cells in primary auditory cortex (A1) in adult mice that were prenatally exposed to fentanyl.
Results: We found that L2/3 cells in perinatal fentanyl-exposed animals display functional hypoconnectivity of both excitatory and inhibitory circuits. We confirmed these results by recording miniature excitatory (mEPSCs) and inhibitory synaptic currents (mIPSCs).
Conclusions: These results suggest a specific reduction in excitatory and inhibitory intralaminar cortical circuits after fentanyl exposure. We speculate that these unbalanced changes in cortical circuits contribute to the functional manifestations after prenatal and neonatal opioid exposure.

Importance of Spectral Versus Temporal Cues for Vocalization Categorization in Guinea Pigs

Manaswini Kar*[1], Marianny Pernia[1], Kayla Williams[1], Satyabrata Parida[1], Nathan Schneider[1], Isha Kumbam[1], Madelyn McAndrew[1], Srivatsun Sadagopan[1]
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Category: Primary Auditory Cortex
Background: Vocal communication sounds (human speech or animal calls) are produced with a high degree of variability in diverse listening conditions. The auditory system can seamlessly discriminate and categorize sounds despite such variability. However, what vocalization features are perceptually important for categorization and how these features are represented in the brain remain poorly understood. As a first step towards exploring the impact of different spectral and temporal cues for call categorization, we developed an appetitive Go/No-Go call categorization task.
Methods: We trained guinea pigs (GPs), highly vocal and social rodents that use complex calls in specific behavioral situations, to discriminate between two call categories with similar low-frequency content but different

temporal modulations to the envelopes. To determine the call features necessary for categorization, we presented the animals with calls with systematically manipulated spectral and temporal features.

Results: GPs maintained robust categorization across a wide range of temporal modulations including changes to tempo, reversal, and changes to inter-syllable intervals. However, categorization performance was affected when the frequency content of the calls was shifted away from the natural range.

Conclusions: These results suggest that spectral cues are dominant for the categorization of some GP calls. To determine if this result generalizes to other call types, in ongoing work, we are performing these experiments using a different pair of calls that are characterized by strong frequency modulations and have non-overlapping frequency content.

Effects of Noise-Induced Hearing Loss on Evoked Responses and Temporal Processing in the Auditory and Frontal Cortex of Mice

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Category: Primary Auditory Cortex

Background: Noise-induced hearing loss (NIHL) is a major cause of auditory processing impairments with recreational and occupational hazard implications. Peripherally, cochlear hair cell damage has been studied extensively in NIHL. Studies on central auditory system effects suggest 'the central gain model' in which the lack of peripheral input (deafferentation) following NIHL increases leading to exaggerated spontaneous responses. These responses may lead to tinnitus and hyperacusis, though currently there is no treatment to reduce tinnitus or hyperacusis following NIHL. The cellular mechanisms underlying the increased gain is unclear. This study uses electroencephalography (EEG) recordings from mice before and following NIHL to quantify response magnitudes and temporal processing.

Methods: Hearing loss after 3-hours of noise-exposure was verified by measuring the auditory brainstem response (ABR) and measured at each subsequent recording day at 1-, 10-, 23-, and 45-days after noise-exposure. The event related potential (ERP) was measured in the auditory cortex (AC) and frontal cortex (FC) of awake mice (FVB, 2.5-4 months at time of noise-exposure; noise-exposed N= 29; control N= 12) to uncover the emergence of central gain. To test temporal processing, we used a gap-auditory steady state response (gap-ASSR) paradigm to test the auditory cortex's ability to detect short gaps embedded in noise.

Results: Despite an absence of an ABR up to 90 dB SPL up to 45-days post-exposure (PE) – indicating permanent hearing loss – ERPs recorded during EEG recordings from the AC and FC showed recovery in ~50% of the mice beginning at 1-day PE. We found that there was an increasing ERP amplitude trajectory beginning 1-day PE that led to central gain being observed at 45-days PE. However, temporal processing as quantified using the intertrial phase coherence (ITPC) to the gap-ASSR was reduced following NIHL in both auditory and frontal cortex in all the mice and showed a significant reduction even at 45-days PE compared to the pre-NIHL recordings. These results suggest increased emphasis on sound detection, and reduced temporal processing in cortical responses in the absence of an ABR.

Conclusions: These EEG findings corroborate the central gain model theory found from previous studies. Although there is no indication of a peripheral signal being detected through the ABR, recordings from both the AC and FC indicate that there is some signal that is being received centrally and perhaps being amplified locally, but temporal processing remains impaired even with strong cortical ERPs. We are currently testing a potentially compensatory mechanism led by upregulated matrix metalloproteinase-9 activity after NIHL, leading to perineuronal net and inhibitory neuron dysfunction leading to enhanced gain and reduced temporal processing.

The Contribution of Perineuronal Nets to Temporal Processing in the Auditory Cortex

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Category: Primary Auditory Cortex

Background: Perineuronal nets (PNNs) are specialized extracellular matrix aggregates that form around neurons, particularly parvalbumin (PV+) expressing GABAergic interneurons. PNNs are suggested to increased excitability of PV+ neurons, and thereby increase temporally precise inhibition in the cortical network. PNNs are made up of chondroitin sulfate proteoglycans (CSPG's) and the maturation of PNNs in the cortex marks the closing of critical learning periods and enhanced stability in the network. Removal of PNNs decreases PV

expression as well as gamma frequency band activity in the neocortex. In the auditory cortex, loss of PNNs have been described in both aging mice as well as mouse models of Fragile X Syndrome, an autism spectrum disorder. The impact of PNN loss on auditory temporal processing, a deficit seen in both aging and in autism, is unclear, and forms the main aim of this study.

Methods: This study tests the hypothesis that removing PNNs would affect cortical temporal processing to sounds. We recorded baseline and sound-evoked epidural EEGs in awake and freely moving mice. We quantified temporal fidelity by measuring inter-trial phase clustering (ITPC) using a stimulus termed '40 Hz gap-induced auditory steady-state responses (gapASSR)'. The gapASSR was induced by presenting a 40 Hz amplitude modulated (75% modulation depth) noise stimulus with varying gap lengths of 3 – 9 ms between each peak of the stimulus. The phase locking of the response across trials at different gap durations provides a stimulus-response space to measure temporal processing. We also recorded event- related potentials (ERP) in response to broadband noise bursts (70 dB). Removal of PNNs was done by injecting chondroitinase ABC (chABC) into the right auditory cortex (AC) with recordings taken from both the right and left AC. ERP and gapASSR recordings were obtained 4 days post chABC injection.

Results: A robust gapASSR was seen in the non-injected auditory cortex for gaps >5 msec. On the ChABC injected side, the gapASSR ITPC was significantly reduced for gaps between 6 and 9 ms. This suggests that removal of PNNs reduces the fidelity of auditory temporal processing and causes a reduced ability of neural generators to detect short gaps in auditory stimuli. The resulting ERP showed a small increase of the N1 peak on the injected side, compared to the non-injected side, but additional mice are needed to examine statistical significance.

Conclusions: Our results suggest that PNN removal causes temporal processing deficits in the auditory cortex. Future studies examine resting EEG, influence of movement on EEG responses as well as quantifying the effect of PNN loss on PV expression in the auditory cortex.

Spectral Modulation Detection Thresholds in the Mongolian Gebil
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Category: Psychoacoustics

Background: Natural sounds are composed of spectral and amplitude modulations (SM and AM, respectively), although the latter cue has been studied far more broadly at the level of behavior or neural processing. One reason to study SM is that this percept appears to be essential to the understanding of speech in noise, a particular problem for those with developmental hearing loss. Here, we describe an SM detection task in the Mongolian gerbil. We measured SM detection thresholds at both 2 cycles/octave and 10 cycles/octave, as well as at different levels (45 and 35 dB SPL). The term goal is to assess the impact of transient developmental HL on spectral processing.

Methods: Male and female gerbils, aged postnatal days P40-50 (juvenile) or P90-100 (young adult), were used for these experiments. Animals were trained to report the presence of SM, using a go-nogo procedure that has permitted us to assess amplitude modulation detection thresholds (Sarro and Sanes, 2010). SM noise stimuli were generated with a Matlab script (Donal Sinex) at 2 or 10 cycles/octave, using a bandwidth of 200-20,000 Hz. After reaching training criterion, animals were tested with a range of SM depths. Behavioral sensitivity (d') was calculated from hit and false alarm rates, and the values were fit with psychometric functions. Threshold was defined as the modulation depth at which animals displayed a sensitivity of d'=1.

Results: Gerbils were tested for 10 successive days, and improved gradually on the task, reaching stable thresholds by approximately 7 days. Animals displayed statistically similar SM detection thresholds at 2 and 10 cycles/octave. Thresholds were 11.0 plus-minus 4.1 dB at 2 cycles/octave and 8.3 plus-minus 2.5 dB at 10 cycles/octave (n = 8 gerbils per group). To assess whether SM detection thresholds were robust to sound level, as is true for AM detection, animals were tested on two different sound levels, 35 and 45 dB SPL. Finally, data will be presented from a group of animals reared with transient hearing loss (i.e., bilateral earplugs from P11-23).

Conclusions: Gerbils learn the SM detection task as efficiently as the AM detection task (Caras and Sanes, 2017), demonstrating the feasibility of a reliable animal model for research into the effects of hearing loss. Achieved thresholds were a few dB worse than found in human studies, but performance on 2 and 10 cycles/octave did not significantly differ, in contrast to humans (e.g., Eddins et al., 2007).

A Comparison of Gain Reduction Estimated From Behavioral Measures and Various Transient-Evoked Otoacoustic Emission Measures as a Function of Broadband Elicitor Duration

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Category: Psychoacoustics

Background: Humans are able to hear and detect small changes in sound across a wide dynamic range despite limited dynamic ranges of individual auditory nerve fibers. One mechanism that may adjust the dynamic range is the medial olivocochlear reflex (MOCR), a bilateral sound-activated system which decreases amplification of sound by the outer hair cells in the cochlea. Much of the previous physiological MOCR research has used long broadband noise elicitors. In behavioral measures of gain reduction, a fairly short elicitor has been found to be maximally effective for an on-frequency, tonal elicitor. However, the effect of the duration of broadband noise elicitors on behavioral tasks is unknown. Additionally, MOCR effects measured using otoacoustic emissions (OAEs), have not consistently shown a positive correlation with behavioral gain reduction tasks. This finding seems counterintuitive if both measurements share a common generation mechanism. This lack of a positive correlation may be due to different methodologies being utilized for the OAE and behavioral tasks, and/or due to the analysis techniques not being optimized to observe a relationship. In the current study, we explored the effects of ipsilateral broadband noise elicitor duration both physiologically and behaviorally in the same subjects, using a forward-masking paradigm.

Methods: The effects of an ipsilateral pink broadband noise elicitor (0.2 - 10 kHz) on TEOAEs and behavioral thresholds were measured as a function of elicitor duration in 19 normal-hearing humans. The elicitor was fixed at 50 dB SPL, which should be below the middle ear muscle reflex threshold, and elicitor durations ranged from 50-400 ms. TEOAEs were measured with and without an elicitor to estimate MOCR strength. TEOAE measures included the change in magnitude, or in magnitude and phase, for different frequency analysis bands. For the same subjects, a psychoacoustic forward-masking paradigm was used to measure the effects of the elicitor on masking by an off-frequency masker for a 2-kHz signal, and for the effect of the elicitor on a signal with 20 ms of silence replacing the masker.

Results: The effects of the precursor on TEOAEs and behavioral thresholds will be compared as a function of elicitor duration for the different TEOAE and behavioral measures. The goal is to determine how duration of a broadband MOCR elicitor affects cochlear gain physiologically and perceptually, and if there is a relationship between these measures.

Conclusions: The current study highlights the importance of choosing appropriate methodology used in estimating MOCR strength, particularly with OAEs.

Acknowledgments:

Assessing the Potential Contribution of Amplitude Modulation Cues to Natural Soundscapes Classification

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Category: Psychoacoustics

Background: Natural soundscapes carry a wealth of biological and geophysical information susceptible to help us identify, map and navigate through our habitat, or provide information about the time of day and year. Unfortunately, our understanding of the human auditory system's ability to extract useful information from these

soundscapes is still fragmentary. Consistent with speech studies, a recent work suggested that relatively slow amplitude-modulation (AM) cues may play a role in the auditory discrimination of terrestrial biomes. It was shown that these biomes could be categorized well above chance by a support vector machine classifier algorithm using only temporal information at the output of a model of the human auditory system. The goal of this research was to replicate and extend these initial results. In particular, a large database including acoustic recordings from terrestrial biomes from five continents (temperate deciduous and temperate coniferous forests, tropical and sub-tropical forests, boreal forest, desert, savannah, chaparral, etc.) was used.

Methods: Our database included soundscape recordings obtained from nine distinct terrestrial biomes spread across the five continents (America, Europe, Africa, Asia, Oceania). Each recording encompassed two contrasting seasons and four moments of the day. They were divided in 2-sec samples. Each sample was processed through a simplified model of the auditory system to compute AM spectra. The two main stages of this model involved a cochlear filterbank followed by a modulation filterbank. The AM spectra were then used to train a simple convolutional neural network.

Results: Preliminary data obtained on a subset of our database (four terrestrial biomes recorded in a biosphere reserve on the North American continent) replicated the results of a previous study and showed that relatively slow AM cues are sufficient to classify habitats well above chance level.

Conclusions: Results obtained on the whole database will also be presented and discussed. Additional simulations will establish the nature of the sensory cues that play a critical role in the classification of our terrestrial biomes.

Measuring Harmonic Benefit in Musicians and Non-Musicians in Several Tasks

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Category: Psychoacoustics

Background: Prior work has established that harmonic sounds are easier to detect in noise and that fundamental-frequency (F0) discrimination in noise is more accurate for harmonic complex tones than for inharmonic complex tones. Musicians have previously been found to have better F0 discrimination than non-musicians but no benefit for the detection of harmonic vs. inharmonic tones in noise. The present study aimed to replicate these basic findings and also extend them to other psychophysical tasks (FM detection and AM detection) for which the effects of harmonicity and musicianship are less well understood.

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Results: We confirmed that inharmonic complex tones were more difficult to detect in noise and F0 discrimination was worse for inharmonic complex tones than harmonic complex tones in noise (but not in quiet). However, different patterns of results were observed for FM and AM detection. For FM detection, thresholds were comparable for harmonic and inharmonic complex tones at all tested SNRs and in quiet. For AM detection, thresholds were consistently worse for inharmonic complex tones at all tested SNRs and in quiet. Preliminary results suggest that musicians have better overall performance in the psychophysical tasks but have no additional "harmonic benefit" over non-musicians, and no selective benefit for the pitch-related tasks of F0 discrimination and FM detection.

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Working Memory for Multifeature Audiovisual Items

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Category: Psychoacoustics
Background: Working memory (WM) reflects the transient maintenance of information in absence of external input, which can be attained via different senses. The prevailing view suggests the dominance of visual WM over other sensory systems. However, this imbalance may be attributed to challenges comparing notoriously disparate representations across modalities. Here, we address this methodological problem by using a multisensory design in which the discriminability of auditory (ripple velocity) vs. visual (spatial frequency) memory content is parametrically equated.
Methods: WM stimuli were adjusted relative to each healthy participant's (n=15) just noticeable differences (JND) in each modality separately, determined using a staircase algorithm before the experiment. In each trial, the participants were presented with a pair of 1 s audiovisual items, accompanied by a retro cue indicating the item to memorize over a pseudo-randomized maintenance period of 2 s or 10 s. A third audiovisual probe was then presented to test recall for the attended item. The task goal was to identify whether the probe and the memorized item matched or did not match. In randomly ordered trials, the probe was either fully matching (match) or differed from the memory item auditorily (auditory non-match), visually (visual non-match), or audiovisually (non-match). Each participant completed 288 trials in total, across 6 runs.
Results: Based on our linear mixed effects model, the subjects correctly rejected a significantly larger number of auditory non-match probes than visual non-match probes (p<0.001).
Conclusions: Our results suggest that, in the case of feature maintenance, auditory WM is at least equally precise as its visual counterpart when complexity of the content is bimodally equated.

Behavioral Estimates of Human Cochlear Tuning With Contralateral Acoustic Stimulation: Effects of Masker Duration
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[1]*Complutense University of Madrid,* [2]*University of Salamanca*

Category: Psychoacoustics
Background: The effect of the medial olivocochlear reflex (MOCR) on human cochlear tuning has been typically assessed by comparing psychoacoustical tuning curves (PTCs) obtained with and without presenting white noise, which isan effective MOCR elicitor, in the contralateral ear. Results, however, are not univocal, probably because of differences in methodological strategies adopted in the different studies. One such difference is the duration of the masker employed to measure the PTCs. Long-duration maskers can elicit an ipsilateral MOCR by themselves, whose effect can be hard to disentangle from the sought effect of the contralateral MOCR. Moreover, effects might be different for different cochlear sites. Here, PTCs are measured in forward masking for signals of 500 Hz and 4.0 kHz in the absence and the presence of contralateral broadband noise at 60 dB SPL, a level capable of activating the MOCR with minimal activation of the middle-ear muscle reflex.
Methods: In the two conditions, PTCs were measured using maskers that were respectively long (250 ms) and short (40 ms) enough to activate or not the ipsilateral MOCR. Masker frequencies ranged from 0.5 to 1.2 times the probe frequency. Five listeners with normal hearing participated in the experiments.
Results: Results revealed clear differences for short and long masker durations at 4 kHz. Specifically, the contralateral MOCR broadened the PTC (decreased tuning) only in the short masker condition, i.e., when the ipsilateral MOCR was presumably absent. At 500 Hz, differences for short and long maskers were minimal.
Conclusions: These results indicate that the interaction between the ipsilateral and the contralateral MOCR might be more complex than originally thought and that these complex interactions might be frequency dependent.

Identification and Optimization of Channelrhodopsin Variants for Optogenetic Hearing Restoration
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Category: Regeneration

Background: In recent years a lot of progress has been made towards the development of optical cochlear implants (oCI) which aim to overcome the limited auditory perception of electrical cochlear implant (eCI) users imposed by the physical properties of electrical stimulation. Using spatially confined light instead of wide spreading current in eCI, the oCI allows for targeted stimulation of smaller spiral ganglion neuron (SGN) populations enabling improved spectral selectivity (Izzo et al. 2007; Moser 2015; Dieter et al. 2020). Optogenetic hearing restoration employs light-gated ion channels found in green algae, called Channelrhodopsins (ChR) (Nagel et al. 2003; Boyden et al. 2005) to render SGN light sensitive. One of the remaining tasks before a clinical application of optogenetic hearing restoration is the selection of the ChR variant most suitable for optogenetic manipulation of SGN. The ChR of choice should combine several properties to ensure natural sound encoding: fast kinetics, large ion conductance as well as a red-shifted action spectrum, stable and selective expression in the plasma membrane and high light sensitivity are some of these requirements (Dieter et al. 2020). In this study, we investigated variants of the previously described red-shifted Chlamydomonas noctigama ChR f-Chrimson (Mager et al. 2018) and cryptophyte ChR ChRmine (Marshel et al. 2019) for their potential for stimulation of SGN in the future clinical optogenetic hearing restoration.

Methods: Characterization of the electrophysiological properties of f-Chrimson and ChRmine variants was performed by whole cell patch clamp recordings of transfected neuroblastoma glioma (NG) cells.

Results: Whole-cell patch clamp recordings of f-Chrimson in NG cells revealed a significant drop in photocurrent density following the removal of the adjacent membrane-bound fluorescent protein enhanced yellow fluorescent protein (EYFP) that can be rescued by modification of the C-terminus. ChRmine-EYFP displayed big peak photocurrents in NG cell recordings rendering it a promising candidate for optogenetic applications. However, its strong desensitization leads to a significantly reduced stationary photocurrent density. We constructed the new ChRmine variants ChRmine-Mutant1(M1)-EYFP, ChRmine-Mutant2(M2)-EYFP and ChRmine-M1-M2-EYFP which feature a significantly reduced desensitization.

Conclusions: We consider f-Chrimson the currently most promising candidate for optogenetic hearing restoration due to the combination of red-shifted action spectrum and fast closing kinetics enabling SGN spiking at near physiological rates. Our results demonstrate significant alterations of the properties of f-Chrimson-EYFP following the removal of EYFP. Rescuing these alterations by modification of the C-terminus further optimizes f-Chrimson for clinical applications by abolishing the need for the additional expression of a jellyfish-derived fluorescent protein with potential adverse effects in the human ear. Furthermore, new ChRmine variants, displaying a red-shifted action spectrum with big photocurrents, a reduced desensitization and high light sensitivity, combine several of the required ChR properties for optimized optogenetic application.

Transcriptomic and Epigenomic Dynamics of Follistatin and LIN28B-Mediated Supporting Cell Reprogramming in the Murine Cochlea

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Category: Regeneration
Background: Cochlear hair cell (HC) loss is a major cause of deafness in humans. Recent studies conducted in mice have revealed limited HC regeneration in the immature cochlea prior to the onset of hearing. The source of newly formed HCs is neighboring supporting cells (SCs), which are competent to fate-inducing cues such as ectopic Atoh1 expression, Notch inhibition, and/or Wnt/β-catenin activation, leading to regeneration of HCs through mitotic and nonmitotic mechanisms. However, the regenerative capacity of SCs decreases after birth and is completely lost by postnatal day 5 (P5). Previously, we have shown that transient overexpression of the RNA-binding protein LIN28B restores the capacity of stage P5 cochlear SCs to generate new HCs in response to HC-fate inducing cues. More recent unpublished data indicates that LIN28B's positive effect on SC plasticity is further enhanced by co-activation of the Activin antagonist follistatin (FST). Based on the strong upregulation of select progenitor-specific genes, we postulated that LIN28B facilitates HC regeneration by reprogramming of SCs into a HC progenitor-like cells.
Methods: To address this hypothesis, we analyzed the transcriptomic and epigenetic landscapes of control and LIN28B and or FST overexpressing stage P5 cochlear epithelial cells (SCs) in organoid culture at the peak of reprogramming. RNA sequencing (RNA-seq) analysis revealed that reactivation of FST and LIN28B reduces the expression key SC transcription factors and SC-specific genes involved in tectorial membrane and extracellular matrix formation. We also observed significant upregulation of cell cycle and stemness genes, as well as upregulation of early HC fate inducers such as Atoh1, Pou4f3 and Gfi1. Furthermore, using Assay for

Transposase-Accessible Chromatin using sequencing (ATAC-seq) to analyze chromatin accessibility, we found that LIN28B overexpression, and to a lesser extent LIN28B and FST overexpression, alters chromatin accessibility on a global scale.

Results: Differentially accessible regions (DARs) between LIN28B and FST overexpressing organoids and wild type control organoids are enriched for binding motifs of transcription factors that are essential in auditory sensory epithelium development, such as the Six, Eya, and Gata families of transcription factors. Additionally, we show significant restriction of chromatin within SC-specific enhancer regions. Comparisons to publicly available progenitor-, HC-, and SC-specific epigenomic datasets enable qualitative assessment of the progenitor-like cell population we observe in LIN28B and FST overexpressing organoids, and integration of RNA-seq and ATAC-seq datasets allows for computational inference of gene regulatory networks which control SC reprogramming and identification of future target genes for increasing SC regenerative capacity and HC production in the adult mammalian cochlea.

Conclusions: Together, these findings support a sequential model of SC reprogramming, HC fate specification, and subsequent differentiation, and reveal similarities between FST and LIN28B-mediated SC reprogramming and auditory sensory epithelium development.

Cell-Type Identity of the Avian Utricle

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Category: Regeneration

Background: The chicken utricle continuously turns over a substantial number sensory hair cells per day (Goodyear et al., 1999). This permanent production of new sensory hair cells contrasts with the regeneration-capable basilar papilla, which is quiescent in homeostasis, and the mature mammalian inner ear's sensory organs that cannot generate new hair cells.

Methods: We used single-cell RNA-sequencing to characterize the spatiotemporal transcriptomic profiles of chicken utricular sensory epithelium cells. Extrastriolar type II, striolar type I, and striolar type II hair cells were identified based on their distinct gene activation patterns, and new gene expression markers were validated using hybridization chain reaction (HCR).

Results: Our study provides the transcriptional landscape for spatially distinct supporting cell populations. We delineated a chronological sequence of gene expression changes that define the natural generation of new hair cells in the utricle and validated our observations in situ using multi-probe HCR combined with identification of cells that passed through the S-phase of the cell cycle. Further, we describe two transitional cell types of dedifferentiating supporting cells to newly generated hair cells.

Conclusions: Our dataset provides an extensive inventory of gene expression dynamics for natural hair cell generation in the avian utricle, as well as hypotheses of signaling pathways that are active during this process. Goodyear, R.J., Gates, R., Lukashkin, A.N., and Richardson, G.P. (1999). Hair-cell numbers continue to increase in the utricular macula of the early posthatch chick. J Neurocytol 28, 851-861.

Clinical Update on a Potential Hearing Restoration Therapeutic, FX-322

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Category: Regeneration

Background: FX-322, a small-molecule combination that demonstrated regenerative effects preclinically, showed an increase in word recognition (WR) and words-in-noise (WIN) testing in a double-blind, placebo-controlled study (Study 201) of 23 subjects with permanent sudden sensorineural hearing loss (SSNHL) or noise-induced hearing loss (NIHL). Follow up testing of responding patients examined the durability of this response. A separate single-dose open label study (Study 111) was performed to compare two different formulation preparations in subjects with permanent SSNHL, NIHL, or idiopathic SNHL. These patients were also assessed for long-term assessment of a response.

Methods: To study response durability from Study 201 in 23 subjects, 5 subjects, including the 4 responders that showed statistical improvement in WR established by Thorton and Raffin (1978) at Day 90, were invited to return for audiometric and speech perception testing 13-21 months after the administration of FX-322 (Study 201-2). To study the effects of FX322 in different formulation conditions, 33 subjects were dosed unilaterally in an open-label study with the contralateral untreated ear serving as a control (Study 111). The trial enrolled all acquired SNHL etiologies and enrolled subjects with pure tone averages at 0.5kHz, 1kHz, 2kHz in the range of 26-90dB. Patients were evaluated for 90 days for otoscopy, tympanometry, pure tone audiometry, speech perception, and adverse events.

Results: In the follow up of Study 201 patients, 3 of 4 responding subjects that had achieved within patient statistically significant improvement in WR at Day 90 did not statistically change outside of test-retest expectations at the 13-21 month visit. One subject that had not met statistical significance at day 90, but attended a 13-21 month follow up, did not show any further benefit.

In Study 111, 5 of the 33 subjects showed within patient statistically significant improvements in WR in treated ears at day 90. Twenty-five of the 33 subjects returned for follow-up testing 8-12 months after the initial dose. Of the 5 responders, the 4 subjects who were able to return for testing trend towards a reduction in correct words, but scores remained above baseline. Four patients that showed a trend for improvement at day 90 reached statistical improvement at their 8-12 month follow up. Interestingly, these data show that the late emerging responders started with better WR performance than the early responders.

Conclusions: These data demonstrate that hearing improvement in FX-322 treated patients can last for 1-2 years or more as measured by improvement in WR.

The second independent single-dose study FX-322 that was associated with statistically significant improvements in WR supports the continued evaluation in multiple populations to determine the ranges of SNHL that FX-322 might address.

Bulk and Single-Cell RNA-Seq Highlight the Transcriptional Response of the Adult Human Utricle After Damage

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Category: Regeneration

Background: In the vestibular system, loss of inner ear sensory hair cells (HCs) leads to permanent balance deficits. This loss is difficult to mitigate because vestibular organs have a limited capacity to regenerate. Nonetheless, supporting cells (SCs) can survive after HC loss, and in newborn mice, SCs can replace HCs by either direct transdifferentiation into HCs or via proliferation followed by transdifferentiation. However, in adults, this process is limited and not sufficient for functional recovery.

Methods: To assess the adult human utricle's genes and transcription factors that are involved in the damage response and potentially in a regenerative response, we used a well-known HC damage paradigm - gentamicin. We damaged, in culture, utricles from patients with acoustic neuroma and evaluated the early response to damage as this might represent a critical time window to set the stage for HCs regeneration. After 24 hrs, we isolated the RNA from the sensory epithelia only and performed bulk RNA-seq in control and treated samples.

Results: We found 47 genes with significant transcriptional changes between control and damage conditions highlighting an immediate cellular response of the adult sensory epithelium to damage. In addition, among these genes, we identified 3 transcription factors: Jun, Tcerg1, and the deafness gene Coch. Furthermore, to profile gene expression changes at higher resolution, we performed single-cell RNA-sequencing (scRNA-seq) on the same cohort of patients. Our bioinformatic analyses have revealed a robust difference in the transcriptional profile between gentamicin-damaged and control samples.

SCs represent an endogenous population of cells that is a prime target for regenerative strategies as SCs survive after HC loss. We used scRNA-seq to elucidate SC heterogeneity in a human utricle. Our data indicate that there are six putative types of SCs in the adult human utricle from patients with acoustic neuroma. Characterization of the different SCs is a first step towards identifying which subtype(s) represent 'stem' cells for HC regeneration.

Conclusions: In conclusion, we successfully performed bulk and scRNA-seq on adult human utricles, delineating the earliest response to ototoxic damage. Furthermore, we are delivering the first human utricle gene atlas. Overall, these discoveries will advance fundamental knowledge in the field of inner ear regenerative medicine and pave the way for developing therapeutics for the treatment of balance dysfunction.

Uchl1 Inhibition Increases the Trans-Differentiation of Supporting Cells Into Hair Cells in the Neonatal Cochlea

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Category: Regeneration
Background: In the cochlear epithelia of adult mammals, non-sensory supporting cells become a subpopulation that can regenerate into hair cells after those are lost. Wnt target gene, Lgr5 is expressed in the supporting cells such as the third Dieter's cells, inner pillar cells and inner border cells, which can serve as hair cell progenitors. Our previous study in the neonatal cochlea showed that deubiquitinating enzyme, Uchl1 has a similar expression pattern to Lgr5 in a third Dieter's cell and pillar cells. In this study, we investigated the potential role of Uchl1 in the trans-differentiation of supporting cells into hair cells in neonatal cochleae.
Methods: The localization and mRNA level of Uchl1 in the Sprague Dawley-rat organ of Corti on embryonic day 17.5 (E17.5), postnatal day 1 (P1), 3, 5, 9 and 14 were evaluated using immunohistochemistry and qRT-PCR, respectively. The effect on differentiation was observed by analyzing the expression of Uchl1 under the conditions of hair cell differentiation using combined application of a γ-secretase inhibitor and a Wnt agonist. The rate of hair cell differentiation was also analyzed using the Uchl1 inhibitor, LDN-57444.
Results: Uchl1 was observed in the greater epithelial and lesser epithelial ridge cells and the site which hair cells will locate, at the cochlea of E17.5. This Uchl1 expression was graualy decreased as hair cells developed. From P2 to P7, the Uchl1 was restricted to the third row Dieter's cells and inner pillar cells. At P9, Uchl1 was only expressed in the efferent nerve fibers. Next, we demonstrated that inhibition of Uchl1 activity by LDN-57444 promoted the hair cell induction in response to γ-secretase inhibitors and Wnt agonist. Mechanistically, Uchl1 inhibitor increased mTORC1 activity and Sox2 expression. This increase accelerated hair cell differentiation of supporting cells into hair cells.
Conclusions: Uchl1-positive cells have the capacity to act as cochlear progenitor cells in neonatal mice through the mTORC1 pathway and proliferation of cochlear supporting cells. Uchl1 might be new therapeutic target for hair cell regeneration.

Supporting Cells Express Early Markers of Hair Cell Fate After Damage, but Cannot Progress to Myosin VIIA

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Category: Regeneration
Background: Spontaneous regeneration of HCs in the neonatal cochlea has been documented. This process occurs by two mechanisms: direct transdifferentiation and mitotic regeneration of supporting cells (SCs). HC regeneration has been defined by the expression of the HC marker, myosin VIIA, expressed by converting SCs. During development there are several transcription factors (TF), notably Atoh1, Gfi1, and Pou4f3, which are expressed in progenitor cells that take a HC fate prior to the expression of myosin VIIA. We investigated the expression of these TFs in SCs after HC damage in the neonatal and juvenile cochlea and observed a significant number of "transitional cells" that expressed one of more of these TFs, but failed to express myosin VIIA.
Methods: Pou4f3DTR mice were used to induce HC damage with diphtheria toxin (DT) (6.25ng/g, IM) injected on postnatal day (P)1 or P7. Cochleae were harvested on P7-P8 (after P1 HC damage) or P14 (after P7 HC damage), post-fixed in 4% paraformaldehyde at room temperature for 2 hours, and stored in 10mM phosphate buffered saline (PBS) at 4°C. Pou4f3DTR negative littermates that lacked HC damage were used as controls. To visualize Atoh1 and Gfi1, we crossed Pou4f3DTR mice with knockin reporter lines (Atoh1GFP and Gfi1GFP). Each cochlea was dissected using the whole mount method and stained with the following primary antibodies: rabbit anti-myosin VIIA, goat anti-Sox2, chicken anti-GFP, and mouse anti-Pou4f3 IgG1 F1016. Alexa-

conjugated secondary antibodies were used at a 1:1000 dilution. Slides were imaged using a Zeiss LSM800 confocal microscope, and processed using Zen Blue software.

Results: Preliminary results show that after HC damage at P7 or P14, many SCs attempt to convert into HCs by expressing Atoh1 and/or Gfi1, but not myosin VIIA. While in control samples without HC damage, both Atoh1 and Gfi1 are only expressed in HCs. Pou4f3 expression after damage at both ages is under investigation.

Conclusions: Regeneration of HCs has been defined by the expression of the HC marker myosin VIIA by SCs, however, our preliminary results suggest that many other SCs attempt to convert into HCs after HC damage, by expressing Atoh1 and/or Gfi1. Previous epigenetic evidence suggests that Atoh1 is maintained in a "poised, but transcriptionally silenced" state in P1 SCs, thus these SCs are able to activate Atoh1 expression in response to HCs damage. However, our results suggest a latent capacity for SCs to express Atoh1 that extends into the second postnatal week, and that downstream targets of Atoh1 and Gfi1 are likely epigenetically repressed which limits SC-to-HC conversion. Results from this study will further elucidate how far the "transitional cells" progress down the HC fate pathway, and may highlight potential therapeutic targets to induce HC regeneration.

Towards a Better Understanding of the Sensitivity of the Pupil Response During Speech Perception and its Relationship With Perceptual Effort Investment

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Category: Speech Perception

Background: Pupillometry is commonly used as an objective measure of listening effort. However, less is known about the sensitivity of the task-evoked pupil response and its relation to perceived listening effort in speech-in-noise perception conditions.

This study investigated the just noticeable differences (JND) of behavioral listening effort and their relationship to changes in pupil responses. The JND in effort was defined as the minimum increase in the speech-to-noise ratio (SNR) necessary for a person to perceive a difference in effort. Pupil responses were also obtained at behavioral JND in SNR, as well as at the just meaningful difference (JMD) in SNR, reflecting the minimum increase in SNR necessary for a person to seek intervention, inspired by earlier results from McShefferty et al. (2016). The pupil responses obtained at the JND in SNR, JND in effort and JMD in SNR were analyzed to explore the potential of pupillometry as a clinical tool.

Methods: Participants listened to blocks of 12 paired sentences consisting of a reference sentence at 0 dB SNR and a target sentence presented at a higher SNR. Five SNR increases were considered, ranging between 0.5 and 8 dB. The presentation level of the sentences was roved in 0.1 dB steps around 63 dB SPL. For each pair of sentences, listeners were asked to identify: (a) which sentence was clearer (to extract the JND in SNR); (b) if they noticed a difference in the effort they allocated between the two sentences (to extract the JND in effort); and (c) if they would change to new headphones that provide an improvement in clarity similar to the one presented (to extract the JMD in SNR). Pupillometry was recorded throughout all experimental blocks. Post-processing of the raw pupil data included blink detection and trial rejection, as well as baseline correction and range normalization.

Results: A psychometric function was fit to each of the participants' ratings, and the respective just-noticeable and just-meaningful differences were extracted. Additionally, changes in pupil responses were analyzed at each of the three SNR thresholds.

This study reports similar levels of JND in SNR and JMD in SNR as previously found in the literature. On average, the JND in effort lies between the JND in SNR and JMD in SNR, but substantial variability is found across individuals. Pupillometry data is expected to show a decrease in the changes of the pupil responses with increasing SNR changes.

Conclusions: This study contributes to a better understanding of the sensitivity of the pupil response during speech perception by extracting the individual changes of the pupil response at SNRs where people first perceive a change in SNR compared to when they first perceive a change in perceptual effort or when they would change their device.

DM Benefit in Adverse Listening Environments in Preschoolers With Hearing Loss

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Category: Speech Perception

Background: Many educational settings present challenges to speech understanding for young children who are deaf/hard of hearing (D/HH), because the auditory signal is degraded by noise in the environment and distance between the listener and the speaker. Most recently, virtual teaching has introduced another source of degradation due to the reduction and distortion of the frequency range transmitted by video conferencing software and computer hardware. One common strategy to improve auditory access in these situations is the use of a DM system. The current study evaluates the benefit of using DM technology in adverse listening environments in 3- to 5-year-old children who are D/HH.

Methods: Data was obtained as part of the annual assessment battery given to all 3- to 5-year-old children enrolled in a specialized listening and spoken language preschool program. The benefit provided by the DM system was measured by comparing performance with and without the DM system in three listening conditions: multitalker babble (+5dB SNR), quiet presentation level (35dB HL) and through video conferencing software (zoom). Speech perception in multitalker babble and at quiet levels was assessed using clinical assessments of speech perception (NU-CHIPS, WIPI,PBK-50). Speech perception through video conferencing software was assessed by measuring consonant discrimination.

Results: Preliminary data analysis indicates that, on average, children's performance improved when the DM system was used by 22% in multitalker babble and by 26% for quiet levels (34 children with bilateral mild- to profound hearing loss). The DM benefit in multitalker babble was similar for children with hearing aids (HA) (21%, n=22) and cochlear implants (CI) (23%, n=12). The DM benefit for quiet presentation level was slightly smaller for children with HAs (25%) than CIs (30%). Children's ability to discriminate consonants presented through video conferencing software improved, on average, by 6.5% when the DM system was used (9 children with bilateral mild- to profound hearing loss). In this condition, the DM benefit was similar for children with HAs (6.9%, n=5) and CIs (7.1%, n=4). In fact, performance with the DM system in all adverse listening conditions was similar compared to performance in the baseline quiet listening condition. The final analysis will include a larger number of children, including children with unilateral hearing loss, and a full description of their demographic, hearing loss and technology related information.

Conclusions: These preliminary results indicate that the use of a DM system can help preschool-aged children who are D/HH overcome the negative impact of signal degradation on speech understanding. The consistent use of DM systems in early childhood settings is therefore a powerful tool for providing clear access to spoken language and supporting learning in preschoolers with hearing loss.

Assessment of Staggered Spondaic Words in Those With and Without Neurocognitive Deficits

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Category: Speech Perception

Background: Neurocognitive deficits in those infected with human immunodeficiency virus (HIV) is a significant public health problem. Auditory tests that tax the central auditory pathway may be able to provide a window into neurocognitive function. Previous studies show that HIV positive individuals develop signs of central auditory processing deficits which cannot be attributed to peripheral hearing loss. Previous cross-sectional studies show a relationship between central auditory and cognitive measures, but sensitive measures of central auditory processing that identify neurocognitive dysfunction do not exist. We have been performing a battery of central auditory and cognitive measures on a cohort of HIV+ and HIV- individuals in Shanghai, China to understand how the central auditory system could be used to identify, track, and potentially predict neurocognitive dysfunction. Specifically we have been using the Staggered Spondaic Words Test (SSW) as a metric of dichotic central auditory processing as a measure of neurocognitive dysfunction in those with HIV. The goal of the project was to determine if the SSW could identify neurocognitive dysfunction in those diagnosed with HIV.

Methods: Cognitive performance on a battery of neurocognitive measures, as well as the SSW, was performed on 58 individuals (30 with HIV and 28 HIV-, age range 20 to 54). Cognitive tests included the Montreal Cognitive Assessment (MoCA), and a custom battery of neurocognitive tests developed by Robert Heaton PhD to assess HIV Associated Neurocognitive Disorder (HAND). Primary analyses focused the relationship between SSW scores (errors) and neurocognitive impairment defined by either global deficit score (>0.5) or MoCA (<26). Data were analyzed using a logistic binomial regression model.

Results: SSW errors were significantly related to neurocognitive impairment on both global deficit score and MoCA (all p<.001). Results showed that as the probability of errors increased on SSW, the probably of cognitive impairment also increased. The interaction of age and cognitive impairment on SSW errors also yielded a significant result (p<.001) of consistent increased probability of cognitive impairment with more errors on the SSW and as age increases.

Conclusions: Results were consistent with a positive significant relationship between SSW errors and cognitive impairment, suggesting that performance on the SSW may be a sensitive measure to screen and identify impairment in HIV+ individuals. The SSW is a quantitively rich central auditory test that can be analyzed in various ways (Decoding, Tolerance Fading Memory, Integrations, and Organization). Further analysis using the SSW could potentially provide a sensitive and specific screening measure to monitor cognitive decline in patients with HIV, making it a useful surveillance tool for this major public health problem.

Impact of Data Quantity and Model Complexity on Temporal Response Function Analyses of Electrophysiological Responses to Continuous Competing Speech
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Category: Speech Perception

Background: Understanding how meaning is extracted from complex speech signals by auditory and language processing hierarchies is a key goal of cognitive and auditory neurosciences. In recent years, methods utilizing electro- and magnetoencephalographic (M/EEG) responses to continuous speech to derive neural signatures of lower- and higher-level speech processing, known as temporal response functions (TRFs), have become increasingly popular. While these methods have led to novel insights about speech processing, less is known about how they perform under various constraints, such as the data quantity and the number of speech features used in the analysis. Here we addressed these practical unknowns in the context of neural processing of continuous dual-talker paradigm.

Methods: Forty one adults aged 18-70 yrs participated in a noninvasive EEG recording from 64 surface electrodes, while attending to one of two diotically presented audiobooks (65 dB SPL each). The audiobooks were characterized both in terms of lower-level features including the acoustic envelopes, word onsets, and word-level audibility, as well as a higher-level feature characterizing relation of each word to preceding context, termed lexical surprisal. These features were used to generate time-resolved regressors aligned with the EEG data, for both the attended and ignored stories. We then used regularized linear regression to iteratively fit models containing subsets of these features using progressively more data ranging between 2 and 41 minutes, resulting in a total of 11 analyses per model. Finally, we compared both the TRFs generated by these analyses and the model goodness-of-fit values across the 11 analyses.

Results: Our results indicated that both lower- and higher-level responses were detectable with just minutes of data, although both the model fit and TRF estimates monotonically improved with more data up to 41 minutes. Moreover, although for sparser word-level features the overall model goodness-of-fit values significantly exceeded zero even for small amounts of data, comparisons to null model performance based on permuted features revealed that veridical feature values only improved goodness-of-fit when >20 minutes of data were included in the fitting stage. This indicates that smaller data quantities may allow for capturing more stereotypical aspects of neural responses, but they miss aspects of responses that vary as a function of the specific feature values. Lastly, we observed that while the envelope TRFs appeared relatively similar across analyses, they grew in amplitude as more data were included, which was attributable to monotonic decrease in the regularization parameter with more data. Due to this interplay between data quantity and regularization, across-study comparisons of TRF amplitudes may not be advisable.

Conclusions: Overall, our results confirm that duration of data acquisition is a critical parameter in multi-feature studies of speech processing that needs to be carefully considered during study design.

The Use of Speech Temporal Cues in Phonetic Processing: An Electrophysiological Study With Infants and Adults
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Category: Speech Perception

Background: Before 10 months of age, infants are not yet attuned to the consonant contrasts of their native language, meaning that, compared to adults, they are sensitive to certain non-native phonological contrasts. The nature of the mechanisms shaped by age and exposure to the native language is yet to be discovered. The current project hypothesizes that auditory mechanisms supporting speech perception may play a crucial role in perceptual attunement. The project aims to explore the interaction between auditory and speech perception abilities during early development by looking at the neural correlates underlying the processing of specific speech acoustic cues.

Methods: This study adopts a psychoacoustic approach suggesting that the auditory system decomposes the spectral and temporal (amplitude and frequency modulations: AM/FM) components of speech. Those acoustic cues can be selectively manipulated using vocoders to assess their role in speech perception. Recent studies have suggested that 6-month-olds with normal hearing weigh fast AM cues (> 8 Hz) more heavily than adults for consonant discrimination. To explore the neural underpinnings of this development, we used electroencephalography (EEG) to measure the cortical auditory evoked potentials (CAEPs) underlying auditory detection of native and non-native consonants in French-learning 6-month-old (N=20), French-learning 10-month-old (N=20), and French adult listeners (N=20). We used vocoders to process three syllables: French-voiced /aba/, French-unvoiced-unaspirated /apa/, and an English-unvoiced-aspirated /apha/. Three vocoder conditions were designed to preserve: i) original FM and AM ("Intact"), ii) original AM ("Fast"), and iii) only the slowest AM ("Slow").

Results: Overall, effects of FM degradation are observed for neural auditory processing of 6-month-olds, 10-month-olds and adults. There is then no further effect of AM degradation on CAEPs for any of the three age groups. This result is counterintuitive to the findings of previous behavioral literature showing that young infants may rely more on faster AM cues than slow one compared to adults. Nevertheless, differences are observed between Phoneme and Vocoder conditions over age. The effect of FM degradation is significant for /aba/ and /apa/ at 6 months, for /apha/ at 10 months, and for /apa/ and /apha/ in adults. The further effect of Fast AM degradation is only present in 10-month-olds and adults for the non-native /apha/.

Conclusions: Taken together, these results demonstrate an effect of temporal degradation on CAEPs, and an interaction between phoneme type and temporal degradation; whether this effect is related to the acoustic properties or the linguistic properties of voicing and aspiration needs to be further investigated.

Deep Neural Networks Effectively Model the Dynamics of Neural Adaptation to Changing Background Noise

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Category: Speech Perception

Background: The human auditory pathway displays a robust capacity to adapt to sudden changes in the statistics of an auditory scene, an ability that helps in many real-world scenarios, such as tracking a conversation while walking through a door from a street to a loud restaurant. While this neural ability has been identified and characterized, its mechanism is not well understood due to the difficulty of interpreting such a nonlinear behavior. In order to understand how the brain achieves this adaptation, an important first step is to analyze a model that can mimic the brain's behavior.

Methods: Traditional models such as the spectro-temporal receptive field (STRF) are highly interpretable, due to their linearity, but fail to capture the nonlinear dynamics of neural adaptation. To overcome this limitation, we employ recent advances in a certain class of convolutional neural network models (CNN). These models have a greatly improved capacity to learn nonlinear transformations and can be easily interpreted as dynamic STRFs (DSTRF), since a linear equivalent function to the CNN can be found for each stimulus instance. This provides a means of interpreting the nonlinear operations of the network as stimulus-dependent linear functions. In this study, we recorded intracranial EEG (iEEG) from neurosurgical patients who were asked to attend to speech overlaid on background noise that kept changing between several categories, and we trained CNN models to predict their neural responses.

Results: We first demonstrate that a CNN trained to predict neural responses can produce the same rapid adaptation phenomenon as the auditory cortex significantly better than a linear STRF model. Additionally, this model can replicate a more complex phenomenon arising from adaptation, where phoneme discrimination momentarily drops and background noise encoding increases following a noise change, after which noise information is suppressed and phoneme discrimination is restored. We further analyze the model's computations and show that some neural regions react to a sudden change in background noise by altering their filters to deal

with the new sound statistics. The model's equivalent filters exhibit gain changes and shape changes in order to deal with the new noise, with filter changes occurring in the first few hundred milliseconds after noise transitions. **Conclusions:** By looking at how these filters change over time particularly when adapting to a new background noise, we provide evidence that can explain how the brain adapts to changing noise conditions. Hence, we show that modeling the auditory cortex with deep neural networks can help us understand the neural computations that underlie noise-robust speech perception in real-world environments.

Distinct Neural Encoding of Glimpsed and Masked Phonetic Features in Multitalker Speech Perception

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Category: Speech Perception
Background: Speech perception in multitalker acoustic environments is a challenging task requiring a listener to extract and group the phonetic features of a target talker from those of non-target talkers. Because speech is a structured sound composed of sparsely distributed regions of energy with redundant information, it is hypothesized that listeners rely on glimpses of spectrotemporal regions in which one talker contains more energy than the others. The glimpsing hypothesis has been tested with computational and behavioral models to show that glimpses contain sufficient information to support speech recognition. While the glimpsing model makes no claims regarding the processing of masked phonemes, one possibility, supported by the phoneme restoration effect, is that masked phonemes may be restored at the acoustic-phonetic level using glimpsed information. Despite the computational and behavioral evidence, the neuroscientific evidence for the glimpsing model is lacking. Specifically, we do not know if glimpsed and masked phonemes of target and nontarget talkers are encoded differently in the human auditory cortical areas. It is also unclear how attention to a talker changes the processing of glimpsed and masked phonemes of that talker.
Methods: Here, we obtained intracranial EEG recordings in primary and nonprimary auditory cortex, including Heschel's gyrus (HG) and superior temporal gyrus (STG) respectively, while subjects attended to one talker in a co-located two-talker mixture. We used banded ridge regression encoding models to predict the high-gamma band envelope of neural responses using acoustic, phonetic, and phonotactic features of the target and non-target talkers.
Results: We confirmed that lexical processing beyond phonetic features is only present for the target talker. In processing phonetic features, neural responses were more accurately predicted using separate glimpsed and masked phonetic representations. In particular, we found a robust encoding of glimpsed phonetic features of both target and non-target talkers in HG, as well as a weaker encoding of masked phonemes of the target talker. We also found that STG partially represents glimpsed non-target phonetic features, as well as both masked and glimpsed phonetic features for the target talker, with masked phonetics encoded with an additional 100ms delay.
Conclusions: Together, these findings provide neural evidence for the glimpsing model, suggesting that the auditory cortex selectively uses glimpses of the target talker to restore masked speech at the acoustic-phonetic level, leading to a complete and invariant representation of target talker phonetic features in STG that can simplify noise-invariant lexical and semantic processing in downstream auditory cortical areas.

Characterization of the Neonatal Frequency-Following Response Elicited to the /oa/ Stimulus

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Category: Speech Perception
Background: The Frequency-Following Response (FFR) is an auditory evoked potential elicited to complex stimuli such as speech or music, which has gained recent interest as it captures with great fidelity the tracking accuracy of the periodic sound features in the auditory hierarchy. The FFR appears disrupted in children with neurodevelopmental disorders and speech and language impairments, and it is sensitive to musical and language exposure. This supports the idea of using the FFR as a possible biomarker for speech processing impairment and literacy achievements. However, characterization of the rich information provided through different FFR parameters in the normal neonatal population has not yet been provided with regard to the encoding of the

stimulus envelope and temporal fine structure of speech sounds. The establishment of a normative database of the neonatal FFR was hence the main objective of this study.

Methods: FFRs elicited by a two-vowel stimulus (/oa/) were recorded in a sample of 95 neonates born healthy and at term. The used /oa/ stimulus was tailored to extract both pitch and formant structure encoding accuracy (Arenillas-Alcón et al., 2021; Sci. Rep., 11, 6660). All of the neonates passed successfully the Universal Hearing Screening Test and a click ABR before the FFR recording.

Results: FFR parameters were extracted in the time and frequency domains to characterize the normality of this response in neonates. Descriptive statistics were depicted for each parameter computed as mean and standard deviation. Results are presented here as an extended database for the FFR parameters normality in neonates born healthy at term.

Conclusions: The present study supports the possibility of recording the FFR as a clinical tool in hospital settings. This measure could be used to assess early abnormalities in their response that could be associated to later language impairments, and to establish early preventive measures during their first days of life when plasticity of the underlying neural tissue is optimal.

Effects of Low-Level Noise Therapy on Perceived Loudness and Objective Auditory Measures: Preliminary Results

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Category: Tinnitus

Background: Background: Hyperacusis is an intolerance to moderately-loud sounds with a prevalence of ~9% in the adult population [1]. Animal studies suggest that hyperacusis may result from enhanced sound-evoked neural activity due to an imbalance in excitatory/inhibitory network control along the auditory neuroaxis [2]. In animals, prolonged exposure to low-level noise (LLN) can reduce sound-evoked activity in sub-cortical and cortical auditory nuclei [3 4]. In humans, using LLN therapy for several weeks has shown to elevate the intensity at which sounds become intolerably loud [5], this conceivably would reverse reduced loudness tolerance in patients with hyperacusis. However, there remains little evidence that links LLN-induced elevated loudness discomfort levels to reduced sound-evoked neural activity in humans. This project objectively assessed sub-cortical and cortical auditory regions before and during LLN noise therapy. The goal was to (1) determine if self-reported change in loudness was linked to reduced sound-evoked activity and (2) evaluate the utility of clinically available objective tools to assess noise therapy effectiveness at treating abnormal loudness symptoms.

Methods: Methods: Seven adults that self-reported abnormal loudness tolerance (hyperacusis questionnaire score) used ear-level noise therapy for a minimum of 12 h/day for 3 weeks. Noise therapy consisted of a 1-4 kHz band-passed noise; the spectrum of noise was confirmed using on-ear probe-mic measures. Consistent usage of noise therapy was monitored by data logging the average hours/day from the device on a weekly basis. A test battery that included subjective questionnaires, auditory brainstem response (ABR), cortical auditory evoked potentials (CAEPs), acoustic reflex thresholds (ARTs) and auditory reaction-time intensity (RT-I) functions were collected before noise therapy began and on a weekly basis for three consecutive weeks.

Results: Results: Participants adhered to the consistent use of noise-therapy (mean ~12h/day). After two weeks of noise therapy participants self-reported a significant decline in the function impact of their loudness intolerance, this remained declined after three-weeks (repeated one-way ANOVA, $p<.05$). There was no significant change in ARTs evoked with .5 or 1 kHz tones, but there was a significant increase in ARTs evoked with a 2 kHz tone after three weeks of noise therapy (repeated one-way ANOVA, $p<.01$). There was no significant change in ABR amplitude or latency (wave V), or CAEP (P1) amplitude input/output functions. However, CAEP latencies were significantly longer after 3 weeks of noise therapy (repeated two-way ANOVA, $p<.05$), primarily those evoked with moderately-high intensities. There was no significant change in RT-I at any time point compared to baseline.

Conclusions: Conclusions: These preliminary findings suggest that noise therapy may improve (increase) loudness tolerance by reducing sound-evoked neural activity in both sub-cortical (ARTs) and Cortical (CAEPs) auditory regions of the brain. With further characterization, these objective tests may serve as a method of evaluating treatment efficacy for patients with hyperacusis.

Hyperacusis Correlates in Noise Overexposed Guinea Pigs

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Category: Tinnitus

Background: Hyperacusis is characterized by steepened loudness growth, collapsed sound intensity tolerance, and faster reaction times to sounds (for review, see Tyler et al, Am. J. Audiol., 2014). Tinnitus, phantom sound perception, is frequently comorbid with hyperacusis, as both commonly arise following cochlear damage. Following noise-overexposure, ventral cochlear nucleus (VCN) bushy cells exhibit firing patterns consistent with psychophysical characteristics of hyperacusis (Martel and Shore, Sci. Rep., 2020). Since bushy cells contribute to the auditory brainstem response (ABR), hyperacusis-related increases in bushy-cell excitability may underlie increased ABR wave amplitudes. Furthermore, increased ABR wave amplitudes correlated with hyperacusis and tinnitus behavioral measures in mice following ototoxicity (Longenecker et al, Front. Neurosci, 2020). Here, we further elucidate the bushy-cells role in hyperacusis and tinnitus in guinea pigs, using a novel hyperacusis behavioral test.

Methods: Acoustic startle (pinna) reflexes (ASR) were measured to assess hyperacusis and tinnitus. Hyperacusis was assessed by measuring changes in startle amplitude and reaction-time latency post-noise-overexposure (Chen et al, JARO, 2013). Broadband noise pulses (BBN; 2ms duration), clicks (100us/phase) and upsweep chirps (100Hz-30kHz; 2.1ms duration) were presented over a range of intensities (60-100 dB SPL; 10 dB steps; 15-20s intertrial interval). One session consisted of five repetitions of each unique intensity-sound combination. Tinnitus was assessed using gap/prepulse-inhibition of the ASR (Berger et al, J. Neurosci. Methods, 2013). For each session, background carrier bands (3-6,8-16kHz) were presented at 65 dB, while the startle reflex was activated using a BBN pulse (2-20kHz; 20ms; 0.1ms rise/fall). Prepulse stimuli were a gap or sound-pulse inserted into the background carrier (50ms; 5ms rise/fall; 50ms delay r.e. startle). Twenty trials were presented for each carrier band.

ABRs were measured using tone-pips (8-20 kHz, 4kHz-steps; 5ms; 0.5ms rise/fall), clicks (100us/phase) and upsweep exponential chirps (100Hz-30kHz; 2.1ms duration). ABRs were measured at baseline to establish normal hearing (N=7). Four weeks of baseline behavioral data were collected. Guinea pigs were anesthetized (ketamine/xylazine) then exposed to unilateral narrowband noise (103 dB SPL; 7kHz centered, quarter octave band) in a temporary-threshold shift paradigm. ABRs (every two weeks) and behavioral assessments (biweekly) continued for an additional twelve weeks.

Results: We found that a single noise-overexposure can induce both tinnitus and hyperacusis (N=3/7; hyperacusis-alone: N=1/7). Preliminary results in hyperacusis animals indicate that ABR amplitude-intensity functions are steeper-and-with greater amplitudes compared to animals with neither hyperacusis nor tinnitus.

Conclusions: Consistent with other studies, we found that hyperacusis and tinnitus are co-morbid following auditory damage. Moreover, ABR enhancements at suprathreshold intensities occurred in hyperacusis animals, suggesting that VCN bushy-cell firing patterns and hyperacusis behavior are linked. Future studies will investigate precisely how VCN bushy-cell firing patterns contribute to hyperacusis-related ABR enhancements.

Acute Tinnitus is Independent of Thalamic Firing Alterations

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Category: Tinnitus

Background: The auditory thalamus exhibits oscillatory neural firing that is implicated in stimulus gating, discrimination, and novelty coding. Intersecting both the sensory and the limbic circuits, auditory thalamic rhythmicity has been suggested as the neural substrate for the affective component of tinnitus. However, other than correlational and brain imaging studies in humans, the "thalamic dysrhythmia" theory has not been studied adequately in animal models.

Methods: Herein, we recorded extracellular unit activity from the ventral and medial divisions of the medial geniculate body (MGB) prior to, during, and up to 8 hours after a unilateral, 2-hour noise exposure at 97 dB SPL. Tinnitus was confirmed using simultaneous recordings in the dorsal cochlear nucleus (DCN), where unit activity was instantaneously classified as tinnitus or no-tinnitus using a supervised machine learning classifier.

Results: In animals that showed a tinnitus phenotype immediately after the cessation of noise exposure, there was increased spontaneous firing and bursting at a larger magnitude than in the DCN. However, both noise-exposed tinnitus and non-tinnitus animals exhibited a slower oscillatory pattern (reduced frequency). Non-exposed controls confirmed that reduced oscillatory frequency was the direct consequence of noise exposure.

Conclusions: These results show that altered thalamic oscillations did not correlate with acute tinnitus, thus questioning the auditory thalamic dysrhythmia theory of tinnitus generation.

Evidence of Tinnitus-Specific Differences in Stimulus Evoked Brainstem Potentials

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Category: Tinnitus
Background: Research on tinnitus, a phantom perception of sound, is limited by the fact that animal models of tinnitus require expensive and time-consuming behavioral tests. A fast, objective test is needed. Here, we tested animals with and without behavioral signs of tinnitus for changes in brainstem electrophysiological activity after application of a novel stimulus paradigm (NSP) for acoustic stimulation (patent pending).
Methods: Tinnitus was induced in awake CBA/CaJ mice by unilateral continuous sound exposure using 2 kHz wide, 16 kHz centered noise at 113 dB SPL. Tinnitus status was determined behaviorally 8 weeks after exposure. Brainstem evoked potentials similar to auditory brainstem responses (ABR) were collected in response to our NSP in anesthetized mice. This included responses to pure tone pips at three or more frequencies delivered to each ear separately. For tinnitus mice, the frequencies were determined by the tinnitus frequency indicated by behavioral testing, plus stimuli ±1 octave. Non-tinnitus and control mice were tested with frequencies matching the sound exposure frequency plus stimuli ±1 octave.
Results: We measured peak and trough amplitudes in the tone-pip evoked potentials produced by our NSP to calculate tinnitus scores for each waveform. When comparing the peak-trough scores, the interaction between tinnitus and exposure was significant (2-way ANOVA, $p=0.0027$). Specifically the non-tinnitus exposed ear (NTE) scores were significantly less than those in the tinnitus exposed ear (TE) (Tukey test, $p=0.0056$). Peak latency in the evoked potentials was also analyzed but was not significantly different.
Tinnitus is associated with increased central gain, so we calculated the V/I ratio in the evoked potentials in response to our NSP. When comparing the average V/I tone-pip responses of TE to the control responses, the V/I ratio is significantly larger than control (Student's T-test, $p=0.04$), consistent with the idea that tinnitus animals have increased central gain.
A bootstrapping method that randomly compares multiple evoked potentials and correlates them was used to compare responses to our NSP. In TE, the standard deviation of the responses was increased only at the tinnitus frequency. Furthermore, in tinnitus mice, the mean R values at the tinnitus frequency and the lower frequency were significantly different. These patterns were not seen in control groups.
Conclusions: Taken together, these data identify quickly attainable metrics that correspond with performance on behavioral tests of tinnitus.

Somatic Maneuvers and Their Effect on Tinnitus

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Category: Tinnitus
Background: Tinnitus is the perception of sound in the absence of external auditory input. The most common factor associated with tinnitus is hearing loss. Up to 80% of tinnitus patients can modulate their tinnitus by movements of the head or neck – a phenomenon termed "somatic or somatosensory tinnitus (Levine, Abel; CRANIO, 2004)." Herein, we examine the relationships between hearing threshold, somatic tinnitus, and perceived tinnitus severity.
Methods: Data from 207 participants were used for this analysis. Modulation assessments were performed either in-person in a standard audiological clinical sound booth, or remotely while participants were in a quiet room. Standard pure tone audiometry was performed, and the Tinnitus Functional Index (TFI; Meikle et al, 2012) questionnaire was utilized to assess reactions to tinnitus. To quantify somatic-induced changes, participants were instructed to report increases or decreases in tinnitus loudness on a 0-4 scale (0 = no change; 4 = greatest change) (Roberts reference/Levine reference). Modulation scores were grouped by cranial nerve (CN) innervation: CN III, IV, VI for eye maneuvers, CN V for jaw, CN VII for cheek maneuvers, CN XI and dorsal column spinal nerves

for neck maneuvers, and CN XII for tongue maneuvers. CN XI and dorsal column spinal nerves are also separated by a subset of maneuvers: Passive, Active, and Active with Resistance.

Results: The mean number of modulations that altered tinnitus loudness per subject was 16.14. Further, more than half of somatic maneuvers increased tinnitus loudness. Somatic maneuvers involving CN V, XI or spinal nerves were the most likely to alter tinnitus loudness (counts/proportions). For CN XI and spinal nerve maneuvers, Active with Resistance maneuvers consistently elicited a change in tinnitus loudness, while Active maneuvers elicited more increases than Passive movements. TFI scores positively correlated with the sum of effective modulations ($r=0.216$; $p=0.004$), suggesting that subjects with more effective modulations had more bothersome tinnitus. Moreover, pure tone average (PTA; 500Hz, 1kHz, 2kHz, 4kHz) and the sum of effective modulations were inversely correlated ($r=-0.118$; $p=0.011$).

Conclusions: The negative correlation between PTA and effective somatic maneuvers suggest that better hearing thresholds were associated with more effective somatic modulations. Furthermore, greater tinnitus severity was associated with an increased ability to modulate tinnitus with a somatic maneuver. Interestingly, jaw and neck movements were associated with more effective modulations, supporting previous work showing that projections from these regions activate the cochlear nucleus neurons linked to tinnitus generation.

Feasibility of an Inertial Measurement Unit Sensor-Based Guiding System for the Treatment of Benign Paroxysmal Positional Vertigo

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Category: Vestibular: Basic Research and Clinical

Background: Although canalith repositioning procedures (CRP) by physicians are effective in treating benign paroxysmal positional vertigo, lower success rates of self-treatment with Epley and Barbeque roll maneuvers is an important issue because of the high recurrence rate of benign paroxysmal positional vertigo.

To validate the feasibility of an inertial measurement unit sensor-based CRP guiding system (IMU-CRP) by analyzing accuracy differences in required rotational angles compared to education-based conventional CRP (EDU-CRP).

Methods: This was a prospective, comparative effectiveness research, conducted between July 2019 and January 2021. We enrolled 19 subjects (15 females, mean age: 62.8 ± 1.9) without active vertigo and no prior knowledge of benign paroxysmal positional vertigo or CRP. After reading and explaining the procedural handouts, all subjects conducted the Epley and Barbeque roll maneuvers with and without auditory guidance of the real-time head monitoring system (EDU-CRP vs. IMU-CRP) twice. Both CRPs were compared based on the accuracies of head rotations. The target criteria of head rotational angles in all CRP steps were determined based on the American Academy of Otolaryngology-Head and Neck Surgery guidelines. Differences between expected and measured angles were considered errors. Errors between EDU- and IMU-CRPs were compared in all CRP steps, and CRP test-retest variations were also evaluated.

Results: For all steps in the Epley and Barbeque roll maneuvers, the absolute errors were smaller for IMU-CRP than for EDU-CRP. Significant differences were observed in steps 2-4 of the Epley maneuver and in steps 3-6 of the Barbeque roll maneuver. A learning effect was not found in the Epley maneuver but was observed in steps 4 and 5 of the Barbeque roll maneuver.

Conclusions: Real-time feedback on the head rotation angle induced more appropriate movements when performing the Epley and Barbeque roll maneuvers which can be used for the treatment of benign paroxysmal positional vertigo. A guiding device based on head monitoring providing real-time auditory feedback will be beneficial for increasing the success rate of self-administered CRPs.

Preliminary Study on the Effectiveness of the Geneva Balance Test (GBT) on Children with Bilateral Vestibulopathy

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Category: Vestibular: Basic Research and Clinical

Background: Vestibular deficits are considered to be rare in children, but the lack of systematic screening leads to underdiagnosis. The few studies made on this topic have demonstrated that chronic vestibular dysfunction impacts

normal psychomotor development of children. Early screening of these children is needed to allow for optimal follow-up and clinical management, ensuring better global development. To this purpose, our research group has developed a screening test aiming to quantify the balance capacity of children over a broad age range, the Geneva Balance Test (GBT).

Methods: The aim of the study was to determine the possibility of quantifying balance deficits in children with bilateral vestibulopathy (BV) using the GBT. We conducted an observational prospective study in a population of 11 children with BV. Two age-matched control groups were included in the study, composed of (1) 15 healthy children without vestibular or auditory disorder (HS) and (2) 11 pediatric cochlear implant recipients (CI) without vestibular disorders (since sensorineural deafness is a frequent comorbidity in children with BV). Results of the three populations have been compared in 3 different age groups (3-5 years, 6-9 years, ≥10 years), and with results of the Bruininks-Oseretsky Test of Motor Proficiency Ed. 2 (BOT-2).

Results: Statistical analyses demonstrated significant differences in the scores of the GBT between children aged 3-5 and ≥10 years with BV and both control populations (HS and CI). A similar tendency was observed in the 6-9 years group, but results did not reach significance for this smaller group. Children in the youngest CI group (3-5 years) showed intermediate balance capacities (worse than HS but better than BV). These seemed to normalize in the 6-9 years group. All the results of the GBT were significantly correlated with BOT-2 results, but the GBT was better tolerated in all patient populations. The GBT scores correlated significantly with subject age.

Conclusions: In this small study, the GBT allowed to quantify balance deficits in children with BV. These results are comparable with the results of the BOT-2, which has been validated by multiple studies and is currently used in the clinic. However, the BOT-2 test is not validated for children <4,5 years of age, and results quickly saturate reaching maximum values already at 6-9 years. The GBT showed potential to be a useful tool to monitor the development of balance capacities with age and as such could be used in the follow-up of children with BV. Interestingly, we observed a significant difference in balance capacities in very young CI children which normalized by 6-9 years, potentially due to hearing rehabilitation with the CI. A study with a larger population is necessary to confirm these results.

Reduction of Severity of Self-Reported Vertigo by Bone Conduction Masking in a Real-World Setting
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Category: Vestibular: Basic Research and Clinical
Background: Vertigo is a common problem, often involving excessive or asymmetric activation of the vestibular system (vestibulogenic vertigo). Current solutions to these forms of vertigo are few, the most common involving the use of pharmaceuticals, which take a while to take effect and when used for motion sickness outlast the cause. OtolithLabs has developed a device, the OtoBand, which we posit masks information from the vestibular system (akin to noise maskers for tinnitus), thereby reducing sensations of vertigo/dizziness. The OtoBand is a small device placed over the mastoid, vibrating at a bone conduction power level within a narrowly defined range. We have shown a previous iteration of the OtoBand mitigated motion and VR sickness.

Methods: In response to all our clinical trials being halted in 2020 in academic centers, we developed a TeleHealth pilot study. Participants (18-70) recruited online, with self-reported chronic vertigo and a Dizziness Handicap Inventory >35 were invited. After consent, they received an OtoBand at their home. The OtoBand was programmed to work for 14 days following first use and had 4 power levels to choose from. Participants were instructed, when they developed an episode of vertigo, to fill out a questionnaire on their vertigo, nausea, standing balance and walking gait. They filled the questionnaire again within 5 minutes of putting the OtoBand on. The OtoBand was limited for any single use to 11 hours of continuous operation. After 14 days, the OtoBand was returned to our company, and participants' use data extracted and compared to questionnaire contents.
As this was a dosing study, there was no sham device. We will also present results from a currently completin cross-over design trial in which participants with a medically documented diagnosis receive in a randomized order a study device, and a sham device.
Results: 87% of participants reported their vertigo, balance and walking gait was helped by the OtoBand. Overall, the mean reduction in vertigo score (measured on a scale from 0 to 6) from "just before" to within "wearing the OtoBand for 5 minutes" was 0.8 across all participants, with a Cohen d (effect size) of 0.83. The mean reduction for the upper quartile of participants was 1.4 (out of 6). When considering the worst episode of vertigo, i.e. episodes with highest vertigo "just before", the mean reduction in vertigo across all participants was 1.83 with an effect size of 1.81.

Participants in our study were representative of all forms of vestibulogenic vertigo, namely BPPV, Migraine Associated Vertigo, Labyrinthitis/Vestibular Neuritis and Meniere's disease.

Conclusions: OtolithLab has developed a non-pharmaceutical intervention that is effective at mitigating vertigo and gait issues in people with vestibulogenic vertigo such as BPPV, Migraine Associated Vertigo, Meniere's disease and vestibulopathies (Vestibular neuritis, Labyrinthitis) and 3PD and MdDS.

Translational Vestibulo-Ocular Reflex in Adults With Central Visual Field Loss
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Category: Vestibular: Basic Research and Clinical
Background: Age-related macular degeneration (AMD) occurs in over 7 million individuals in the United States alone (Klein et al. 2011), and is the most common cause of central field loss (CFL). Loss of vision in general, and foveal vision in particular, has significant implications for patients' quality of life, and presents risks such as falling. The translational VOR (tVOR) compensates for linear motion through the environment and is needed whenever an individual translates in space (e.g., walking, driving). The velocity of the tVOR is scaled with movement direction and viewing eccentricity relative to the fovea, with the amplitude of the response being smallest for gaze along body motion axis and increasing with gaze eccentricity relative to this axis (Hess and Angelaki, 2003). The problem is more complex in individuals with CFL who may have an eccentric eye position for a central gaze position (where gaze is defined as the orientation of the eccentric preferred retinal locus used in individuals with a damaged central retina). One possibility is that individuals with CFL will have compensatory eye velocities that are more appropriate for foveal than PRL eccentricities for all viewing locations.
Methods: To investigate whether individuals with CFL have tVOR comparable to individuals with foveal vision and how it might be scaled, we vertically translated 3 participants with binocular or monocular CFL (1F) and 4 age-matched controls (4F) at a maximum peak-to-peak amplitude of 5 cm and two motion frequencies: fast (0.76 Hz, vestibular-only) and slow (0.2 Hz, visual) while seated on a 6-degree of freedom motion platform (DOF Reality, USA) with their heads fixed to the chair with a foam strap. Eye movements were tracked using a head-mounted, binocular eye tracking goggles (Pupil Core, Pupil Labs, Germany) and head motion was tracked using a 6-axis inertial measurement unit (IMU, LPMS-Research, Japan). Participants viewed a target at a 30 cm viewing distance, placed either centrally, or eccentrically at 10°.
Results: For central targets, participants with CFL had comparable tVOR (fast) and smooth pursuit (slow) to controls. For the fast motion and eccentric viewing condition, the two CFL participants with near-central binocular fixation (<2°) performed similarly to controls and to the central viewing condition. All participants with CFL had a slight increase in eye velocity with eccentric viewing for the slow condition. Interestingly, the participant with greatest viewing eccentricity (6° fovea-PRL distance) had a significant increase in eye velocity for eccentric, compared to central fixation during the fast (tVOR) condition.
Conclusions: While this change is consistent with prior work showing an increase in eye velocity for increasing target eccentricity, additional participants are needed to confirm this relationship. Should it hold, this relationship would suggest that tVOR eye velocity is scaled to the foveal, not PRL location.

Influence of Visual Field Motion on Vestibular Evoked Myogenic Potentials While Standing
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Category: Vestibular: Basic Research and Clinical
Background: While navigating through the environment, we constantly receive multisensory arrays informing us about our relationship in the surrounding environment. Visual-vestibular integration is particularly important for self-motion perception. Vestibular-evoked myogenic potentials (VEMP) are highly plastic to changes in human-environment dynamics (i.e., video gaming, visual field motion). Prior work has focused on the pre-/post-exposure modulation of VEMP testing with the standard procedure in supine. We attempted to examine the modulation during visual field motion (VFM) and its relationship to balance control.
Methods: Twelve neurotypical adults (8F; 28.9±5.4 years) without concussion history participated. CVEMP was measured (Interacoustics EP25; Denmark) while subjects stood inside of an immersive virtual reality environment consisted of a 1m-radius dome (Bertec CDP/IVR; Columbus, OH). While standing inside of the dome, subject's frontal plane was perpendicular to the meridian. To carry out cVEMP, subjects turned only their heads in yaw

allowing their heads to turn away from the cVEMP testing side while maintaining sufficient SCM basal contraction. VFM was presented as pitching up (relative to the trunk; backward) at 0°/s, 5°/s, 15°/s, 30°/s. Air-conducted sound stimuli consisted of 500Hz, 100dB nHL tone-burst (2ms rise/fall, 2ms plateau), a repetition rate of 5.1Hz, and 100 sweeps/trial. Electromyographic activity was obtained from sternocleidomastoid; referenced to sternum; grounded to the forehead. Latency and normalized amplitude of P13 and N23 were identified. Additionally, center-of-pressure (COP) data were recorded and analyzed for posturography and stabilogram diffusion analysis (SDA) parameters. Repeated-measures ANOVA tested condition effects on cVEMP and COP parameters. Pearson correlation tested the correlation between cVEMP and COP parameters.

Results: Significant condition effect emerged in P13 normalized amplitude (nAmp) only in left ears (ps<.01); suggesting vestibular laterality. Specifically, the P13 nAmp was significantly reduced in the 15°/s condition compared to 0°/s. No condition effect emerged in any of the cVEMP parameters obtained from the right ears. Condition effects emerged in the RMS distance, sway area, and pathlength (ps<.05); with increased VFM perturbation, subjects became less stable in their balance. Condition effects also emerged in critical point coordinates in time, diffusion coefficient, and scaling exponent. As VFM velocity increases, subjects tend to switch from open-loop to closed-loop control strategies quicker, exhibit less stable and more stochastic sway patterns. When P13 nAmp from the left ears dropped significantly during 15°/s, N23 nAmp from the right ears stepped in to fine-tune the response; subjects switched to closed-loop control quicker if their N23 nAmp during 15°/s had a much higher increase from that during 0°/s.

Conclusions: Our findings around VFM's modulation of cVEMP while maintaining an upright stance are novel. Vestibular laterality seems to corroborate with the previous report. The current study showed the direction for future studies in advancing knowledge around the underlying mechanism of multisensory processing for balance control.

Vestibular Aberrations in an Animal Model of Autism Spectrum Disorder

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Category: Vestibular: Basic Research and Clinical

Background: In utero exposure to the antiepileptic valproic acid (VPA) is associated with an increased risk of autism spectrum disorder (ASD) in humans. Accordingly, timed exposure to VPA is utilized as a clinically relevant and validated animal model of ASD. Previous work in our lab has revealed drastic structural, functional and connectivity changes in the auditory brainstem of VPA-exposed animals. Additionally, we have shown that VPA exposed animals have smaller brains, smaller cerebellar Purkinje cells, reduced expression of calbindin (CB) in Purkinje cells and significant gait ataxia. Based on these findings, we suspected additional involvement of brainstem vestibular pathways in VPA-exposed animals. Specifically, we hypothesized that VPA exposure will result in fewer neurons in the vestibular nuclei, reduced CB immunoreactivity in vestibular nuclei and their afferent projections, postural instability and poor gait performance after vestibular challenge.

Methods: Herein we used a combination of behavioral testing using motor tasks, recordings of vestibular evoked myogenic potentials and morphometrics to examine vestibular structure and function.

Results: We found that VPA-exposed animals had significantly fewer neurons in the spinal, lateral and superior vestibular nuclei and larger neuronal cell bodies in the spinal and lateral vestibular nuclei. In the lateral nucleus, we found significantly more stellate and fusiform neurons but fewer round/oval neurons. VPA-exposed animals had fewer CB+ synaptic terminals in vestibular nuclei. Motor testing suggests VPA-exposed animals are more severely impacted by vestibular challenge than control animals. Finally, recordings of vestibular-evoked myogenic potentials revealed significantly longer latency responses in VPA-exposed animals.

Conclusions: Together, these findings support vestibular dysfunction in VPA-exposed animals and provide a foundation for further investigation of vestibular function in human subjects with ASD.

Vestibular Function Predicts Prefrontal and Sensorimotor Cortical Gray Matter Volumes in a Cross-Sectional Study of Healthy, Older Adults

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Category: Vestibular: Basic Research and Clinical

Background: The vestibular system, composed of five end-organs housed in the inner ear, senses head motion and orientation. This information is transmitted through the brain stem and cerebellum, to the thalamus, and then to the sensorimotor and prefrontal cortices [1]. Previous clinical research found that patients with vestibular impairment had reductions in gray matter volumes in the postcentral gyrus [2], in the right superior frontal gyrus [3], and in the right precentral gyrus and left dorsolateral prefrontal cortex [4]. However, little is known about how sub-clinical vestibular impairment impacts cortical structure. This study investigates whether age-related vestibular end-organ functions predict volumes of the prefrontal cortex, comprised of the superior, middle, and inferior frontal gyri, and volumes of the precentral and postcentral gyri of the sensorimotor cortex.

Methods: The data is a subset from the Baltimore Longitudinal Study of Aging (BLSA) involving 117 participants ≥ 60 years old who had MRI brain scans and vestibular testing in the same visit between 2013 and 2015. All participants provided informed consent and were free of neurological disease. 3T T1-weighted volumetric MRI scans acquired at the National Institute on Aging Clinical Research Unit were automatically parcellated using MRICloud (https://www.mricloud.org/). Functions of three vestibular end-organs, the saccule, utricle, and horizontal semi-circular canal were evaluated using the cervical vestibular evoked myogenic potential (cVEMP), ocular VEMP, and video head impulse testing, respectively. Multiple log-linear regression adjusted for age, intracranial volume, and sex was used to investigate the relationship between mean regional volume and vestibular function. Hypothesis testing was performed using permutation testing and confidence intervals were calculated using bootstrapping.

Results: Higher canal function was associated to larger volumes of the prefrontal cortex (bilateral-mean: 11.8%, p~0.04; left: 11.9%, p~0.045; right: 11.6%, p~0.048), of the postcentral gyrus (bilateral-mean: 14%, p~0.048; left: 16.8%, p~0.033), of the superior frontal gyrus (bilateral-mean: 13.4%, p~0.038; left: 16.6%, p~0.015), and of the middle frontal gyrus (right: 14.5%, p~0.041). Higher saccular function was associated to lower volumes of the postcentral gyrus (bilateral-mean: -3.68%, p~0.014; left: -4.35%, p~0.0088) and precentral gyrus (left: -2.99%, p~0.044). Higher utricular function was associated with larger middle frontal gyrus volume (right: 0.23%, p~0.029). Having a bilaterally present saccular response was associated with reduced superior frontal gyrus volume (bilateral-mean: -4.48%, p~0.043; right: -5.08%, p~0.038). Having unimpaired canal function was associated with larger inferior frontal gyrus volume (left: 10.1%, p~0.04). Having a bilaterally present utricular response was not associated with the volumes of the prefrontal cortex, precentral gyrus, or postcentral gyrus.

Conclusions: Age-related vestibular functions predict volumes of the prefrontal cortex and precentral and postcentral gyri. This work not only furthers the understanding of the role of the vestibular system in cortical structure changes, but also may anatomically link vestibular function to cognition.

Vestibular and Audiometric Impact of Transmastoid Semicircular Canal Plugging

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Category: Vestibular: Basic Research and Clinical

Background: Surgery of the vestibular system is only indicated in selected cases. Some patients with disabling superior canal dehiscence syndrome (SCDS) can be treated with transmastoid plugging of a semicircular canal. This procedure may also help certain patients with intractable benign paroxysmal positional vertigo (BPPV). However, the impact of surgical procedures on the vestibular labyrinth, including plugging of a semicircular canal is largely unknown. Although only one semicircular canal is manipulated, this may effect other parts of the labyrinth. Reports on the effect on hearing and on vestibular function vary and research with structured follow-up during several months after plugging is lacking.

This study aimed to systematically investigate the evolution of vestibular function and hearing after transmastoid plugging of one semicircular canal.

Methods: Pre- and postoperative vestibular and audiometric tests were performed in six patients undergoing transmastoid plugging of a semicircular canal. Four patients underwent plugging of a superior semicircular canal for SCDS and two patients underwent plugging of a posterior semicircular canal for BPPV. Patients underwent postoperative testing at one week, two months, and six months follow-up. Testing included caloric irrigation test, video Head Impulse Test (vHIT), cervical and ocular Vestibular Evoked Myogenic Potentials (VEMPs), and audiometry.

Results: All patients showed initial decrease of caloric response and four patients showed immediate decrease of vHIT vestibulo-ocular reflex (VOR) gain of all ipsilateral semicircular canals. At six months follow-up, caloric

response recovered to >60% of the preoperative value in four patients and vHIT VOR gain was restored to >85% of the preoperative value for both ipsilateral non-plugged semicircular canals. This gain of the plugged semicircular canal decreased in four of the five patients with a residual preoperative VOR gain, but recovered to >50% of the postoperative value at six month follow-up. Four patients retained cervical VEMP responses and five patients retained ocular VEMP responses. Bone conduction hearing deteriorated 10 dB or more in half of the patients. This recovered within two months postoperatively, but one patients had a persistent loss of 15 dB at 8 kHz.

Conclusions: Transmastoid plugging of a semicircular canal can impact vestibular function and hearing. After initial deterioration, most patients show substantial recovery during follow-up, although vestibular loss and/or high-frequency hearing loss can still persist after six months. These risks should be taken into account during selection and counseling of potential candidates for this surgery. Additionally, this study displays the resilience that the inner ear may carry, as well as its limitation. This provides insights regarding other vestibular surgeries, such as the potential impact of an intralabyrinthine vestibular implant on residual function.

Calretinin Enriched Vestibular Ganglion Neurons Have Distinct Biophysical and Ion Channel Properties That Favor Rapid Transmission

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Category: Vestibular: Basic Research and Clinical

Background: Vestibular sensory information is transmitted to the brainstem by distinct neuronal sub-groups. Rapid head movements are believed to be encoded by a morphologically and molecularly distinct sub-group of afferent neurons that innervate the central zones of vestibular epithelia, so-called "calretinin positive pure-calyx afferents." Here we combined patch-clamp electrophysiology, immunohistochemistry, and modeling to test the hypothesis that this sub-group of neurons have distinct ion-channel properties that contribute to their rapid responses.

Methods: We recorded firing patterns and whole-cell currents from the cell bodies of disassociated and overnight-cultured vestibular ganglion neurons (VGN). VGN came from post-natal rats ranging in age between post-natal days 15 and 19. Recordings were made with perforated-patch methods to preserve the intracellular concentration of endogenous second messengers. Cultured cells were fixed and immunolabeled against β-3 tubulin to label all neurons and calretinin to label only the cell bodies of "calretinin-positive pure-calyx afferents." We inferred cell size from capacitance measurements and by light microscopy. Cell size and enrichment for the calcium-binding protein calretinin were used as indirect markers for VGN somata belonging to the pure-calyx afferents from the central zones of vestibular epithelia.

Results: Results from our preliminary data show that approximately 31 (10%) out of 302 cultured cells are enriched with calretinin. Consistent with previous reports from mature VGN, cells that are enriched for calretinin are significantly larger than cells that lack calretinin (calretinin positive = 509.1 ± 35.2 µm2, calretinin negative = 221.3 ± 34.7 um2, $p < .0001$). We, therefore, used size as an indirect measure of calretinin enrichment for our electrophysiology data. Large VGN (>30 pF) had distinct membrane properties, including lower input resistance (0.17 ± 0.04 GΩ in large cells compared to 0.35 ± 0.03 GΩ in small cells), greater current threshold (263.3 ± 23.1 pA in large cells to 73.3 ± 13.4 pA in small cells), and a depolarized voltage activation range for HCN channels (-88.8 ± 2.9 mV in large cells to -100.1 ± 1.7 in small cells). Notably, large cells have lower spike latencies than small cells (8.4 ± 0.6 ms in large cells compared to 12.9 ± 1.5 ms in small cells).

Conclusions: These data are consistent with the notion that calretinin-positive neurons have biophysical properties that prioritize the rapid responses associated with the afferent neurons in the central zone. Ongoing work is focused on developing a conductance-based model of VGN that captures the biophysical properties of the large cell-bodied neurons. Our goals are to test the impact of large surface area and depolarized HCN channel properties on the excitability of vestibular afferent neurons.

Effects of DARC Deficiency on Age-Related Hearing Loss

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Background: Resident cochlear macrophages are the primary immune cells in the sensory epithelium. It has been documented that the number and morphological features of cochlear macrophages undergo drastic change after the

inner ear injury due to either noise exposure or ototoxic challenge. Regarding the age-related hearing loss, previous reports focused mainly on the immune cells in the cochlear lateral wall. Here, we attempted to assess the influence of the resident and the migrated macrophages in the sensory epithelium of aged cochleae between two different strains of mice, DARC mutant and C57BL/6.

Methods: Aged DARC and C57BL/6 mice, at the age of 37 and 48 weeks, were selected. ABR thresholds, wave 1 amplitudes were measured to determine the hearing sensitivity, while DPOAE was acquired to assess the outer hair cell (OHC) function, at the frequencies from 4.0 to 64.0 kHz in 0.1 octave steps. Mice were sacrificed at either 37- or 48-week-old, temporal bones removed, and cochleae decalcified and immune-processed for wholemount dissection. OHCs were labeled with phalloidin, resident macrophages were identified with an anti-IBA1 antibody, and migrated macrophages with a Ly6C antibody.

Results: At either investigated age, age-related hearing deterioration is more evident in C57BL/6 mice compared to DARC mutants. Thirty-seven-week-old C57BL/6 mice presented with ABR thresholds that significantly elevated at the low and mid frequency locations, and undetectable thresholds were recorded at higher frequency locations. However, ABR thresholds in DARC mutant demonstrated preserved hearing throughout the cochlear at all frequencies. At 48 weeks, ABR thresholds were undetectable across the entire cochlea in C57BL/6 mice, in contrast to the DARC mutant, in which only mild elevation of ABR thresholds was observed.

Averaged DPOAE responses were compared between groups and indicated significant hearing decline in C57BL/6 mice. Although electro-physiological consequences didn't match the morphological results in mid-frequency region of the cochlea in C57BL/6 mice, it could indicate that OHCs lost their function before death. In contrary, OHC survival in DARC mice was in accordance with DPOAE outcome across the entire cochlea. Morphologically, longitudinal reduction of resident macrophages from apex to base was observed in DARC mutants and migrated immune cells appeared more in numbers compared with C57BL/6 mice.

Conclusions: DARC deficiency delayed hearing deterioration in aged cochleae, demonstrated by the correlated hearing sensitivity and hair cell survival. Moreover, immune cell infiltration and the reduction of resident macrophages in the base of the cochlea in the DARC mutant indicated ongoing efferocytosis, a process generally promotes anti-inflammatory environment and offers hair cell protection. The identification of cochlear factors in DARC deficiency mice controlling the differentiation into pro- and anti-inflammatory macrophages and determining the mechanisms regulating cytokines production will be future step and could open promising therapeutic options for patients with presbycusis.

Macrophage Activation is Augmented by Smoke Exposure in a Murine Model of Age-Related Hearing Loss

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Background: Presbycusis or age-related hearing loss (ARHL) occurs in about 30 percent of adults over the age of 65 years old. ARHL is a multidimensional disease, many factors such as noise damage, immune system dysregulation, and smoking, in addition to aging alone can lead to hearing loss. Smoking causes vasculature damage, immune cell recruitment and responses such as macrophage activation. Macrophage activation has often been characterized by cellular morphology. Ameboid (round) shaped cells are considered active while more processed cells with elongated filopodia are considered non-activated or surveillance macrophages. Here we tested the hypothesis that smoke enhances macrophage activation leading to auditory nerve degeneration in a mouse model of age-related hearing loss.

Methods: Three-month-old C57BL/6J mice received daily secondhand smoke for either 6 months or were kept at room air as controls. To control for aging, young 2-month-old, room air raised animals were included. Post exposure, mouse cochleas were collected, fixed in 4% paraformaldehyde and immunohistochemistry was performed on either sections or whole-mount preparations. These tissue preparations were examined for macrophage activity and interaction between macrophage and strial microvasculature. Iba1 and isolectin B4 or CD31 were utilized to label macrophage and microvasculature, respectively. Quantitative analysis of Iba1+ macrophages and associated microvasculature has been performed with confocal microscope and 3-D reconstruction using Imaris Cell Imaging Software.

Results: Preliminary results revealed an increased number of macrophages in the basal cochlear lateral wall of smoked mice compared to young controls. Light microscopy observation of the whole-mount preparations from mouse cochlea showed increased morphologically activated macrophages and less vascular networking in the lateral walls of smoke-exposed ears compared to age matched room air controls.

Conclusions: Our data suggest that macrophage activation and vascular changes are augmented by smoke in an ARHL mouse model.

Glutamate Decarboxylase mRNA Has Altered Expression With Age in the Low Frequency Inferior Colliculus in Fischer Brown Norway Rats

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Background: GABAergic downregulation is well documented in the aging inferior colliculus (IC) and contributes to age-related hearing loss. We sought to determine whether GAD1 mRNA is downregulated in the central IC (ICc) at higher/lower frequencies and in larger/smaller cells during middle and old age.
Methods: We assessed 3-4-, 19-, and 28–29-month-old Fischer Brown Norway (FBN) rats, which develop low frequency presbycusis at ~24 months of age. Unfixed brains were extracted, flash frozen in liquid nitrogen and sectioned coronally at 12 µm. IC sections were processed using single molecule fluorescent in situ hybridization using the Fluorescent Multiplex Reagent Kit according to the manufacturer's instructions (320850, 1:50; ACDBio) to express GAD1 mRNA. Sections were counterstained with NeuroTrace to obtain neuronal profile area. We used Paxinos and Watson ('98) rat brain atlas and Neurolucida (MBF Bioscience) to contour and divide the ICc into three equal regions across the dorsomedial-ventrolateral axis to represent low, middle and high frequencies. Montages were taken at 0.3 µm z-steps throughout the entire tissue thickness across each ICc region. GAD1 mRNA were manually and automatically quantified with Puncta Detection (Neurolucida 360). We analyzed 939 cells; profile areas ranged from 37.4 µm2 to >1000 µm2; with the majority (77%) falling between 100-400 µm2. Quantities of GAD1 mRNA puncta ranged from 9 to 1,451. We implemented a general linear model in R to quantify variation in GAD1 mRNA across ages, ICc location and/or profile area.
Results: Our first finding was that the total number of GAD1 mRNA was significantly reduced in old age at low frequency. The second finding was that smaller low frequency cells significantly (p=0.0055) downregulated GAD1 mRNA at 19 months. However, this downregulation did not continue into old age. We found that larger low frequency cells significantly (p=0.0035) upregulate GAD1 mRNA at 19 months and continue to do so (p=0.0012) into old age.
Conclusions: We conclude that age-related changes to GAD1 mRNA expression in the dorsomedial ICc may contribute to the low frequency age-related hearing loss that occurs in the FBN rat. We also conclude that GAD1 mRNA is differentially regulated during age according to cell size. The downregulation of GAD1 mRNA observed in smaller cells, at an age when presbycusis is uncommon, may reflect the downregulation of GABA in the IC that is often considered a compensatory response to age-related peripheral loss of excitation. The upregulation of GAD1 mRNA in the larger cells is perhaps surprising as previous studies examining GAD immunohistochemistry demonstrated a reduction of GAD expression in IC GABAergic cells. Thus, our findings may indicate a disruption between the transcription of GAD mRNA and the subsequent translation of the GAD enzyme/protein in the largest GABAergic IC cells during aging.

Predicting Pathology: Towards Using Sift-Ms to Facilitate Early Intervention for Age Related Hearing Loss

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Background: Age related hearing loss (ARHL) affects the majority of those over 65 years old, with existing treatments unable to offer continued improvement as the illness progresses. However, research suggests where degradation of cochlear fibrocytes leads to downstream damage, biological interventions may be possible. Thus, an early detection strategy for fibrocyte damage is required to enable timely intervention. This research presents progress towards such a strategy, laying groundwork for a method wherein selected ion flow tube mass spectrometry (SIFT-MS) of ear wax (a biofluid known to indicate patient health) may be used to ascertain health of cochlear fibrocytes non-invasively.
Methods: This research examined levels (parts per billion) of volatile organic compounds (VOCs) in the headspace of murine cochlear fibrocyte cultures (healthy and inflammatory states) and human ear wax samples in real time using SIFT-MS. Murine cochlear fibrocytes were isolated from tissue explants, with cell type characterised via immunocytochemistry (NaKATPase, S100B, Aqp1). Inflammation was induced via the addition of IL-1β to culture media. Inflammatory state was confirmed by examination of IL-6 and IL-8 production. Fibrocytes and ear wax swabs were analysed via SIFT-MS (Transpectra Profile 3) to obtain measures of VOCs in

the headspace of samples, with observations across H3O+ and NO+ precursors for compounds of m/z 1-180. Statistical analysis of multi ion mode samples was performed via Mann Whitney U test.

Results: Cochlear fibrocytes were successfully explanted to culture with type indicated as II, IV or V. SIFT-MS shows cellular samples indicate unique compounds (suggested as: pyrrole, hexyl acetate and menthone) even at low, biologically relevant, numbers (15,000 cells per sample), with significant differences between conditions identified in means of compounds: acetaldehyde (U(Nmedia=N15000=3)=2, z=-2.193, p<0.05), butyric acid (U(Nmedia=N15000=3)=2, z=-2.193, p<0.05), benzaldehyde (U(Nmedia=N15000=3)=0, z=-2.611, p<0.05) and pyruvic acid (U(Nmedia=N15000=3)=0, z=-2.611, p<0.05) for H3O+ precursor multi ion monitor data. SIFT-MS of inflamed cells demonstrates detectable differences between undosed and dosed cultures, with notable differences identified in the levels of significant compounds (noted above). SIFT-MS of ear wax similarly demonstrates unique compounds, with known wax markers identified. Results suggest distinct, detectable metabolic profiles for cochlear fibrocyte and ear wax samples.

Conclusions: The suggested novel method for SIFT-MS based measurement of cochlear fibrocyte cultures and ear wax samples appears successful thus far, with research demonstrating progress towards the establishment of cochlear fibrocyte health profiles and the use of ear wax as a biofluid for their detection. The next stage of research, therefore, will focus on the detection of poor auditory health via ear wax. Overall, though this research represents only the initial development of the technique, it may be reasonably stated that, if successful, the capacity to non-invasively detect early auditory issues in real time via ear wax may well be a breakthrough for the practice of Otolaryngology.

Initial Improvements in Cochlear Stria Vascularis Cell Lines With 3D Cell Culturing
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Background: Three-dimensional cell culture systems allow for recapitulation of in vivo models that closely represent the tissue microenvironment. Studies using these 3D systems have shown improvements in morphology, response to stimuli, drug metabolism, and gene or protein expression. A stria vascularis cell line SV-k1 was recently used to study the key cells involved in maintaining the endocochlear potential, or "cochlear battery." Treatment of age-related hearing loss known as presbycusis includes the use of promising drug candidates during in vivo studies, and considerable differences from the preliminary results are often found. The present study aims to develop and characterize a 3D culturing method for the SV-k1 cell line. The objective is to shorten the gap between in vitro and in vivo studies and create a novel model for studying the cellular basis of the endocochlear potential and changes during presbycusis.

Methods: SV-k1 cell line is cultured on Gelatin Methacryloyl (GelMA) as the artificial extracellular matrix. 5% w/v of 2,4,6-trinitrobenzene sulfonic acid (TNBSA) assay is used to examine the degree of functionalization of the hydrogel. The alamarBlue colorimetric assay is used to determine cell viability and metabolism. Briefly, SV-k1cells are manually seeded on a 24-well plate coated with 500 µl of crosslinked GelMA at 5,000 cells per well. Measurements of cell viability are taken daily for 7 days.

Results: The synthesis of GelMA in the laboratory was proven to be successful by the TNBSA assay, and it can support healthy cells as shown by the alamarBlue assay. However, the hydrogel was stained when assessing cell viability, requiring further experimentation to improve this step of the procedure. Brightfield microscope images revealed that the morphology of the cells is altered by the hydrogel, and possibly cell migration was inhibited.

Conclusions: Even though a relatively simple way to 3D culture the SV-k1 cell line was achieved, where the cells survive and grow, further experiments need to be performed to fully characterize the new hydrogel and 3D culturing system, and understand its advantages and drawbacks when compared to the standard cell line cultures and in vivo functional morphology. Future steps include carrying out experiments that account for hydrogel staining, measuring the cell growth rate in more detail, and analyzing differences in protein expression, focusing on proteins with high functional significance, such as cell membrane ion channels like NKCC1 and NaK-ATPase.

Loss of Strial Phenotype in Genetically Recovered lpr (Lupus) Mouse Model
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Background: The MRL-Faslpr mouse develops an autoimmune disease resembling lupus with a phenotype that has been reported to include hearing loss caused by strial pathology and a reduced endocochlear potential (EP). This mouse model putatively provides for the study of cochlear mechanics in the presence of a low EP with no outer hair cell pathology. Previous literature has indicated exclusively strial pathology in this model by 20 weeks, with significantly elevated auditory brainstem response (ABR) thresholds and reduced EP in comparison to wild types. We evaluated currently-sold MRL/MpJ-Faslpr stocks and have found that the current phenotypes do not reflect previously published observations. Genetic drift in stocks maintained at the Jackson Laboratory (JAX) may have reduced the utility of this model for auditory research.

Methods: Homozygous mutant (MRL/MpJ-Faslpr) and wild type (MRL/MpJ) breeding pairs were obtained from JAX and bred at Washington University School of Medicine. Heterozygous and wild type mice (+/lpr, +/+) were compared with mutant mice (lpr/lpr). Genotypes were identified using quantitative polymerase chain reaction. Initial sample sizes included 13 homozygous mutants (5 female, 8 male), 5 heterozygous mutants (3 female, 2 male), and 9 wild types (5 female, 4 male). ABR thresholds were collected in response to tone-burst stimuli at 6, 12, 18, 24, 28.3, 40, and 56.6 kHz. EP and ABRs were measured at 7 and 40 weeks.

Results: The baseline EP for 7 week heterozygotes ranged from 92 to 104 mV (n=6). The 40-week EPs for male homozygotes ranged from 77 to 99 mV (n=3, with only males surviving to 40 weeks) and 67 to 90 mV for wild types (n=5, 4 female, 1 male). Overall, ABR thresholds were relatively stable in both groups from 7 to 40 weeks, particularly in the mid frequencies. By 40 weeks, ABR thresholds in the mid frequencies (18, 24 kHz) increased up to 10 dB for male homozygotes (n=3), up to 5 dB for the surviving male wild type, and up to 20 dB for female wild types (n=4). In comparison, thresholds in the high and low frequencies showed greater variability within groups.

Conclusions: While our results for lpr mutant mice are presently limited due to added mortality, particularly in females, the absence of threshold elevation or decreased EPs in the mutants by 40 weeks of age appears to contradict previous findings. Our observations suggest that the auditory profile of this strain, as sold by JAX, has changed, perhaps due to loss of modifying alleles on the MRL/MpJ background. Our updated observations on the MRL-Faslpr mouse model pose caveats for researchers considering the use of this mouse as a model for presbycusis or autoimmune hearing loss.

Relationships Between Co-Modulation Masking Release, Speech-In-Noise Perception, and EEG Measures of Temporal-Coherence Processing

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Background: Navigating "cocktail-party" environments is particularly challenging for those with hearing loss. A key component of parsing these complex acoustic scenes is grouping of acoustic elements to form perceptual auditory objects or streams. This figure-ground segregation is thought to occur via processing of temporal coherence. Here, we designed a noninvasive neural metric of temporal-coherence processing using EEG. To test whether this metric can provide a neural correlate of figure-ground segregation, we   compare this metric to behavioral comodulation masking release (CMR), which is thought to be simple measure of coherence-based scene analysis, and to speech perception in noise.

Methods: We employed a novel comodulation-figure-ground stimulus where the comodulation statistics of a set of 20 tones can be parametrically manipulated without changing the modulation statistics of individual tones. The frequency spacing between the tones was 50% wider than established cochlear tuning bandwidths, such that peripheral interactions between the tones are minimal, and sensitivity to the co-modulation statistics would have to arise primarily from temporal coherence processing in the central auditory system.

We measured CMR using a stimulus consisting of three bands of noise with the middle band centered at 4 kHz, and the spacing between bands being 50% wider than cochlear tuning bandwidths. The CMR task involved detecting a 4 kHz tone embedded in the noise while random aperiodic envelopes with varying co-modulation statistics (i.e., temporal coherence) were applied to the three bands of noise.

Lastly, we measured scores in two speech-in-noise tasks with a different balance of energetic and informational masking, and with differing cognitive demands.

Results: Preliminary data shows that our neural metric is robust and sensitive to the coherence manipulations within the stimulus. Behavioral measures showed a CMR in the range of 6-10 dB. Early trends suggest that CMR and the EEG neural metric developed here can account for a portion of the individual differences in the speech-in-noise tasks.

Conclusions: Preliminary data suggests our novel EEG metric can capture temporal-coherence processing at the individual level, and that this metric along with a CMR measure may be useful in explaining variance in speech-in-noise tasks.

Cortical Encoding of Discrete Prosodic Features in Continuous Speech

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Background: Prosodic patterns carry crucial information for disambiguating the linguistic and affective cues to convey a coherent structure of the talker's intended message to the listener. These prosodic patterns provide important contextual cues and help bind information across different segmental speech units for efficient perception. Prior neuroimaging work using intracerebral recordings shows that sites in the superior temporal gyrus (STG) and middle temporal gyrus (MTG) encode intonational categories at sentential levels. However, the sentential level prosody consists of short timescale (~<300 ms) pitch inflections tied to the stressed syllables that form discrete prosodic categories of pitch accents. These pitch accents are fundamental components of prosody in languages such as English. These pitch accents follow a specific grammatical structure and form the building blocks of prosody in naturally produced speech. However, there is very little research on how these pitch accents in continuous speech are encoded in the brain. We leveraged the high spatio-temporal resolution of human intracerebral recordings to study if the pitch accents in continuous speech are encoded as discrete categories in the brain, distinct from the lower-level spectrotemporal features.
Methods: Eight individuals undergoing intracerebral recordings to localize seizure foci participated in the study. Participants listened to 30–45 mins of the narrative Alice in Wonderland during local field potential recording. We extracted the high-gamma activity and estimated multivariate encoding models to track cortical representations of the pitch accent and spectrotemporal acoustic cues such as absolute pitch, relative pitch, and the amplitude envelope. We used a partial-correlation approach to partition the unique variance explained by the pitch accents beyond what can be explained by the spectrotemporal acoustic cues.
Results: Pitch accent categories accounted for unique variance in the high gamma activity in 34/701 electrodes. At least two electrodes in each participant showed significant unique variance explained by the pitch accent categories. The cortical areas encoding the pitch accent categories were spatially dissociated from the regions encoding spectrotemporal features (Heshcl's gyrus, and sites around the sylvian fissure, and STG). These regions ranged from the planum temporale to the STG and MTG, and were also diffusely distributed across the cortex.
Conclusions: The pitch accents in continuous speech are encoded as discrete categories beyond linear veridical representations of the spectrotemporal cues of prosody. These results indicate that human brain transforms the spectrotemporal cues and builds higher-order invariant categorical representations of pitch accents, which are then utilized to weave the melodic structure in continuous speech.

Spatial Decorrelation of Sound Representations Throughout the Auditory System

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Background: Auditory perception relies on the separation of the complex spectrotemporal patterns. If the transformations of auditory information that enable this process have been long studied, it remains debated what the key compuZonnal steps are. For example, influencial work suggests that specific representations of complex spectro-temporal motifs (e.g. direction of frequency modulations) exist already in the inferior colliculus. However, causal manipulations of the cortex, thalamus and inferior colliculus in animals indicate that auditory cortex representations are required for discriminating these spectro-temporal motifs. This suggests that some distinctive features of cortical representations remains to be identified.
Methods: To address this, we systematically compared population representations of diverse sounds across a detailed biophysical model of the cochlea by Bourien et al. and extensive two-photon calcium imaging and electrophysiological recordings in the inferior colliculus (>10000 neurons), thalamus and auditory cortex (>60,000 neurons) of awake mice listening to an array of 140 pure tone and more complex time-varying sounds (AM, FM,

Chords, 500ms). To compare the spatio-temporal representations of these sounds across auditory system structures, we developped a noise-corrected similarity measure that removes biases from the neural variability differences across structures and the recording techniques used. This measure was used also to compare representations based on both temporal activity sequences and cell identity (spatio-temporal code), or on cell identity only (spatial code). We also developed a reinforcement learning model that simulates sound discrimination tasks based on neuronal representation data recorded in vivo.

Results: We observed that spatio-temporal patterns remained equally informative along the auditory system for temporal resolutions of few 100ms. Representations were denser in the thalamus without loss of information. However, in cortex, we evidenced a convergence of the spatial and spatio-temporal code, without loss of temporal resolution. This convergence is due to a spatial decorrelation which renders individual neurons more specific for particular combinations of acoustic features. Spatial decorrelation of temporal features was strongest in cortex where time-averaging had little effect on the discriminability of time-varying sounds, a population-scale property that was much weaker subcortically.

Based on our reinforcement-learning model, we showed that this spatial decorrelation of temporal features boosts discriminative learning of spectrally overlapping time-varying sounds. We show that this effect explains why auditory cortex is specifically required to distinguish more complex sounds behaviorally.

In addition A similar decorrelation process is observed in artificial deep neural networks if trained to identify a broad enough set of sounds and sound features.

Conclusions: Our results show together that spatial decorrelation is a key computation culminating in cortex, which serves to separate temporal cues such that they can be more easily associated to behavioral decisions and perceptual classes.

Bilateral in Vivo Widefield Calcium Imaging of Mouse Auditory Cortex

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Background: In vivo widefield calcium imaging is a well-established method to visualize neural activity in the mouse cortex, particularly to understand spatial organization of responses, uncover correlated regions, and locate topographic maps. In the auditory system widefield imaging has been used to study tonotopic maps. As of now, widefield imaging experiments of the auditory cortex solely include data from one hemisphere; however, normal auditory processing involves coordinated function of both hemispheres. Human fMRI studies show bilateral activation of the auditory cortex in response to unstructured tones, frequency modulated tones, reversed speech, pseudowords, and words (Binder et al., 2000). Imaging studies in human subjects have also shown functional lateralization of the auditory cortex: left auditory cortex is more involved in temporal processing, while right auditory cortex is more involved in spectral processing (Zatorre et al., 2001). Understanding lateralization of functional connectivity between the hemispheres can provide insight into normal function as well as auditory processing deficits. For example, structural reorganization causing atypical lateralization of the auditory cortex in children with unilateral cochlear implant may lead to speech processing and sound localization difficulties (Gordon et al., 2013) and abnormal cross-hemisphere communication in the auditory cortex is thought to contribute to auditory hallucinations in schizophrenia (Steinmann et al., 2019).

Methods: In order to investigate in vivo how the left and right auditory cortex work in synergy, we developed a novel bilateral widefield calcium imaging microscope that utilized hardware synchronized cameras. We used this microscope to simultaneously image auditory cortex of both hemispheres in awake mice transgenetically expressing either GCaMP6s or jRGECO1a.

Results: Using these methods, we were able to simultaneously visualize functional tonotopic maps in both hemispheres. Tone evoked changes in fluorescence were similar between the hemispheres. Simultaneously imaging both hemispheres using a diverse set of stimuli allows us to investigate both spontaneous and tone evoked correlated activity between matching and non-matching regions of the two auditory cortices.

Conclusions: In conclusion, we have developed a novel bilateral widefield microscope that allows us to reveal the pattern of coordinated activation across hemispheres in awake behaving mice.

Connectivity of Long-Range Projecting Inhibitory Neurons in Primary Auditory Cortex

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Background: Central auditory circuits adapt to changes in our sound environments throughout life. Identifying neural mechanisms that control central auditory plasticity will have far-reaching impact, offering potential ways to restructure neural circuitry following hearing loss. Our work and that of others has identified groups of cortical GABAergic inhibitory neurons that express vasoactive intestinal peptide (VIP) and neuron-derived neurotrophic factor (NDNF) as key hubs for cortical plasticity. While previous studies have focused on the short-range connections of these GABAergic neurons with neighboring cortical neurons, our results show that a subset of these neurons send long-range projections to distant cortical and subcortical regions.
Methods: To survey the columnar and laminar organization of the long-range projecting VIP and NDNF neurons in mouse primary auditory cortex (A1), we employed the AAV-Brainbow technology that uses Cre-loxP recombination for stochastic combinatorial expression of fluorescent proteins. This technique allowed us to label individual VIP or NDNF neurons with distinct colors and examine their axonal projections and postsynaptic targets. To further examine the prevalence and distribution of the GABAergic neurons in A1 that project to MGB, we used several complementary retrograde tracing methods combined with immunohistochemistry and in situ hybridization.
Results: Here, we show that NDNF and VIP neurons in A1 send long-range projections, extending beyond auditory cortex into subcortical regions including the medial geniculate body (MGB). Ongoing studies combining AAV-Brainbow technology, tissue clearing, and 3-D whole brain mapping techniques are examining the diverse distal outputs of these long-range projecting NDNF and VIP neurons. Our retrograde tracing studies demonstrate that about 2% of all cortico-thalamic neurons are GABAergic, and subsets of these neurons express NDNF and neuropeptide Y (NPY). Ongoing in vitro electrophysiology studies are evaluating the intrinsic and synaptic properties of these long-range projecting inhibitory neurons.
Conclusions: Together, these findings extend our understanding of cortical GABAergic neurons beyond local circuitry. The characterization of long-range projecting GABAergic neurons in auditory cortex could provide insight into novel auditory plasticity mechanisms and potential therapeutic strategies to promote adult plasticity and learning.

Long-Term Shifts in Membrane Potential of Neurons in the Basolateral Amygdala Affecting Background and Vocalization-Evoked Firing May Indicate State Changes

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Background: The amygdala participates in acoustic communication through its analysis of the meaning of social vocalizations and the organization of emotional responses. This role is supported by mechanisms acting within the basolateral amygdala (BLA) on a moment-by-moment basis to integrate auditory and other sensory information with limbic and neuromodulatory inputs reflecting internal state. We previously showed in extracellular recordings that vocalizations with both positive or negative emotional valence evoke spiking responses in BLA neurons, and that vocal responses could be altered by other sensory stimuli or conditions that change internal state. We hypothesize that these state-related effects on spiking responses may be apparent in resting properties and subthreshold responses obtained through intracellular recording. To test this hypothesis, we recorded intracellular responses in BLA neurons in bats in response to their communication calls.
Methods: Three adult big brown bats (Eptesicus fuscus) were used in the study. Intracellular responses of BLA neurons were recorded in unanesthetized animals in response to different vocal signals previously recorded from freely behaving bats (Gadziola et al., 2016). Free-field stimuli were presented pseudorandomly with an interstimulus interval of 1 s or 3 s and a level of 70 or 50 dB SPL. Quartz micropipettes filled with 2 M KCl solution having impedances in the range from 100 MΩ to 300 MΩ were used for recording. Postmortem histological evaluation of recording sites was performed using iontophoretic injection of the fluorescent tracer Fluoro-Gold.

Results: Intracellular responses of 148 BLA neurons were recorded in response to three different social vocalizations. The vast majority of BLA neurons (85%) selectively responded to a particular vocal stimulus. These responses were typically subthreshold rather than suprathreshold (spiking). They lasted up to several hundred milliseconds and, depending on communication call, were excitatory (depolarizing) or inhibitory (hyperpolarizing). Unexpectedly, we found that resting membrane potentials (RMPs) in a majority of BLA neurons were hyperpolarized when a sequence of social vocalizations.was repeated multiple times. As a result, the mean value of RMPs of these neurons during the hyperpolarized state was on average 10 mV more negative compared to the initial RMPs. The hyperpolarized state in these BLA neurons could last for several minutes and could revert back to the depolarized state by a change in the acoustic stimulation pattern.

Conclusions: BLA neurons in bats exhibit substantial shifts in resting membrane potential and spiking responses during repeated patterns of acoustic stimulation. These presumptive changes in state can be modulated by stimulus novelty, i.e, a change in the presentation of social vocalizations.

The Sound of Silence: Neuronal Responses to Omitted Tones in the Auditory Brain

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Background: Mismatch negativity (MMN) was first recorded in the 70s using EEG in the context of the oddball paradigm and is currently interpreted in terms of predictive processing, that is probably one of the most powerful and comprehensive theories of neural function and it is claimed by some authors for providing a great unified theory of the brain. According to this framework, the brain does not respond passively to incoming inputs but it extracts the regularities and uses them to actively predict what should happen next. Using a similar experimental approach, previous studies have demonstrated that neurons in the auditory system adapt to repeating sounds and resume their firing to new, unexpected sounds. This phenomenon seen at the neuronal level is referred to as stimulus-specific adaptation (SSA) and has been suggested to be the neuronal contributor of MMN. MMN has also been found to occur using an omission deviant but to date neuronal responses to stimulus omissions have been elusive.

Methods: Here, we recorded single neurons from the inferior colliculus and auditory cortex in anesthetized rats and awake mice using an oddball paradigm in which the standard tone has been replaced by an omission deviant. These omitted tones occurred in periodic and randomized sequences, to study the influence of expectancy. We also presented the tones with different SOAs (125ms, 250ms and 500ms) which allowed to observe non-overlapping responses.

Results: Our results reveal a subset of neurons in the auditory pathway that appear to respond to omitted tones. Omission responses shown by auditory cortex neurons are stronger than those from inferior colliculus, showing a higher probability of occurrence with shorter SOAs. Omission responses in awake animals are similar but with a higher response magnitude suggesting that anesthesia may affect the level of predictions. Moreover, response latencies in auditory cortex are similar for the tone and omission responses, but significantly longer for the omission responses in the inferior colliculus suggesting that these subcortical responses may be inherited from the cortex through the corticofugal pathways.

Conclusions: Our findings suggest that the auditory system does not need an external stimulus trigger to detect a deviation from expectations. The predominance of omission responses at an SOA of 125ms aligns with the highest probability of omission responses at short latencies in humans. This supports that the rodent brain can generate predictions and the existence of predictive coding in animal models with varying degrees from subcortical to cortical stages and anesthetized to awake conditions.

Round Window Assays of Noise-Induced Cochlear Synaptopathy in Gerbil

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Background: Our prior work in animal models of noise- and kainate-induced hearing loss and excitotoxicity shows that synapses between inner hair cells and auditory nerve fibers (ANFs) are highly vulnerable to insult and suggests that some fibers may be more susceptible than others. To facilitate the study of this deafferentation, we established a model of noise-induced cochlear synaptopathy in gerbil, a species with well-characterized distributions of ANFs by spontaneous rate (SR) and a frequency range of hearing significantly overlapping that of humans. Building on this work, we have now extended our investigation to include higher-level noise and longer post-exposure holding times, and we have expanded our functional testing to include assays providing additional insights on the relative contributions of ANFs by SR subtype.
Methods: Young adult gerbils (female and male, 14 weeks) were exposed to noise (2.8-5.6 kHz, 100 or 103 dB SPL, 2 hours) and held for post-exposure durations ranging from 24 hours to 10 weeks. Cochlear function was then assessed by distortion product otoacoustic emissions (DPOAEs) and by recordings at the round window of auditory nerve compound action potentials (CAPs), spontaneous activity, peri-stimulus time responses (PSTRs), and envelope-following responses (EFRs). Cochleae were immunolabeled for quantification of inner and outer hair cells and synaptic structures (pre-synaptic ribbons and post-synaptic glutamate receptor patches).
Results: After a single 100 dB SPL exposure, DPOAE and CAP thresholds were temporarily elevated, with maximum shifts at 16 kHz, but returned to control levels by 2 weeks as did DPOAE amplitudes. As expected from this pattern of recovery, there was no hair cell loss. In contrast, CAP amplitudes showed some initial recovery but remained reduced from pre-exposure values at 4 and 10 weeks post-exposure. Synapse loss was maximum at 16 kHz and outpaced the neural amplitude declines at extended post-noise times. Acute reduction in spontaneous round window-recorded neural noise suggests acute involvement of high-SR neurons. However, reduction in PSTR plateau, but not peak, amplitudes in chronic ears points to persistent dysfunction of low-SR ANFs following 100 dB SPL noise exposure. In response to higher level exposure (103 dB SPL), larger acute threshold elevations extended over a broader range of frequencies and recovered less completely. Synapse losses grew and showed limited recovery. Exaggerated and persistent declines in spontaneous activity and stimulated PSTRs suggest that losses after higher-dose noise may include medium- and high-SR fibers as well.
Conclusions: Declines in neural activity suggest noise-dose sensitive involvement by SR subtype. Results will inform our understanding of the underlying injury and the declines in function that result from noise, leading, in turn, to the goals of improved clinical diagnosis and treatment.

Model-Based Hearing Restoration Strategies for Cochlear Synaptopathy Pathologies
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Background: With age, our hearing ability starts to decline: communicating in noisy environments becomes challenging, and hearing faint sounds difficult. Part of this decline stems from outer-hair-cell (OHC) damage and a second factor relates to damaged auditory-nerve (AN) fibers, i.e., cochlear synaptopathy (CS). CS occurs before OHC damage in the progression of sensorineural hearing loss, and has a suspected high prevalence among the older or noise-exposed population. However, conventional hearing-aid algorithms do not specifically compensate for the functional deficits associated with CS.
Methods: Here, we present and evaluate several CS-compensating algorithms that maximally restore sound processing in three different CS types. Using a biophysically-inspired auditory periphery model, we designed audio signal-processing functions that minimize the difference between simulated CS and normal-hearing (NH) AN responses. The developed algorithms operate on the signal waveform and modify its temporal envelope modulations to increase the resting periods between stimulation. The remaining AN fibers are thus able to recover faster from prior stimulation and subsequently generate stronger onset responses and more synchronized AN activity. Our model simulations show that our audio processing restores the AN responses to amplitude-modulated (AM) tonal stimuli, as well as to high-pass-filtered speech. We tested whether our algorithms enhanced envelope-following responses (EFRs), AM detection sensitivity and speech intelligibility (Flemish matrix test) in participants with or without suspected age-related CS. Volunteers with normal audiograms and ages between 18-25 (N=16; 5.2 dB-HL PTA) or 45-65 (N=15; 12.5 dB-HL PTA) y/o participated.

Results: Our data shows enhanced EFRs and AM sensitivity in both young and older listeners when using our CS-compensation algorithms. We saw a small improvement in speech intelligibility that was not consistent across participants: in the young group, those with high EFR magnitudes to speech or good AM detection sensitivity were able to benefit from processed speech. In the noisiest scenario, a median word recognition improvement of 5% and 8.3% was measured for broadband and high-pass-filtered speech, respectively. The applied processing affected each subject differently, with the inter-subject variability increasing as the processing compensated for more severe CS types.

Conclusions: Our proposed CS-compensating algorithms go beyond conventional hearing-aid strategies that typically apply dynamic-range compression to compensate for OHC loss. Our processing either preserved or extended the dynamic range of the temporal speech envelope and thereby increased the compromised dynamic range of temporal envelope coding by the remaining AN fiber population after CS. Because the algorithms were easy to implement and fast to execute, our CS-compensating sound processing may extend the application range of present-day hearing aids to improve temporal envelope processing while leaving sound amplification unaffected.

Relations of Correlation Index and Vector Strength for Bimodal Phase Distributions

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Background: Phase-locked action potentials (spikes) underlie various types of acoustic information processing in the auditory system. The degree of phase-locking of a neuronal spike train is often quantified with the metric called vector strength (VS), which was first introduced by Goldberg and Brown (1969, J. Neurophysiol.). The VS is the absolute value of the Fourier component of the spike train normalized by the total number of spikes. More recently, another metric called the correlation index (CI) was introduced by Joris et al. (2006, Hear. Res.). The CI is defined as the peak value of the shuffled autocorrelogram (SAC), and quantifies the temporal reproducibility of spike sequences driven by repeated presentation of the same acoustic stimulation. In the last ARO meeting (Kessler et al., 2021), we reported mathematical relationship between VS and CI under the assumption that the spike train data are well approximated by the von Mises distribution. While our previous results were generally confirmed with physiologically and numerically generated spike train data, theoretical predictions of CI deviated considerably from empirical CI values for non-unimodal phase distributions.

Methods: In the present study, we derive mathematical relationship between VS and CI for phase distributions that are represented with a sum of two von Mises distributions. This assumption covers skewed and bimodal phase histograms that can be seen in neurons driven, for example, by high-intensity, low-frequency sounds. We then test our theoretical results with an auditory periphery model that can be used with any arbitrary sound stimulus (Bruce et al., 2018, Hear Res).

Results: Consistent with physiological data in cats (Johnson, 1980, J Acoust Soc Am), simulated phase histograms were skewed and finally became multimodal when the modeled auditory nerve fiber was stimulated with increasingly high-intensity tones (typically above 70 dB SPL) at low frequencies (below 500 Hz). Fitting with unimodal (single) von Mises distribution failed for these cases with R^2 values often falling below 0.8 and errors in CI estimation greater than 20%. Introducing a second peak in the theoretical distribution largely improved the fitting, resulting in R^2 values above 0.9 and CI estimation errors below 10% in most cases.

Conclusions: Our results provide general relations between the two measures of phase-locking, VS and CI, for wider conditions than those that can be described by a symmetric, unimodal distribution. They are relevant for characterizing the spike response properties of a wide range of auditory neurons. Confirming these theoretical results with physiological data will be a subject of a future study.

Probing Adaptation and Spontaneous Firing of the Auditory-Nerve Fibers With Far-Field Peri-Stimulus Time Responses

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Background: Sound-level coding in the auditory nerve is achieved through the progressive recruitment of auditory nerve fibers (ANFs) that differ in threshold of activation and in the stimulus level at which the spike rate saturates. To investigate the functional state of the ANFs, the electrophysiological tests routinely used in clinics

only capture the first action potentials firing in synchrony at the onset of the acoustic stimulation. Assessment of other properties (e.g. spontaneous rate and adaptation time constants) requires single-unit recordings directly from the nerve, which for ethical reasons is not allowed in humans.

Methods: By combining neuronal activity measurements at the round window and signal-processing algorithms, we constructed a peri-stimulus time response (PSTR), with a waveform similar to the peri-stimulus time histograms (PSTHs) derived from single-unit recordings in young adult female gerbils.

Results: Simultaneous recordings of round-window PSTR and single-fiber PSTH provided models to predict the adaptation kinetics and spontaneous rate of the ANFs tuned at the PSTR probe frequency. The predictive model derived from gerbils was then validated in female mice, and finally applied to humans by recording PSTR from the auditory nerve in normal-hearing patients who underwent cerebellopontine angle surgeries. A rapid adaptation time constant of ~3 milliseconds and a mean spontaneous rate of ~23 spikes/s in the 4 kHz frequency range were found.

Conclusions: This study offers a promising diagnostic tool to map the human auditory nerve, thus opening new avenues to better understanding auditory neuropathies, tinnitus, and hyperacusis.

Cochlear Neurodegeneration in Deafened Rats Involves Innate and Adaptive Immune Responses Not Requiring Complement

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Background: Spiral ganglion neurons (SGNs) transmit auditory information from cochlear hair cells, the auditory receptor cells, to the CNS. SGNs slowly degenerate after hair cell loss but the reason for this degeneration is unclear because neurotrophic support for SGNs remains available. Our previous RNA-seq and histological studies have provided evidence that an immune response, including complement activation, occurs in the spiral ganglion after hair cell loss. To further elucidate which specific immune response mechanisms may be contributing to SGN loss, we assessed whether complement activation contributes to SGN death using a rat model in which complement component 3 (C3) was knocked out. We assessed SGN survival in hearing and deafened C3+/+ and C3-/- rats. Additionally, we quantified infiltration and activation of immune cells in the spiral ganglion.

Methods: Male and female Sprague-Dawley rats were intraperitoneally injected with kanamycin once daily from postnatal day 8 (P8) through P16 to destroy hair cells. Cochleae from hearing and deafened C3+/+, C3+/-, and C3-/- rats were at collected P70 and cryosectioned parallel to the midmodiolar plane at 25 μm thick. Sections were labeled with antibodies to identify neurons (Tuj1), macrophages (Iba1), activated macrophages (anti-CD68), CD45, and/or CD4 immunoreactive cells. Sections were then imaged using confocal microscopy and the number of cells was counted at several locations along the base to apex axis in the ganglion.

Results: Our previous RNAseq results identified an upregulation of early complement component genes (C1-4) and genes associated with an adaptive immune response (i.e., Ptprc (CD45), Cd4, and MHCII genes), along with other immune response-related genes. Similar to our previous studies, histological analysis revealed infiltration of macrophages and increased expression of CD68, a marker of phagocytic activity and cell activation, in the deafened ganglia. Moreover, the immune response includes not only monocytes but also CD45+ and CD4+ lymphocytes.

Conclusions: Many immune response genes, including complement system genes, are upregulated in the spiral ganglion after hair cell loss, consistent with infiltration and activation of monocytes and lymphocytes. However, SGN death and IBA1+ cell infiltration were similar in C3+/+ and C3-/- rats, implying that complement is not necessary for the immune activation or for SGN death after deafening. The data suggest that adaptive, as well as innate, immune responses are involved.

Vibrotactile Music Enhancement for Cochlear Implant Users

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Background: Music listening can be enhanced by vibrotactile stimulation (e.g., Merchel and Altinsoy, 2018). Therefore, vibrotactile stimulation might offer a novel way to enhance music for people with cochlear implants. While many commercial devices exist which are designed to enhance music with vibrotactile stimulation, it is not known which perceptual features of vibration are relevant for this music enhancement to occur. Further, since CI users do not perceive musical dimensions in the same way as non-CI users, it is important to understand how

congruence between the vibrotactile stimulation and music supports this enhancement in CI users compared with non-CI users. In this experiment vibrotactile music enhancement was evaluated using a series of perceptual congruences between the vibrotactile stimulation and music in both CI users and non-CI users. The results could support the development of a vibrotactile device for CI users in the future.

Methods: Fifteen subjects with CIs and twenty-two subjects without CIs participated in this study. A simple melody was delivered through direct audio input or supra-aural headphones, while vibrotactile input was delivered via a small haptic actuator held between the thumb and forefinger. The perceptual dimensions of music – pitch, intensity, and rhythm were mapped to the perceptual dimensions of the vibration – frequency, intensity, and timing. Congruent and incongruent versions were created, and test subjects were asked to rate their preferences across the different versions in a MUSHRA-like set-up.

Results: Subjects without CIs tended to prefer audio-tactile stimuli which were aligned in intensity and time, while subjects with CIs were much more variable. Test subjects with CIs could be separated into two groups: the first group, 6 out of 15 subjects, consistently rated stimuli misaligned in time lower than stimuli aligned in time while the second group did not. The first group also rated stimuli with incongruent intensities lower than stimuli with congruent intensities, like test subjects without CIs.

Conclusions: For subjects without CIs, time alignment and intensity congruence between music and vibrotactile stimuli appear to be the most important components of vibrotactile music enhancement. For certain subjects with CIs, time alignment and intensity congruence between music and vibrotactile stimulation are similarly important as shown in subjects without CIs. However, most subjects with CIs showed no congruence condition favored over the others. The results suggest while some CI users may benefit from the same perceptual congruences between vibrotactile stimulation and music as non-CI users, other CI users may prefer a different strategy for vibrotactile music enhancement than congruence between the two modalities. These results could assist in determining who could benefit from vibrotactile stimulation to enhance music.

Short and Long Incus Process Coupling of an Active Middle Ear Transducer in Human Temporal Bones

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Background: In contrast to conventional hearing aids which produce sound, active middle ear implants transmit vibratory motion directly to an ear structure e.g. using a floating mass transducer (FMT) of the Vibrant Soundbridge system. The original FMT was constructed with a pre-fixed clamb for connection to the long process of the incus. Several studies followed applying the FMT to other locations in the middle ear. Today, in case of sensorineural hearing loss, there is the choice between two different titanium devices (so called couplers) to connect the FMT either to the short- or long process (SP or LP) of the incus. The operative approach is different and implantation surgery as well as hospital stays are shorter for SP coupling, while the audiometric improvement is comparable. Still, the choice of coupler remains mainly to the surgeon and a direct comparison of the performance in the same temporal bone is missing.

Methods: This study directly compared the electro-mechanical performance of the SP- and LP-coupled FMT of the VSB in the same temporal bone specimen (n=10). The surgical approach for each temporal bone included an extended antrotomy and a posterior tympanotomy to sequentially connect both couplers. Laser Doppler vibrometry (LDV) was used to measure stapes and round window motion. For magnitude evaluation, a sweep was applied in frequencies between 0.1 to 8 kHz in 50 logarithmic steps. To determine total harmonic distortions (THD), FMT stimulation was measured at nine audiometric frequencies corresponding to 100 dB hearing level equivalent.

Results: Comparison shows a maximally 10 dB higher magnitude for the LP coupler at stapes and round window for frequencies below 600 Hz whereas the SP coupler shows a maximally 20 dB higher magnitude at the stapes and round window for frequencies above 600 Hz. THD show similar behavior with less distortion at 500 Hz for the LP coupler and less distortions for the SP coupler in higher frequencies, whereat 7/10 LP-coupled and only 1/10 SP-coupled ears show at least one outlier of >10% THD. Assuming that the device is perfectly connected, the main difference between the two couplers is the distance of the vibrating FMT to the rotational axis of the whole ossicular chain. With a greater lever, the LP coupler raises more torque, which is especially effective in low frequencies with the piston-like motion of the stapes.

Conclusions: Our experiments showed that the SP coupling may be mechanically favorable, in terms of magnitude and distortion, for the transmission of FMT vibrations at higher frequencies (e.g. in presbyacusis).

Musical Interval Perception in Single-Sided Deafened Cochlear Implant Users

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Background: Pitch perception through a cochlear implant (CI) is problematic. Although CI users can identify pitch change direction, they generally do not hear musical intervals correctly. This results in perceiving melodies as out-of-tune and harmonic structures as distorted. It is unknown if this interval distortion is a direct result of the signal processing in the CI, or if intervals cannot be accurately represented with electric stimulation. The answer has implications for the nature of pitch and interval perception and could help develop changes to signal processing to better preserve musical relationships.

Evaluation of perceived intervals through a CI is challenging as reporting of interval size depends on auditory memory of intervals from when the individual had normal hearing (NH). Additionally, it is difficult to verify that their reporting is accurate as the relationship between physical stimulation and pitch intervals is unknown. To address this, we measured perceived intervals with a CI in single-sided deafened listeners with their CI ear alone and included testing with their NH ear alone as a within-subject control. Furthermore, we investigated the effect on interval perception with the combination of a CI and NH ear.

Methods: Ten Single-Sided Deafened CI users participated. Listeners adjusted the fundamental frequency of each note in the song "Happy Birthday" until it was perceived as being "in-tune." All notes consisted of prerecorded sung vowels. The process was repeated for 2 voices (one male, one female), and for 3 listening conditions (CI ear, NH ear, and CI+NH ear).

Results: With the NH ear, performance was highly variable. Some listeners identified all intervals correctly, while no significant correlation was observed with the physically correct intervals for others. Significant correlations were detected between the physical and CI interval settings for most listeners for whom significant correlations were detected between the physical and NH interval settings. Some correlations were strong between the CI perceived interval and the physically correct interval. However, even with the strong correlations, the perceived interval magnitude was often smaller than the physically correct intervals. When the NH+CI ears were combined, performance was similar with the NH ear alone. However, the variability in perceived interval size may have been reduced in the NH+CI condition for listeners who were not already performing at ceiling.

Conclusions: Variability with the NH ear alone suggests caution is necessary in interpreting CI interval tasks in that there is variability on these tasks that is not related to auditory deficits. Listeners may be able to perceive intervals through the CI, but the interval size may be distorted. It is therefore hopeful that modifications for signal processing may improve interval representation. The combination of NH+CI listening is not interfering with interval perception, but may not be beneficial, either.

Modeling and Characterization of a PVDF-TrFE Intracochlear Hydrophone for Totally Implantable Cochlear Implants

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Background: Assistive hearing systems combining a fully-implantable microphone and electronics with a cochlear implant would enhance directional and focused hearing by taking advantage of ear mechanics. Implantable microphones would be usable in almost all environmental conditions throughout the day and night. Current implantable microphones suffer from unstable mechanics, poor signal-to-noise ratio (SNR), and low bandwidth. We used analytical modeling, a finite-element model, and experiments to design a polyvinylidene fluoride-trifluoroethylene (PVDF-TrFE) intracochlear hydrophone and amplifier system for high-bandwidth sensitivity, surgical viability, and improved SNR by electrical shielding and circuit design.

Methods: We derived an analytical model for the PVDF-TrFE hydrophone using the governing equations of piezoelectricity. We verified the analytical with a finite-element model of the sensor created in COMSOL Multiphysics. We designed a charge amplifier using circuit and noise analysis to maximize SNR. We verified the amplifier performance via LTSpice and electrical measurements. To characterize our fabricated hydrophones, we employed a vibrating water column method to generate a known pressure distribution. Finally, we demonstrated sensor performance in a cochlea through preliminary temporal bone experiments.

Results: We found the measured response of our sensor and amplifier perform are in alignment with our analytical models. For the current design, we achieved a sensitivity of 0.2 fC/Pa (or 20 mV/Pa after amplification), a bandwidth of 470 Hz to 16 kHz, and a RMS output noise of 20 mV for a 40-micron-thick sensor of size 15 mm by 0.5 mm. The analysis suggests that the copolymer PVDF-TrFE should be used due to its higher hydrostatic sensitivity, area of the sensor should be maximized to maximize gain, and length should not exceed a maximal value determined by the bandwidth requirement. A short-circuit topology charge amplifier maximizes the SNR of the sensor by minimizing thermal and amplifier noise.

Conclusions: We characterized the design of an intracochlear hydrophone and amplifier system and demonstrated the validity of our analytical models for design optimization. We believe our approach to be a promising candidate to bring fully-implantable assistive hearing systems closer to reality. Using these results, we will further optimize the design of our sensor for improved performance.

Characteristics of Sequential Stream Segregation in Cochlear Implant Users

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Background: The ability to perceive pitch is crucial for both music perception and for understanding speech in the presence of competing talkers. In recipients of cochlear implants (CIs), pitch perception is degraded relative to that of individuals with normal hearing, with performance on both pitch-perception and speech-recognition varying widely across individuals. It is less clear, however, the extent to which good pitch perception in recipients of CIs can facilitate speech recognition in the presence of competing talkers via stream segregation. This study examines the psychoacoustic basis of stream segregation for pure and complex tones in cochlear implant users and tests for relationships with stream segregation on a pitch-based speech comprehension task. The motivating hypothesis is that stream segregation on a psychophysical task is predictive of masking release for speech comprehension.

Methods: Cochlear implant users and normal-hearing listeners—matched in age and musicianship—completed a battery of psychoacoustic and speech comprehension measures. The psychoacoustic measures include frequency discrimination of pure tones (near 1 kHz) and fundamental frequency discrimination of complex tones (near 110 Hz). Stream segregation abilities were also measured for these pure and complex tones. In this task, the target tone stream was comprised of eight brief (100 ms) tones occurring every 400 ms. The last tone in the series is presented early or late, and the listener was asked to determine the relative timing of that tone. Adaptive procedures were used to vary the timing of that tone without a distractor stream and with distractor streams comprised of tones 12, 6, 3, or 0 semitones higher than the target. Finally, speech reception thresholds are measured for spondee words in comparable conditions with background noise comprised of the target spondee words having altered pitch shifts of 12, 6, 3, and 0 semitones.

Results: Preliminary results indicate deficits in frequency and fundamental frequency resolution in cochlear implant users compared to their normal-hearing peers. These results extend to stream segregation with pronounced deficits in segregation performance for both pure and complex tones. CI users receive less masking release for speech reception when the competing speech is shifted in vocal pitch. Data collection is continuing, and results will be analyzed for relationships between psychophysical abilities and masking release for speech reception. Results will be further analyzed to consider the effects of musicianship.

Conclusions: Frequency and fundamental frequency resolution are poor in CI users compared to their normal-hearing peers. This study characterizes differences between groups and between individuals within groups on psychophysical and speech reception measures. Preliminary results support the conclusion that individuals with better frequency and fundamental frequency resolution have better speech comprehension, but further data is needed to draw conclusions concerning how segregation of tonal streams might mediate speech comprehension in the presence of competing speech.

Electrocochleography Compared With Angular Insertion Depth and Speech Perception Outcomes in Children With Cochlear Implants

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Background: It has previously been demonstrated that Total Response (TR) can account for 48% of variance in monosyllabic word score outcomes in adults cochlear implant (CI) recipients (Fontenot et al., Ear and Hearing, 2019, 40:577–591). TR is an across-frequency measure of the cochlear responses to sound, in this case using intraoperative round-window Electrocochleography (ECochG) just prior to CI insertion. When Angular Insertion Depth (AID) and electrode array type (straight vs. precurved) are included with the TR in a multivariate linear regression 73% of variance could be accounted for. However, for children under the age of 6, the TR only accounted for 15% of variance in speech perception scores. For this study, we included information about AID and electrode type to determine if these can contribute information beyond the TR alone as was the case with adults.

Methods: The study included 31 children under the age of 6 receiving a CI. Straight electrode arrays were implanted in 4 subjects, and precurved arrays in 27 subjects. TR was defined as the sum of responses to a series of tones. The AID was determined from X-rays according to the procedures of Giardina et al. (Otol Neurotol 41:e686–e694, 2020). The subjects were a subset of a larger group of 44 that had X-rays of sufficient quality for the technique to be used. The data obtained from the participants were analyzed using forward selection linear stepwise regression. The outcome measure was PB-k word scores taken after at least 1 year of implant use, and up to 4 years for children implanted as infants.

Results: In this subset of subjects, the TR alone accounted for approximately the same amount of the variance as in the larger sample. When combined with AID and electrode type the variance accounted for did not increase significantly.

Conclusions: Unlike in adults, the variance in word score outcomes accounted for by TR alone is small and does not improve when AID and electrode type are added. An explanation for the difference may lie in the different types of hearing loss, i.e., congenital in children vs. progressive in adults. The TR is primarily from hair cells, and apparently predicts the underlying neural substrate available for electrical stimulation. In contrast, congenital hearing loss can be due to 1) loss of function in the cochlea, which reduces the TR while the nerve may still be fully functional with good CI outcomes, or 2) because of auditory neuropathy where the TR is large but outcomes may be poor because of loss of neural function.

Evaluating Fixed and Individualized Channel Interaction Coefficients for Speech Perception in Dynamically Focusing Cochlear Implant Strategies

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Background: Prior findings on the speech perception benefits of dynamic focusing cochlear implant strategies have been mixed. In these studies, channel interaction coefficients (K), determining the rate of change for the degree of current focusing (σ) according to input level, were fixed across participants and channels. K increases with electrode-to-neuron distance, and if the fixed K differs from what would be optimal, then loudness growth could differ from the expected acoustic-to-electric map. Therefore, fixing K may result in suboptimal loudness growth, and poorer speech perception, compared to when K is individualized. This study tested the hypothesis that individualized K strategies will improve speech perception relative to fixed K strategies.

Methods: Twelve ears from ten adult cochlear implant recipients (aged 23 – 75) participated. Participants were programmed with three 14-channel strategies matched for pulse duration, pulse rate, filtering, and loudness. The strategies were monopolar, dynamic partial tripolar ($\sigma = 0.8$ at threshold and 0.5 at most comfortable level) with a fixed K of 0.9, and the same dynamic partial tripolar strategy with K individually estimated for each participant and channel using most comfortable loudness levels. Participants had 15 minutes of listening experience with each strategy. Sentence recognition, scored for key words, and vowel identification was measured with each strategy at 60 dB SPL equivalent in quiet and four-talker babble noise. The signal-to-noise ratio was adjusted so that monopolar performance was between 40-60% for word recognition, and 60-80% for vowel identification. Ratings on clarity and ease of listening were gathered.

Results: Word recognition and vowel identification in quiet were similar between the strategies. While word recognition in noise was slightly better with the fixed (M = 52.7 RAU; 95% CI = 45.4 – 59.9) and individual (M = 53.2 RAU; 95% CI = 45.5 – 61.0) K strategies compared to monopolar (M = 50.0 RAU; 95% CI = 45.8 – 54.2), the effect sizes were minor. Vowel identification in noise was better with the individual K strategy (M = 71.1 RAU; 95% CI = 66.6 – 75.7) compared to the fixed K (M = 63.8 RAU; 95% CI = 55.7 – 71.9) and monopolar (M = 67.1 RAU; 95% CI = 64.8 – 69.5) strategies. On average, there were little differences in clarity and ease of

listening ratings of the strategies. As in previous research, there were considerable individual differences in dynamic focusing benefit between participants. Vowel identification in noise benefit was positively correlated with duration of hearing loss for dynamic focusing strategies relative to monopolar.

Conclusions: These initial results demonstrate little clear benefit to individualizing channel interaction coefficients in dynamic focusing strategies. Future studies evaluating novel cochlear implant strategies will employ take-home field trials to facilitate adaptation, including self-assessments of intelligibility and impact on lifestyle.

Binaural Cochlear Implants Must Encode Interaural Time Differnce Cues Through Pulse Timing, Not Envelopes

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Background: Interaural time difference (ITD) discrimination with binaural cochlear implants (biCIs) is notoriously difficult. Particularly prelingually deaf patients exhibit ITD thresholds too large to provide any useful information, which led to the hypothesis that a lack of binaural experience during an early critical period may be responsible for the poor biCI ITD sensitivity. However, most late deaf biCI patients also have quite poor ITD thresholds, and recent work from our research groups has demonstrated that neonatally deafened (ND), adult implanted rats can be trained to lateralize ITDs with remarkably low thresholds (~50 μs), suggesting that technical issues are probably the main limitation for biCI ITD perception. An obvious problem is that practically all clinical CI processors employ pulstaile stimulation in which pulse timing is not well synchronized between ears, so that biCI users normally only experience ITDs in pulse-train envelopes. Some psychoacoustic work suggests that there may be a small amount of envelope ITD sensitivity in biCI users, and digital signal processing theory may suggest that envelopes should be fully encoded in pulse train carriers with sufficieintly high sampling rates. Nevertheless we hypothesized that the (uninformative) ITDs of the electric CI puses would likely swamp any ITDs encoded in envelopes, given that there are no obvious reconstruction filters in the CI implanted ear.

Methods: We tested this hypothesis by training ND biCI rats to lateralize sinusoidally enveloped pulse trains in which envelope ITDs (envITD) and pulse-timing ITDs (ptITDs) co-varied, and then tested the animals in probe trials in which the envITDs and ptITDs were drawn independenty from the set {-100, 0, +100} μs, where negative denotes left-ear leading. Various combinations of two pulse rates (900 and 4500 pps) and three envelope modulation rates (5, 20 and 100 Hz) were tested. Probit analysis was used to quantify how strongly the animals based their lateralization judgments in probe trials on envITDs or ptITDs at each of the various pulse and modulation rates.

Results: The animals learned the task quickly and lateralized ITDs with high accuracy when envITDs and ptITDs were congruent. In probe trials, where envITDs and ptITDs conflicted, ptITDs completely dominated the animals' lateralization judgments. At none of the conditions tested did envITDs significantly influence lateralization behavior.

Conclusions: Our results strongly suggest that the mammalian auditory pathway simply isn't set up to process envITDs under biCI stimulation. Our ears can be highly sensitive to acoustic envelope ITDs but the situation in biCI stimulation is radically different. If CI manufacturers wish to make rich binaural hearing available to their customers they must begin to pay much greater attention to interaural pulse timing.

Beyond the Phantom: Unroofing the Scala Vestibuli in a Fresh Temporal Bone as a Model for Cochlear Implant Insertion Experiments

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Background: Cochlear implants are the standard of care in bilateral profound sensorineural hearing loss. An electrode array is inserted into the scala tympani (ST) where it stimulates the cochlear nerve within the modiolus in order to restore partial audibility. Advancements in atraumatic technique and electrode designs have greatly improved outcomes from cochlear implantation, expanding indications for use. During development, investigation of electrode array characteristics, dynamic behavior, and traumatic forces is typically performed in transparent acrylic 3D-printed models or via retrospective analysis of insertions performed in cadaveric temporal bones using radiographic and histologic techniques. However, acrylic models are limited in their ability to simulate the tissue

properties of the human temporal bone. Traditional insertions performed in cadaveric temporal bones more closely simulate real-world conditions, but without the ability to visualize the three-dimensional dynamic behavior of the electrode in real-time. Here we present a cadaveric temporal bone preparation method in which the bone overlying the scala vestibuli (SV) is removed so that the basilar membrane (BM) and ST remain unviolated. Electrode insertions can be performed in the micro-dissected cochlea and the electrode can be visualized through the semi-transparent BM.

Methods: Electrode insertions were performed in four micro-dissected fresh cadaveric temporal bones: one manual insertion of a straight electrode, two robotic insertions of straight electrodes, and one robotic insertion of a precurved electrode in which an intentional tip fold-over was induced. The first three temporal bones were further evaluated with micro-CT and histology.

Results: In all scenarios the model provided excellent visualization of the electrode behavior during insertion (compelling photos and videos will be displayed). Micro-CT and histologic findings were in agreement with the final electrode positioning that was directly observed and confirmed that the ST was unviolated during dissection and electrode insertion.

Conclusions: The micro-dissected cadaveric cochlea is an excellent tool for real-time evaluation of cochlear implant electrode behavior, providing the visualization benefits of an acrylic phantom with the better representative tissue properties of a cadaveric temporal bone. To our knowledge, this is the first time a tip fold-over has been observed directly in a cadaveric model. Micro-CT and histology provided further confirmation that this is a valid model for evaluation of electrode characteristics. Overall, we believe this will be a valuable tool for electrode development and surgical training.

Evaluating Hearing Performance With Cochlear Implants Within the Same Patient Using Daily Randomization and Imaging Based Fitting - The Elephant Study
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Background: Prospective research in the field of cochlear implants is hampered by methodological issues and small sample sizes. The ELEPHANT study presents an alternative clinical trial design with a daily randomized approach evaluating individualized tonotopic CI fitting.

Methods: A single blinded, daily randomized clinical trial was implemented to evaluate a new imaging based CI mapping strategy. 14 participants were included from the start of the rehabilitation process with a 1-year follow-up period. Based a post-operative cone beam CT scan (CBCT), an experimental CI program was created whereby the frequency mapping of electrical stimulation was aligned to the natural place-pitch arrangement in the individual cochlea. A randomization scheme was implemented whereby the blinded subject crossed over between the individualized imaging based map and the standard 'one size fits all' map on a daily basis. Hereby the subject effectively acted as his own control. This was followed by a period of free choice between both maps to incorporate patient preference. With this new approach the occurrence of a first-order carryover effect and a limited sample size was addressed.

Results: Subjects showed discriminative learning abilities for both the experimental and the standard CI mapping. Learning curves were unique for control and intervention despite concurrent wearing. Overall compliance to the daily randomization scheme by study subjects was high.

Conclusions: The novel trial design proved to be a suitable method for evaluation of an experimental CI fitting intervention in a prospective trial setup. First results of the experimental imaging based CI mapping will be presented.

Reduced Loss of Spiral Ganglion Neurons After Cochlear Implantation Through Pre-Treatment by Near-Infrared-Light
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Background: Electrode array insertion into the cochlea can initiate the loss of hair cells and spiral ganglion neurons (SGN) in both animals and humans. It is assumed that direct mechanical injury, along with the expression of intracochlear inflammatory cascades are detrimental to the survival of cochlear structures. Various approaches

have been investigated for the prevention of such damage. One simple, local and effective option is the perioperative application of near-infrared light (NIR). Specific wavelengths within the NIR-spec¬trum are known to influence cytochrome-c-oxidase activity, which leads in turn to a decrease of apoptotic and inflammatory mechanisms. Our group has previously shown that NIR can significantly decrease the auditory threshold shift and cochlear hair cell loss if ap¬plied as a single pre-treatment, immediately before the electrode insertion. The present study investigated the efficacy of a single NIR pre-treatment on SGN density in an animal model of cochlear implant (CI) surgery.

Methods: During a CI surgery, normal hearing adult guinea pigs had one cochlea pre-treated with NIR for 15 minutes. Immediately after NIR exposure, a specifically designed guinea pig electrode array was inserted through a cochleostomy into the scala tympani of the first cochlear turn. The contralateral ear received a similar treatment (insertion) but was sham-exposed only.

Four weeks after implantation, SGN density was determined in histological cochlear samples and data were compared between the two ears (with or without NIR pre-treatment) for each animal.

Results: The data demonstrated that SGN density was significantly higher in NIR-pre-treated ears compared to the contralateral side (implanted only) (p = 0.013). The main protective effect were found in the apical region were most SGN were missing upon cochlear implant electrode insertion. Detailed results will be prevented showing SGN cell counts at various cochlear regions.

Conclusions: Our results suggest that a very effective protection of cochlear structures is possible during cochlear implantation by a single NIR pre-treatment. Given the extremely limited side effects, such a treatment should be possible to deploy in clinical practice.

Auditory Model Based Recommendations for Evaluation of Cochlear Health Using the Inter-Phase Gap Effect
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Background: Measurements of electrically evoked compound action potentials (eCAPs) provide means to assess the status of the electrode-neuron interface inside the cochlea. Previous investigations have demonstrated that the eCAP thresholds decrease and the slopes of the eCAP amplitude growth function (AGF) become steeper when the inter-phase gap (IPG) of a symmetric charge-balanced pulse is prolonged and that this so-called IPG effect is pronounced in healthy cochleae (Prado-Guitierrez et al., Hear. Res., 2006; Ramekers et al., JARO, 2014). Problems in translating findings from animal studies into benefits for cochlear implant users have raised questions about how much the eCAP-based metrics are influenced by non-neural aspects and how the IPG effect should be defined (Schvartz-Leyzac and Pfingst, Hear. Res., 2016; Brochier et al., JARO, 2021).

Methods: A computational modeling approach was applied to investigate effects of neuron survival as well as of non-neural aspects on eCAP AGFs. A 2D model was designed to simulate how the electrical current spreads inside the cochlea, evoking neurons distributed along the cochlea to respond, which then results in eCAP responses being recorded at specific electrode contacts. Electrode locations were derived from computerized-tomography scans (Yoshimura et al., Acta Otolaryngol., 2020). Scala-tympani height data (Avci et al., Ear. Hear., 2016) was used to approximate the electrode-neuron distance at different positions along the cochlea. Neural responses were obtained using a phenomenological model (Takanen and Seeber, submitted) to predict neurons' unique spiking activities and convolving the spiking outputs with a unitary response function (Vernsel et al., Hear. Res., 1992). Both the electrical input to the neurons and the neural responses were attenuated by 3 dB/mm. Two IPGs (2.1 and 30 µs) and two electrode-neuron distances as well as four recording electrodes were included in the evaluations. The AGFs were inspected on both linear and logarithmic axes, and the AGF slopes were used to determine the IPG effect describing neural health to identify its dependency on neural and non-neural aspects.

Results: Model predictions were found to agree with existing data: reduced neural survival and increased stimulating-recording site distance resulted in shallower eCAP AGF slopes. Increased electrode-neuron distance resulted in larger eCAP thresholds. Prolonged IPG resulted in steeper eCAP AGF slopes and in smaller eCAP thresholds. Only when the IPG effect was computed as the difference between the slopes of the two AGFs expressed on a linear input-output scale, did the IPG effect depend on the neural survival. Other means of determining the IPG effect eliminated this dependency along with interferences of non-neural aspects.

Conclusions: Model predictions demonstrate how the IPG effect of cochlear health depends not only on the neural survival, but as well as on non-neural aspects and how the metric is computed. This provides insights into applying the metrics in clinical practice.

Human Sensitivity to Interaural Phase Difference Declines More Abruptly than Previously Thought.

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Background: It is well established that in normal-hearing humans the threshold of interaural time differences (ITDs) for pure tones increases dramatically above about 1250 Hz, only to become unmeasurable above 1400 Hz. However, the common understanding is that the actual decline in sensitivity is more gradual and only appears to be abrupt because it dips below the threshold correct rate near 1400 Hz. Published data only reports thresholds at certain correct rates but does not report the correct rates decline with increasing frequencies.
Methods: Here we present pure tone behavioral data obtained with a constant stimulus procedure.
We present correct rates of nine normal hearing subjects for seven different IPDs at frequencies between 1300 and 1500 Hz.
Results: Seven of nine subjects show correct rates above 90% at 1300 Hz. The data indicate virtually no sensitivity at 1500 Hz (correct rate within 7% of guessing rate), and consequently an even steeper decline in IPD sensitivity than previously assumed. This corresponds to a low-pass filter order of at least 14, which to our knowledge is unparalleled in psychoacoustics.
Conclusions: The steep decline cannot be explained by the decline of phase locking of auditory nerve fibers or bushy cells in the cochlear nucleus and not by any model of binaural processing.

Informational Masking Release Based on Interaural Level Difference Cues

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Background: Cochlear-implant (CI) users' inabilities to solve the cocktail party problem are well-known. This perceptual processing deficit is due predominantly to the lack of temporal fine-structure (TFS) cues present in signals processed by CIs, including interaural time differences cues. The limited TFS information in turn limits the amount of informational masking release available to these users.
Methods: While naturally-occurring interaural level difference (ILD) cues can provide benefits to speech understanding in the form of signal-to-noise ratio (SNR) improvements due to the head-shadow effect, they are not thought to contribute to informational masking release.
Results: It has recently been demonstrated that larger-than-typical ILD clues can provide significantly greater speech understanding in noise by bilateral CI users (Brown, 2018), but this study did not establish the extent to which the masking release observed was informational or energetic. Informational masking has been shown to be associated with activity in the superior temporal gyrus (STG; Zhang et al., 2021).
Conclusions: The current study examines the possibility that magnified ILD cues alone can facilitate informational masking release by using functional near infrared spectroscopy to quantify STG activation during a cocktail-party like task in which the target talker is spatially-separated from the maskers.

Frequency Importance Function in Simulated Bimodal and Electric Acoustic Stimulation Hearing

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Background: Bimodal and electric acoustic stimulation (EAS) hearing require integrating spectral information, processed by a hearing aid and a cochlear implant. The answer to the question of how isolated or disparate spectral holes influence spectral integration is important to understand the mechanisms of bimodal and EAS hearing. The goals of this study were, using simulation of bimodal and EAS hearing with normal hearing (NH), to examine the effect of the location and size of spectral holes on sentence perception and to derive a frequency importance function for spectral integration in bimodal and EAS hearing.
Methods: Two separate groups of adults with NH were recruited for the simulated bimodal group and the simulated EAS group each. The acoustic high-frequency hearing loss was created using low-pass filters with a fixed cutoff frequency of 500 Hz and a slope of 48 dB/octave. For the electric simulation, 6-channel sinewave

vocoder was used with a fixed output frequency ranges (1000-7938 Hz) with three different input frequency ranges to create typical frequency maps found in bimodal and EAS patients: overlap (188-7938 Hz), meet (500-7938 Hz), and gap (750-7938 Hz), relative to the cutoff frequency (500 Hz) used for the acoustic simulation. A total of 22 spectral hole conditions were created in the electrical stimulation. Six single hole conditions were created by setting the amplitude of each of the six channels to zero. Fifteen two-hole conditions were also created by removing two adjacent channels or disjoint, alone with a baseline condition in which we did not remove any frequency channels. For the bimodal hearing, the acoustic and electric stimulations were delivered to opposite ears, whereas for the EAS hearing, both acoustic and electric stimulations were presented to the same ear. Sentence perception was measured in quiet condition, with simulated acoustic stimulation alone, simulated electric stimulation alone, and combined acoustic and electric stimulations as a function of spectral hole and frequency map (overlap, meet, and gap). These procedures were administered for bimodal and EAS hearing each.
Results: For both groups, performance was decreased the most when a single hole was introduced in middle-frequency regions of the spectrum. When two spectral holes were introduced, performance was sensitive to the location of the holes, particularly in the middle-frequency ranges. The frequency-importance function was relatively flat for EAS hearing, while unequal weighting across the various channels for bimodal hearing.
Conclusions: The finding that the location and pattern of holes affects mostly performance suggests that in cochlear implants, neuron survival ought to account for some of the variability in vowel recognition performance among cochlear implant listeners. The shapes of the frequency-importance functions were found to be different between bimodal and EAS hearing, suggesting that integration process might be different.

Development of a Cost-Effective, Open-Source Device for EEG-Based Online Auditory Attention Detection in the Real Listening Condition
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Background: Auditory attention detection (AAD) is to identify an attended speaker-based on the brain signals in a cocktail-party situation. Despite the necessity of applying AAD to real life, most AAD studies have been conducted using lab-based devices in soundproof environments. In the present study, we propose a cost-effective device using open-source software that can be used for online EEG-based AAD experiments in real listening conditions.
Methods: We developed a device consisting of an OpenBCI board, WAV Trigger and an Arduino UNO board. This device can be used for EEG signal acquisition (16 channels), auditory stimuli presentation, and trigger synchronization. EEG signals and trigger pulses are sent to the PC via a Bluetooth dongle and recorded with python-based Brainflow data-acquisition software. The online AAD model was built by the correlation-based method proposed in the previous study. Specifically, the AAD model was built through the first 14 trials and the remaining 16 trials were used to evaluate the trained decoder.
 To improve decoder accuracy, we searched for the optimal frequency range by simulating with data from our previous study. We also compared the performance of the decoder after applying an exponential moving average (EMA) to the envelope correlation values. To test the performance of the proposed device, we recruited nine participants (age range 25 to 34; all native Korean speakers) and conducted an auditory attention experiment following the previous study [1]. During the experiment, two different speech stimuli were dichotically presented through the conventional in-ear earphone. The experiment was conducted in a quiet meeting room (46-47 dB). Participants were asked to maintain attention to one speech stimulus (attention direction is randomly chosen). The direction of target speech is fixed during the first 26 trials (attention-fixed) but randomly switched at the last 4 trials (attention-switch). At the end of each trial, four questions were presented to confirm the attention of the participant.
Results: We found that decoder accuracy was relatively high when the low-frequency range (0.5-2 Hz) was used. Based on this result, all EEG signals were band-pass filtered between 0.5-8 Hz.
As a result of decoding for test trials (16 trials), the average decoder accuracy was 72.18% and increased to 76.81% when EMA was applied. For the attention-fixed trials (12 trials) included in test trials, the average decoder accuracy was 73.53% and improved to 77.86% after applying EMA. However, for the attention-switch trials (4 trials), the average decoder accuracy was 68.12% and improved to 73.86% when EMA was applied.
Conclusions: In this study, we attempted to implement the online AAD using a cost-effective device and showed the possibility of applying online AAD in the real listening conditions.
[1] Baek et al. (2021). sensors.

Spectral Weighting in Sound Localization: Effects of Simulated Hearing Loss With Diffuse Threshold-Elevating Noise

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Background: For listeners with normal hearing (NH), spatial hearing relies upon cues distributed across the frequency spectrum. Low-frequency interaural-time-differences (ITDs) tend to dominate–particularly those near the 600-800 Hz "ITD dominance region" [Bilsen and Raatgever 1973, Acustica 28:131-132]. Folkerts and Stecker [2018, ARO 42:505] quantified this phenomenon by measuring spectral weighting functions (SWFs) during localization and lateralization by listeners with NH. As expected, SWFs revealed weight maxima around 800 Hz. However, it is not known whether impacts of sensorineural hearing loss (SNHL)–such as reduced audibility at some frequencies–could alter this process. Here, we measured NH localization SWFs in the presence of a spatially diffuse threshold-elevating noise masker, intended to simulate a sloping high-frequency SNHL. This simulation approach mimics some–but not all–aspects of SNHL, including elevated hearing thresholds, loudness recruitment, and reduced sensitivity to high-frequency ITDs [Steinberg and Gardener 1937, J Acoust Soc Am 9:11-23]. A previous study [Folkerts and Stecker 2020, ARO 43:865] found that reducing component audibility via attenuation resulted in SWF shifts favoring the loudest components. It is therefore expected that SWFs in threshold-elevating noise will similarly favor lower frequencies.
Methods: The threshold-elevating noise masker was derived using similar methods to Desloge and colleagues [2010, J Acoust Soc Am 128:342-359]. For each of the 23 1/3-octave noise bands from 80 Hz, the spectrum level was calculated by subtracting the critical ratio [Hawkins and Stevens 1950, J Acoust Soc Am 22:6-13] from the desired threshold level (for a moderate sloping high-frequency SNHL [Bisgaard et al. 2010, Trends in Amp 14:113-120]). Independently derived noise maskers were presented from a 64-channel array of loudspeakers (2-m radius, 5.625□ azimuthal separation) for 1100-ms, 500-ms before the target stimulus. Ten listeners with NH were asked to localize 100-ms complex tones containing seven frequency components. On each trial, the components were randomly presented across an azimuthal variation of 0°, ±5.625°, or ±11.25° around a "base" azimuth selected within a ±56.25° range. Multiple linear regression of the rank-transformed azimuthal response estimated the SWF weights as in Folkerts and Stecker [2018, ARO 42:505].
Results: Elevation of free-field detection thresholds by addition of the noise was confirmed to match target levels (i.e. simulated moderate sloping SNHL). SWFs obtained in noise conditions were compared to threshold-frequency functions and to SWFs obtained in quiet. Overall SWFs in noise favored cues in the vicinity of the ITD dominance region (i.e. 400-800 Hz). Individual differences in the effects of noise on SWFs were also observed.
Conclusions: Threshold-elevating noise offers a promising model of weighting changes anticipated to result from altered cue audibility in SNHL. Future studies will evaluate further aspects of auditory dysfunction (i.e. beyond audibility) and their impacts on SWFs of listeners with non-simulated SNHL.
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Abnormal mGluR5 and mGluR1 Expression in the Cochlear Nucleus in the Fragile X Syndrome Mouse Model during the Auditory Critical Period.

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Background: Fragile X syndrome is the most common hereditary cause of autism spectrum disorder and is associated with auditory hypersensitivity. The well-studied fmr1 knock-out mouse model exhibits many features of Fragile X and also exhibits auditory seizures. mGluR1, a metabotropic glutamate receptor (mGluR), which mediates excitatory responses, is known to be abundantly expressed in neurons of the adult cochlear nucleus. Thus, we sought to examine the expression patterns of all mGluRs in the fmr1 knock-out mouse during the auditory critical period (a time when the brainstem undergoes significant change in its neuroplasticity responses to afferent deprivation).
Methods: mRNA and proteins were isolated from cochlear nuclei. RNA-seq next generation sequencing, was performed for postnatal day 8 and adult mice (n=3 each). The levels of all mGluRs transcripts were compared. mGluR1 and mGluR5 protein levels were measured by Western blotting at various ages from P8 to adult, and between fmr1 knock-out and wildtype mice (n=3).

Results: Although mGluR1 is the most abundant mGluR in the mature cochlear nucleus as previously described, it was unexpected to find mGluR5 to be the most abundant receptor expressed in early development. The transition from mGluR5 to mGluR1 expression occurs around the critical period. However, this transition is slightly delayed in the fmr1 knock-out mouse.

Conclusions: We present new data that there is a shift from high mGluR5 to mGluR1 expression in the auditory brainstem that occurs during the critical period and this shift is delayed in the fmr1 knock-out mouse, a model for Fragile X syndrome.

Development of the 'Click Plus' Stimulus for Simultaneous Recording of the Auditory Brainstem Response and the Frequency-Following Response

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Background: In children who cannot be reliably tested behaviorally, the gold standard for assessment of hearing thresholds is the auditory brainstem response (ABR). While the ABR provides excellent information about hearing acuity, it is far less sensitive to suprathreshold deficits in neural encoding than other electrophysiologic measures, such as the frequency-following response (FFR). The ability to identify such deficits is crucial because they have been purported to influence language development and understanding speech in noise. Unfortunately, current clinical practice does not allow sufficient time to measure the ABR and FFR separately. Such time pressures could be alleviated, however, with use of a stimulus that allows for simultaneous recording of the ABR and FFR. As a preliminary step towards addressing this issue, our current goal was to validate a signal called a 'click plus' which can allow both responses to be recorded simultaneously.

Methods: Here, we recorded the ABR and FFR with different experimental conditions. First, we recorded the ABR to a click stimulus with varying stimulus intensities. Second, we recorded the FFR using four different stimuli (100 Hz harmonic complex, 200 Hz harmonic complex, 100 Hz tone, and a synthetic /a/). Finally, we recorded the ABR and FFR simultaneously to a 'click plus' stimulus; this stimulus consisted of a click followed by 15 ms of silence, and then one of the stimuli used to elicit the FFR. The 'click plus' was recorded with each of the four stimuli used to elicit the FFR alone, using the same stimulus intensities as the ABR. Across all three conditions, recordings were made from young adults with normal hearing using clinical recording montages. Data were analyzed in two stages. First, we compared the latency and amplitude of Waves I-V for the ABR-alone and the 'click plus' conditions. Second, we compared the RMS amplitude of the FFR waveforms for the FFR-alone and 'click plus' conditions.

Results: While preliminary, our results indicate no differences in ABR waveforms elicited by a click stimulus presented either alone or as part of the 'click plus' stimulus. Notably, the RMS amplitude of the FFR appeared to be slightly larger in the 'click plus' condition than in the FFR-alone condition. No other differences were noted between FFRs recorded alone or as part of the 'click plus' stimuli.

Conclusions: Taken together, these results suggest that the ABR and FFR can be reliably recorded simultaneously using a combined 'click plus' stimulus with only a minimal increase in time. Such work provides an important step to increase the feasibility of clinical recording of FFR, thereby allowing suprathreshold measures of auditory function to be widely assessed in neonates and young children.

Effects of Cochlear Synaptopathy on Tone-In-Noise Coding in the Cochlear Nucleus

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Background: Hearing impairment in the absence of threshold shifts is characterized by permanent damage to synapses between inner hair cells and high-threshold auditory nerve fibers (ANFs). Cochlear synaptopathy is a potential major health issue in humans, as many listeners with clinically normal audiograms still have difficulty hearing in noisy settings and aged human temporal bones demonstrate widespread synapse loss despite no explicit otopathology. Previous behavioral studies have shown no effect of synaptopathy on tone-in-noise detection thresholds, but this has not been confirmed by neural recordings, nor has the effect of synaptopathy been shown on

suprathreshold neural responses in noise. Here, we examine the effect of synaptopathy on tone-in-noise coding on the direct recipients of ANFs – cells in the cochlear nucleus.

Methods: Guinea pigs received a unilateral sound overexposure to the left ears (7 kHz centered, third-octave noise at 102 dB SPL for 2 h), producing unilateral temporary threshold shifts. At 4 weeks post-exposure, loss of auditory nerve synapses and reduced ABR wave 1 amplitudes were observed specifically on the left side. A separate group of animals received sham noise exposures. Single unit responses were recorded from several cell types in the cochlear nucleus. Puretone stimuli (2-24kHz; 0-90 dB SPL) were used to generate receptive fields and rate-level functions in the presence of either 0, 40 or 60 dB SPL continuous broadband noise.

Results: The synaptopathy-inducing noise exposure did not affect mean single-unit tone-in-noise threshold , nor the lowest tone-in-noise thresholds in each animal, demonstrating equivalent tone-in-noise detection thresholds compared to sham animals. However, synaptopathy reduced neural responses in response to suprathreshold tones in the presence of background noise.

Conclusions: These results demonstrate that despite not altering tone-in-noise thresholds, synaptopathy results in reduced activity in the cochlear nucleus in response to suprathreshold tones in the presence of background noise. Overall, these data have implications for hearing in the presence of background noise and offer a new method to objectively test for synaptopathy.

Bushy Cell Dendrites Degenerate During Age-Related Hearing Loss

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Background: Bushy cells are one of the principal neurons of the ventral cochlear nucleus that are characterized by their elaborate dendritic arborization. It receives auditory nerve (AN) inputs via large axosomatic synapses called the endbulb of Held. Our previous study showed that AN synapses onto bushy neurons degenerate during age-related hearing loss (ARHL), and the degeneration is more profound in non-calretinin-expressing synapses (presumably from low/medium spontaneous rate spiral ganglion neurons). It remains unclear whether different subtypes of innervating AN synapses correlate with specific bushy cell morphologic features, and how bushy cell morphology changes during ARHL.

Methods: To investigate these questions, we imaged and reconstructed the 3D structure of dye-filled bushy cells from young (2-5 months) and aged (28-32 months) CBA/CaJ mice. We further identified the subtype-specificity of their innervating AN synapses using immunohistochemistry with antibodies against calretinin and VGluT1. Both calretinin-expressing and non-calretinin-expressing synapses were quantified by the volume of their vGluT1-labeled puncta. The morphologic features of reconstructed bushy cells were evaluated by Sholl analysis.

Results: We found no correlation between the subtype-specificity of AN synapses and the cellular morphology of the postsynaptic bushy neurons. However, the number and complexity of dendritic arborizations of bushy cells in old mice were significantly decreased compared to those in young mice, indicating that bushy cell dendrites degenerate during aging. In addition, the degeneration was more profound in bushy cells predominantly innervated by non-calretinin-expressing synapses.

Conclusions: The results suggest that bushy cells innervated by different subtypes of AN inputs are differentially impacted by aging which may contribute to the development of ARHL.

Place Specificity of the Parallel Auditory Brainstem Response

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Background: The frequency-specific auditory brainstem response (ABR) is commonly used for clinical and research purposes, but it is limited by its long test times. To speed up ABR acquisition, our lab has recently developed the parallel ABR (pABR) exam. The pABR utilizes randomized stimulus timing to present multiple frequencies in both ears all at once, producing waveforms comparable to standard ABR paradigms in shorter test times. Parallel presentation of stimuli may provide the additional benefit of improved place specificity. Higher-intensity stimuli can excite broad regions of the cochlea such that responses to frequency-specific stimuli can contain significant contributions from regions with characteristic frequencies below and (especially) above the stimulus frequency. With standard ABR methods, this spread of excitation can be reduced by using masking noise. However, adding masking noise further increases test times, so it is rarely used in practice. Since the pABR

paradigm presents stimuli in parallel, each stimulus can also act as a masker so additional masking noise is not needed to obtain place-specific responses.

Methods: Our previous work using models of the auditory periphery and nerve has shown parallel presentation improves place specificity. Here, we complement that investigation with data from 12 human subject recordings. EEG was recorded while subjects were presented with serial or parallel toneburst stimuli, along with high pass masking noise at varying cutoff frequencies. The masking noise allowed us to examine different frequency regions of the auditory system to assess the effect of parallel presentation on place specificity. We also tested a secondary hypothesis that stimulus rate and frequency would affect the efficacy of masking in parallel presentation, and thus the place specificity, with stronger masking at lower frequencies and higher stimulus rates. This was accomplished by testing two frequencies for the serial condition (500 and 2000 Hz) and three stimulus rates.

Results: At the low stimulus frequency (500 Hz), we find smaller responses with longer latencies at higher masking cutoff frequencies, indicating parallel presentation improves place specificity. However, we see no differences in responses at the higher stimulus frequency (2000 Hz). This is likely due to a very small effect size at higher frequencies, where even serial responses are more place specific than for lower frequency stimuli.

Conclusions: The pABR provides responses faster than existing methods, and these responses more directly link stimulus frequency and cochlear place. This link between stimulus frequency and cochlear place at high levels, in conjunction with more complete testing made possible by faster test times, means the pABR may provide a more accurate assessment of hearing impairments.

Electrophysiological and Morphological Properties of Inhibitory and Excitatory Principal Neurons of Mouse Lateral Superior Olive

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Background: The lateral superior olive (LSO) nucleus in the brainstem is critical for horizontal sound localization. LSO principal neurons (PNs) compare excitatory inputs driven by the ipsilateral ear with inhibitory inputs driven by the contralateral ear. The textbook version of LSO function is encoding ongoing interaural level differences. However, recent findings and deductions based on the extreme timing fidelity provided by the calyx of Held suggest that a major function of the LSO lies in extraction of interaural timing differences particularly for transient broadband sounds, but also for amplitude modulations. These modes of sound localization place disparate demands on the cellular properties of LSO neurons. Furthermore, there is cellular diversity in the LSO that is not well understood that may underlie specialization for certain functional roles.

Methods: We examined inhibitory and excitatory LSO PNs in mouse brain slices using whole-cell patch-clamp and two-photon imaging. To target specific cell types, we used knock-in reporter mice that co-express the fluorescent protein tdTomato with vesicular glutamate transporter 2 (vGlut2). PNs were selected based on size and electrophysiology. Glutamatergic PNs had prominent somatic fluorescence while other PNs exhibited a distinct lack of fluorescence in their soma, rendering these putative inhibitory cells "black holes." Immunostaining for glycine and in situ hybridization suggest no overlap between glutamatergic and glycinergic LSO neurons.

Results: Compared to excitatory PNs (n=48), inhibitory PNs (n=41) had 5mV lower resting membrane potential (I:-69.81, E:-64.91mV) and 47% higher input resistance (I:56.55, E:30.24MΩ). Inhibitory PNs also had correspondingly slower membrane time constants and lower rheobase (I:325.49, E:490.21pA). These findings suggest that despite their more hyperpolarized resting membrane potential, inhibitory LSO PNs are more excitable.

Threshold was not different between the groups, however, inhibitory PNs had lower AP peaks (I:-3.88, E:7.24mV) suggesting differences in voltage gated sodium channels. AP half-width was wider in inhibitory PNs (I:0.216, E:181ms). Excitatory PNs exhibited larger sag potentials in response to hyperpolarizing current injections (I:12.83, E:17.57mV) suggesting higher HCN channel density.

In both LSO PN types, we observed two action potential firing modes. Onset-burst responses were more common and produced 3 to 5 spikes at higher current injection levels. Multi-spiking neurons capable of sustained firing rates up to 550Hz were found throughout the body of the LSO. Neither group exhibited tonotopic bias. Multi-spiking was less common in inhibitory neurons (I:27%, E:50%).

Inhibitory and excitatory PNs had similar maximum dendritic extension, soma volume, and primary dendrite diameter, however, excitatory PNs had more complicated dendritic arbors with larger total dendritic length (I:426.90, E:603.70µm), number of dendritic branch points (I:2.41, E:3.83), and number of primary dendrites (I:3.52, E:4.41).

Conclusions: These data show that inhibitory and excitatory LSO PNs differ in electrophysiological and morphological properties that would impact integrative processes involved in sound localization.

Kv1 Channels Regulate Variations in Spike Patterning and Temporal Reliability in the Avian Cochlear Nucleus Angularis

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Background: Diverse physiological phenotypes in a neuronal population can broaden the range of computational capabilities within a brain region. The avian cochlear nucleus angularis (NA) contains a heterogeneous population of neurons whose variation in intrinsic properties results in electrophysiological phenotypes with a range of sensitivities to temporally modulated input. The low-threshold potassium conductance (GKLT) is a key feature of neurons involved in fine temporal structure coding and interaural time difference computations in the timing pathway of the brain stem but a role for these channels in intensity or spectrotemporal coding has not been established.

Methods: To determine whether GKLT affects the phenotypical variation and temporal properties of NA neurons, we applied dendrotoxin (DTX), a potent antagonist of members of the Kv1 family of potassium channels, to chick brain stem slices in vitro during whole-cell patch clamp recordings from NA neurons. In order to mimic spectrotemporal activity in vitro, we used noisy current injections that simulate the fluctuation of input that NA neurons receive during periods of temporally modulated auditory input.

Results: We found that only a subset of NA neurons were sensitive to DTX, suggesting cell-type specific expression of the Kv1 channels. As expected, single-spiking NA neurons were most profoundly affected, but a subset of tonic firing neurons were also DTX-sensitive. Among these, both tonic I and tonic II neurons, which can be differentiated based on their phasic onset bursting or delayed bursting firing patterns in vitro, showed DTX sensitivity in their firing rate and phenotypical firing pattern. A third subset, tonic III neurons, were unaffected. When spike time reliability and fluctuation sensitivity was measured in DTX-sensitive NA neurons, we found that the temporal response sensitivity to rapidly fluctuations in their inputs was reduced with the drug. Finally, we show that DTX reduced spike threshold adaptation in these neurons.

Conclusions: These results suggest that variations in Kv1 channel expression are present in tonic NA neurons as well as single spiking NA neurons. Differential expression of these channels may be a key factor in functional diversity in the avian cochlear nucleus. Increased firing rate, diminished spike timing and fluctuation sensitivity indicated that Kv1 channels increased selectivity to temporally modulated input. Reduced spike threshold adaptation suggests that Kv1 channels interact with other ion channel mechanisms, such as Na channel inactivation, to produce the temporal properties that allow coding of rapid changes in their inputs. Taken together, these results suggest Kv1 channels are important for the reliable encoding of spectrotemporal input.

Effects of Noise Damage on Auditory Nerve Inputs to T-Stellate Cells and Subsequent Modification of Nitric Oxide Activation

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Background: Little is known about how noise damage affects the central processes of auditory nerve (AN) axons or their synapses in ventral cochlear nucleus (VCN). We previously provided specific details about the number and size of AN inputs to T-stellates (Cao and Oertel, 2010), making it possible to compare/evaluate changes generated by noise exposure.

Methods: Mice were exposed to broad-band noise (2-32 kHz, 105 dB SPL, 3 hrs). 2-14 days after exposure auditory brainstem responses (ABRs) were measured, followed by patch clamp recordings from T-stellates in slices. Single and repetitive shocks were applied to the AN.

Results: In control mice, increasing AN shocks elicited 5-6 synaptic events in T-stellates. 2 days after noise exposure eliminating ABRs, shocks elicited 2-3 events. Individual input amplitudes were similar to controls but had smaller combined amplitudes due to reduced numbers. In demyelinating diseases like multiple sclerosis (MS), patients given low doses of 4-aminopyridine (4-AP, voltage-sensitive K-channel blocker) show improved motor function thought to arise from restored spike conduction in affected axons by blocking exposed voltage sensitive K channels at damaged nodes of Ranvier. We reasoned that 4-AP might have similar effects on damaged AN fibers restoring conduction. 4-AP applied to noise damaged slices did not restore lost AN inputs to T-stellates.

Although synapse number stayed constant, 4-AP did increase the remaining synaptic event amplitudes presumably by widened action potentials generating enhanced glutamate release from still-functioning AN terminals. Remarkably, despite reduced synaptic numbers un-recovered by 4-AP, repetitive shocks to the remaining AN inputs in 4-AP generated post-stimulus spike responses not seen in control slices. We previously showed such post-stimulus responses to be mediated by nitric oxide (NO) synthesis and release. The present post-stimulus events are also NO mediated in the noise damaged preparation. 14 days after noise exposure the ABR was still absent but synaptic input number returned to normal and this NO mediated event was no longer elicited. **Conclusions:** 2 days after noise damage, functional AN inputs to T-stellates are reduced generating a reduction of the maximum summed excitatory input. 4-AP does not restore these lost inputs but does generate a NO mediated post-stimulus response not elicited in control slices. By day 14 the ABR has not recovered but the central AN processes apparently have. Despite this recovery the NO mediated event is no longer evoked. This indicates that by day 2 the system had compensated or perhaps over-compensated for the input loss by what appeared to be an upregulation of the remaining AN input's ability to activate the T-stellate NO system. By day 14 AN central processes recover and NO upregulation is lost. The persistent ABR loss likely arises from cochlear damage peripheral to the spiral ganglion cell body.

Quality of Life in Classical (Infratentorial) Superficial Siderosis

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Background: The most common symptoms of classical (infratentorial) superficial siderosis (iSS) are hearing impairment followed by imbalance which gradually decline over time. Multifunctional involvement (particularly hearing) is likely to adversely impact quality of life (QoL) in iSS. This has not been evaluated previously. The aim of this study was to assess QoL in iSS.
Methods: This anonymous online survey included generic QoL questionnaires: Health Utilities Index-Mark 3 (HUI3) and EuroQoL-5D (EQ5D-5L, UK tariff). Following Ethics approval, the study information was distributed to dedicated patient groups, organisations and charities. Participants were asked to confirm they were of ≥18 years old, confirmed they had been diagnosed with iSS, and consented to participating in the study. They were then able to proceed to study-specific questions and questionnaires, presented in fixed order.
Results: We included 51 participants: 31 (61%) were male; mean (± standard deviation, SD) age was 57.9 (±12.1) years; median (interquartile range, IQR) disease duration (time from likely causative event to survey, known in 33 cases) was 22.0 (28.0) years. The multi-attribute scores (n=50) were: mean (±SD) 0.36 (±0.45) for HUI3 and median (IQR) for EQ5D-5L 0.64 (0.33) and were statistically significantly different between the two instruments (mean ranks, z=4.85; p<0.001). Disease duration correlated (assessed by Kendall's tau-b, T_b) with HUI3 scores ($T_b(33)$=-.268, p=0.033), but not EQ5D-5L scores ($T_b(33)$=-.245, p=0.051). The HUI3 scores in the "Hearing" domain correlated with multi-attribute scores for both HUI3 ($T_b(50)$=.650, p<0.001) and EQ5D-5L ($T_b(50)$=.323, p<0.001). Most frequently affected domains (moderate or worse category) were "Hearing"/"Pain" for HUI3 (32 (64%) and 24 (48%), respectively) and "Mobility"/"Pain" for EQ5D-5L (27 (54%) and 25 (50%), respectively).
Conclusions: The QoL scores in iSS are low, indicating a major adverse impact comparable to Motor Neuron Disease (EQ5D-5L). HUI3 QoL instrument that includes "Hearing" domain – correlates with disease-duration (HUI3) and possibly better reflects the QoL in iSS, by capturing lower multi-attribute scores. We suggest that QoL measure such as HUI3 should be used in future clinical and research studies of iSS.

Comparison of Spiral Ganglion Neuronal Counts in Human Temporal Bones Harboring Sporadic and NF2-Associated Vestibular Schwannomas

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Background: Vestibular schwannomas are benign Schwann cell tumors of the vestibular nerve and are frequently associated with progressive sensorineural hearing loss (SNHL). Tumors can be caused by sporadic mutations in the NF2 gene, or by congenital loss of NF2, which is associated with Neurofibromatosis Type 2.

To this day, the etiology of the hearing loss is poorly understood. Our previous work in a mouse model of NF2 demonstrated that SNHL and cochlear phenotype were associated with loss of auditory synapses and preserved spiral ganglion neuron architecture, which could in part explain the sensorineural hearing loss in patients with vestibular schwannomas. In this preliminary human temporal bone study, we compared numbers of spiral ganglion neurons (SGNs) in human temporal bones specimens associated with sporadic vestibular schwannomas and NF2-associated vestibular schwannomas.

Methods: We scanned our National Temporal Bone, Hearing and Balance Pathology Resource Registry for cases of unilateral or bilateral vestibular schwannomas without additional cochlear or retrocochlear pathology. A total of 28 cases of unilateral or bilateral schwannomas were identified. For our preliminary analysis, we selected 5 cases of sporadic schwannomas and 3 cases of NF2 that had sufficient H and E imaging for quantification, as well as audiometric data available for analysis. Age matched controls were obtained and quantitative analysis of neurons and glial cells was performed per 100 µm2 area in midmodiolar sections of Rosenthal's canal (apex, mid-apex, mid-base, base for each) and Scarpa's ganglion in 3 separate areas of 3 serial sections.

Results: All NF2 cases demonstrated longstanding, severe to profound hearing loss in the affected ear(s). Histological analysis of these cases demonstrated severe neuronal loss in both the cochlea and Scarpa's ganglion. Proliferation of glial cells was clearly evident in Scarpa's ganglion. In contrast, numbers of spiral ganglion neurons in patients with sporadic schwannomas and moderate to severe SNHL did not significantly differ from age-matched controls.

Conclusions: In this preliminary human temporal bone study, we report that NF-2 associated vestibular schwannomas appear to be associated with greater SGN loss than sporadic vestibular schwannomas. We discuss the implications for sensorineural hearing loss in the setting of vestibular schwannomas.

Influence of Auditory Brainstem Implant (ABI) Position on Perception: A Multicenter Study

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Background: Variable perceptual outcomes in auditory brainstem implant (ABI) patients can be explained by a number of factors that include electrode array position. Our group has studied the relationship between perception and ABI array position using quantitative three dimensional (3D) image analyses of postoperative computed tomography (CT) described by Barber et al. Here, we extend the use of this technique to correlate 3D ABI position and perceptual outcomes across two centers.

Methods: Retrospective cohort study of adult NF-2 and non-tumor ABI users from two tertiary medical centers with similar devices (Cochlear Corp. ABI24, ABI22 and ABI541). Adult ABI subjects (both NF-2 and non-tumor) from the Medizinische Hochchule Hannover (MHH, Hannover, Germany) were compared to adult subjects from Massachusetts Eye and Ear (MEE, Boston, MA). For all subjects, post-operative 3D CT reconstructions were used to measure the ABI position. The vertical distance (D1), horizontal distance (D2) and angles (T, V) of the ABI array were measured in the lateral and posterior views (Barber, 2017). ABI position, perceptual outcomes, and side effects were compared across patients from both medical centers.

Results: A total of 12 subjects (10 NF2, 2 non-tumor)(83% suboccipital craniotomy) from MHH and 20 (18 NF2, 2 non-tumor)(55% suboccipital craniotomy) from MEE were analyzed. From the lateral and posterior view most arrays were posteriorly and medially oriented. There was no significant difference (p>0.05) in the ABI position between the MHH and MEE. The mean angle in the lateral view (V) was 43.86˚(SD, 32.31) versus 29.21˚(SD, 30.50), and the posterior view (T) was 40.59˚(SD, 34.98) versus 77.32˚(SD,91.76) for MHH and MEE, respectively. The D2 distance in the posterior view was 1.04 cm (SD, 0.42) vs 1.11 cm (SD, 0.30), the D2 distance in the lateral view was 1.34 cm (SD, 0.41) vs 1.30 cm (SD, 0.46), and the D1 distance in both views was 1.85 cm (SD, 0.39) vs 1.73 cm (SD, 0.42) for MHH and MEE, respectively. Patients with better speech perception (open set word-recognition) at both centers had lower charge thresholds compared to other patients (p<0.05) and similar array orientations. However, most active electrodes for MHH ABI users were in the proximal part of the array while these were found in the middle portion of the array in MEE patients. Almost 60% of electrodes were

associated with side effects from both centers, although there were differences in the type of side effects (mostly vertigo for MHH and tingling in the head and neck for MEE.

Conclusions: The best performing ABI users shared similar 3D array orientations and low charge electrode thresholds. There was a range of active electrode patterns and side effects across medical centers. Future efforts will include a prospective study of ABI position and perception across multiple centers.

Factors in Control and Optimization of Extracochlear Electrocochleography Recordings

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Background: Advances in cochlear implant (CI) surgery have established electrocochleography (ECochG) as an effective intraoperative method to monitor and potentially mitigate insertion-related intracochlear trauma. Some ECochG systems utilize intracochlear recordings from the CI electrode array, but because the electrode array position is constantly moving during insertion, stationary extracochlear recording may provide a more consistent signal for determining intracochlear condition. In this study we evaluated ways to optimize extracochlear ECochG recording signal quality through electrode design and location assessment in a large animal in vivo CI model.
Methods: The effects of recording electrode type and location on the signal quality of an extracochlear ECochG recording system were evaluated during electrode insertion in a sheep in vivo model. Recording electrodes tested included a ball tip, bare wire, conductive mesh, and the presence or absence of a saline-soaked sponge in various locations (round window (RW), promontory, and facial recess). A main effects analysis of electrode type and location was completed for impedance measures and ECochG amplitude of each permutation.
Results: The effect of electrode tip type on recording impedance was insignificant except in the case of the conductive mesh, which significantly reduced impedance. However, the tip type significantly affected the ECochG magnitude; a ball tip and sponge provided significantly higher amplitudes than all others. Additionally, recordings taken closer to the RW exhibited significantly higher amplitude than other locations. All locations and tip types tested were able to detect ECochG changes during CI insertion into the cochlea of live sheep.
Conclusions: The effect of electrode tip type on recording impedance was insignificant except in the case of the conductive mesh, which significantly reduced impedance. However, the tip type significantly affected the ECochG magnitude; a ball tip and sponge provided significantly higher amplitudes than all others. Additionally, recordings taken closer to the RW exhibited significantly higher amplitude than other locations. All locations and tip types tested were able to detect ECochG changes during CI insertion into the cochlea of live sheep.

Cochlear Implantation in Ski-Slope Hearing Loss Considering Molecular Etiology and Residual Hearing

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Background: Patients with ski-slope hearing loss are at a unique situation because hearing aids do not provide adequate amplification to mid-to-high frequencies necessary for speech perception. In these patients, cochlear implantation (CI) with or without electric-acoustic stimulation (EAS) is a good option.
Methods: We identified ski-slope hearing patients, defined as hearing thresholds of 40 dB or less at 250 Hz, 70 dB or more at frequencies higher than 2kHz, and a sudden decline between 250 Hz and 2 kHz, from our registry of hearing loss patients. Genetic diagnosis was made whenever possible. The percentage of those using hearing aids and cochlear implants were reviewed. The hearing preservation rate, and threshold shift were analyzed in patients with CI.
Results: Forty-six patients with ski-slope hearing loss were recruited in this study. Forty-five agreed to undergo genetic testing. Pathogenic/likely pathogenic variants were identified in 15 (33.3%) patients in which TMC1, LOXHD1, and TMPRSS3 variants were the most common. Thirteen patients continued to wear hearing aids and 24 chose to undergo CI. The pure tone average at 500, 1k, 2k, and 3k Hz were better in the hearing aid users (right ear, 64.1 ± 15.9 dB; left ear, 67.4 ± 15.2 dB) than the CI users (right ear, 85.0 ± 15.8 dB; left ear, 87.2 ± 15.9 dB) ($p<.001$). As for the CI users, at 1-year post-implantation, threshold shifts at 250 Hz and 500 Hz were 30 ± 20.5 dB and 28.3 ± 21.1 dB, respectively. The proportion of subjects who met the criteria for either complete or partial hearing preservation (according to the HEARRING classification) was 87.5%. The hearing preservation rate was not different between the genetically diagnosed group and the undiagnosed group ($66.2\pm30.8\%$ vs $48.5\pm22.9\%$). The proportion of implantees maintaining functional low-frequency hearing (better than 85 dB) was 48.5%.

Conclusions: The detection rate of molecular etiology in ski-slope hearing loss was low compared to other types of sensorineural hearing loss. Low frequency hearing after CI was well preserved, allowing acoustic hearing and even natural hearing in some patients.

SU55. Developmental Determination of Central Auditory Physiology by the Inner Ear

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Background: Neurons in auditory nuclei express topographically distributed specializations enabling precise computations in frequency-specific channels. The degree to which expression of tonotopic features arises independently or in response to topographically patterned input from the inner ear in development is unknown. Previous studies have established central neurons' dependence on cochlear afferents for survival, connectivity, and refinement by utilizing methods that profoundly disrupt hearing (Parks, 1979; Mostafapour et al., 2000, Sanes and Takacs, 1993; Leao et al., 2004). Clearly, ablation and deafening studies demonstrate that peripheral input is required for central neuron survival and connectivity, but it is unknown how tonotopic refinement is regulated in the cochlear nucleus (CN) during the peri-hearing onset period. In particular, the influence of peripheral patterning on determination of intrinsic biophysical features in central neurons has not been investigated. Here, we successfully induce molecular repatterning of peripheral tonotopy, and evaluate the developmental outcomes of this treatment on patterning of central phenotypes in the avian CN, nucleus magnocellularis (NM).
Methods: Unilateral tonotopic rearrangement of the basilar papilla (BP) was induced through overexpression of Bmp7 in the developing ear prior to tonotopic patterning (Mann et al., 2014). We then investigated hallmarks of tonotopic identity in both hair cells and in neurons of the NM using electrophysiological, molecular, and anatomical methods.
Results: Tonotopically distributed phenotypes in the periphery were systematically altered, indicative of an altered tonotopic representation. Overexpression of Bmp7 in the BP resulted in disruption of the hair cell phenotypic gradient, such that hair cells along the length of the basilar papilla showed morphologies typical of low frequency representation. In control ears, strong BK channel current kinetics vary tonotopically and contribute to electrical frequency tuning. In contrast, following Bmp7-treatment, voltage clamp recordings and qPCR confirmed a dampening of the gradient of BK activation kinetics and an associated alteration in BK subunit expression along the BP. Together these results strongly suggest that our Bmp7 method generates BPs that are biased toward low frequency representation. Centrally, NM neurons located in "high frequency" positions acquired low frequency phenotypes ipsilateral to treatment. For example, reduced expression of two classes of voltage gated potassium channel resulted in increased excitability in the "high frequency" NM, a feature typically indicative of low frequency processing.
Conclusions: These results strongly suggest that Bmp7 treatment generates BPs with overrepresentation of low frequencies and little to no representation of high frequencies. The repatterning of the periphery during a critical period of development induces tonotopic reorganization of the cochlear nucleus that is expressed at the ion channel level. This work provides new insights into the contributions of cochlear organization to brain development. These findings may inform interventions that aim to preserve central auditory function following early stage deafness or cochlear aplasia.

Sox2 Deletion From Native or Regenerated Type II Vestibular Hair Cells Causes Conversion to Type I-Like Cells and Increased Motor Activity in Adult Mice

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Background: Around 22% of vestibular hair cells (HCs) is regenerated when HCs are destroyed by diphtheria toxin in adult Pou4f3DTR mice. All regenerated HCs are type II. Vestibular function does not recover. In adult mice, conditional knock-out (CKO) of Sox2 from type II vestibular HCs causes them to convert into type I-like HCs. We hypothesized that 1) manipulation of the type I:II proportion via Sox2 CKO would disrupt vestibulo-motor behaviors and 2) conversion of regenerated type II HCs to type I HCs would ameliorate vestibulo-pathic behaviors resulting from HC damage. We also deleted Atoh1, which acts downstream of Sox2, from regenerated type II HCs and hypothesized that type II HCs would also convert into type I-like HCs.

Methods: We administered tamoxifen to Atoh1-CreERTM::Sox2loxP/loxP:Rosa26tdTomato adult mice, with or without the Pou4f3DTR allele, to delete Sox2 from undamaged (homeostatic) type II HCs or from regenerated type II HCs. Using tdTomato labeling to identify cells with Sox2 deletion, we examined HC markers, morphology, ultrastructure, and innervation for several months post-tamoxifen. In these mice, we examined open field behaviors, rotarod performance, and the vestibulo-ocular reflex (VOR). In addition, we administered tamoxifen to Pou4f3DTR:Atoh1-CreERTM::Atoh1loxP/loxP:Rosa26tdTomato adult mice and examined HC markers, morphology, and innervation.

Results: Sox2 CKO from regenerated type II HCs resulted in loss of calretinin (a selective marker of type II HCs), elongation of stereocilia to type I-like proportions, and increased numbers of HCs with a calyceal afferent terminal (a type I-specific feature) in utricles by 4 months post-tamoxifen. Deletion of Atoh1 from regenerated HCs also caused them to acquire some of these type I-like features by 4 months. Mice with Sox2 CKO from native type II HCs exhibited increased motor activity relative to Sox2 wildtype controls, as reflected by total distance travelled and average velocity. Mice with regenerated HCs after damage had increased motor activity (distance travelled, average velocity, spinning, and climbing) compared to mice without HC damage, and these behaviors were increased following Sox2 CKO. We noted no changes in rotarod performance after Sox2 deletion in either group. The VOR was essentially lost from mice with HC destruction and not regained after Sox2 deletion from regenerated HCs.

Conclusions: Induction of native type II HCs to acquire type I-like features via Sox2 deletion produced abnormal motor activity. Deletion of Sox2 from regenerated HCs also caused them to acquire some type I features and resulted in altered motor activity. This latter finding does not support the hypothesis that the current approach toward inducing conversion of regenerated HCII to HCI is sufficient to ameliorate vestibulo-pathology. Further studies are required to understand the mechanisms underlying the alterations in motor activity. We are assessing the impact of conversion of native HCII>I upon other organs and the VOR.

Deletion of Sox2 from Adult Vestibular Type II Hair Cells Partly Shifts Properties of Outward K+ Currents From Type II-Like to Type I-Like

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Background: Mammals have two types of vestibular hair cells (HC), type I and type II, which differ morphologically and functionally. Type I HCs are enwrapped by calyceal afferent terminals and have a low-voltage-activated potassium (K) conductance (gK,L) and therefore low membrane resistance, Rin. Type II HCs are contacted by afferent bouton terminals, have K channels that activate positive to resting potential and therefore high Rin, and, unlike type I HCs, express the transcription factor Sox2. Deletion of Sox2 causes type II HCs to acquire type I-like features (Stone et al. 2021, J Neurosci 41:6217). To functionally characterize these Sox2-deleted type I-like HCs, we recorded membrane currents in adult mice at different time points after Sox2 deletion.

Methods: Experimental and control mice are described in Stone et al. (ibid.). In 6-week-old Sox2-CKO (conditional knockout) mice (Atoh1-CreERTM:Rosa26tdTomato:Sox2loxP/loxP), tamoxifen injections drove deletion of Sox2 expression selectively from td-Tomato-labeled type II HCs. Control Sox2-WT littermates (Atoh1-CreERTM:Rosa26tdTomato:Sox2+/+) received the same treatment. From 1 - 11 months post-tamoxifen, we recorded HC currents using whole-cell patch-clamp in the semi-intact utricle.

Results: Sox2 deletion changed Rin and properties of the dominant outwardly rectifying K+ currents in type II HCs to values intermediate between WT type II and type I HCs, consistent with partial transdifferentiation from type II toward type I. No differences were observed in resting potential and cell surface area. Midpoint of the activation voltage range (V1/2), voltage sensitivity (slope factor) and maximum conductance density differed significantly between WT type I, WT type II, and Sox2-CKO type II HCs, with Sox2 deletion moving the values toward type I values. For Sox2-WT utricles, V1/2 was -30 ± 1 mV (27 type II) and -85 ± 1 mV (8 type I). For Sox2-CKO utricles, V1/2 was -40 ± 2 mV (37 Sox2-deleted type II), and -83 ± 1 mV (8 type I). Thus, deleting Sox2 from type II HCs shifted V1/2 significantly more negative than WT ($p = 2.5e{-}4$), though less negative than in type I hair cells.

Conclusions: The voltage dependence of outwardly rectifying K+ current in WT type I and type II HCs differs strikingly, with consequences for receptor potential gain and frequency dependence as well as mechanisms of afferent synaptic transmission. Sox2 is a marker for vestibular type II HCs, and its deletion from adult hair cells shifted the voltage dependence and size of the KV current, as well as Rin, in the direction of type I values. These

results offer promise for the effort to regenerate type I HCs in adult vestibular epithelia, which naturally regenerate only type II HCs (González-Garrido et al., J Neurosci 41:7779, 2021).
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Analysis of Temporally Distinct Roles of the Notch Ligand Jagged1 During Sensory Development of the Cochlea

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Background: The sensory region of the cochlea, the organ of Corti, contains several critical cell types, including hair cells (HC), supporting cells (SC), and neurons. Notably, in mammals, these cells cannot be regenerated or repaired. Understanding the factors involved in the development of these critical cell types will aid future studies in cell regeneration/replacement. Studies have shown the Notch ligand, Jagged1 (JAG1), is important for sensory formation in the inner ear. However, JAG1 has a dynamic expression pattern during inner ear development, suggesting Jag1 may have several temporally distinct roles. Here, we investigated several possible roles of JAG1 based on its dynamic expression pattern. During early otic development (~E10.5) JAG1 is expressed ventrally in a broad domain and then becomes localized to the ventral region of the cochlea including the future organ of Corti (E12.5). This early expression pattern suggests that JAG1 may be involved in initial sensory region specification. Later in development (E14.5), JAG1 becomes localized to a medial region adjacent to the organ of Corti, indicating it may be involved in boundary formation.
Methods: To dissect the potential roles of JAG1 during different otic time points, Jag1 was conditionally deleted via Foxg1+/Cre (~E10.5), and Sox2+/CreER (E12.5 and E14.5). These cochleae were analyzed at E17.5/E18.5 or postnatal day (P)6. We primarily analyzed the cochlear basal regions via immunohistochemistry in frozen sections and whole mount to avoid overlap in temporal developmental windows.
Results: Upon deletion of Jag1 during early sensory development (~10.5), there was widespread loss of HCs and SCs, indicating JAG1 is involved in early specification of the sensory progenitors. Upon Jag1 deletion at E12.5, there was loss of outer hair cells (OHC), along with disorganization of IHCs. Interestingly, there was an expansion in p75ntr expression and PROX1+ nuclei in the outer hair cell region, suggesting progenitors are present but may be delayed in differentiation. In contrast, when Jag1 was conditionally deleted at E14.5, there was no observed disorganization of the IHC region, suggesting Jag1 does not have a role in boundary formation region at this time point. However, the disorganization in IHCs observed when Jag1 is deleted at E12.5 may still reflect a medial boundary loss.
Conclusions: Taken together, our results indicate Jag1 is required for early sensory progenitor specification (~E10). Later (E12.5), JAG1 is required for proper temporal differentiation of the outer hair cell region and may delineate a medial boundary for proper inner hair cell patterning. Surprisingly, Jag1 does not appear to play a significant role in pattern formation in the cochlea after E12.5. Our results indicate that JAG1-Notch signaling plays a complex role in early sensory patterning in the cochlea.

Characterization of FGF5 During Development of Inner Ear Neurons

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Background: During development of the inner ear, neuron progenitors delaminate from the otic epithelium forming the cochleovestibular ganglion (CVG). These neuronal progenitors will undergo cell fate decisions and differentiate into cochlear and vestibular neurons. The Fibroblast Growth Factor (FGF) family has been shown to contribute to many developmental processes in the inner ear. Previous research has shown that FGF5 is expressed in the otic vesicle and CVG very early in development.
Methods: To explore whether FGF5 might play a role in cell fate specification of inner ear neurons, we characterized the spatiotemporal expression of FGF5 in chick auditory and vestibular neurons, by performing immunohistochemistry from E3-E6.5, at every half day. The observed FGF5 expression was compared to several well-known neuronal labels NeuroD1, GATA3, and TuJ1.
Results: At E3-4.5, FG5 labeled a subset of cells within the TuJ1 labeled cells, and this population was NeuroD1 negative. These data suggest that FGF5 is present in postmitotic cells and may be involved in differentiation of neurons. GATA3 and FGF5 were co-expressed in the neuronal population indicating a role in differentiation of cochlear neurons.

Conclusions: Together these results indicate a role for FGF5 in differentiation of inner ear neurons.

Visual Deprivation Alters Spontaneous and Sound-Driven Activity in the Auditory Cortex of Newborn Mice

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Background: Sensory experience facilitates structural and functional maturation of the developing nervous system and sensory deprivation severely impairs them. Although the time course for experience-driven sensory development is specific for each modality, the developing brain acts as a whole, and therefore, sensory perturbation in one modality results in adaptive reorganization of neural pathways within the unaffected, spared modalities, a phenomenon known as "crossmodal plasticity". Although a large body of literature has demonstrated adaptive crossmodal changes during the classic critical period, it is still not known how early in development crossmodal changes emerge. Based on recent findings that peripheral perturbations can alter cortical circuits and activity during the first two postnatal weeks of newborn mice, we hypothesized that sensory manipulation results in crossmodal changes in cortical activity earlier than the onset of the critical period.
Methods: To test the hypothesis, we deprived newborn mice of visual inputs after birth and investigated the activity of the auditory cortex during the first two postnatal weeks. We performed bilateral enucleation (or sham surgery) on postnatal day (P) 0-1 in mouse pups expressing calcium indicator GCaMP6 in excitatory cortical neurons. Later, we performed in vivo imaging in auditory cortex in unanesthetized, awake pups on P8-9 and on P12-14.
Results: We found that enucleation significantly alters both spontaneous and sound evoked activity of the auditory cortex in an age-dependent manner.
Conclusions: To our knowledge, this is the first demonstration of crossmodal functional changes in sensory cortices before the onset of the critical period. Our results will shed light onto novel therapeutic interventions for the recovery of function of deprived senses in infants with sensory disorders, e.g., hearing impairment.

Early Life Stress Impairs Behavioral and Neural Temporal Processing Across the Auditory Pathway

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Background: Stress induced during adulthood can affect sound-evoked responses and synaptic elements in the auditory pathway. Yet despite increased plasticity of the ascending auditory system during critical periods of development, it is largely unexplored whether developmental stress (early-life stress, ELS) affects the periphery or central auditory system, and whether these effects last into adulthood. Our lab has shown that ELS impairs gap detection behaviorally and in primary auditory cortex, a region necessary for detection of short gaps. Yet ELS may have more widespread effects on the auditory pathway and even the auditory periphery; for example, a corticotropin-releasing factor signaling system is active in the cochlea (Vetter and Yee, 2018).
Methods: To study how ELS affects processing of complex sound stimuli across the auditory pathway and across development, we measured gap-in-noise ABRs (GIN ABR, reflecting auditory nerve to inferior colliculus inputs), middle latency responses (MLR, reflecting cortical responses) and frequency following responses (FFR, reflecting brainstem temporal synchrony) within the same animals at three ages across development. To induce ELS, gerbils were maternally separated and restrained for 2 hrs/day at unpredictable times from postnatal day (P)9 to P24. GIN ABRs, FFRs at several modulation frequencies (MFs: 35, 50, 125, 250, 500, and 1000 Hz), and MLRs were recorded in awake animals (sedated with dexdomitor) at three developmental time points: P25, P40 and P90. GIN ABRs were also measured at P40 in a separate group of ketamine-xylazine-anesthetized gerbils.
Results: GIN ABRs revealed higher gap detection thresholds in ELS animals, driven by a reduced amplitude to the second sound burst in ABR waves I, II, and III (auditory nerve and brainstem). In FFRs, the response power across MFs matured gradually in both groups, with a better signal to noise ratio (SNR) and lower noise floor in adults (P90 vs both P40 and P25). This pattern was altered by ELS, which reduced SNR and increased the noise floor in comparison to controls, at all MFs and ages tested. ELS unexpectedly increased response power at slow but not fast MFs, but this higher power was not sufficient to overcome the higher noise floor. Finally, the developmental pattern of middle latency responses was altered by ELS, which caused shorter latencies but lower amplitudes, indicating reduced response synchrony; these changes were largely resolved by P90.

Conclusions: Our data reveal ELS effects on temporal processing in both the periphery and central auditory system, throughout development and into adulthood. This suggests that the known effects of ELS on learning, memory, and attention may arise in part from poor sensory input. It is possible that ELS may directly induce auditory temporal processing deficits in children, causing difficulty processing rapid transitions in speech, particularly in noisy environments.

Sema5a Modulates the Spontaneous Activity in Developing Neurons in Cochlea
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Background: In the auditory system, sensory neurons exhibit spontaneous activity in the absence of external stimuli before the onset of hearing. Spontaneous activity in the auditory system originates in the periphery following ATP mediated excitation of cochlear hair cells, and propagates through the ascending auditory pathway. In the developing cochlea, spiral ganglion neurons (SGNs) from late embryonic stages in mice show infrequent and uncoordinated firing activity, which by the early postnatal days become more frequent and coordinated. The molecular factors and mechanisms that initiate and refine the nature of spontaneous activity in SGNs is not well known.

Semaphorins control different aspects of the development of neural circuitry, and are most commonly known in axon guidance. In the cochlea, spontaneous activity occurs concurrently with a number of important events related to axon guidance like SGN branch refinement, SGN differentiation, and ribbon synapse development, pruning and maturation. These axon guidance events are crucial for refinement of developing cochlear neural circuit. In this study, we explored the role of Semaphorin-5a in cochlear innervation, and have discovered a role for it regulating spontaneous activity.

Methods: Immunohistochemistry was performed to determine the expression of Sema5a in developing cochlea. To examine activity, cochlea with GcAMP6s-expressing SGNs from postnatal day 2 mice were cultured briefly then recorded by time lapse imaging. Movies were recorded immediately and ten minutes after the application of Sema5A-Fc protein. The data were analyzed to compare the Sema5A-induced frequency and coordinated activity changes. Effects of Fc were controlled by comparing activity using human IgG alone. In this ongoing research, we are evaluating spontaneous activity characteristics in Sema5a KO mice.

Results: Immunostaining results show that Sema5A is expressed at P0 exclusively on the SGN fibers and the expression diminishes greatly by P3. Human IgG has no effect on the activity pattern of developing cochlea whereas SEMA5A-FC protein had significant effects immediately after application: the area of activity, frequency, and coordinated events were all significantly reduced. Although the activity resumed after ten minutes (in constant presence of Sema5A-Fc) with increased active area, the overall frequency and coordinated events were comparable with the baseline activity.

Conclusions: Our data show that Sema5A may regulate spontaneous activity in SGNs during early cochlear development. In ongoing work, we are investigating the mechanism by which Sema5A achieves this.

Building "Cochlear Spiral Ganglion on a Chip" Bioelectric Model
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Background: A key limitation of cochlear implant (CI) performance is the electrical interface with spiral ganglion neurons (SGNs). There is a pressing clinical need for a reliable model for testing strategies for electrical stimulation of SGNs. Current in vivo clinical measures are very limited and animal models do not replicate the anatomical structure of the human cochlea.

Therefore, we propose the development of an in-vitro Spiral Ganglion-on-a-Chip model that combines 3D replication of the anatomy and core structures of the cochlea, along with embedded SGNs and microelectrode arrays (MEAs) to record neural responses. This combines electrical stimulation using real implants generating realistic electrical fields, with detailed probing of neural responses in order to generate a more complete picture of the implant-neural interface. This testbed enables the evaluation and optimisation of both current and future CI stimulation strategies and treatments, to answer key questions in the field and directly improve patient outcomes.

Methods: Spiral Ganglion-on-a-Chip aims to replicate in vivo neural electrical responses expected in humans in a controlled and measurable in vitro system. The model consists of 3 main elements: 1) Rat SGNs and human induced pluripotent stem cell (hiPSC)-derived SGNs (in separate models) 2) Custom microelectrode arrays

(MEAs) to measure cellular electrical activity and 3) Custom-designed 3D printed microfluidic chips to replicate the structure of the human cochlea.

Currently, we have been focusing on developing both the cellular and device aspects of the Spiral Ganglion-on-a-Chip model separately. Several versions of the PDMS microfluidic device design have been iteratively optimised in order to ensure: 1) accurate anatomical and electrical conductivity representation of the scala tympani (where CIs sit in the cochlea), 2) reproducible 3D printing and subsequent casting of the precise features of the casted model, and 3) reliable fluid input into the device, needed for ionic current spread and cell growth.

Results: A casted PDMS device from a 3D-printed mould demonstrated good casting of all required microfeatures. Rat SGNs and glial cells were seeded in initial Cochlea-on-a-Chip prototypes and survived in the SGN channel for over a month. SGNs extended neurites through the microchannels toward the CI channel. We also developed human auditory neuron-like cells from human induced pluripotent stem cells (hiPSCs) derived from human fibroblasts. These hiPSC-derived SGNs displayed both a similar morphology to rat SGNs and express a neuronal marker TUJ1. We were also able to culture these neurons onto commercial MEAs and measure both their spontaneous ability to develop action potentials, and their response to electrical stimulation, and show that the action potential profiles are very similar to rat SGNs also grown on MEAs.

Conclusions: The Spiral Ganglion-on-a-Chip model will enable the rapid evaluation of existing technologies and the development of new CIs and hearing loss treatment strategies.

Studying the Role of Gpr156 in Hair Bundle Orientation and Afferent Selectivity in Zebrafish

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Background: Within neuromasts of the lateral line system, sensory hair cells (HCs) with two opposing orientations allow fish to detect two directions of water flow. Afferent neurons selectively innervate HCs of the same orientation to ensure directional information is transmitted to the brain. How afferent selectivity is accomplished is not completely understood. Previous studies in zebrafish have shown that the transcription factor Emx2 acts in HCs of just one orientation to mediate HC orientation. In addition, Emx2 also mediates afferent selectivity in the lateral line. Recent work in mice and zebrafish indicates that the G-protein coupled receptor, Gpr156, acts downstream of Emx2 in HCs to mediate orientation, but the role of Gpr156 in afferent selection has not been examined.

Methods: My research examines the role of Gpr156 in afferent selection. To study afferent selectivity, I used immunohistochemistry to assess synaptic pairing in gpr156 zebrafish mutants. In addition, to study afferent selectivity, I performed single afferent neuron labeling. For this labeling, I microinjected a neurod:tdTomato construct into zebrafish embryos to label individual lateral line afferent neurons in mutants and wildtype animals. After microinjection, I performed immunohistochemistry on these injected animals to label HC orientation and Emx2. This labeling allowed me to assess how well afferent neurons select HCs based on orientation and the presence or absence of Emx2.

Results: My immunohistochemistry results suggest Gpr156 is not required for synapse formation. While I observed synapse mispairing in emx2 mutants as previously reported, I observed no mispairing in gpr156 mutant HCs compared to wildtype HCs. By following the innervation of single afferent neurons, I also found that gpr156 mutant afferent neurons innervate HCs based on the presence or absence of Emx2 with a similar accuracy compared to their respective siblings.

Conclusions: While a previous study found that Gpr156 acts downstream of Emx2 in HC orientation, my results indicate a Gpr156 independent mechanism functions downstream of Emx2 in synapse formation and afferent selectivity. In the future, I plan to investigate additional molecules that may function downstream of Emx2 in synapse formation. Overall, this work provides insight into the molecular mechanisms underlying how this sensory system assembles to detect bi-directional water flow.

Immature Spectral Modulation Sensitivity, but Mature Frequency Resolution in 6-Month-Old Infants

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Background: Acoustic spectral resolution, the ability to perceive peak and trough patterns of energy across frequency, is essential for speech perception. Spectral resolution has been proposed as a proxy measure of device

efficacy in pre- and post-lingually deaf cochlear implant users. Measures of spectral resolution, however, are likely impacted by young-listeners' immature sensitivity to across-spectrum intensity modulation, from here referred to as spectral modulation sensitivity (SMS). In contrast, previous literature would suggest that, by 6-months of age, resolution of the modulation peaks across frequency, from here referred to as frequency resolution (FR), should be mature. These individual factors have not generally been studied independently when investigating development of spectral resolution in young listeners. In the present study, we used a two-threshold method to assess FR from SMS in normal-hearing infants. First, we measured SMS as the lowest intensity modulation depth at which the listener could perceive spectral modulation. Second, we measured FR as the highest modulation density that could be discriminated from a high-density referent at a fixed modulation depth relative to SMS. The hypothesis was that SMS would be immature while FR would be mature in 6-month-old infants.
Methods: Participants included 46 6-month-old infants and 12 adults. All listeners reported normal hearing, no risk factors, and passed otoacoustic emissions screening. Stimuli were 1-second pure-tone-complex carriers with spectrotemporally-modulated envelopes. Temporal rate was fixed at 5 Hz for all stimuli and spectral peak density varied from 1 to 20 "ripples" per octave (RPO). The listeners' task was to respond behaviorally to "target" trials with spectral density <20 RPO and not to respond to "no-target" trials with spectral density = 20 RPO. A single-interval forced choice observer-based procedure was used to estimate thresholds adaptively. First, modulation depth in dB was varied at fixed target density = 1 RPO to determine SMS. Then modulation density in RPO was varied at fixed target depth (twice the first threshold) to determine FR.
Results: Two thresholds were obtained from 66% of infants and 100% of adults. Data from infants who did not provide two thresholds was excluded. Mean spectral modulation sensitivity was significantly worse in infants than adults by an average of 3.3 dB. FR was not significantly different between infants and adults.
Conclusions: The findings support the hypothesis that FR is mature by 6-months of age, on average, in normal hearing children. A high degree of variability in FR was noted in both age groups. Our next step will be to use this 2-point spectral modulation paradigm to study development of SMS and FR in children who use cochlear implants and to examine whether early FR is a predictor of clinical outcome.

Adeno Associated Virus Transgenes Under Control of the SLC6A14 Proximal Promoter Drive Vestibular Supporting Cell-Specific Transgene Expression

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Background: Long-term success in gene therapy and its application to regenerative medicine relies on the ability to restrict transgene expression to specific cells. One such application is the overexpression of Atoh1 in the damaged vestibular sensory epithelia to promote transdifferentiation of supporting cells into hair cells. Off-target expression of Atoh1 in hair cells, neurons, glia, and other resident cell types is highly undesirable as it could disrupt transcription and function of these cells.   Using our single-cell transcriptomic data, we have identified a promoter that restricts transgene expression to the vestibular supporting cells of the inner ear and demonstrated its ability to drive the induction of hair cells in damaged maculae through expression of Atoh1.
Methods: scRNA-seq profiling of murine cochlear and vestibular sensory organs was analyzed to identify SLC6A14 as a candidate gene promoter., Promoter sequences were synthesized and subcloned to regulate GFP and/or Atoh1 in AAV transfer plasmids. GFP-encoding transfer plasmids and AAV were delivered into HepG2 cells to confirm promoter function. AAV was delivered on utricle explants and injected into the semicircular canal of both naïve and IDPN-damaged mouse models to confirm specificity of expression and the ability of Atoh1 to drive hair cell transdifferentiation. AAV was also delivered into macaque semicircular canals to assess specificity of the promoter in primates.
Results: The HepG2 cell model showed low levels of transgene expression from all SLC6A14 promoters tested in both plasmid and AAV delivery confirming the promoters were capable of driving transgene expression. Screening the AAV SLC6A14-GFP constructs in utricle explants allowed us to identify the promoter construct with the highest GFP expression to carry forward to in vivo testing. Delivery of an AAV SLC6A14-GFP reporter virus via direct injection into the murine posterior semicircular canal produced a clear restriction of expression in Sall2+ vestibular supporting cells without expression in cochlear supporting cells or in Pou43+ hair cells. Similar

specificity was observed in the ears of AAV-treated non-human primates. Delivery of AAV SLC6A14-ATOH1 showed a dose-dependent increase in Pou4f3+ hair cells in murine IDPN-damaged maculae.

Conclusions: The SLC6A14 promoter restricts transgene expression to the vestibular supporting cells of the inner ear and will be a useful tool for continued development of gene therapies aimed at treating vestibular dysfunction.

A Triple Line of Defense: The Role of the Group 1 Rfx Transcription Factors in the Inner Ear

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Background: Successful development of therapeutics for hearing and balance disorders depends on detailed knowledge of the transcriptional regulatory networks involved in hair cell (HC) development and function. Our laboratory previously identified a striking over-representation of the RFX transcription factors (TFs) binding motif in the promoters of HC expressed genes, suggestive of an important role for this family of ciliogenic TFs in HC development. The RFX TFs are divided into four groups based on their functional domains, where group-1 consists of RFX1, RFX2, and RFX3. Our laboratory subsequently showed that conditional deletion of both Rfx1 and Rfx3 (Rfx1/3) from HCs results in profound hearing loss, secondary to an abrupt loss of all outer hair cells (OHCs) shortly after the onset of hearing, and a mild, late onset vestibular phenotype. However, the Rfx1/3 conditional knock out (cKO) mutants did not exhibit kinocilia or planar cell polarity (PCP) defects. Due to significant homology in functional domains, similar role in ciliogenesis, and expression in both cochlear and vestibular HCs, we hypothesize that RFX2 functions to compensate for the loss of RFX 1/3 in the inner ear HCs. Here, we explored the role of RFX2 in the inner ear throughout development and adulthood as well as the compensatory role of RFX2 for RFX1/3 within the vestibular system.

Methods: To determine the spatiotemporal expression of Rfx2 within the cochlea and vestibular system, we used an Rfx2 knock out mouse model (Rfx2Gt) containing a lacZ gene trap cassette within the Rfx2 transcript. X-gal staining was performed on Rfx2Gt control and heterozygous inner ears, from postnatal day (P) 1 to 6 months of age. The role of RFX2 in hearing and vestibular function was measured using Auditory Brainstem Responses (ABRs) and Vestibular Sensory Evoked Potentials (VsEPs). Lastly, to assess the compensatory role of RFX2 in the vestibular system, we created a triple conditional knockout mouse, Rfx1/2/3 cKO, by crossing the Rfx2Gt mouse with our already existing Rfx1/3;Gfi1Cre cKO mouse and measured vestibular function using VsEPs.

Results: Rfx2 is specifically expressed in all cochlear HCs at P1, apical cochlear HCs at P7, and in all vestibular HCs from P1 to 6 months of age. Despite the continuous expression of Rfx2 in vestibular hair cells from development into adulthood, ABR and VsEP thresholds of Rfx2Gt mutant mice are not significantly elevated compared to wild type controls. However, a loss of Rfx1/2/3 together causes significantly elevated VsEP thresholds as early as 1 month of age.

Conclusions: RFX2 alone does not have an overt functional role in the auditory or vestibular system but has a compensatory role for RFX1/3, revealing the essential role of group 1 RFX TFs in vestibular function.

Single Cell RNA Sequencing Analysis of Mouse Cochlear Supporting Cell Transcriptome With Activated ERBB2 Receptor, a Candidate Mediator of Hearing Restoration Mechanisms

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Background: In mammals, hearing loss due to loss of cochlear hair cells (HCs) cannot be restored. In contrast, in birds lost auditory HC are regenerated from supporting cells (SCs) through proliferation and transdifferentiation. We hypothesize that these processes may be driven in part through epidermal growth factor (EGF) receptor family signaling. Using transgenic mice harboring a constitutively active (CA)-ERBB2 transgene, we have previously shown that ERBB2/3 signaling in SCs can stimulate proliferation in vitro, and in vivo ectopic HC differentiation in undamaged neonatal cochlea. In young adult mice, activation of the ERBB2 signaling after traumatic noise damage drove partial low-frequency auditory recovery and increased survival of HCs. In both studies, gene lineage tracing indicated that ERBB2 activation had non-cell autonomous effects, indicating the presence of a signaling cascade. In this study, we used single-cell RNA sequencing (scRNA-Seq) analysis to examine a transcriptome profile of SCs and identify molecular mechanisms induced by ERBB2 signaling that promoted in mice long-term auditory recovery.

Methods: To examine the transcriptional changes that occur after induction of CA-ERBB2 signaling, we used transgenic mice harboring inducible CA-ERBB2 and eGFP transgenes that are expressed in SCs. Cochlea were

collected from P3 mice, and dissociated cochlear cells were used in FACS analysis to capture single GFP+ cells. About 300 single cells were collected from CA-ERBB2+ mice (Ca-ErbB2+/GFP+) and from control mice (Ca-ErbB2-/GFP+) and used in subsequent scRNA-Seq analysis using SMART-Seq2 platform.

Results: Unbiased clustering identified at least 5 groups of cells with discrete patterns of gene expression. We identified numerous genes that were upregulated in CA-ERBB2+ SCs, including genes described for HC progenitors. One distinct group of cells identified only among CA-ERBB2+ SCs had enriched transcripts of cell cycle genes, signaling pathway genes, and secreted proteins genes that might be involved in regulating SCs proliferation and their differentiation into HCs. In this group we identified some markers of Deiters' cells, Outer Pillar cells, but particularly markers of Greater Epithelial Ridge cells. The latter population was recently shown in mice to have proliferative potential and robust ability to generate hair cell marker-positive cells. We focus our analysis and validation on these genes, particularly on genes for secreted proteins that are important in short- and long-range signaling.

Conclusions: Our preliminary results indicate that inducing of signaling through ERBB2 in mouse cochlea results in upregulation of signaling pathway genes and secreted proteins genes, suggesting potential short- and long-range signaling effects. Future experiments will determine if these pathways are important in promoting cochlear regeneration and hearing restoration.

Early Pathological Changes in Cochlear Microvasculature Precede Hair Cell and Hearing Loss in a Mouse Model of Norrie Disease

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Background: Norrie disease is a rare recessive X-linked disorder, manifesting as congenital blindness and progressive hearing loss. It is caused by mutations in the NDP gene, which encodes Norrin, a secreted Wnt-analog that induces canonical Wnt/β-catenin signaling through a Fz4/Lrp5/6/Tspan12 complex. Currently, there are no treatments for Norrie disease and the role of Norrin in the cochlea is not well understood. This study sought to identify the early sequence of pathological events leading to the onset of hearing loss in mice with a loss-of-function mutation in Ndp.

Methods: Cochlear tissue and auditory function of Ndp knockout mice (auditory function of male and female Ndp knockout mice (allele Ndptm1Wbrg; Ndp-KO) were analysed from postnatal day 10 to 2 months. Vasculature morphology and perivascular cell distribution in the cochlear lateral wall were analysed by TEM and by confocal microscopy. Integrity of the blood-cochlea barrier formation was assessed by qRT-PCR, immunohistochemistry, and vascular tracer assays. At 1 and 2 months inner and outer hair cells survival was mapped in full-length organ of Corti whole mounts, and Endocochlear Potential (EP), Distortion Product OtoAccoustic Emmission (DPOAE) and Auditory Brainstem Responses (ABR) were recorded.

Results: The cochlear microvasculature in the spiral ligament showed an abnormal morphology as early as postnatal day 10 in the Ndp-KO, prior to the onset of hearing. Vascular barrier function was defective by P20 showing leakage of the fluorescent tracer and dysregulation of Cldn5, Plvap, Cav1 gene expression in the lateral wall. Subsequent marginal cell pathology, significant reduction of EP and onset of outer hair cell degeneration in the mid-frequency range was found from 1 month onwards. DPOAE and ABR readouts of Ndp-KO differed significantly from control littermates at 2 months.

Conclusions: Cochlear microvascular pathology and reduction of EP in the Norrie disease mouse model has an early postnatal onset and precedes the degeneration of outer hair cells and detectable hearing loss indicating it is a primary site of Norrie disease pathology. Identifying this sequence of pathological events in the cochlea helps provide outcome measures for the evaluation of targeted therapeutic interventions in the in vivo model. Acknowledgements: NewLife, Sparks, Royal National Institute for Deaf People, GOSH-ICH NIHR BRC, GOSHCC and the Norrie Disease Foundation. In Memory of Professor Maria Bitner-Glindzicz.

Defining the Genetic Landscape of STRC-Related Hearing Loss

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Background: Mutations in STRC are the most common cause of autosomal recessive mild-to-moderate hearing loss. Due to a pseudogene with high homology, copy number variants (CNVs) are the most frequently seen genomic changes in STRC. The most prevalent CNV is a contiguous whole gene deletion with the neighboring CATSPER2 gene. Mutations in STRC result in nonsyndromic hearing loss; DFNB16. Biallelic STRC-CATSPER2 deletions result in Deafness Infertility Syndrome (DIS) in males (DFNB16 for females). Here we present the largest study to date exploring the spectrum of STRC-related mutations and phenotypes.
Methods: We used targeted genomic enrichment and massively parallel sequencing to screen all known deafness-associated genes in a large ethnically diverse cohort with hearing loss. All variants were discussed in the context of clinical and familial history data.
Results: Of 5756 patients tested, a genetic cause of hearing loss was identified for 2461 individuals (43%). Variants in STRC accounted for ~13% (311) of the positive cases. Of the 311 STRC cases, DFNB16 was diagnosed in 246 patients (79%, 113 males, 133 females) and DIS in 65 males (21%).
Within the STRC positive cohort we identified 497 CNVs of which ~71% (351) were contiguous STRC-CATSPER2 deletions, including 116 homozygous patients (51 females, 65 males). Twenty-nine percent (145) of the CNVs were gene-to-pseudogene conversions. At least ten unique conversion events were identified within this cohort, the most prevalent involves exons 19-28 (108 alleles). Contrary to previous studies, STRC whole gene or partial gene deletions are ultra-rare, as we only identified one case (0.2%).
About 66% of the total diagnoses of DFNB16 and DIS were due to CNV/CNV, 28% were due to CNV/SNV, and 6% were due to SNV/SNV. Patients with available clinical data and audiograms were categorized by STRC genotypes to determine if any differences in audiological profiles existed. We saw no significant differences in the severity of hearing loss between genotypes.
Conclusions: Our findings show that not only are CNVs the most prevalent genetic abnormality in STRC positive diagnoses, but they are also overwhelmingly limited to contiguous STRC-CATSPER2 deletions and gene-to-pseudogene conversions. These data assert that pseudogene sequencing is required for comprehensive genetic testing in persons with hearing loss.
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Heritability of Audiometric Phenotypes Using Multigenerational Pedigrees

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Background: A considerable number of hearing studies focus on auditory disease phenotypes. The collection of non-disease auditory phenotypes provides an avenue to study how genetic variation impacts phenotypic variability. Audiometric data coupled with familial and genomic data affords us the ability to estimate the heritability of hearing acuity, the mode of inheritance, and which genes contribute to phenotypic variability. Historically, heritability is estimated using twin studies, which produce biased estimates due to violations of assumptions about shared environment and gene-environment interactions. We hypothesize that twin studies overestimate hearing acuity's heritability and that using three-generational familial and phenotype data will produce a more accurate estimate of hearing acuity heritability. Additionally, we hypothesize that genetic variation significantly impacts phenotype variability and specific loci are responsible for this phenotypic variability.
Methods: Our analysis utilizes a cohort of thirty-three, three-generational families, referred to as the Utah CEPH families. The cohort consists of 604 individuals who have whole-genome sequencing data and 200 phenotypic measurements are available. Four hundred and thirty have audiometric data, supplying us with two different quantitative phenotypes; pure-tone and high-frequency hearing acuity. We employed the Sequential Oligogenic Linkage Analysis Routines (SOLAR) algorithm and the Mendel software package to estimate hearing acuity heritability in the Utah CEPH families. The packages each use pedigree data intersected with phenotype data to estimate heritability. In addition, the packages can analyze the mode of inheritance for a phenotype and identify quantitative trait loci.
Results: The results from SOLAR and Mendel indicate a polygenic model of inheritance for pure-tone and high-frequency hearing acuity. SOLAR and Mendel estimate the heritability of high-frequency hearing acuity at 23 and 22.6 percent, respectively. Similarly, SOLAR and Mendel's pure-tone hearing acuity (frequencies at which human voices are heard) heritability estimates are 27 and 28.6 percent, respectively.   We also have preliminary evidence indicating that loci in chromosomes 1, 3, and 16 may impact phenotypic variability.
Conclusions: Recent twin studies of hearing acuity provide a range of heritability estimates, 45 to 63 percent for standard audiometric measures, and a greater range for hearing loss phenotypes. Our results provide evidence that

previous twin studies overestimate standard hearing acuity heritability. In addition, they suggest that the heritability of disease-causing loss of hearing acuity may have been overestimated in twin studies, and further analysis is needed. Our analysis has shown that using three-generation familial and phenotype data produces a more accurate estimate of hearing acuity heritability, and that hearing acuity has a polygenic mode of inheritance. This suggests a new model for assessing non-hearing phenotypes hearing. Moving forward, we will identify loci in the genome that contribute to hearing acuity variability. Additionally, we plan to use the fourth generation of the CEPH pedigrees, which is currently being collected, to improve our study.

An Investigation Into the Effects on Hearing of the CAPOS Syndrome Mutation in a Mutant Mouse

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Background: CAPOS syndrome is a rare ATP1A3-related neurological disorder, inherited with autosomal dominance, characterised by cerebellar ataxia, areflexia, pes cavus, optic atrophy and sensorineural hearing loss (SNHL). Presentation of the symptoms typically begins after a fever-related illness, although pregnancy and delivery can also trigger episodes. Here, we investigate hearing in mice carrying a E818K substitution mutation in the Atp1a3 gene, known to evoke CAPOS syndrome in humans.
Methods: We have compared auditory brainstem responses (ABRs), distortion product otoacoustic emissions (DPOAEs) and measurements of endocochlear potential (EP) in male and female (post-parturition and no-offspring groups) carrying a single copy of the mutation, compared to littermate control mice that carry the wildtype allele.
Results: Homozygotes for the E818K mutation are prenatal lethal in the mouse so all mutant analysis was carried out on heterozygotes. Comparison of repeated ABR thresholds at ages up to 13 months old revealed no differences between heterozygotes and wildtype littermates. Therefore, we tested mice after they had produced litters in an attempt to trigger hearing impairment, as reported in human CAPOS syndrome. ABR thresholds at 6 months old showed no differences between groups of wildtype and heterozygous males, wildtype and heterozygous females that had previously produced litters, and heterozygous females that had not produced litters. However, post-parturition female heterozygotes exhibited an abnormally small ABR wave 1. DPOAEs were largely comparable across all groups of mice aged 6 months old. EPs were normal, irrespective of sex, genotype or whether females had borne litters.
Conclusions: These data support the observations in human CAPOS syndrome that auditory anomalies can be triggered by pregnancy in females carrying a single copy of a pathogenic mutation. The hearing impairment appears to be sensorineural in nature, affecting auditory nerve function (ABR observations), whereas outer hair cell function remains largely normal (DPOAE observations). EP, an indicator of function of the stria vascularis, is normal in hearing impaired mice suggesting no contribution of a metabolic hearing impairment to the phenotype observed. Further work remains to be done to investigate in more detail the nature of the auditory deficit.

Novel KCNQ4 Variants in Different Functional Domains Confer Genotype- And Mechanism-Based Therapeutics in Patients With Nonsyndromic Hearing Loss

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Background: Loss-of-function variant in the gene encoding the KCNQ4 potassium channel causes autosomal dominant nonsyndromic hearing loss (DFNA2), and no effective pharmacotherapeutics have been developed to reverse channel activity impairment. Phosphatidylinositol 4,5-bisphosphate (PIP2), an obligatory phospholipid for maintaining KCNQ channel activity, confers differential pharmacological sensitivity of channels to KCNQ openers.
Methods: Through whole-exome sequencing of DFNA2 families, we identified three novel KCNQ4 variants related to diverse auditory phenotypes in the proximal C-terminus (p.Arg331Gln), the C-terminus of S6 segment (p.Gly319Asp), and the pore region (p.Ala271_Asp272del). Potassium currents in HEK 293T cells expressing each KCNQ4 variant were recorded by patch-clamp, and functional recovery by PIP2 expression or KCNQ openers was examined.
Results: In the homomeric expression setting, the three novel KCNQ4 mutant proteins lost conductance and were unresponsive to KCNQ openers or PIP2 expression. Loss of p.Arg331Gln conductance was slightly restored by a

tandem concatemer channel (WT-p.R331Q), and increased PIP2 expression further increased the concatemer current to the level of the WT channel. Strikingly, an impaired homomeric p.Gly319Asp channel exhibited hyperactivity when a concatemer (WT-p.G319D), with a negative shift in the voltage dependence of activation. Correspondingly, a KCNQ inhibitor and chelation of PIP2 effectively downregulated the hyperactive WT-p.G319D concatemer channel. Conversely, the pore region variant (p.Ala271_Asp272del) was nonrescuable under any condition.

Conclusions: Collectively, these novel KCNQ4 variants may constitute therapeutic targets that can be manipulated by the PIP2 level and KCNQ-regulating drugs under the physiological context of heterozygous expression. Our research contributes to the establishment of a genotype/mechanism-based therapeutic portfolio for DFNA2.

Surface Expression of KCNQ4 is Mediated by Binding of HAP1 to C-Terminal Tail of KCNQ4

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Background: In the mammalian cochlea, K+ recycling and homeostasis is important for the auditory function and voltage-gated channel subfamily Q member 4 (KCNQ4) plays a crucial role in the K+ recycling. Mutations in KCNQ4 cause non-syndromic sensorineural deafness type 2 (DFNA2), an autosomal dominant form of progressive hearing loss. KCNQ4 consists of six transmembrane domains with a long cytoplasmic C-terminal tail. This C-terminal tail engages in functional regulation of KCNQ4. For example, calmodulin (CaM) decreases KCNQ4-mediated currents by binding to the C-terminal tail of KCNQ4. In this study, we assumed that there will be additional proteins which interact with the KCNQ4 C-terminus, thereby regulating KCNQ4 activity.

Methods: To identify interactors of KCNQ4, we performed yeast two-hybrid (Y2H) screening with murine adult inner ear cDNA library as a prey and KCNQ4 C-terminal tail as a bait. The Y2H revealed HAP1 and MMP14 as novel interactors in addition to CaM, a known interactor. We confirmed this novel interaction by co-immunoprecipitation in HEK 293 cells. GST pull-down assay with purified interaction domain of HAP1 and KCNQ4 C-terminal tail corroborates the direct interaction of KCNQ4 and HAP1.

Results: To investigate the functional effect of the interaction, we performed electrophysiology and found that HAP1 overexpression decreased KCNQ4-mediated currents, whereas MMP14 did not affect KCNQ4-mediated currents. Since HAP1 was previously reported to involve in vesicular trafficking and to interact with membrane proteins including GABA A receptor, InsP3R1, EGFR, and IP3 receptor, we performed surface biotinylation assay to examine whether HAP1 affects the membrane trafficking of KCNQ4. Consequently, we identified that the surface KCNQ4 expression was decreased by HAP1 overexpression. To verify if the decrease of surface KCNQ4 under HAP1 overexpression results from intercellular endocytosis defects, we conducted endocytic assay in HEK 293 cells. Endocytic Assay confirmed when HAP1 co-expressed with KCNQ4, endocytosed KCNQ4 level remained unchanged while KCNQ4 gradually endocytosed by time-dependent manner in the absence of HAP1. We generated HAP1 knockout cell lines by utilizing CRISPR/Cas9 system. HAP1 KO cell lines showed elevated membrane expression of KCNQ4 and increased KCNQ4 currents. Moreover, we found that the HAP1 is expressed in the mouse outer hair cells and co-localized with KCNQ4.

Conclusions: These findings suggest that HAP1 regulates surface KCNQ4 through its interaction with KCNQ4 C-terminal tail in the inner ear.

Investigating the Characteristics of Genes and Variants Associated With Self-Reported Hearing Loss in Older Adults in the UK Biobank

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Background: Age-related hearing loss is a common, complex disease with a strong genetic component, but it is not clear how genetic variation contributes to age-related hearing loss; whether it is the result of individual high-impact rare variants or multiple common low-impact variants which interact with each other. Most of the variants reported to underlie adult-onset hearing loss are rare, high-impact variants, identified through family studies and candidate gene screening of patient cohorts, but the contribution of common variants to age-related hearing loss has been harder to assess.

Methods: Here we have investigated variants associated with self-reported hearing loss in the 200,000 UK Biobank participants with exome sequence data. We filtered the participants for those who reported either hearing difficulty, hearing difficulty in noise, or hearing aid use. Participants younger than 55 years old were excluded, as were those with other hearing-related diagnoses such as Meniere's disease, or cochlear implant users. Our final cohorts consisted of 45,581 older adults with self-reported hearing loss (24,237 men and 21,344 women), and 48,731 older adults with no self-report of hearing loss (18,235 men and 30,496 women). Variants were filtered based on quality, call depth, missingness, and excess heterozygosity (which are all indicators of sequence quality), and annotated using the Ensembl Variant Effect Predictor. We compiled lists of variants based on their minor allele frequency (MAF < 0.1, "common", and MAF < 0.005, "rare"), and predicted pathogenicity (high impact or low impact), and carried out an outlier analysis of the variant load observed in older adults reporting hearing loss compared to those who did not report hearing loss.

Results: We found 37 genes had a high load of rare, high-impact variants in older adults with self-reported hearing loss, including 7 known deafness genes (eg TECTA, COL11A1 and MYH14). When we looked at common variants with a high impact, we found 206 genes with a high variant load, 21 of which were known deafness genes. However, when we tested variants with low impact, there were very few genes with a high variant load. We carried out functional enrichment analysis on the lists of high variant load genes and investigated the expression of candidate deafness genes using publicly available transcriptome data from the gEAR.

Conclusions: From this study, we have identified candidate genes involved in self-reported hearing loss in older adults. Our results support the hypothesis that genes underlying early-onset, severe deafness may also contribute to age-related hearing loss. They also suggest that allele frequency may be a less useful filter than predicted impact when it comes to assessing variants for involvement with a common, heterogeneous disease such as age-related hearing loss.

De Novo Mutation is a Common Cause of Genetic Hearing Loss

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Background: De novo mutations (DNMs) are a well-recognized cause of genetic disease. The contribution of DNMs to hereditary hearing loss (HHL) is unknown.

Methods: Targeted genomic enrichment (TGE) and massively parallel sequencing (MPS) were used for molecular testing of all exons of known HHL genes, with no exclusions on the basis of type of hearing loss or other clinical features. Segregation analysis was recommended to all families in which one or more potentially causative variants were detected. We retrospectively reviewed all trios in which samples were available from both parents to characterize the rate, distribution, and spectrum of DNMs.

Results: Samples from both parents of 280 probands were available and in all cases, non-paternity was excluded. 22/280 (7.8%) showed non-transmission consistent with DNM, and DNMs were causative in 18/91 (19.8%) probands who underwent segregation analysis for autosomal dominant variants. 22 DNMs were detected in 14 known HHL genes, including 8 novel variants in ACTG1, ATP2B2, CDH23, GATA3, MITF, MYO6, and NR2F1. DNMs were most frequently detected in MITF (22.7% of DNMs), followed by GATA3 (13.6%), ACTG1 (9.1%), and STRC (9.1%). Strategies for identification of putative DNM were developed for future prioritization of variants for segregation analysis to detect DNM.

Conclusions: DNMs are a common cause of genetic hearing loss and must be considered in sporadic hearing loss and cases in which a proband's phenotype is inconsistent with the phenotype of affected relatives.

Effects of High Fat Diet on Aminoglycoside-Induced Hearing Damage and Regeneration in Zebrafish

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Background: Obesity affects almost half of all people in the United States. The consumption of a high-fat diet (HFD) and large amounts of processed foods not only results in weight gain but can lead to a host of other

comorbid conditions, including diabetes and heart disease. One unrecognized comorbid condition that can present alongside obesity is hearing loss. Epidemiology data demonstrate a correlation between obesity and hearing loss in human populations, but the underlying mechanisms are unknown. Here we examine the correlation between diet-induced obesity and hair cell damage in a zebrafish model as a key step for future mechanistic studies.

Methods: We fed both male and female zebrafish for up to two months using either a control diet of dry food, dry food mixed with 20% lard, or dry food mixed with 20% egg yolk powder; the latter two diets contain high proportions of fat. We used aminoglycoside antibiotics to damage hair cells, then assessed hair cell numbers immediately after damage and after 48 hours of regeneration. We also collected fecal samples before and after the HFD was administered and used 16S sequencing to quantify microbial populations based on taxon-specific DNA signatures.

Results: We found zebrafish fed the yolk diet had significantly increased baseline hair cell counts after one month when compared to controls, but this increase was not present after two months. We also found the fish fed the lard HFD seemed to experience greater hair cell susceptibility to damage after two months when compared with controls. Surprisingly, hair cell regeneration was similar between all diets for both time points. While our sample sizes were too small to determine significance, there were interesting sex-based differences in hair cell susceptibility to aminoglycoside damage that warrant future study. There were also no significant differences in weight gain across groups.

Conclusions: We found no significant difference in the neomycin and regeneration conditions among groups, in contrast to our prior work showing that hair cells in HFD fish exhibited greater sensitivity to aminoglycoside damage. In our previous study we successfully induced obesity, which was not replicated here. The failure of the diet to induce obesity will be addressed in future studies, starting with an increased amount of food. The continually increasing prevalence of obesity in modern society calls for a greater emphasis on research into the mechanism behind the relationship of diet-induced obesity and hearing. Once we have induced obesity, we believe zebrafish are an excellent model to study comorbid obesity and hearing loss.

Nuclear Translocation Triggered at the Onset of Hearing in Cochlear Inner Hair Cells of Rats
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Background: Nuclear position is precisely orchestrated during cell division, migration, and maturation of cells and tissues. Emerging evidence from several systems suggests that nuclear position is important for maintaining the normal function of differentiated post-mitotic cells. Hearing, vision, and muscle pathologies result from misplaced nuclei (reviewed in Razafsky and Hodzic 2015). In cone photoreceptors, post-mitotic nuclear movement is essential for achieving mature synaptic function (Xue et al., 2020). Here, we report an analogous movement of the nucleus in rat cochlear inner hair cells at the onset of hearing.

Methods: Rat cochleae between post-natal day (P)3 and P62 were harvested, fixed, and dissected into whole mount preparations. Hair cells, ribbons, and nuclei were immunolabeled with Myosin VI and CTBP2. Hair cell length and the lengths of the sub- and supra-nuclear compartments were tracked to quantify the morphological development of inner hair cells. Cell morphology was examined in Long-Evans rats and in wildtype and knockout mice with mutations targeted to the tectorial membrane (TECTA C1409G) and stria vascularis (MITF-M).

Results: Normally-maturing inner hair cells experience a period of sustained growth during the first two post-natal weeks, reaching a stable length by the onset of hearing (~P12/P13 in rats and mice). Before the onset of hearing, nuclei are positioned close to the base of inner hair cells, in proximity to efferent and developing afferent synapses. Nuclei move away from synapses toward the cuticular plate around the onset of hearing. Nuclear migration begins in the middle turn and is complete throughout the cochlea within 2-3 days. Nuclei remain polarized to the basal pole of outer hair cells at all ages examined. Mouse and rat inner hair cells grown in organotypic culture failed to mature in length and nuclear position, suggesting that the morphological maturation of inner hair cells depends on signals available only in the in vivo environment. Inner hair cells in adult TECTA mice, which receive abnormal mechanical input, exhibit normal morphology and nuclear position. This suggests that morphological development proceeds even in the presence of abnormal sound input. Inner hair cells in adult MITF-M knockout mice, which lack endolymphatic potential (EP), have immature morphologies in cell length and nuclear position.

Conclusions: Our results show that the onset of hearing triggers a dramatic morphological transformation in inner hair cells. We hypothesize that nuclear migration is triggered by the onset of endolymphatic potential and linked to the final phase of inner hair cells' functional development. The work points to nuclear movement as a

phenotypic marker for a functionally mature inner hair cell. Understanding the sequence and significance of these developmental milestones may inform therapeutic strategies that aim to regenerate/reprogram functional hair cells.

The Ratio of β- and γ-Actin is Maintained during the Mechanotransduction-Dependent Remodeling of the Stereocilia Cytoskeleton in Mammalian Auditory Hair Cells

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Background: The stereocilia actin cytoskeleton consists of polarized actin filaments that contain both β- and γ-actin isoforms (Furness et al. 2005; Perrin et al. 2010). In the absence of either actin isoform, auditory hair cell stereocilia develop normally but exhibit premature degeneration (Belyantseva et al. 2009; Perrin et al. 2010). In humans, mutations in these genes are associated with syndromic and non-syndromic forms of hearing loss.
In normal conditions, auditory hair cell stereocilia exhibit cytoskeleton remodeling only at their tips (Zhang et al. 2012; Narayanan et al. 2015). However, changes to the resting influx of calcium through the mechano-electrical transduction (MET) channels, which are located at tips of stereocilia in the shorter rows, affects the stability of the stereocilia cytoskeleton and leads to changes in stereocilia thickness and height (Velez-Ortega et al. 2017). When bound to calcium, γ-actin exhibits slower polymerization and depolymerization kinetics than β-actin (Bergeron et al. 2010). Therefore, while β- and γ-actin differ from each other only by four amino acids, calcium influx through the MET channels may have a variable effect on these isoforms. Thus, we evaluated MET-dependent changes in the distribution of β- and γ-actin along stereocilia lengths in mouse auditory hair cells.
Methods: Organ of Corti explants were isolated from C57Bl/6 and CD-1 mice at early postnatal days and cultured in control conditions or in the presence of the MET channel blockers tubocurarine (60 µM) or benzamil (30 µM). Explants were fixed at specific time points during MET channel blockage, or after drug washout and recovery time. Samples were either immunostained with fluorescently-labeled antibodies against β- and γ-actin isoforms and imaged via confocal microscopy, or processed for scanning electron microscopy (SEM) imaging.
Results: We first evaluated any culturing effects on stereocilia morphology or actin composition. In outer hair cells, SEM images showed no changes to the height of the tallest row of stereocilia but a temporary (~5-12h) decrease between the staircase "steps" between rows (which could indicate an increase in the heights of the transducing stereocilia). In contrast, we did not observe any significant changes to the ratios of β- and γ-actin isoforms along the stereocilia lengths up to 48h in vitro. As expected, MET channel blockers led to the thinning and shortening of transducing stereocilia, which were fully recoverable upon blocker washout. However, no changes in the ratio of β- and γ-actin were observed during these MET-dependent cytoskeleton rearrangements.
Conclusions: During MET-dependent remodeling, the stereocilia cytoskeleton maintains the proportions of β- and γ-actin. This indicates that calcium-dependent differences in polymerization and depolymerization rates between β- and γ-actin are not the main mechanism driving stereocilia cytoskeleton rearrangements upon changes in intracellular calcium concentrations.

Mechanical Gating of the Auditory Transduction Channel TMC1 is Sensitive to Mutations in the Fourth and Sixth Transmembrane Helices

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Background: The transmembrane channel-like (TMC) proteins play a central role in auditory transduction, forming ion channels that respond to force and initiate the conversion of sound-induced mechanical stimuli into electrical signaling. The channels assemble as dimers, and each subunit is thought to have a distinct pore composed of transmembrane helices TM4-8. However, the molecular mechanism of gating is unclear—how do conformational changes of the TMC protein lead to opening or closing of the permeation pathway? Here, using predicted structural models of TMC1 as a guide, we probed the effects of a dozen mutations on mechanical gating of the transduction currents in mouse hair cells in vivo.
Methods: To identify possible gating movements, we drew on structures of TMC1 predicted from cryo-EM structures of the related TMEM16 channel, from molecular dynamics relaxations of a homology-based structure of TMC1, and from de novo protein-folding algorithms. These suggested that a separation of transmembrane helices TM3 and TM4 from helix TM6 might enlarge a permeation pathway. We identified several residues in TM4 and

TM6 that might regulate this movement, and generated point mutations in them. Twelve AAV9-PHP.B viral vectors were constructed that encoded these mutant TMC1s, along with one that encoded wild-type TMC1. As we did previously (Pan et al., 2018), we injected the AAV vectors into P1 mice lacking both TMC1 and TMC2 wild-type channels. Cochleas were placed in culture at P5-7, and hair cells were studied with whole-cell patch clamp recording at P10-14. Bundles were stimulated with a stiff, blunt glass probe. Activation (I-X) curves were measured with step stimuli and compared to those for wild-type TMC1 also expressed in the double-null background.

Results: We found that mutations of most but not all of these residues reduced the force sensitivity or shifted the open probability of the channels, or both. For many, the slope of the I-X curve was reduced, indicating that it required a larger stimulus to open the channel. For some, the I-X curve was shifted, consistent with the mutation changing the relative energy of the open state. In some cases, these gating changes were accompanied by a change in single-channel conductance, measured with nonstationary noise analysis, consistent with TM4 and TM6 also contributing to the permeation pathway (Pan et al., 2018).

Conclusions: While a limited set of mutations can only begin to probe the mechanism of gating, these observations are in line with a model in which helices TM4 and TM6 are involved in the mechanical gating of the TMC1 transduction channel.

Akyuz, Karavitaki and Pan contributed equally and are listed alphabetically.

Using Light to Study Sound: Stimulating Hair Cells With Photonic Pressure

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Background: The sense of hearing relies on specialized sensory cells in the inner ear—the hair cells—to convert sounds into electrical signals that the brain can interpret. Each hair cell is equipped with a mechanical antenna, a cluster of stiff, enlarged microvilli that pivots at its base in response to sound stimuli. Pivoting of this hair bundle toward its tall edge opens mechanosensitive ion channels and triggers a cascade of events that results in a signal travelling to the brain.

Although our understanding of hearing has improved significantly through the development of techniques for mechanically stimulating hair bundles, these techniques pose serious limitations. Two methods are commonly used to apply force to a hair bundle. The first uses a piezoelectric actuator to deflect the bundle with a glass fiber attached to its top. The second consists of a fluid jet that displaces the hair bundle through a puff of liquid driven by the action of a piezoelectric diaphragm. The inability of both methods to reach very high frequencies limits our quantitative understanding of hair-cell mechanics over 95 % of the range of mammalian hearing, which extends to 20 kHz in humans and at least 150 kHz in some species of bats and whales. Furthermore, common methods are susceptible to artifacts that often lead to conflicting results.

Methods: To overcome these challenges, we developed a new method to move individual hair bundles with photonic force. We etched an optical fiber in hydrofluoric acid to taper its tip to a diameter of a few micrometers. By controlled melting, the fiber's tip was endowed with a custom-built ball lens to focus the light beam and thus to selectively irradiate individual hair bundles. We delivered polarized laser light onto hair bundles of the bullfrog sacculus and rat cochlea while tracking their resulting movements with a dual photodiode.

Results: Because photonic force arises when photons are absorbed, reflected, or refracted upon interaction with an object, intense illumination applied substantial force to a hair bundle. We deflected bundles in the mammalian cochlea and in the bullfrog sacculus with a variety of stimuli: ramps, pulses, sine waves, and frequency sweeps up to the kilohertz range. We further demonstrated that the requisite irradiation did not damage the hair bundles.

Conclusions: We have developed a method that allows the stimulation of hair bundles with photonic pressure at previously inaccessible timescales, for the delivery time of the stimulus can accommodate the full frequency range of mammalian hearing. At the same time, this approach avoids the artifacts that bedevil current methods by providing evenly distributed forces and reducing the effects of hydrodynamic drag.

Establishment of Ischemic Hearing Loss Animal Model Induced by Rose Bengal and 532nm Laser

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Background: Sudden sensorineural hearing loss (SSNHL) occurs for a variety of reasons, but its etiology is not well identified. Ischemic change of microcirculation of cochlea is thought to be one of the important mechanisms

of SSNHL. Ischemic hearing loss animal models using photochemical reactions have been devised in various ways. This research is designed to establish an animal model that generates thrombus using rose bengal (RB) and 532nm laser in more effective way than other animal models which is useful for further research.

Methods: RB was administered to male Spraque-Dawley (SD) rats weighing 200~220g at 6 weeks of age in two ways: intraperitoneal injection or intravenous injection. 5 minutes after rose bengal injection, 532nm laser was irradiated for 5, 10 and 15 minutes through external auditory canal. Auditory brainstem response (ABR) thresholds were obtained by performing a test before the procedure for all rats and the results of ABR threshold of 1, 3 and 7days after the procedure were compared. On the 10th day, cochlea were obtained to make tissue sections and pathohistological examination was done.

Results: With all 9 rats in RB intraperitoneal-injection group, hearing loss was not observed in all 9 different conditions and additional pathohistological examination didn't reveal any destruction of inner-structure of cochlea. With rats in RB intravenous-injected group, hearing loss was not observed in conditions where 532nm laser was radiated with 150 mW for 5 and 10 minutes. In the case of rat radiated for 15-minute, hearing loss of more than 70 dB was checked at all frequencies 1 day later, and hearing loss of more than 50 dB remained 10 days later. And additional pathohistologic examination discovered destruction of structure of organ of Corti and stria vascularis on H and E staining and also immunofluorescence staining.

Conclusions: In this study, we induced the ischemic change of microcirculation in the cochlea, one of important mechanisms of SSNHL through the intravenous injection of RB and transcanal irradiation of 532 nm laser. By establishing an less-invasive ischemic hearing loss animal model with photochemical reaction, it can be applied to further study for treatment of SSNHL such as photobiomodulation.

Greater Working Memory and Speech Perception Scores in Cochlear Implant Users Are Associated With Better Subjective Quality of Life and Hearing: Results From an Online Test Paradigm

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Background: A common concern in individuals with cochlear implants (CIs) is difficulty following conversations in noisy environments and social settings. The difficulty may result from listening in a noisy environment, long-term effects of hearing impairment, listening with a CI, or a combination of all three. Listening in complex acoustic environments engages cognitive processes, such as working memory and attention to interpret the auditory, visual and contextual cues in order to make sense of the degraded stimuli. The ability to accomplish these listening tasks relies on the individual's working memory abilities and draws upon limited cognitive resources to accomplish successful listening. For some individuals, this can result in long-term detriments to quality of life.

Methods: For some individuals, this can result in long-term detriments to quality of life. For this study, 43 CI users completed a series of online behavioral tests and quality of life surveys, in order to investigate the relationship between visual and auditory working memory, clinical and behavioral measures of speech perception, and quality of life and hearing. Behavioral tests included reading and listening (quiet and with noise-masking) working memory and a speech perception (quiet and with noise-masking) task based on Hagerman's matrix sentence test. The surveys include the CI Quality of Life (CIQOL) and Speech, Spatial, and Quality of Hearing (SSQ).

Results: Results showed that recall performance on the three working memory span tests decline from visual reading span to auditory listening in quiet and then listening in noise. Speech perception was predictably worse when presented with noise maskers. Correlation analysis revealed that memory recall and speech perception ability were significantly correlated with sections of CIQOL and SSQ surveys along with clinical speech perception scores.

Conclusions: These results confirm that speech perception is related to working memory ability and that working memory ability is correlated to the quality of life. Importantly, we demonstrate that online testing can be used as a tool to assess hearing, cognition, and quality of life in CI users.

Learning from Model-Based Approaches for Hearing Loss Compensation: Which Speech Features are Enhanced in Machine-Learning-Based Audio Algorithms for Cochlear Synaptopathy Compensation?

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Background: Auditory models have been used for decades to develop audio signal processing algorithms in hearing aids. Typically, the difference signal between a normal-hearing (NH) and hearing-impaired (HI) model is used to design such algorithms, but only recently machine-learning (ML) methods have made their entry in this field. Specifically, when adopting differentiable descriptions of biophysical models of hearing-impairment, it is possible to fully backpropagate through the models and design a new type of ML-based audio signal processing that compensates for different aspects of SNHL (hair cell damage or cochlear synaptopathy, CS). Here, we investigate which sound and speech features are modified when letting the ML-algorithms decide the most-optimal solution.

Methods: We used a biophysically-inspired auditory model, in a differentiable convolutional neural network (CNN) description (CoNNear), to train different ML-based algorithms that maximally restore CS-affected auditory-nerve (AN) responses. The NH CoNNear model parameters were adjusted to obtain individualized HI models simulating different degrees of outer hair cell loss and/or CS-related AN fiber loss. Based on the reference NH model and a HI model, we used backpropagation to design ML-based audio signal processing algorithms that optimally compensate for CS. We designed several CS-compensating algorithms using the same CNN encoder-decoder architecture (8 encoder, 8 decoder layers) but constrained their training using different loss functions. These functions focused on minimizing different aspects of the AN responses (e.g. free training, using more or less cochlear channels, limiting the frequency range). After training, we processed pure tone stimuli and a battery of words in quiet and noise to evaluate the auditory feature restoration capabilities of the ML-algorithms using transfer functions and auditory model simulations.

Results: CS-compensating algorithms enhanced the AN responses to both low and high frequency pure tones, and to vowels in quiet and noise, but responses were usually not restored to the NH level. Consonant enhancement was only obtained when using a loss function with a low AN-response threshold. The algorithms generally sharpened the onset response to speech and improved the stimulus dynamic range. Transfer functions and excitation patterns showed that in an unconstrained operation, the ML-algorithms added more energy to the higher frequencies. This created audible high-frequency tonal components that degraded speech quality and intelligibility, but the effect could be reduced by applying a frequency weighting to the loss functions.

Conclusions: This work shows how ML-based end-to-end CS-compensating algorithms can be designed through backpropagation in a fully automatic way, without the need for prior assumptions on the signal processing. The constraints in the loss functions of the trained algorithms cause differences in restored auditory features to compensate for CS. In future work, we will objectively assess the effect of these compensation algorithms on sound quality and speech intelligibility in clinical experiments.

The Pre- And Postnatal Exposure to PCBS Inhibits the Recovery From Noise Induced Hearing Loss Later in Adulthood.

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Background: Previous studies have shown that the developmental exposure to polychlorinated biphenyls (PCBs) causes a hearing deficit to the offspring in rats. However, it is unknown if noise-induced hearing loss later in adulthood could be exacerbated following the pre and early postnatal exposure to the PCB.

Methods: To address this question, female Swiss Webster mice were dosed with 0, 6, or 12 mg/kg PCBs daily starting 28 days before breeding with PCB-unexposed male mice and continued until weaning. The auditory brainstem response (ABR) was recorded from two-month-old mice before and after exposing the mice to broad band noise at 110 dB SPL for 45 minutes to induce a temporary hearing threshold shift.

Results: Compared to the control, the developmental exposure to 6 mg/kg PCBs initially resulted in a significant sex-dependent hearing deficit to low-frequency pure tones only in male mice, which were therefore used in the

subsequent study. Although there was no exacerbation of noise-induced hearing loss by PCB exposure, the developmental exposure to the PCB inhibited the recovery of the animals' hearing compared to the animals exposed to noise only after one week of acoustic trauma.

Conclusions: Ongoing two-photon imaging and molecular experiments are being done to characterize the effect of the PCBs and noise-induced hearing loss on the functions of the inferior colliculus.

Glucocorticoid and Mineralocorticoid Receptors Expressed in Cochlear Support Cells Play Different Roles in Recovery of Cochlear Function Following Noise Exposure

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Background: Corticosterone signals through lower-affinity glucocorticoid receptor (GR) and higher-affinity mineralocorticoid receptor (MR) to modulate homeostatic functions. GC signaling effects within the cochlea may control cochlear inflammatory responses associated with noise exposure. GR activity improves auditory recovery from noise trauma potentially through modulation of inflammatory processes. MR activity, potentially through effects on ionic balance, improves autoimmune outcomes and age-related hearing loss. Using conditional gene knockout mouse lines (cKOs) for GR and MR, we investigated whether support cell GR and MR signaling alters physiological (ABR thresholds) and morphological (afferent synapse loss) or inflammatory response dynamics following noise exposure.

Methods: We used mouse lines carrying tamoxifen-inducible Cre recombinase to conditionally ablate MR or GR expression in the population of support cells in the inner sulcus and lateral sulcus of the cochlea. Two- to five-months old mice were daily injected with tamoxifen (cKO) or corn oil (WT) for five days. Noise exposures were either 2h 94dB (non-neuropathic) or 100dB (neuropathic) at 8-16kHz. Auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE) from 5.66 to 45.25kHz and up to 21 days post-noise were collected. Sensory epithelium preparations were immunolabeled for presynaptic ribbons (CTBP2) and postsynaptic glutamate receptors (GluA2). Cochlear cryosections will be immunolabeled for immune cells (CD45), and specifically for macrophages (Iba1 or F4/80), at day-7 post noise exposure.

Results: After 100dB noise exposure, WT and cKO MR mice (n=8 each) had similar threshold shifts of ~20dB at 22.6kHz without permanent threshold shift (PTS). In cKO MR mice, ABR P1 amplitude was transiently decreased at day-1 post-noise. Endpoint ribbon synapse counts per IHC were unaffected in noise-exposed WT and cKO MR mice compared to controls (n=3-4). After 94dB noise exposure, WT and cKO GR mice (n=9-12) had similar threshold shifts of ~45dB without significant PTS. However, delayed recovery of ABR threshold and P1 amplitude after noise exposure was detected in GR ablated mice. Interestingly, both GR ablated (f/f) and knockdown (f/+) mice (n=3) had reduced ribbon synapse counts at endpoint recovery times after 94dB noise exposure compared to control mice (n=2-3).

Conclusions: These data suggest a differential role for corticosterone in the mouse cochlea dependent on the receptor bound among support cells near the basilar membrane. Our data indicate that GR ablation from support cells delays threshold recovery after noise exposure (94dB, 2hr) but MR ablation from the same support cells does not alter recovery dynamics. Greater ribbon synapse loss and delayed recovery of ABR thresholds in cKO GR mice indicates vulnerability to noise-associated damage in mice lacking support-cell GR expression. But the same seems not to be true of the support cell MR expression.

Three-Dimensional Organization of the Gerbil Spiral Ganglion

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Background: Knowledge of the three-dimensional (3D) organization of the cochlea and its internal structures has been a goal of otolaryngology research for over a century. Recently we, and others, have used the method of light sheet fluorescent microscopy (LSFM) to advance our understanding of the cochlea's 3D structure. Previously we investigated geometrical relationships defining the gerbil organ of Corti with LSFM, here we report our initial observations on the nature of the 3D organization of spiral ganglion cells (SGCs) in the gerbil.

Methods: Fixed cochlear specimens were prepared for LSFM after immunohistochemical processing with primary antibodies to TUJ1 alone (for SGCs) or in combination with Myosin VII (for hair cells) followed by AlexaFluor conjugated secondary antibodies. Specimens were dehydrated though a series of alcohols, cleared with Spalteholz solution and imaged with a LaVision UntraMicroscope II, and viewed in 3D with Bitplane Imaris 9.7.

Results: TUJ1 antibodies label SGCs within Rosenthal's canal (RC), their dendrites to hair cells and their axons coursing to the auditory nerve (AN). It also labels other neural elements including efferent fibers to outer hair cells and the intraganglionic spiral bundle. Overall, the gerbil SGC column follows the general mammalian plan in that the basilar membrane and organ of Corti extend further apically than do SGCs. In gerbil, the most apical SGC lies at the level of approximately 300 Hz on the basilar membrane. When viewed in 3D there are regional differences in geometry of the SGC column. For example, when viewed from the scala vestibuli side, the SGCs form a continuous cell layer – but when viewed from the scala tympani side the SGCs take on a "glomerulus" like appearance, a form in 3D reminiscent of a string of light bulbs. Some SGCs reside outside of RC among axon fascicles en-route to the AN. These can be in excess of 400 microns from RC. Prior to the apex, there is a large extrusion of SGCs away from the RC, creating an 'apical bulge' with a complex geometrical structure. A similar large extrusion of cells was recently reported for SGCs in humans (Li et al 2020).

Conclusions: The 3D organization of SGCs in gerbil, particularly with an apical arrangement so similar to the human, provides an excellent model to study details of the distribution of SGCs and their frequency representation. A hypothesis for the arrangement in the apical bulge region is that hair cells representing frequencies lower than 300Hz are innervated by SGC's at some distance below the most apical extent of the basilar membrane, i.e., the frequencies represented by these SGCs has a downward bend near the apex. If so, this arrangement poses a challenge to frequency selective electrical stimulation in this region containing such a large number of SGCs.

Seasonal Plasticity of Utricular Hair Cell Auditory Sensitivity in Female Plainfin Midshipman, Porichthys Notatus

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Background: The plainfin midshipman, Porichthys notatus, is a seasonally breeding soniferous marine teleost fish that generates acoustic signals for intraspecific social and reproductive-related communication. Females rely upon their highly adapted auditory inner ear (saccule, utricle and lagena) to detect and localize the multiharmonic advertisement calls of reproductive males during the breeding season. Previous work showed that the female saccule exhibits reproductive state-dependent changes in auditory sensitivity such that summer reproductive females are better suited than winter non-reproductive females to encode the dominant harmonics of the male advertisement call. However, it is unknown whether the utricle or lagena in females exhibit reproductive state-dependent related changes in auditory sensitivity.

Methods: Here, we measured evoked hair cell receptor potentials in the utricle of summer reproductive (n = 17, 30; # of animals, # of records) and winter non-reproductive females (n = 16, 30) to determine if seasonally related changes in utricular hair cell auditory sensitivity occur in response to behaviorally relevant acoustic stimuli. Utricular potentials were recorded using glass microelectrodes
that were positioned near the sensory epithelia of the utricle. Since the midshipman utricular end organs reside primarily within the xy-plane and the sensory hair cells are oriented in the horizontal plane, the underwater speaker was positioned upright in the experimental tank with the speaker being submerged 2 cm below the water's surface. Acoustic stimuli consisted of single 500 ms pure tones that were randomly presented at the following frequencies: 105, 125, 145, 165, 185, 205, 245, 285, 305, 405, 605, 705, 805, 905 and 1005 Hz. The tested frequencies were chosen because they encompass the range of dominate frequencies contained within type I male midshipman advertisement vocalizations and do not overlap with resonance frequencies of the experimental tank.

Results: Evoked utricular hair cell potentials were recorded in response to sound pressure levels that ranged from 103 to 154 dB re: 1 μPa. Hair cell potential recordings revealed that evoked potentials were significantly greater among summer reproductive females when compared to winter non-reproductive females. Additionally, the utricular hair cell tuning curves of reproductive females had threshold that were 7 - 10 dB lower (i.e., more sensitive) than non-reproductive females across a broad range of frequencies that encompass the dominant harmonics of the male advertisement call (105 – 505 Hz).

Conclusions: These seasonal changes in utricular sensitivity indicate that reproductive state may play an important role in modulating auditory utricular sensitivity for the enhanced detection, recognition, and localization of mates during the breeding season.

Changes on the Otic Capsule Throughout Life: A Temporal Bone Study

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Background: The otic capsule refers to the dense primary bone of the petrous temporal bone that surrounds the membranous labyrinth of the inner ear. To date, it is widely known that this rigid structure presents during early maturation of fetal development and does not show bone turnover throughout life. In disease states, such as otosclerosis, abnormal bone turnover leads to demineralization and can result in both conductive and sensorineural hearing loss. In non-diseased states, it is unknown whether the otic capsule area changes as a function of age or differs between sexes. Herein, using a large cohort of human otopathologic specimens, we analyze the otic capsule area as a function of age and sex.

Methods: The National Temporal Bone Database was reviewed for cochleas of patients without bone disorders during life. A single, representative mid-modiolar section stained in hematoxylin and eosin was taken for quantitative analysis under light microscopy for each individual. Images were obtained at 4x magnification (low power) and cochlear area measurements were made in ImageJ software (National Institutes of Health). The specific boundaries of the otic capsule were pre-defined as the dense lamellar petrous bone extending circumferential to the membranous labyrinth. Endosteum and periosteum were included in the area measurement. Two blinded researchers individually analyzed the areas to ensure consistency of measurement using Alpha Crohnbach analysis. Otic capsule area measurements were then assessed for differences as a function of age and gender.

Results: 200 unique mid-modiolar sections were analyzed. 91 male ears and 109 female ears were examined. Age range observed was from 2 months - 96 years. Overall, there was a significant decrease in otic capsule area as a function of age ($p < 0.0001$). There was no statistically significant difference when the mean otic capsule area of all females was compared to all males (0.204 ± 0.112 mm2 vs 0.205 ± 0.108mm2). A steady decrease in otic capsule area was observed in females as a function of age ($p < 0.0001$). In women 50 years of age and younger, a large, steep decline in otic capsule area was revealed ($p < 0.0060$). A relatively stable otic capsule area was found in women over the age of 50. Women over the age of 50 have a significantly smaller average otic capsule area when compared to males in the same age range (0.164 mm2 vs 0.191 mm2, $p = 0.02$). Males presented a decrease in otic capsule area ($p < 0.0008$), however this decline was steady over time.

Conclusions: The strongest decrease in otic capsule area occurred in women under the age of 50, while the area remained constant in women over the age of 50. Although there was an overall decrease in otic capsule area over time with men, the decline was more steady.

Sex Differences in AMPA Receptor Subunit mRNA With Fast Gating Kinetics in the Mouse Cochlea

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Background: Physiological differences in the auditory processing of healthy females and males have been described in multiple mammalian species including humans. For example, females have larger auditory brainstem response (ABR) wave I amplitudes than males which is a proxy for spiral ganglion neuron (SGN) synchrony (Lev and Sohmer, 1971). The contributing molecular mechanisms, however, remain unresolved (Lin et al., 2021). Understanding these sex differences in healthy inner ears will be important for tailoring protection from and treating sensorineural and age-related hearing loss in men and women.

Type I SGNs are the afferent fibers that synapse with inner hair cells (IHCs) to relay sound information to the brain. Ionotropic AMPA receptors (AMPARs) located on SGN dendrites respond to glutamate released by IHCs to mediate excitatory information. In mature SGNs, postsynaptic AMPARs are tetramers of three subunits GluA2, 3, and 4. Studies in the CNS have reported that higher levels of mRNA for GluA3 and 4 (GRIA3 and GRIA4 genes, respectively) correlate with faster AMPAR gating kinetics, while higher levels of GluA2 (GRIA2) correlate with slower gating kinetics (Geiger et al., 1995; Angulo et al., 1997). In addition, alternative splice variants (flip/flop) of each subunit's mRNA affect gating kinetics with flop isoforms (especially GRIA3- and GRIA4-flop) increasing the gating kinetics of the AMPARs when compared to flip (Mosbacher et al., 1994). Fast channel gating kinetics of AMPARs are important for highly synchronous and fast transmission of auditory information due to more reliable and precise excitatory post-synaptic currents and shorter SGN refractory periods (Trussell, 1997).

Methods: We hypothesized that increased expression of GRIA3 and GRIA4 mRNA overall, and increased levels of the flop splice variants contribute to larger ABR wave I amplitudes due to faster gating kinetics of AMPARs. Therefore, we predicted that SGNs of females have higher levels of GRIA3 and GRIA4 overall and/or more relative abundance of the flop splice variant compared to males. To test our predictions, we quantified levels of GRIA2,3, and 4 mRNA along the tonotopic axis of the cochlea using in situ hybridization (RNAScope) and relative abundance of GRIA2,3, and 4 flip/flop isoforms in the entire cochlea using qRT-PCR in healthy adult female vs. male C57BL/6J mice.

Results: We found that females have overall more GRIA3 mRNA in the base and middle turns, with smaller differences in mRNA levels of GRIA2 and GRIA4. Furthermore, females have greater relative abundances of GRIA3-flop while males have more GRIA3-flip.

Conclusions: Together, our data suggest that increased GRIA3, specifically in the flop isoform, may in part mediate larger ABR wave I amplitudes in females, and is to our knowledge the first study to propose a molecular mechanism contributing to this known physiological difference in mammals.

A Numerical Study of Cochlear Properties That Predict the Distinct Differences in Nonlinear Cochlear Responses Between Gerbil and Guinea Pig
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Background: Gerbils and guinea pigs exhibit clear differences in the amount of cochlear nonlinearity as indicated by measurements of basilar membrane (BM) and reticular lamina (RL) displacement, fluid pressure within the scalae, and outer hair cell extracellular voltages called the local cochlear microphonic (LCM). Gerbil cochleae are relatively more nonlinear and measurements of the RL and LCM indicate gerbil cochleae are more hypercompressive than those of the guinea pig. Data from each species also show distinct differences in the amplitude and phase morphology of the RL, BM, and LCM that imply contrasts in cochlear physiology. The aim of our study was to use our numerical rodent cochlear model to identify two sets of parameters that predict the global and local cochlear responses of both species and provide mechanisms to explain these differences. A potential source for the differences observed between these species could be that their subtectorial regions are anatomically different. Hence, we hypothesize that fluid-mechanical losses could explain the species dependence.

Methods: A physiologically based mathematical model of the guinea pig cochlea that produces realistic dynamic and kinematic cochlear responses to sound was used for all experiments. The model incorporates parameters for the electrical, fluid, and mechanical properties of the organ of Corti and surrounding tissue and fluids. Nonlinear responses were computed with an iterative alternate-time-frequency method. Model parameters such as subtectrorial damping stiffness and mass, tectorial membrane mass, and basilar membrane damping, were systematically varied. Global and local-intracochlear responses were analyzed and will be reported.

Results: We found that the parameters damping, associated with the motion of the tectorial membrane (TM), basilar membrane, and the relative shear of the RL and TM controlled the high and low frequency rate of change (in the frequency domain) of the response and the nonlinear gain, while TM mass controlled the frequency where nonlinearity in the BM motion initiated.

Conclusions: Hypercompression of the RL could be due to the viscous damping in the organ of Corti.

Prestin Derived OHC Surface Area Reduction Underlies Age-Related Rescaling of Frequency Place Coding
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Background: Outer hair cell (OHC) is key to the mammalian cochlear amplifier. The functional change of OHC is assumed to play a major role in age-related threshold elevation. To better understand the functional changes of OHCs due to aging, we evaluated the nonlinear capacitance (NLC) changes of OHCs at fixed cochlear region and quantified the total number of Prestin (Q_{max}), charge density (Q_{sp}), voltage sensitivity (V_h) and cell sizes (C_{lin}) as a function of age. Age-matched in vivo measurements of DPOAE group delay were carried out to determine whether there is a change in these properties that could have altered traveling wave properties.

Methods: Auditory Brainstem Responses (ABRs) were used to find age-related hearing threshold elevations, latency prolongations and monitor recovery after acoustic lesion. We explored age-related OHC changes by measuring nonlinear and linear membrane capacitance under whole-cell patch-clamp. Age-related stereocilia length changes of inner hair cells (IHCs) and OHCs were quantified by scanning electron microscopy (SEM).

DPOAE group delays were used to compare traveling wave time between young and aged mice. To verify frequency place coding shift, acoustic lesion experiments were performed in young and aged mice. The lesions were quantified by confocal imaging for loss of ribbon synapse throughout the length of the cochlea.
Results: When compared at the fixed cochlear location, aging OHCs show progressively reduced total Prestin levels that contribute to surface area reduction but maintain Prestin's density. This change started right after the end of development around P21, following an exponential decay and the trend continued throughout the time period of our measurement (up to 16 months). In aged OHCs, voltage sensitivity of Prestin became less responsive to holding potential change manifested in reduced pre-pulse effect. Patch-clamp measurements revealed both aged IHCs and OHCs had reduced cell sizes (Clin), while elongated first row stereocilia are only observed by SEM in aged IHCs. DPOAE group delays are prolonged in the aging ear, suggest prolonged traveling wave time in the aged cochlea. Replot of ABR latency vs. level curves across different frequencies also revealed that prolonged latency exists in the aged cochlea and, coupled with a narrowed time gap for the one-octave band, suggesting that the cochlear frequency place coding rescaled during aging. Acoustic lesions demarcate a shifted damage boundary accompanied by a broader damage transition in aging cochleae.
Conclusions: Overall, the cell-based findings suggest that OHCs may improve their frequency responsiveness over aging through improved low pass Prestin's responses and reduced cell sizes that mechanically better couple to higher frequency tuning. In vivo measurements suggest that in the aging cochlea, a shift in frequency place coding could occur due to the changes in cochlear active and passive mechanics.

Modeling the Interaction of Stimuli and Adaptive Rate Constants on OHC Hair Bundle Response
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Background: The current and displacement response of the HB of the OHC exhibit rate dependence attributable to both viscous effects (fluid damping) and the adaptation of the gating mechanism. Further, any rise time associated with the applied force will be reflected in the response. To study this, we simulated the in vitro response of an isolated HB to varied mechanical loads to determine its operating range (OR) using the simple HB adaptation model of Tinevez, et al. (Biophys. J., 93(11), 4053-4067 (2007)), we will denote it the TMJ model.
Methods: We subjected the TMJ model of the HB and adaptation motor to applied forces that achieve a constant value after an initial exponential rise time (τ_F). Using a Boltzmann function for channel open probability, we solved the nonlinear system using the 4th-order Runge-Kutta method on MATLAB. Further, we linearized the system about different resting open probabilities to obtain closed-form solutions, revealing the current and force evolution dependence on τ_F and adaptation time constants.
Results: We analyzed the solutions for resting channel open probabilities ranging from 5% to 40%. We noticed a delayed saturation to steady-state for HB motion, a decline accompanied by a rightward shift in the current peak, and a broadening of the HB's OR with increasing τ_F. When τ_F increased to near 0.4 ms, the current peak disappeared, and the OR saturated to its maximum value. The nonlinear and linearized system responses conformed well. From the linearized model, we obtained both a fast (τ_FA) and slow (τ_SA) time constant for adaptation motor response. We observed that for $\tau\_F < \tau\_FA$, current peaked due to the fast adaptation mechanism, and for $\tau\_FA < \tau\_F < \tau\_SA$, the stimulus controlled the peak time and value. Finally, when τ_F surpassed τ_SA, the force was too slow for fast adaptation to affect the current, and we saw a slow adaptation to the steady-state value. We also observed that the OR was constant as τ_F exceeded τ_SA. The OR for an instantaneous force ($\tau\_F = 0$) was found to be ~ 34 nm increasing to ~ 340 nm (similar to experimental values) for $\tau\_F = 0.4$ ms. Lastly, we found that the model only qualitatively corresponded to the experimental force-displacement relations and underestimated the force at the peak current using τ_F matching experimental conditions.
Conclusions: The rise time of the applied force alters the predicted OR and the associated force induced by HB as found by Nam, et al. (Biophys. J., 108, 2633-2647 (2015)). We find that 30 nm - 50 nm is a lower limit for the OR using the TMJ model. Finally, the relation of τ_F to τ_FA dramatically influences the quantitative characterization of peak currents and forces. This work was supported by NIH-NIDCD 04084.

Is the Long Outer-Hair-Cell Time Constant a Bug or a Feature?
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Background: Measured outer-hair-cell (OHC) membrane resistor-capacitance (RC) time constants are significantly longer than the shortest characteristic oscillatory period of cochlear mechanical responses. The long time constant implies that the transmembrane potentials that drive the OHC electromotile response decrease by several orders of magnitude across the frequency range of mammalian hearing. The presence of this low-pass filtering has both called into question the role of high-frequency OHC electromotility for cochlear mechanics and prompted the search for compensating mechanisms that might somehow extend the OHC bandwidth.

Methods: Here, we show that the magnitude of this so-called "RC problem" has been inflated by (i) overextension of the analogy with low-pass filters and (ii) over-reliance on cochlear mechanical models of low spatial dimensionality that require unrealistically sharp OHC tuning. A simple "worst-case-scenario" RC low-pass filter model of the OHC membrane—where all possible compensating mechanisms are excluded by design and with parameter values intentionally unfavorable for high-frequency operation—reveals that OHC motile responses are comparable to or larger than high-frequency (>50 kHz) BM vibrations up to moderate sound levels.

Results: The simple RC model accounts for recent mechanical data collected in the mouse cochlea as well as the OHC action postulated by both recent and (to a certain extent) classic theories

Conclusions: Analogies with existing electronic circuits suggest that the long RC time constant is actually a beneficial design feature and not an annoying biophysical bug of the cochlear amplifier. Analyses of existing theories and recent experiments indicate that arguments against a role for high-frequency OHC electromotility often mistake the parts for the whole.

Vibration Maps Across the Cochlear Partition Reveal the Mechanical Interplay Between Different Structures Within the Organ of Corti Complex

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Background: Active cochlear mechanical processing of sound (still) is the subject of intense scientific research because it underlies both the frequency selectivity and sensitivity of the auditory system. Most of our knowledge comes from direct measurements of basilar membrane (BM) responses in the base of the cochlea. Recent application of optical coherence tomography (OCT) revealed the responses from structures within the organ of Corti complex (OCC: comprising the organ of Corti, BM and tectorial membrane (TM)), which improved our views on the fundamental processes involved in the transduction of high-frequency sound. Auditory nerve fiber responses, otoacoustic emissions, and direct intracochlear observations suggest that sound is processed differently in the base and apex of the cochlea, and OCT gives access to more apically located cochlear regions. This study presents mechanical responses of the gerbil OCC located near the apex of the cochlea. By combining data from multiple locations within a single cross-section of the cochlear duct we reconstruct amplitude and phase response maps that describe the frequency dependent motion across the entire OCC.

Methods: Acoustically evoked vibrations within the OCC, which includes the organ of Corti, the basilar membrane (BM), and the tectorial membrane (TM), in the second turn of adult gerbil cochleae were measured using OCT. The care and use of animals were approved by the Institutional Animal Care and Use Committee (IACUC) of the VA Loma Linda Healthcare System.

Results: Within the OCC, several regions are visible that differ in their frequency selectivity, input-output characteristics, as well as their relative phase. These regions seemingly correlate with distinct anatomical structures that include the BM, the OHC region, the lateral support (Hensen's/Claudius) cell region, and the TM. Tuning and gain are qualitatively like those observed in the cochlea's base. We observe nonlinear responses over an extended range in the OHC region, as well a vibratory "hotspot" in the OHC region that turns "cold" following death. In addition, phase differences between regions cause shearing motion within the OCC and cochlear amplification: BM and OHC vibrations are ~90-degrees apart, while OHC and TM move in antiphase (i.e., there is shearing motion between them).

Conclusions: By combining amplitude and phase responses across the OCC obtained over a range of stimulus frequencies and intensities, the data provide an extensive description of the sound-induced vibrations of the cochlear partition that reveals substantial deformation of the OCC. Despite these deformations, the BM responses (i.e., a structure "far away" from the inner hair cell) provide a good description of the low-frequency neural tuning curve. We hypothesize that the relative phase of the vibrating OCC components plays a pivotal role in achieving the frequency selectivity and sensitivity within the inner ear.

Minipigs as a Large Animal Model for Cochlear Implantation with Similar Inner Ear Dimensions to Humans

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Background: Animal models are indispensable in hearing research. This applies especially to cochlear implantation research, where the majority of experiments are performed in rodents. However, relatively small anatomical inner ear dimensions in rodents complicate the transfer of experimental results to the clinic. Larger animal models with similar inner dimensions to humans are sparse. Therefore, we evaluated piglets and Aachen minipigs for the feasibility of cochlear implantation with commercially available cochlear implants, which are commonly used in humans. Additionally, we wanted to determine the induced trauma in implanted animals and evaluate this animal model for applicability in hearing research.
Methods: After the establishment of a step-by-step surgical approach in the cadaver model, Flex 20 or Flex24 cochlear implant electrodes were inserted intraoperatively in seven domestic piglets and six Aachen minipigs. Anatomical common features and differences were defined in comparison to the human skull. Electrophysiological measurements were performed before, during and after implantation up to a follow-up period of two months. These included auditory brainstem responses (ABR) as well as electrically and acoustically evoked compound action potentials. After euthanasia and extraction of the cochlea, histological analysis was performed in representative samples for fibrosis determination and electrode insertion trauma in addition to the electrode position in the cochleae. Extracted cochleae of pigs and minipigs were scanned via micro-CT to determine cochlear dimensions.
Results: A retroauricular single incision approach was established. Micro-CT scans show a larger round window membrane in pigs and minipigs compared to humans and a relatively voluminous first cochlear turn. X-rays of implanted animals showed successful electrode insertion within the first two cochlear turns. Baseline click ABR thresholds in minipigs were 27 ± 2.8 dB SPL (mean ± SEM) and frequency specific ABRs ranged between 30 and 80 dB SPL. Functional deafness was determined within the first two weeks after surgery, but hearing recovered partially to a threshold of 80 dB SPL in ABR responses. Electrically evoked compound action potential thresholds and eABR thresholds increased within the first week after surgery. Histological analysis after cochlear extraction in implanted ears revealed fibrosis in Masson's trichrome stain compared to unimplanted inner ears. Further electrode in-situ histological analysis confirmed electrode location within the first two cochlear turns.
Conclusions: Cochlear implantation with clinically applied cochlear implants in piglets and minipigs is feasible. Moreover, the inner ear dimensions of this animal model are comparable to humans. We confirm the minipig as a representative animal model in hearing research, which may close the existing gap between rodents and humans. Important long-term findings such as partial hearing recovery and overall cochlear health are presented after cochlear implant insertion and may lay the ground for further research questions.

The Role of ATF Signaling in Response to PTS-Inducing Noise

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Background: The ATF signaling pathway has known roles in regulating stress response and cell survival within a variety of tissue types. In a recently published paper from our laboratory, we identified the ATF signaling pathway as uniquely upregulated in the type 1A spiral ganglion neurons (SGNs) following traumatic noise exposure. This finding is intriguing because of the relative resilience of type 1A SGNs in response to noise, in comparison to type 1C. Here we explored the spatiotemporal expression of the ATF transcription factors and some of their canonical target genes in the spiral ganglion, organ of Corti, and lateral wall. To determine whether the observed Atf upregulation was a transient or long-lasting response to traumatic noise, expression was evaluated 6 hours, 24 hours, and 7 days following a permanent threshold shift (PTS)-inducing noise exposure.
Methods: Male and female B6CBAF1/J mice were divided into sham-exposed and noise-exposed groups at 10 weeks of age. Noise trauma was induced with an 8-16kHz octave band of noise at 105 dB SPL administered for 2 hours, and the cochleae from both groups were collected 6 hours, 24 hours, and 7 days following exposure. Using fluorescent in situ hybridization, expression of Atf3, Atf4, Gadd45a, and Ddit3 were assessed at each time point

with their respective RNAscope probes. Changes in both localization and level of expression were quantitatively evaluated between sham- and noise-exposed mice across all time points.

Results: In response to PTS-inducing noise exposure, we identified a statistically significant and highly cell type-specific upregulation of the ATF signaling pathway within different domains of the cochlea. Consistent with our earlier single cell RNA-seq analysis of the spiral ganglion neurons, Atf3, Atf4, Gadd45a, and Ddit3 exhibited a slight increase in expression within the SGNs beginning 6 hours after noise, with robust upregulation occurring in the type 1A SGNs at the 24-hour time point before returning to baseline expression at 7 days. Although no changes in expression were seen within the sensory cells or supporting cells of the organ of Corti, transient upregulation of all four Atf targets were observed within the spiral limbus and lateral wall 6 hours following noise, which returned to baseline by 24 hours. The transient upregulation of Atf3, Atf4, Gadd45a, and Ddit3 was primarily localized to the basal cells of the stria vascularis, with only one ATF target, Gadd45a, displaying expression in the fibrocytes of the lateral wall.

Conclusions: Using a combinatorial approach of transcriptomics and in situ hybridization, we have identified the ATF signaling pathway as a potentially significant contributor in the stress response against traumatic noise. These results underscore the importance of identifying key signaling regulators of cell survival for the development of targeted therapeutics to prevent and treat noise-induced hearing loss.

An in Vivo Biomarker for Evaluating the Biologic Characteristics of Ototoxic Drugs and Novel Therapeutics That Mitigate Ototoxicity

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Background: A biomarker is a reliable and efficient method to determine the presence or progress of a disease that can be used for either diagnostic purposes or to evaluate the effects of treatment. We previously presented proof of concept data highlighting the dose and time relationship between systemically administered aminoglycosides and neonatal mouse cochlear hair cell accumulation. Here, we present two applications of this biomarker: evaluating differential ototoxin accumulation in sensory and non-sensory cell populations and investigating the time- and dose-dependent effects of ORC-13661, a hearing protection drug.

Methods: Neonatal mice (P5) were given systemic injections of geneticin (G418), a gentamicin congener, conjugated with Texas Red (G418-TR) either alone or in conjunction with ORC-13661 pretreatment. Dosage, injection schedules, and survival times of both drugs were systematically varied. Blood samples were taken, followed by euthanasia, otic capsule dissection with fixation by local perfusion, and dissections of whole mount preparations in addition to cryostat sections. Tissue was immunolabeled with anti-myosin7a and/or phalloidin and imaged with confocal microscopy. Utilizing myosin7a as a hair cell mask, G418-TR fluorescence was quantified within hair cells.

Results: Here, we show that neonatal hair cells accumulate G418-TR over 6 hours, and then retain nearly unchanged levels of G418-TR fluorescence over 72 hours after a single injection. When evaluating other tissue types from within the cochlea, we find that the stria vascularis shows an early peak in G418-TR accumulation before returning to near baseline levels. Both supporting pillar cells and spiral ganglia neurons demonstrate minimal G418-TR uptake. Finally, we demonstrate that ORC-13661 blocks G418-TR accumulation in a time-dependent manner. ORC-13661 demonstrates robust G418-TR uptake inhibition when animals are pretreated for 2 hours and then exposed to G418-TR for 3 hours, but when the paradigm is shifted to 2 hours pretreatment followed by G418-TR for 6 hours, only the highest doses demonstrate an inhibitory effect.

Conclusions: We previously highlighted that neonatal hair cell uptake of aminoglycosides serves as a proxy for in vivo mature hair cell uptake. When evaluating uptake and retention within the neonatal cochlea, we find that aminoglycosides are retained in the sensory epithelium but not other cell populations, suggesting that both uptake and prolonged retention of aminoglycosides contribute to hair cell ototoxicity. Finally, we demonstrate that ORC-13661 pretreatment blocks accumulation of aminoglycosides in vivo in a time-dependent manner, suggesting that either serial dosing or enteral sustained release could enhance otoprotective effects. We believe that this preparation will serve as a valuable translational tool to better understand mechanisms of ototoxicity and as a rapid model to assess biologic characteristics of therapeutic strategies designed to mitigate ototoxicity.

Supporting Cell Ablation Leads to Robust Proliferation and Regeneration in the Greater Epithelial Ridge in the Neonatal Cochlea

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Background: While the adult mammalian cochlea does not proliferate or regenerate, the non-mammalian cochlea can regenerate following damage by conversion of supporting cells to hair cells through either mitotic regeneration or direct transdifferentiation. Prior work has shown that hair cell damage induces a limited degree of both proliferation and regeneration in the neonatal mouse cochlea and that certain supporting cell subtypes, such as inner phalangeal cells (IPhCs) and inner border cells, can regenerate after damage. However, the mechanisms by which supporting cells are regenerated remain unknown. Here, we characterized a mouse model that allows for specific ablation of supporting cells, which leads to robust proliferation and regeneration of IPhCs. Using this model, we will test whether proliferation is involved in supporting cell regeneration.

Methods: In Lgr5DTR/+ mice, the human diphtheria toxin (DT) receptor is expressed specifically in inner pillar cells, inner phalangeal cells (IPhCs) and the third row of Deiters' cells. DT (4ng/g) was administered on postnatal day 1 (P1) to selectively ablate cochlear Lgr5+ supporting cell subtypes. Cochleae were harvested at P4, and P7. Additionally, cochleae were dissected and cultured as whole organs at P2 after damage and cultures fixed at 48hr and 72hr in vitro. The number of IPhCs, marked by Fap7, that had undergone proliferation (EdU-, Ki67-positive) was quantified. Lgr5DTR/+; GLASTCre/+; R26RTdTomato/+ mice were used to fate-map GLAST+ cells in the greater epithelial ridge (GER). Mice were injected with DT and tamoxifen (0.2mg/g) at P1. EdU was injected into mice at P3, P4, and P5 or was added to the culture media.

Results: After DT injection at P1, Lgr5DTR/+ cochleae had significant loss of IPhCs in apical (80.6%∓3.9%), middle (79.2%∓3.7%), and basal (70.9%∓4.1%) turns at P4 in vivo compared to controls. At P7, IPhCs significantly regenerated to control levels in all three turns of the cochlea (81.9%∓1.89% in apical, 91.3%∓2.1% in middle, and 86.4%∓3.6% in basal). A subset of regenerated IPhCs had undergone proliferation, as labelled by EdU and/or Ki67, with proliferation occurring in an apical-to-basal gradient. Fate mapping studies revealed that a large proportion of regenerated IPhCs arose from GLAST+ cells in the GER region. Organotypic cultures of P2 Lgr5DTR/+ cochlea (after DT at P1) had a loss of IPhCs after 48hr, and regeneration of IPhCs at 72hr. We will use a mitotic inhibitor to assess whether proliferation is essential for supporting cell regeneration.

Conclusions: After selective supporting cell damage in the neonatal cochlea, cells in the GER undergo proliferation to regenerate lost supporting cells. Future directions will further investigate the mechanisms of regeneration and whether proliferation is critical in this process in vitro and in vivo.

The Role of Pou4f3 Over-Expression in the Protection of Adult Mouse Utricle Hair Cells From Aminoglycoside Induced Cell Death

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Background: The irreversible loss of hair cells (HCs) caused by multiple insults including traumatic noise, ototoxic drugs, inner ear infection, or aging in the mammalian inner ear results in permanent hearing loss and vestibular dysfunction. Inner hair cells (IHCs) in the cochlea are generally more resistant to all of these insults as compared to outer hair cells (OHCs) and studies have shown that the transcription factor Pou4f3 is expressed at a higher level in IHCs than OHCs. Using a noise damage model, we previously demonstrated that overexpression of Pou4f3 in cochlear OHCs could largely protect hair cells from noise exposure in a subset of targeted mice. Here, we investigated whether overexpression of Pou4f3 offers protection to utricular HCs exposed to aminoglycosides.

Methods: We used tamoxifen inducible Fbxo2CreER or Atoh1-CreERT2 mice to target adult utricular type I or type II HCs, respectively. Pou4f3 was over-expressed using CAG-loxP-stop-loxP-Pou4f3-IRES-mCherry (Pou4f3 OE) mice and tamoxifen injection give at 4 weeks of age. Cre-negative or Pou4f3 OE-negative littermates with normal levels of Pou4f3 expression were used as controls. One week after tamoxifen induction, utricle explant cultures were established, and treated with 4mM neomycin one day later. The utricle explants were fixed one day post-neomycin treatment and type I and type II HCs were manually quantified from confocal images of whole utricles immuno-stained with anti-myosin VIIa and anti-Sox2 antibodies.

Results: Sox2 immunostaining was used to distinguish type I versus type II hair cells. Preliminary results from Atoh1-CreERT2 mice suggest that in utricles with Pou4f3 overexpression, there are increased numbers of type II HCs after neomycin treatment compared to controls. There was no difference in the number of type I HCs

between utricles with Pou4f3 overexpression and controls which makes sense since the Atoh1-CreERT2 line only targets type II HCs. Studies on the protective effect of Pou4f3 OE on type I HCs from neomycin-induced damage are in progress using the Fbxo2CreER   line which specifically targets type I HCs.

Conclusions: Preliminary data suggest that Pou4f3 overexpression protects type II HCs from neomycin induced-damage. Studies are underway to validate these findings, and to investigate the effect of Pou4f3 overexpression on type I HCs. In addition, we are also investigating whether HC loss caused by the explant method (in the absence of neomycin), can be reduced when Pou4f3 is overexpressed in type I or II HCs.

No Effects of BDNF Treatment on the Survival of Hair Cells and Supporting Cells After Ototoxic Deafening in Vivo

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Background: Degeneration of mechanosensory hair cells (HCs) and supporting cells (SCs) in the organ of Corti is often followed by progressive degeneration of the afferent spiral ganglion cells (SGCs) as well. Here, we investigated whether treatment with brain-derived neurotrophic factor (BDNF), which is known to protect SGCs (PMID: 26354903, 33126525, 33271438), could also protect the HCs and SCs in a guinea pig model of sensorineural hearing loss.

Methods: We reanalyzed cochlear tissues from guinea pigs used in previous studies (PMID: 26354903, 33126525). Hearing loss was induced by administration of kanamycin and furosemide in young adult albino guinea pigs. Two different BDNF treatment approaches were used. (1) BDNF treatment by means of gelfoam (BDNF-GF) (6.67 mg/ml) administered two weeks after deafening, with histological assessment four weeks after treatment. (2) BDNF treatment through a mini-osmotic pump (100 µg/ml; 0.25 µl/hour) (BDNF-OP) administered two weeks after deafening together with a chronic CI; treatment cessation four weeks after implantation and euthanasia/histological processing eight weeks thereafter. Cell counting of HCs and SCs was performed over all cochlear locations (from base to helicotrema) on midmodiolar sections. The various subpopulations of cells were identified: outer HCs (OHC), inner HCs (IHC), Border, Phalangeal, Pillar, Deiters', and Hensen's cells. Finally, the total number of SCs was correlated with SGC packing density of the same animals.

Results: The BDNF-GF group had significantly fewer OHCs compared to the untreated group, as resulted from the overall difference in OHC number between untreated and treated ears of individual animals across the cochlea (11% fewer OHC in treated than untreated, p=0.019). Conversely, the IHC and SCs number averaged by experimental group did not vary between treated and untreated ears for each cochlear turn. In the BDNF-OP study, the comparison of the number of sensory cells and non-sensory cells showed no statistically significant differences between the experimental groups. As reported previously, the SGC packing density was significantly higher in the BDNF-treated ears compared to the untreated ones. Notably, BDNF administered through mini-osmotic pump led to an increased packing density over all cochlear locations (p<0.001); while BDNF administered through gelfoam protected SGCs only in the base (p<0.001). No correlation between SGC survival and the number of SCs was found for either BDNF treatment paradigm.

Conclusions: Our data suggest that: (1) BDNF is likely not to be effective in protecting the organ of Corti, and that rather the protection of SGCs could be a direct targeting by BDNF without a role for SCs or HCs; (2) a different function/ activity of the remaining cells in the organ of Corti (which could be independent from cell number) of the BDNF-treated cochleas could mediate SGC protection and needs to be investigated in the future.

Local Cisplatin Delivery in Mouse Reliably Models Sensorineural Ototoxicity Without Systemic Adverse Effects

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Background: Cisplatin is a lifesaving chemotherapeutic drug with marked ototoxic side effects. Cisplatin-induced hearing loss affects a significant part of cancer surviving patients and is an unmet clinical need with important socio-economic consequences. Unfortunately, in current pre-clinical animal models of cisplatin ototoxicity, which are mainly based on systemic delivery, important morbidity is observed leading to premature sacrifice or death. This methodology not only raise obvious animal welfare concerns, but also increase the numbers of animals employed in ototoxicity studies to compensate for dropouts related with early sacrifice.

Methods: To overcome these important limitations, we developed a local delivery model based on the application of a cisplatin solution directly into the otic bulla through a retroauricular approach. The effects of this approach were analysed by immunohistochemistry and platinum mass-spectometry.

Results: The local delivery model reliably induced significant hearing loss with a mean threshold shift ranging from 10 – 30 dB, strongly affecting the high frequencies (22 and 32 kHz). Importantly, mice did not show visible stress or distress indicators and no significant morbidity in comparison to a traditional systemic delivery control group of mice, injected intraperitoneally with 10 mg/Kg cisplatin, where significant weight loss >10 % in all treated animals (without any recovery) led to premature abortion of experiments at day 3. Mass spectrometry confirmed the absence of relevant systemic uptake following local delivery, with platinum accumulation restricted to the cochlea, whereas important platinum concentrations were detected in liver and kidney of the systemic cisplatin group. A clear correlation between the cochlear platinum concentration and the auditory threshold shift was observed. Immunohistochemistry revealed statistically significant loss of outer hair cells in the basal and apical turns of the cochlea and an important and statistically significant loss of auditory neurons and synapses in all cochlear regions.

Conclusions: In conclusion, local cisplatin delivery induces robust hearing loss with minimal morbidity, thereby offering a reliable rodent model for human cisplatin ototoxicity, reducing the number of animals required and showing improved animal welfare compared with traditional systemic models.

Female C57BL/6J Mice Are Less Susceptible to Systemic Kanamycin-Furosemide-Induced Hearing Loss Than Males

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Background: Sensorineural hearing loss (SNHL) is mainly caused by irreversible damage to sensory hair cells. Ototoxicity is one of the leading causes of hair cell damage, together with noise exposure. Animal models represent an important tool for analyzing novel therapeutic opportunities for treating patients with SNHL. We have recently observed that endogenous stem cells in the cochlea of adult male mice survive ototoxicity (Smith-Cortinez et al., 2021, Front. Mol. Neurosci., 10.3389/fnmol.2021.729625). Ototoxic trauma in mice can be induced with systemic administration of furosemide and kanamycin (Jansen et al., 2013, Otol Neurotol, 10.1097/MAO.0b013e318291c610), however effects of different doses have been reported in males only. Several other deafening models have shown differences between females and males in response to an ototoxic trauma. Hence, we examined gender differences in in susceptibility to ototoxicity in the adult mouse cochlea.

Methods: Adult female and male (postnatal day 40) Lgr5-eGFP-IRES-creERT2 heterozygous (Lgr5GFP) mice were used (C57BL/6J background). Animals were either normal-hearing or deafened with a single dose of 100 mg/kg i.v. furosemide and kanamycin (low dose: 700 or high dose: 900 mg/kg s.c). Seven days after deafening, auditory brainstem responses (ABRs) were recorded to click and tone stimuli. Cochleas were harvested and processed for histology in order to evaluate hair cell loss.

Results: Male mice (Lgr5GFP) showed increased ABR thresholds 1 week after ototoxic treatment with the low dose of kanamycin (44 dB threshold shifts for clicks, n=6), consistent with previous findings. However, female mice (Lgr5GFP) showed no significant hearing loss in response the same dosage of kanamycin (700 mg/kg) as observed by ABRs 1 week after ototoxic treatment (9 dB threshold shift, n=9). When treated with the high dose of kanamycin (900 mg/kg) female mice showed increased ABR thresholds 1 week after ototoxic treatment (39 dB threshold shift, n=8).

Conclusions: Female mice (Lgr5GFP transgenic, C57BL/6J background) are less susceptible to kanamycin-induced hearing loss than males and hence need higher doses of kanamycin to reach the same (reduced) hearing performance and hair cell loss as males. This is in line with the prevalence of hearing loss in humans, which is 7.3% in males and only 4.8% in females, where estrogens have been linked to increased hearing performance and protection against hearing loss (Shuster et al., 2019, J Acoust Soc Am, 10.1121/1.5111870).

Hearing Damage Associated With Transcranial Ultrasound Stimulation for Neuromodulation and Blood-Brain-Barrier Opening

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Background: Ultrasound (US) research has grown rapidly in the past decade for multiple transcranial applications including noninvasively modulating brain regions with high spatial resolution and performing targeted drug delivery by transiently opening the blood-brain-barrier (BBB). Studies have also quickly moved into humans citing established tissue safety ranges for diagnostic US imaging in an FDA guidance document (Docket: FDA-2017-D-5372) with some parameters having additional histological safety data in animal models. While investigating US neuromodulation, our lab discovered that US applied transcranially readily activates the auditory system through vibrations of cerebrospinal fluid that then directly vibrate fluids within the cochlea (Guo et al., Neuron, 2018). Our lab has also demonstrated that neural responses to US stimuli closely resemble those to air-conducted stimuli with frequency specific activation of auditory regions possible using amplitude-modulated ultrasound. Due to the potential applications of US induced auditory activation for novel therapeutic devices, our group was interested in characterizing safe levels of US pressure for the hearing system and to investigate parameters used in current transcranial US studies.

Methods: In anesthetized guinea pigs, we collected auditory brainstem responses (ABRs) and electrocochleography (ECochG) in response to air-conducted acoustic pure tones (2, 4, 8, 12, 20, and 30 kHz) and broadband noise at varying levels (10-70 dB SPL) before and after US stimulation with specific parameters that have been used in previous studies for either neuromodulation or BBB opening experiments. Control data was also collected for characterizing the stability of the recording protocol and for noise-induced hearing loss (NIHL) at various levels (90-110 dB SPL) and presentation times (10-30 min). We assessed ABR and ECochG thresholds, amplitudes, and latencies over time to identify any changes that occur.

Results: Many tested US neuromodulation and BBB opening parameters showed neurophysiological changes associated with hearing damage including threshold shifts, waveform amplitude reduction, and increased latencies. Threshold shifts were most prevalent in the high frequencies with some more severe cases of US stimuli causing threshold shifts in the middle frequencies with similarities to hearing loss patterns with NIHL. The amount of measured hearing damage varied depending on the combination of US parameters used with some severe losses occurring.

Conclusions: US parameters of interest for hearing damage in addition to pressure level are duration, duty cycle, and center frequency. Caution is urged when performing transcranial US stimulation studies in humans, especially when targeting locations closer to the regions of the cochlea or if perception of sound is noted by individuals during stimulation. Future studies will include an in-depth characterization of the US parameters of interest and evaluation of parameters in large animal models that better mimic the head size in humans.

Cochlin Deficiency Protects Against Noise-Induced Hearing Loss

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Background: COCH (Coagulation factor C Homolog) is the most highly expressed gene in the cochlea and its encoded protein, cochlin, is the most abundant protein detected in the inner ear. Mutations in COCH cause the adult-onset progressive sensorineural hearing loss (HL) and vestibular disorder, DFNA9.

Methods: To study cochlin's function in response to noise trauma, we exposed adolescent wild-type (Coch+/+) and cochlin knock-out (Coch-/-) mice to noise (8-16 kHz, 103 dB SPL, 2h) that causes a permanent threshold shift and hair cell loss, tested auditory function with auditory brain stem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs), sampled perilymph, and collected cochlear tissue for qRT-PCR and histological analysis.

Results: We show quantitatively that the pro-inflammatory component of cochlin, LCCL, is upregulated 6 hours after noise exposure in perilymph of wild-type mice compared to unexposed mice, as is the enzyme catalyzing LCCL release, aggrecanase1, encoded by Adamts4, in cochlear soft-tissue. Two weeks after noise exposure, Coch-/- mice had substantially less elevation in noise-induced auditory thresholds and hair cell loss than Coch+/+ mice, consistent with cochlin deficiency providing protection from noise trauma. We further show that upregulation of pro-inflammatory cytokines in perilymph and cochlear soft-tissue after noise exposure is lower in

cochlin knock-out than wild-type mice. Comparison of pre-noise exposure thresholds of ABRs and DPOAEs in Coch-/- mice and Coch+/+ littermates revealed a small and significant elevation in thresholds of Coch-/- mice, overall consistent with a small conductive hearing loss in Coch-/- mice.

Conclusions: Taken together, our data demonstrate that cochlin deficiency results in conductive hearing loss and protects against physiologic and molecular effects of noise trauma.

Role of Pyroptotic Cell Death in Cochlear Implantation Trauma

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Background: Electric-acoustic stimulation cochlear implants (EAS-CI) enable hearing restoration in patients with severe to profound hearing impairment. However, the insertion of a cochlear implant electrode array may also contribute to the loss of residual acoustic hearing. Following cochlear implant (CI) insertion, an acute inflammatory response promotes sensorineural cell degeneration throughout different molecular and cellular pathways such as apoptosis, necrosis, and necrosis-like programmed cell death. More recently, pyroptosis has been identified as a unique form of programmed cell death. Mechanistically, pyroptosis cell death is described by inflammasome and caspase-1 (Casp-1) activation leading to gasdermin-induced pore formation on the membrane, cell swelling and lysis, as well as the release of pro-inflammatory cytokines, interleukin-18 (IL-18) and interleukin-1β (IL-1β). Although the study of cell death in CI trauma has received much attention in the last decades, the role of abnormal inflammasome signaling leading to pyroptosis in the inner ear remains uninvestigated.

Methods: In the current study, we performed western blot, RT-PCR, and immunohistochemical evaluation to quantify the role of pyroptosis cell death in a preclinical rodent model of cochlear electrode insertion injury. The University of Miami Institutional Animal Care and Use Committee approved all procedures. Mechanistic studies were performed on adult female Brown Norway rats. Gene and protein expression levels of selected factors and immunohistochemistry labeling of specific markers were quantified in CI injured and untreated cochleae at 6, 24, and 48 hrs post-electrode insertion

Results: Western blot and RT-PCR showed that CI trauma increased the expression of inflammasome proteins and pyroptosis cell death markers NLRP3, GSDMD (Gasdermin-D), ASC, Casp-1, IL-18, and IL-1β compared to non-injured cochleae. In addition, immunohistochemical data suggest that the involvement of GSDMD and Casp-1 is not confined to the cochlear hair cells.

Conclusions: The pathomechanisms underlying the loss of residual hearing following CI electrode insertion are complex and may include multiple injury and cell death pathways. Our results provide clear evidence for the involvement of pyroptosis in the pathophysiology of hair cell death by CI injury. Targeting abnormal inflammasome activation resulting in pyroptosis cell death may reveal potential therapeutic strategies to limit hearing loss and sensorineural cell degeneration in CI patients.

Evaluating the Efficacy of Two Novel Compounds for Providing Otoprotection Using Ex Vivo Model of Cochlear Implant Trauma

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Background: The insertion of electrode array during cochlear implantation induces oxidative stress and inner ear trauma leading to sensory cell damage and loss of hair cells. The objective of this study was to investigate and compare the efficacy of two compounds (compound A and compound B) in providing otoprotection for loss of sensory cells in response to electrode insertion trauma (EIT) and determine appropriate dosage using an ex vivo model.

Methods: The organ of Corti (OC) was harvested from post-natal day 3 rats and divided into four groups: 1) control; 2) EIT; 3) EIT and identified compound A (different concentrations) 4) EIT and identified compound B

(different concentrations). For EIT, a custom designed electrode was introduced into the inner ear through the small cochleostomy located next to the round window area, allowing for an insertion of between 110° and 150°.The explants were subjected to FITC phalloidin staining to determine the number of viable hair cells. In addition, CellROX and cleaved caspase 3 immunostaining was performed on explants to determine the levels of oxidative stress and apoptosis, respectively.

Results: There was significant loss of hair cells in OC explants subjected to EIT. The identified compound A was better than compound B to prevent hair cell loss due to EIT in our ex vivo model. The number of surviving hair cells were significantly higher in OCs treated with the identified compound A compared to those treated with compound B. In addition, OC explants subjected to EIT exhibited activation of oxidative stress and apoptosis pathways. The mean fluorescence intensity values for CellROX and cleaved caspase 3 immunostaining in OCs subjected to EIT were significantly higher than control group. Compound A was more efficacious than compound B in downregulating these oxidative stress and apoptosis pathways as indicated by mean fluorescent intensity values.

Conclusions: Our findings suggest that identified compound A should be explored for developing therapeutic strategies for hearing preservation following cochlear implantation. The identified compound A provide otoprotection against loss of sensory cells in response to EIT that will promote hearing preservation and hence better clinical outcomes in implanted individuals. The ex vivo model used in this study can be explored for developing novel effective therapeutic interventions for hearing preservation following cochlear implantation. We will determine the efficacy of compound A in providing otoprotection in a preclinical animal model of cochlear implantation in future studies.

Inflammatory Monocytes Infiltrate to the Spiral Ligament and Transform to Macrophages After Noise Exposure
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Background: The macrophages are known to increase in the population after noise exposure. This increment is regarded result of the transform of the infiltrated monocytes to the macrophages, rather than the proliferation of cochlea resident macrophages. However, there is lack of study that observed infiltrated monocytes in the cochlea. Thus, we tried to investigate the infiltrated monocytes after noise exposure.

Methods: Wild type and CX3CR1+/GFP C57/B6 mice were used. Immunofluorescence of mouse cochlea was performed to identify the infiltration of inflammatory monocytes and the transformation of infiltrated monocytes into macrophages. The flow cytometry analysis also performed to confirm and quantify the result.

Results: The monocytes were identified in the spiral ligament at 1 day after noise exposure. The flow cytometry analysis confirmed that the monocyte population peaks at 1 day post-noise and decreases after. The amoeboid type macrophages were increases in crista basilaris at 3 days post-noise. After 5 days post-noise, they look spreading to the basilar membrane.

Conclusions: The monocytes infiltrated to the spiral ligament from 1 day after noise exposure, transform to macrophage within 3 days post-noise, and then spread to the basilar membrane.

N1-Methylnicotinamide Protects High-Fat Diet- And Age-Induced Hearing Loss via Stabilization of Sirtuin 1 Protein
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Background: Age-related hearing loss (ARHL) is the common form of hearing loss associated with aging. Sirtuin 1 (SIRT1) is associated with the most complex physiological processes, including metabolism, cancer onset, and aging. SIRT1 proteins are stabilized by the conversion of nicotinamide to N1-methylnicotinamide (MNAM), independent of its mRNA levels. Moreover, MNAM has implications in increased longevity achieved through its mitohormetic effects. In this study, we aimed to determine the relationship between diet, hearing function, SIRT1 and SIRT3 expression levels in the inner ear, and cochlear morphology.

Methods: Mice fed with a high-fat diet (HFD), HFD + 1% MNAM, and low-fat diet (LFD) were monitored for age-related auditory-evoked brainstem responses, and changes in cochlear histology, metabolism, and protein and mRNA expressions were analyzed.

Results: Our results revealed that the HFD- and aging-mediated downregulated expression of SIRT1 and SIRT3 promoted hearing loss that was obfuscated by MNAM supplementation-induced upregulated expression of cochlear SIRT1 and SIRT3.

Conclusions: Our results suggest that MNAM can be used as a therapeutic agent for preventing ARHL.

Sequestration of Cisplatin by Nano-Based SPION Glutathione for Cisplatin Ototoxicity

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Background: Cisplatin is the first-line treatment for many types of cancer, yet the high rate of cisplatin-induced ototoxicity can interrupt or alter the use of the chemotherapeutic or cause a significant decrease in quality of life if treatment persists. Cisplatin-induced ototoxicity represents an important challenge to patient care because of the lack of effective treatment.

Methods: In this work, we developed a novel system to sequester cisplatin by using nano-based glutathione (GSH). The system consisted of superparamagnetic iron oxide nanoparticles (SPIONs) and the biodegradable copolymer poly(ethylene glycol)-b-poly(caprolactone) (PEG-PCL) with a GSH covalently attached to its PEG end. This nano system had a PEG hydrophobic core that can be used to encapsulate hydrophobic SPIONs, leading to the formation of a GSH-containing and magnetic field-responsive micelles.

Results: The dynamic light scattering (DLS) results indicate that the average sizes of micelles were around 120 nm for the concentration of 0.25 mg/mL (mg/mL as determined by the Fe concentration) and the micelles were stable over a period of 96 hours, when continuously stirred. ICP-OES measurements show that for a 0.25 mg/mL GPP-SPION micelle solution, the sequestration of cisplatin with an initial concentration of 1.04 mM was ca. 64% after 96 hours of reaction. The MTT cell proliferation assay results demonstrate that the GPP-SPION micelles had no significant negative effect on the cell proliferation. In addition, the cochlear organotypic culture studies show that the cochlear hair cell morphology was neither altered nor damaged in the presence of the proposed micelles, after 5 days.

Conclusions: This biocompatible nano-based SPION glutathione has great potential to sequester and magnetically extract cisplatin in the inner ear to alleviate cisplatin-induced ototoxicity. The current study lays an important foundation on establishing a novel inner ear nano-dialysis system for ototoxicity.

iTRAQ-Based Quantitative Proteomic Analysis of Ex-Vivo Model of Cochlear Implant Trauma

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Background: Cochlear implantation (CI) is widely used to provide auditory rehabilitation to individuals having severe to profound hearing loss. However, electrode insertion during CI leads to inner ear trauma resulting in activation of host inflammatory and apoptotic molecular pathways and consequently loss of residual hearing. There is very limited information regarding the target proteins involved in electrode insertion trauma (EIT) following CI and damage to sensory cells especially differential expression in the apical, middle, and basal turns of the cochlea. The aim of our study was to identify target proteins and host molecular pathways involved in cochlear damage following electrode insertion trauma utilizing the iTRAQ™ (isobaric tags for relative and absolute quantification) technique.

Methods: The organ of Corti (OC) explants were dissected from postnatal day 3 rats and placed in serum-free media. Explants were divided into control and experimental groups: 1) untreated control and 2) EIT group. In the EIT group, a custom designed electrode (0.28-mm diameter) was introduced through the small cochleostomy located next to the round window area, allowing for an insertion angle between 110° and 150°. After induction of EIT, explants were further dissected into apical, middle and basal turns and cultured in serum free media followed by washing and lysing. The proteins were extracted, purified, and prepared. This was followed by labelling and running through high-performance liquid chromatography/mass spectrometry (HPLC/MS) to identify target proteins involved in cochlear damage. The expression of target proteins was confirmed by confocal microscopy.

Results: We identified distinct molecular pathways involved in EIT induced cochlear damage compared to the control group. Novel networks were discovered in the basal, middle and apical, turns that were not previously identified in the literature. Confocal microscopy confirmed the expression of these identified proteins in OC explants subjected to EIT. By separating the basal, middle and apical turns of the cochlea within our control and

EIT groups, we deciphered a topographic array of host molecular pathways that extend from the base to the apex of the cochlea which are activated post-trauma following CI.

Conclusions: The identification of host pathways implicated in EIT is a novel approach to identify the effectors of cochlear damage following CI. The results of the present study have significant clinical implications. The identification of target proteins involved in cochlear damage will provide novel therapeutic targets for the development of effective treatment modalities for the preservation of residual hearing in implanted individuals.

Pericytes Mediate Vascular Stability and Neuronal Viability via VEGF–VEGFR2 and Dngf- Signaling

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Background: The inner ear contains a rich population of pericytes, multi-functional mural cells that wrap around small blood vessels. However, the role of pericytes in hearing health is largely unknown.

Methods: In this study, we use an inducible and conditional pericyte depletion mouse model (Pdgfrb-CreERT2/iDTR) as well as an in vitro SGN tissue explant model, to demonstrate that pericytes in the adult ear are critical for the stability of mature vessel beds and essential for supporting the health of spiral ganglion neurons (SGNs).

Results: Our in vivo data showed that depletion of pericytes causes vascular regression, loss of SGNs, and decreased hearing sensitivity, indicating that pericytes have a significant effect on blood vessels and auditory peripheral neurons. Pharmacological depletion of pericytes markedly decreasing vascular sprouting and SGN nerve fiber growth in vitro. In contrast, adding pericytes derived from young mouse cochleae to the SGN tissue explants promotes both neuronal dendritic fiber- and vascular branch- growth. Moreover, we found that pericyte-controlled neural growth is strongly associated with pericyte-released growth factors, including vascular endothelial growth factor-A (VEGF-A), and exosomes. Blockage of either the VEGF-A signal with a selective VEGF-A blocker, SU 5408, or the NGF signal with a selective NGF blocker, GNF 5837, dramatically arrests SGN fiber growth.

Conclusions: This study provides the first clear-cut experimental evidence of the critical role pericytes play in vascular and neuronal health in the adult inner ear. Without a normal population of pericytes, hearing is gradually lost.

Blast Induced Tympanic Membrane Perforation Mitigates Peripheral Cochlear Synaptopathy but Does Not Affect Central Auditory Synapses

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Background: The tympanic membrane (TM) is a vulnerable tissue when exposed to blast overpressure. TM perforation (TMP) has been considered as a potential indicator of the severity of blast-induced neurological damages. On the other hand, TMP, resulting in a decrease in harmful blast wave transmission to the cochlea and brain, might exert protective roles against blast-induced auditory dysfunction (BAD). Thus, little is known about the relationship between TMP caused by blast overpressure and BAD. In this study, we aimed to evaluate the auditory pathophysiology after blast exposure in animal models with or without TMP.

Methods: CBA/J mice at 7 weeks of age were exposed to blast overpressure using a blast-tube and allowed to survive for 2 months. The auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) were measured to evaluate cochlear function before and after blast exposure. Cochleae were examined to measure the survival of hair cells (HCs), spiral ganglion neurons (SGNs), and synaptic and neural properties. Transverse sections of the brainstem through the cochlear nucleus (CN) were studied for the expression of synaptic markers, including excitatory VGLUT-1 and inhibitory GAD65.

Results: TMP was observed in 61.9% of ears after blast exposure. The average period until TMP closure was 2.2 weeks, and all TMP ears exhibited complete closure. The thickness of healed TM in TMP(+) ears was significantly greater than in control ears. At 2 months after blast exposure, the ABR and DPOAE thresholds were significantly elevated in exposed ears. However, although there was no significant difference in ABR thresholds between TMP(−) and TMP(+) ears, the DPOAE thresholds of the TMP(+) ears showed significantly higher than that in the TMP(−) ears, indicating TMP(+) ears had some conductive pathology. The survival of HCs, SGNs and

myelination profile were not changed in exposed mice compared to unexposed mice. Disruption of the stereociliary bundle was observed in blast-exposed ears. However, there was no significant difference between TMP(−) and TMP(+) mice in terms of stereocilia disruption. In contrast, the number of peripheral cochlear synapses was significantly lower in TMP(−) ears than in TMP(+) ears. In the CN, although the excitatory synaptic expression of VGLUT-1 was significantly decreased in blast-exposed ears, TMP(−) and TMP(+) ears exhibited comparable levels of VGLUT-1 and GAD65 expression.

Conclusions: Mice exposed blast wave showed auditory dysfunction, regardless of the presence or absence of TMP. Although no significant difference between TMP(+) and TMP(−) ears was observed in ABR hearing threshold, TMP(−) ears exhibited significantly evident cochlear synaptopathy compared to TMP(+) ears, despite the comparable survival of HCs and SGNs. However, central synaptic changes in CN were not affected by the presence of TMP. Therefore, our results suggested TMP would have protective roles against peripheral cochlear synaptopathy.

High-K+ as Noise Trauma Causes Cochlear Synapse Degeneration and Attenuated by Bk Channel Blockers

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Background: As the first synapse in the auditory afferent system, ribbon synapses between inner hair cells (IHCs) and auditory nerves in the inner ear convey auditory information to the brain and have a critical role in hearing. Ribbon synapse can be degenerated by noise leading to hidden hearing loss (HHL) and difficulty in hearing speech in noisy environment, which is also associated with age-related hearing loss. However, the underlying mechanism for ribbon synapse degeneration remains unclear.

Methods: Mouse (CBA/CaJ) cochlea was incubated with high-K+ (5-50 mM) extracellular solutions for 2 hr with or without K channel blocker TEA (20 mM) or BK channel blocker Iberiotoxin (Cat. #401002, Millipore). For noise exposure, adult CBA/CaJ mice were exposed to 95-98 dB SPL white noise for 2 hr, one time. Ribbon synapses were examined by immunofluorescent staining for CtBP2. The ribbon synapses under IHCs and outer hair cells (OHCs) were quantified under confocal microscopy.

Results: As a physiological consequence of noise exposure, elevation of extracellular K+ could cause IHC ribbon degeneration. Blockage of K+ and Ca2+ channels could attenuate this ribbon degeneration. In particular, blockers of Ca2+-dependent K+ (BK) channels could attenuate ribbon synapse degeneration. However, glutamate, which is a neurotransmitter of ribbon synapses, and glutamate receptor (GluR) agonists could cause ribbon swelling but not degeneration. Also, GluR antagonist had no effect on the ribbon degeneration. Finally, the K+-induced ribbon degeneration was similar to the ribbon degeneration in noise-induced HHL and was dose-dependent.

Conclusions: These data reveal a new mechanism that K+ can cause IHC ribbon synapse degeneration in the cochlea. This study also demonstrates that BK channels may be a potential target for prevention and treatment of noise-induced hearing loss and other hearing disorders.

Protective Effect of Agmatine Against Cisplatin-Induced Cellular Apoptosis in an Auditory Cell Line

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Background: The aims of this study were to evaluate the protective effects of agmatine against cisplatin-induced cellular apoptosis in an auditory cell line, and to prove the protective mechanism of agmatine.

Methods: HEI-OC1 cells were co-treated with agmatine at different concentrations and 15 µM cisplatin for 48 h. Cell viability and proliferation were measured. Annexin V-fluorescein isothiocyanate /propidium iodide staining was performed to analyze apoptosis. The levels of intracellular reactive oxygen species (ROS) were measured using flow cytometry. The expression of BCL2-associated X protein (BAX) and the enzymatic activity of caspase-3 were measured to examine the pathway of apoptosis induction.

Results: In normal conditions, the maximal protective effect occurred with 10 mM agmatine. However, in the presence of cisplatin, the maximal protective effect was observed from 8 mM agmatine. Thus, 8 mM was chosen as the ideal agmatine concentration for analysis of the protective effects against cisplatin-induced cytotoxicity. Agmatine exerted a significant protective effect against 15 µM cisplatin when applied for 48 h and reduced the proportion of necrotic and late apoptotic cells. Agmatine did not significantly reduce the cisplatin-induced increase in ROS but decreased the expression of BAX and the activity of caspase-3.

Conclusions: Agmatine protected against cisplatin-induced cellular apoptosis in an auditory cell line. These effects were mediated by the protection of mitochondrial function and inhibition of apoptosis.

A Single Cisterna Magna Injection of Aav Leads to Binaural Transduction in Mice

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Background: Viral-mediated gene augmentation, silencing, or editing offers tremendous promise for the treatment of inherited and acquired deafness. Inner-ear gene therapies require a safe, clinically useable, and effective route of administration to target both ears while avoiding damage to the delicate structures of the inner ear.

Methods: We examined the possibility of using a single cisterna magna injection as a new cochlear local route for initiating binaural cochlear transduction by different serotypes of the adeno-associated virus (AAV2/8, AAV2/9, AAV2/Anc80L65). The results were compared with those following posterior semicircular canal injection, one of the existing standard inner ear local delivery routes.

Results: Our results demonstrated that a single cisterna magna injection of AAVs enables high-efficiency binaural transduction of inner hair cells (IHC) with a strong basal-apical pattern and of large numbers of spiral ganglion neurons of the basal portion of the cochlea, without affecting auditory function and cochlear structures

Conclusions: Our results demonstrate for the first time the successful use of cisterna magna injection as a novel delivery route for binaural therapeutic gene transfer in mice. Therapeutic AAV injection into the cisterna magna for neurodegenerative diseases is already reported in patients. To use this route for inner ear diseases in humans, molecular strategies have to be developed to avoid off-target side effects. In addition, extensive testing will be required before translation beyond mouse models.

Rescue of Balance Function in a Mouse Model of Usher 1B

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Background: Usher disease is one of the most devastating inherited forms of syndromic hearing loss. In its severest form, it affects hearing, balance, and vision leading to significant disability. Usher 1B is a recessively inherited disorder that presents with congenital hearing loss and later presents with loss of vestibular function presenting the possibility that these patients could have a gene therapy intervention targeting their vestibular dysfunction.

Methods: The shaker 1 mouse carries a point mutation in MYO7A, and homozygous mice present with circling behavior between P14 and P21. Evaluation using actimeter testing and rotarod demonstrates a progressive loss of function during the next 6 weeks. Homozygous mutant mice were treated with a third-generation lentiviral vector expression native MYO7A via an injection into the posterior semicircular canal at P4 or P16-17. Mice were followed with serial actimeter and rotarod measurements over 60 days post-delivery.

Results: Delivery of MYO7A in one ear resulted in maintenance of balance function for the duration of the study period. The treatment time (P4 or P16-17) did not affect the degree of rescue

Conclusions: The balance system in Usher 1B may be viable treatment target for treatment with gene therapy.

Assessment of AAV-Mediated Innate Immunity in the Mammalian Inner Ear

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Background: Adeno-associated virus (AAV) is a safe and effective viral vector that has been widely used in gene therapy studies. However, it has been shown that the host immune response to AAV may affect its potency, efficacy, and persistence. Following the recent FDA approval for AAV-based therapies for Leber congenital amaurosis and spinal muscular atrophy, as well as the increasing number of proof-of-concept studies showing that gene therapy is effective at improving the auditory function in various mouse models of hearing loss, inner ear gene therapy is now closer to clinical application than ever before. Therefore, it is critical to evaluate the immune response triggered by AAV in the mammalian inner ear. In this study, we examine the innate immune response triggered by AAV-mediated gene delivery in the mouse inner ear.

Methods: The B6.129P2(Cg)-Cx3cr1tm1Litt/J (termed CX3CR1GFP) mouse model is used in this study. CX3XR1 is a fractalkine receptor expressed in immune cells such as monocytes, dendritic cells and NK cells. In cochlea, CX3CR1GFP/GFP mouse expresses eGFP signals in resident macrophages by which we could follow its migration and trafficking. We injected AAV2.7m8-CAG-TdTomato or the viral suspension buffer (5% glycerol in PBS) into the inner ears of CX3CR1GFP/GFP mice using the posterior semicircular canal approach between 8 to 12-weeks old. In 4 weeks, the activation of resident macrophages was assessed with immunofluorescences by counting the number of GFP-expressing macrophages in cross-sections of cochlea.

Results: Inner ear delivery of AAV2.7m8-CAG-TdTomato caused an increase in the number of macrophages in the cochlea. In addition, morphological changes of these macrophage were observed. The number of macrophages peaked by 14 days after the inner ear injections. Injection of the viral suspension buffer into the inner ear also increased the number of macrophages, which also peaked by 14 days after the procedure. The magnitude of the increase in macrophage activation was similar between animals that were injected with AAV2.7m8-Cmv-TdTomato and vehicle.

Conclusions: Both AAV2.7m8-CAG-TdTomato and viral suspension buffer injections into the mouse cochlea caused similar increases in the number of macrophages in the cochlea. This suggests that the surgical procedure may be the main contributor to macrophage activation within the mouse cochlea following AAV-mediated inner ear gene delivery.

The Otoprotective Efficacy of Transtympanic Probucol Gel, in Guinea Pigs
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Background: There are over 600 categories of drugs that have the potential to cause ototoxicity. Ototoxicity is considered an otologic urgency because there is less recovery of functional damage when a treatment plan is not implemented promptly. Despite the increasing number of studies investigating new otoprotective drugs, to date, no single treatment has been shown to be highly effective, nor been translated into clinical practice. Probucol (Pb) is a lipid-lowering agent with potent antioxidative and anti-inflammatory effects. To date, Pb has not yet been applied to the inner ear or trialed as an otoprotective drug.

Our aim was to perform preliminary studies testing the oto-protective efficacy of Pb, in both the in vitro and in vivo environment, with the hypothesis that Pb can provide significant oto-protection in the case of Kanamycin and Furosemide induced ototoxicity in guinea pigs.

Methods: A major limitation of Pb is its very poor water solubility. To improve Pb solubility, biocompatibility, permeation through the round window membrane, and distribution throughout the inner ear, here, Pb was solubilized and stabilized by forming a nanoparticle complex with the oligosaccharide 2-hydroxypropyl- β-cyclodextrin, which is commonly used in pharmaceuticals to increase drug availability. To further enhance Pb delivery into the inner ear, 2% deoxycholic acid was added to the nanoparticles, given its ability to act as a permeation enhancer. The Pb nanoparticles were mixed in a water-soluble gel, to aid delivery. Our Pb nanocapsules, in different formulations, were applied to a mouse cochlear hair cell line (HEI-OC1) exposed to various concentrations of cisplatin. Following this, our optimal Pb formulation was applied to the round window membrane of guinea pigs, which were then treated with 200mg/kg Kanamycin (S.C.), and 50mg/kg Furosemide (I.V.), and thereafter allowed to recover for 2 weeks.

Results: Our in vitro studies, using Pb to protect HEI-OC1 cells from cisplatin toxicity, demonstrated a significant protective effect at a Pb concentration of 20 to 50uM. Our preliminary in vivo results demonstrated Pb provided significant otoprotection, with round window compound action potential thresholds being 10-20dB better in Pb treated ears, re. control ears. Our post-mortem 3D morphological analysis of the cochleae demonstrated that spiral ganglion cell numbers in the basal turn were significantly higher in Pb treated ears.
Conclusions: Our preliminary evidence suggests Pb can act as a potent otoprotective drug, if delivered to the round window membrane prior to ototoxic insults.

In Vitro Characterization of Gene Silencing Methods With the Potential to Treat Autosomal Dominant Hearing Loss

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Background: Autosomal dominant hearing loss (ADHL) is estimated to represent 30% of all genetic hearing loss and is caused by mutation in one copy of the gene. Current interventions, including hearing aids and cochlear implants, do not address the underlying genetic cause of hearing loss. The development of adeno-associated viral (AAV) vectors as an intracochlear drug delivery platform poses a unique opportunity to treat genetic forms of hearing loss by developing precision medicines that are selectively designed to target the affected gene. Because most individuals affected by ADHL harbor toxic gain-of-function and/or dominant negative mutations, one therapeutic strategy is to reduce the expression of the mutant allele and maintain functional levels of the healthy allele. However, to address target genes with no mutation hotspot, an alternative strategy is to reduce expression levels of both alleles and simultaneously deliver a modified healthy copy which is resistant to gene silencing. Several molecular tools enable allele reduction, including RNAi-mediated gene silencing and CRISPR/Cas9-mediated gene editing. Here we present efforts to screen potential candidates to treat ADHL using AAV vector delivering RNAi gene silencing or CRISPR/Cas9 gene editing techniques, with simultaneous re-introduction of the healthy allele.
Methods: We first explored RNAi techniques, including engineered microRNAs, for their gene silencing efficacy. We designed over 50 engineered microRNA constructs and screened them for their ability to reduce endogenous target gene levels in vitro. We assessed gene silencing using fluorescence microscopy, luciferase-based reporter assays, RT-qPCR, and protein immunoblotting analyses. We also employed NGS analysis of small RNA variants to assess the efficacy of microRNA processing. We also explored gene editing using CRISPR/Cas9-based methodologies. We designed and tested six different CRISPR/Cas9 constructs and screened them for their ability to edit target gene levels in vitro. We measured gene editing in DNA level using tracking indel decomposition (TIDE), and in protein level using fluorescence microscopy and protein immunoblotting analyses.
Results: Luciferase reporter plasmid screening showed that most microRNA constructs effectively reduced target RNA levels but had varying levels of off-target and passenger strand effects. All CRISPR/Cas9 constructs exhibited efficient knockout at the protein level and varying degrees of indel creation using TIDE analysis. To ensure we can simultaneously deliver the healthy-modified copy of the target gene, our data also demonstrated a much greater reduction effect in wild type sequence levels of the target gene, compared to the codon-modified version.
Conclusions: Our results, both with RNAi gene silencing and with CRISPR/Cas9 gene editing, build a foundation which may be used to develop potential future AAV vector-mediated therapies for many types of ADHL and other inner ear conditions.

Low Intensity Ultrasound Enhances Drug Uptake in Vitro by Cochlear Hair Cells

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Background: Ultrasound has now been widely used in medical applications. In contrast to high intensity ultrasound, low intensity ultrasound has little thermal effect and tissue destruction. In this study, we developed an in vitro chamber for ultrasound modulation of cochlear explants, and explored the possibility to enhance drug delivery efficacy in the inner ear using low intensity ultrasound.
Methods: Cochlear explants harvested from postnatal C57BL/6 pups were cultured in a medium containing fluorescence-labeled cisplatin (Texas Red-Cisplatin, ~1000 Da) to emulate drug delivered to the endolymph. We

designed an in vitro stimulation chamber with a focused transducer including an aluminum spherical lens with 12 mm focal distance. A disc-type 1 MHz transducers was attached to the focus lens. With an input voltage of 900mVpp and 1% duty factor (with a repetition rate 1kHz), the measured ultrasound intensity at the cochlear explant location was around 10 mW/cm2. The cochlear explant were sonicated and be fixed to be processed for further observation.

Results: We first tested the cochlear explants with different ultrasound intensities to establish a safety range. The duty factors were tested up to 20% (ISPTA = 200 mW/cm2) for 3 min without damaging the hair cells. With a safety factor of 4 in mind, all our experiments were carried out at an intensity of 50 mW/cm2 (ISPTA) or less. Our results demonstrated the exposure to ultrasound stimulation enhanced effectively cisplatin intake by hair cells. The intake started from the apical side of the hair cells and progressed inward as the exposure time increased.

Conclusions: Ultrasound at a low intensity (50 mW/cm2 or less) can influence cochlear hair cells without causing any damage. Using cisplatin as a model drug, we further demonstrate direct modulation of hair cells by low intensity ultrasound can enhance drug intake by hair cells. These findings suggest potential applications of low intensity ultrasound to the therapeutics of hearing loss.

Magnetically-Assisted Delivery of Custom-Synthesized Nanoparticles Across Guinea Pig Round Window Membrane: A Benchtop Model

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Background: Safe and effective delivery of therapeutics into the inner ear pose a significant clinical challenge in the treatment of labyrinthine disorders such as hearing loss. Magnetic nanoparticles (MNPs) are a novel and promising delivery platform that may allow magnetically-assisted transport of nanoparticles with therapeutic payloads into the cochlea after intratympanic injection. However, the relationship between nanoparticle physico-chemical properties and consequent transport kinetics has not been studied. We investigate the transport efficiencies of custom-synthesized, precisely-tuned MNPs across differential membrane structures using a microfluidic chamber and an external magnetic field gradient.

Methods: Mono-core MNPs suitable for drug delivery were synthesized with approximately 7nm Fe3O4 core size and coated with polyethylene glycol (PEG) (Relative Molecular weight (Mr) 3000)) . Physical and chemical characterization were performed using Transmission Electron Microscopy and Zetasizer. A two chamber microfluidic device designed to accommodate a range of tissue inserts was manufactured using 3-D printing. Using this benchtop model, the time-dependent transport of MNPs across Porcine Sub-mucosal Small Intestine (SIS) membrane and freshly explanted guinea pig RWM were quantified at 1, 2, and 4 hours. Magnetic field gradient was applied using a 1.48 Tesla Neodymium (N52 grade) permanent magnet. MNP concentration was determined by quantifying iron content using Inductively Coupled Plasma- Mass Spectrometry (ICP-MS) and spectrophotometrically using Ferene-s assay.

Results: Mono-core, PEG-coated MNPs custom-synthesized for drug delivery applications demonstrated precise size tuning with core size of 7nm, hydrodynamic size of 145± 15 nm, and polydispersity index of 0.25± 0.1. Avg. MNP dose delivered across SIS membrane was 0.21%, 1.49%, and 2.67 % at 1, 2, and 4 hrs., respectively, without magnetic field gradient; and 7.40%, 13.82%, 16.19% at 1, 2, and 4 hrs., respectively, when magnetic field gradient was applied. Across guinea pig RWM, avg. MNP dose delivered was 1.74%, 17.60%, and 19.43% at 1, 2, and 4 hrs., respectively, without magnetic field gradient; and 2.60%, 24.58%, 27.34% at 1, 2, and 4 hrs., respectively, when magnetic field gradient was applied. Magnetically-assisted transport resulted in 6 and 1.4-fold increase in MNP delivery across SIS and guinea pig RW membranes, respectively.

Conclusions: Magnetically-assisted delivery of MNPs represent a promising approach to overcome diffusion barriers and enhance local penetration of high-molecular weight therapeutics into the inner ear for treatment of conditions such as sensorineural hearing loss. Precisely-tuned MNPs demonstrate differential transport efficiencies across SIS and RW membranes that are enhanced by application of a magnetic field gradient. The benchtop model developed here will be relevant for elucidating the relationship between nanoparticle physical-chemical properties and transport across ear tissues to enable the rational design of MNPs for inner ear drug delivery applications.

Towards Molecular Treatment Strategies: A Human Cochlear Atlas on Protein Expression

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Background: The inner ear is a highly and complex organized organ. Dysfunction of the different cells within the cochlea leads to loss of function resulting in sensorineural hearing loss. Despite rapid progress, the underlying cellular and molecular mechanisms of hearing loss are still poorly understood. Sampling and molecular analysis of perilymph may not only improve the understanding of the pathological processes within the inner ear but may also enable novel molecular treatment strategies.

Methods: To this aim, an indepth shot-gun proteomics approach was performed by mass spectrometry coupled with liquid chromatography (LC-MS) to identify cochlear proteins in perilymph samples from patients undergoing cochlear implantation. For initial cluster analysis, label-free quantification (LFQ) data for each identified protein were uploaded into the Qlucore Omics Explorer and a principal component analysis (PCA) was carried out. Variance filtering using the standard deviation was done to identify a subset of proteins that maximized the PCA projection allowing assessment of relatedness of the individual samples. These data were then converted to a heat map. Individual groups of patient data could then be selected to identify the protein data that characterizes the particular selected group.

Results: A multitude of proteins and patterns of protein expression that allow the characterization of patients into subgroups were revealed with the herein presented approach. Expression of single proteins were related to individual cochlear cell types like inner and outer hair cells, supporting cells, spiral ganglion cells, and the stria vascularis allowing the generation of a detailed cochlear atlas of protein expression. In addition, druggable targets within the perilymph proteome were identified by a bioinformatic data analysis approach.

Conclusions: A human cochlear atlas of protein expression could be provided based on perilymph analysis of cochlear implant recipients. Such an approach allows an improved understanding of the molecular pathophysiology in the cochlea and the modulation of the human perilymph proteome that could open novel diagnostic and treatment avenues for targeting inner ear diseases at a molecular level.

A Spiking Neuron Model of the Inferior Colliculus: Deriving Synaptic Inputs From Spike Trains

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Background: Understanding how neurons encode diverse acoustic information is a complex, yet important, topic of interest in auditory neuroscience. A variety of computational models have shed light on this process as they can predict neuronal responses, both at the single-cell and population level. Such models range from Hodgkin and Huxley (HH) style models with numerous biological parameters to phenomenological integrate-and-fire (IF) models consisting of combinations of linear filters that simulate excitatory and inhibitory synapses. However, processing of sensory information within a cell depends on the nonlinear interplay between inhibitory and excitatory inputs. This 'push-pull' balance between opposing synaptic inputs strongly shapes observed neuronal responses. Spiking Neuron (SN) models bridge the gap between HH style and IF models and overcome each model's respective pitfalls as they have a limited number of parameters and use a nonlinear combination of inhibitory and excitatory input conductances. Further, SN models allow for more realistic simulation of cells having a large number of synaptic inputs.

Methods: We propose a SN model of the inferior colliculus (IC) with an intermediate cochlear nucleus (CN) stage that receives model auditory-nerve (AN) inputs. The SN model simulates statistical properties of neuronal responses while maintaining simulation of biophysical processes such as conductances. The general framework of the SN model includes a conductance kernel that filters input spike trains. Then, the resulting post-synaptic conductance traces are used to derive the time course of the cell's membrane potential. Lastly, the membrane potential is mapped to instantaneous spike rate via a nonlinear function, followed by a spike generator. Parameter-fitting strategies were refined using responses from prior CN and IC models. SN model responses were then fit to physiological responses recorded in the rabbit midbrain using the interior-point method to minimize the summed

squared error between model and neural rate functions. This nonlinear optimization procedure tuned conductance kernel shapes and provided estimates of excitatory and inhibitory conductance traces.

Results: Preliminary results suggest that for some IC neurons, the SN model can be used to derive underlying components of neural responses (i.e., the underlying inhibitory and excitatory conductance traces) from IC spike trains in response to contralateral wideband gaussian noise. The SN model for a given neuron will be tested by predicting IC responses to noise samples not used in model fitting. Additional tests will include predictions of other responses, such as modulation transfer functions and frequency response maps.

Conclusions: The SN model of the IC provides a more realistic mechanism for combining multiple synaptic inputs than previous IF models. Further, a reduced number of parameters allows for simpler fitting procedures than used for HH models. Overall, the combination of these two features provides a model of IC responses to contralateral stimuli that can predict a range of midbrain responses.

Characterizing Spontaneous and Sound Evoked Activity of Neurons in the Central Nucleus and Cortex of the Inferior Colliculus

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Background: Distinguishing neurons of the central nucleus of the inferior colliculus (CNIC) from neurons in the cortex of the IC (IC cortex) based on their response properties alone remains challenging. While small differences exist, there is substantial overlap between the distributions of response properties of neurons from these two populations. Creating a classifier that can reliably identify neurons as CNIC vs IC cortex could provide real-time feedback during in-vivo recordings and could potentially provide insight into which stimulus features are processed by different IC subdivisions. The present study aims to characterize the response properties of CNIC vs IC cortex neurons and test whether these populations can be separated using three different models.

Methods: Extracellular recordings were made under isoflurane anesthesia in 5 CBA/CaJ mice (n=7 IC) using a 64-channel electrode array. Single units were sorted using JRClust. We obtained responses to a stimulus battery that included pure tones (4-32 kHz, 25-85 dB SPL), two-tones, amplitude-modulated tones and dynamic random chords. We used a logistic regression model (LR), linear support vector machine (SVM), and a random forest (RF) classifier on pure-tone responses to determine if CNIC and IC cortex responses could be reliably separated based on simple response properties. The models were trained on a subset of recorded data, with anatomical probe location ground-truth determined post-hoc using DiI and cytochrome oxidase labeling.

Results: We observed several small differences in basic response properties between CNIC and IC cortex neurons. These included spontaneous firing rate (CNIC: 0.02 spk/s, IC ctx: 0.08 spk/s, p=0.006), spontaneous firing rate variability (CNIC: 0.49 spks/s, IC ctx: 1.03 spks/s, p=0.001), threshold (CNIC: 55dB SPL, IC ctx: 40dB SPL, p<0.0001), onset latency (CNIC: 8ms, IC ctx: 7ms, p=0.001), firing rate gain with sound intensity (CNIC: 1.3 spks/s/dB, IC ctx: 0.26 spks/s/dB, p=0.13), bandwidth increase with sound intensity (CNIC: 0.01 oct/dB, IC ctx: 0.05 oct/dB, p<0.0001), and change in best frequency with sound intensity (CNIC: 0 oct, IC ctx: 0.2 oct, p<0.0001). Using these variables as classifier features, all three models were capable of identifying CNIC vs IC cortex neurons with high sensitivity and specificity, with the random forest classifier resulting in the best performance (AUC LR= 0.80, SVM=0.81, RF=0.90).

Conclusions: Small but significant differences exist in the response properties of neurons located in the CNIC vs the cortex of the IC under anesthesia. While individually these differences provide some insight into the location of the neuron, combined they allow for robust, reliable classification. Ongoing analysis will further analyze spectro-temporal receptive fields, gap detection thresholds, temporal and rate modulation transfer functions, and extent and magnitude of lateral inhibition in these two populations.

NPY Neurons Provide Local and Ascending Inhibition in the Auditory Tectothalamic Pathway

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Background: Inhibitory synapses are essential to shaping auditory computations, impacting most aspects of auditory processing. The inferior colliculus (IC) contains many GABAergic neurons that project locally and/or to the auditory thalamus (MG). However, despite the essential role of inhibition in the IC-MG pathway, the cellular organization of local and long-range inhibitory neural circuits remains largely unknown. The ability to selectively manipulate distinct classes of IC GABAergic neurons is key to understanding how inhibition shapes sound

processing in the IC and MG. We recently identified Neuropeptide Y (NPY) expression as a marker for the first molecularly identifiable class of inhibitory neurons in the IC. NPY neurons are labeled in the NPY-hrGFP reporter mouse and send long-range inhibitory projections to the MG. Because most techniques for manipulating distinct neuron types target neurons that express Cre or FlpO transgenes, here we validate an NPY-FlpO mouse line and show that with this mouse we can use viral transfections to investigate how the local and long-range projections of NPY neurons shape inhibition in the auditory tectothalamic pathway.

Methods: To selectively target NPY neurons, we crossed NPY-IRES2-FlpO mice with Ai65F reporter mice, allowing us to visualize NPY neurons by the expression of tdTomato fluorescence. To investigate local and long-range NPY projections, NPY-FlpO x Ai65F mice were injected in the right IC with a FlpO-dependent adeno-associated virus to selectively express the excitatory opsin Chronos in NPY neurons.

Results: Using GAD67 immunolabeling, we found that 92.3% of tdTomato+ neurons are GABAergic. Additionally, tdTomato-expressing neurons exhibited sustained firing patterns and stellate morphology, consistent with the properties of NPY neurons labeled in the NPY-hrGFP mouse. To test if activation of NPY terminals using Chronos would evoke inhibitory postsynaptic potentials (IPSPs) in postsynaptic targets, we recorded from IC and MG neurons in acute slices, using flashes of blue light to activate Chronos-expressing terminals. Our data show that optogenetic activation of NPY terminals elicited IPSPs in postsynaptic neurons in the ipsilateral IC and MG.

Conclusions: The NPY-FlpO x Ai65F mouse line labels the same population of NPY neurons previously validated in the NPY-hrGFP mouse. Using NPY-FlpO mice we can selectively target NPY neurons for expression of viral payloads and evaluate their synaptic physiology and postsynaptic targets using optogenetics. We found that activation of NPY terminals leads to IPSPs in postsynaptic targets in the ipsilateral IC and MG. Our data provides the first functional evidence of how a distinct class of GABAergic IC neuron provides local and long-range inhibition in the central auditory pathway.

Anterograde and Retrograde Tracing of Cholinergic Input to the Inferior Colliculus in Mouse

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Background: Physiology and receptor binding studies indicate that the inferior colliculus (IC) is densely innervated by cholinergic axons across mammalian species. Transgenic mice provide opportunities for selective labeling of cholinergic circuits, but so far there has not been a systematic identification of cholinergic inputs to the IC in mice. Here, we use traditional retrograde tracers and chemically-selective viral anterograde tracers to characterize cholinergic inputs to the IC in normal-hearing adult mice.

Methods: We used adult mice of either sex with normal hearing (ChATCre,Cdh23WT; Beebe et al. 2020; doi.org/10.1016/j.heares.2020.107896). We injected fluorescent retrograde tracers into the IC then identified retrogradely-labeled cells that immunolabeled for choline acetyltransferase (ChAT) or vesicular acetylcholine transferase (VAChT), markers of cholinergic cells. In a second series of experiments, we injected adeno-associated virus containing a Cre-dependent fluorescent protein gene into specific brainstem cholinergic nuclei. After 4 weeks to allow for gene expression, brains were fixed by perfusion and sectioned for fluorescence microscopy.

Results: Following retrograde tracer injections, we observed retrogradely labeled cells in the pedunculopontine and laterodorsal tegmental nuclei (PPT and LDT). Each of these regions contain cholinergic and non-cholinergic (glutamatergic and GABAergic) cells. Both ChAT+ and ChAT-negative cells were retrogradely labeled bilaterally in PPT and LDT, with more cells ipsilateral and, on each side, more cholinergic cells in PPT than in LDT. We also noted a small number of retrogradely labeled cells in the lateral paragigantocellular nucleus (LPGi), a nucleus in the reticular formation of the rostral ventrolateral medulla. Both ChAT+ and ChAT-negative cells were tracer labeled in the LPGi. The superior olivary complex (SOC) contained many retrograde cells, all of which were ChAT-negative.

Anterograde labeling of cholinergic cells with AAV confirmed projections to the IC from each area. Injections into the PPT/LDT labeled axons with en passant and terminal boutons bilaterally across all subdivisions of the IC. Injections into the LPGi labeled axons throughout the ipsilateral IC and in central and rostral parts of the contralateral IC. Injections confined to the SOC failed to label any axons in the IC.

Conclusions: The IC in mice receives substantial cholinergic innervation from the PPT and LDT, and a smaller projection from the LPGi a small nucleus in the reticular formation of the rostral ventrolateral medulla. Cholinergic cells in the SOC do not appear to project to the IC. Projections from different cholinergic sources may

serve different functions. Cholinergic cells in the PPT and LDT have been associated with a wide range of functions, including arousal, reward, sleep-wake cycle, sensory gating and cortically-driven plasticity. Insight into the functions of LPGi projections will require more information on LPGi functions in general. Thus, it appears likely that cholinergic projections to the IC support a wide diversity of functions.

Delivery of Antibiotics Through an Intact Tympanic Membrane via Peptide-Mediated Active Transport

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Background: Otitis media (OM) is a bacterial infection of the middle ear (ME) that frequently occurs in young children. While systemic antibiotics are not recommended for the treatment of uncomplicated acute OM in children over two years of age, they are useful in the treatment of younger children and for complicated forms of the disease. Local delivery has been shown to be efficacious in treating complicated OM, but it requires surgically breaching the tympanic membrane (TM). Using phage display, we previously discovered a family of peptides that are actively transported across the intact TM, providing a potential mechanism for noninvasive ME delivery. Since these peptides can deliver bacteriophage that are 1 µm in length to the ME, we reasoned that they should also be able to deliver drugs.

Methods: As a first attempt at peptide-mediated drug delivery, single amoxicillin or ciprofloxacin molecules were linked to synthesized trans-TM peptide with high transport characteristics and applied to the rat TM in vivo post infection. In addition, using EDC chemistry, neomycin, amoxicillin or ciprofloxacin were conjugated to M13 bacteriophage displaying one of the same trans-TM peptides. The linked antibiotics were separately applied to the external TM surface of rat MEs infected with nontypeable Haemophilus influenza (NTHi) for 8 hours. The ME contents were then harvested and the bacteria titered.

Results: Linkage of antibiotics to peptides or phage did not affect antibiotic activity. However, when amoxicillin or ciprofloxacin linked to peptides in a one-to-one ratio were applied to the TM, insufficient antibiotic entered the ME to affect bacterial titers. In contrast, neomycin linked to the peptide-expressing bacteriophage in a several hundred-to-one ratio entered the ME via peptide-mediated transport and was effective at killing NTHi. Control MEs, inoculated with NTHi and receiving antibiotic linked to WT phage on the TM, showed no reduction in bacterial titers.

Conclusions: These findings suggest that peptide-mediated active transport across the TM can be used to deliver antibiotics at pharmacologically effective levels, using drug packages. This discovery can be used to develop a noninvasive treatment option for OM.

Tympanic Membrane Shape and Impulse Response Measured With High Speed Holography in Live Chinchilla Ear With Otitis Media With Effusion

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Background: Otitis Media with Effusion (OME) is a common middle ear disease in children, which causes hearing loss and learning disability. Several mechanisms have been proposed for OME associated hearing loss, including viscous damping of middle ear motions, reduction of middle-ear air space, static-middle-ear-pressure induced changes in tympanic membrane (TM) shape and compliance, and increased TM thickness and stiffness due to chronic inflammation. However, the frequency dependence of the hearing loss and the effect of fluid levels remain unclear. Furthermore, most mechanical measurements of middle ear responses with OME are based on single-point laser measurements either at the umbo or the stapes, or middle-ear admittance (a measure of average TM motion) yet changes in umbo displacement or admittance do not fully explain the hearing loss in a clinical population. We developed a High-speed Digital Holographic (HDH) system to measure the shape and impulse response of the entire TM in live chinchillas with OME.

Methods: Six chinchillas were studied. The left ear of each animal was injected with Lipopolysaccharide (LPS) to induce OME; the right ear served as the control ear. Auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE) were measured before the LPS injection, and 5 days post injection just prior to holographic measurements. The middle ear infection was monitored daily via endoscope. After the second DPOAE and ABR measurements, the external ear of the chinchilla was removed to expose the TM, the TM was

painted with a reflective paint, and HDH used to record the shape and the click-induced displacement of the entire TM. Frequency and impulse analyses were used to compare TM mechanics between the OME and the control ears.

Results: The shapes of the TM in OME and normal ears are similar, even though significant amounts of fluid were observed behind the TM in OME ears. Large regions of the OME TM surface showed reduced motion, and the averaged frequency response functions (FRFs) of the TM from OME ears show significantly smaller amplitudes across a broad frequency range between 2 kHz and 10 kHz. The reductions in the mechanical responses are correlated with elevated ABR and DPOAE hearing thresholds measured in OME ears.
The resonance frequencies from the averaged FRF peaks are different between normal and OME ears. The RMS displacement magnitude maps within the first 3 ms of the TM impulse response show different motion patterns between normal and OME ears. The impulse responses measured in OME ears tend to have longer rising time and a shorter decaying time compared to the control ears.

Conclusions: Our high-speed holography measurements show significant changes of TM mechanical parameters and impulse response in the OME ear, consistent with the measured hearing losses in these ears.

Advance in the Use of Gold Nanoclusters in the Treatments for Chronic Suppurative Otitis Media

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Background: Chronic suppurative otitis media (CSOM) affects more than 330 million people worldwide and is the most common cause of permanent hearing loss among children in the developing world. Despite intensive topical fluoroquinolone treatment, Pseudomonas aeruginosa often persists in the middle ear of CSOM patients for decades. Repeated topical antibiotic therapies are likely to lead to the long-term development of bacterial resistance.

Methods: Eliminating persister cells is critical to limiting recurrence and resistance in patients with CSOM

Results: We demonstrate that gold nanoclusters augment the antimicrobial effect of fluoroquinolone to a level where persister cells are killed at antibiotic concentrations that can easily be achieved in vivo. In addition, these nanoclusters help to mitigate the development of antibiotic resistance.

Conclusions: This approach could make it possible to develop new fluoroquinolones formulation for the treatments for CSOM.

Organization of Mongolian Gerbil Social Vocalizations

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Background: Vocal communication is central to the social lives of humans and other animals. Mongolian gerbils — a favorable animal model in auditory neuroscience due to its low frequency hearing — use a wide variety of audible and ultrasonic calls that vary with social context. Prior studies documenting the gerbil vocal repertoire have either been limited to behavioral observation over (a) short time periods of minutes to hours, or (b) dyadic social interactions contrived in the laboratory. Thus, it is uncertain whether the known vocal repertoire is representative of those that occur in a social group during uninterrupted daily life, or whether social vocalizations are organized probabilistically over biologically relevant time cycles (e.g., minutes, hours, circadian, across development).

Methods: We address these questions by monitoring families of gerbils (adult male and female with 4 pups) with audio and video recordings continuously for 20-days. Animals lived in an enclosure outside of the animal facility, on a 12:12 hour light cycle, such that extraneous cues were eliminated.

Results: By restricting our analysis only to putative vocalizations, we found that gerbil vocal activity was organized around a diurnal cycle. There was an early vocal peak beginning around the time of light onset, and a larger peak later that occurred just prior to light offset. This diurnal pattern was similar across the developmental period examined (P13-30) and consistent across three gerbil families, suggesting a conserved cycle of daily vocal activity. To quantitatively assess differences in vocal types, we trained a variational autoencoder on vocalization spectrograms and use latent features from the autoencoder for clustering analysis. The results suggest that different types of vocalizations are deployed at distinct periods of development, time of day, and over seconds-to-minutes time scales. In addition, we performed two behavioral perturbations — removing the pups at P30, then

subsequently removing the adult male. Each caused a significant drop in the number and diversity of vocalizations and the lone female largely ceased to vocalize.

Conclusions: Taken together, these findings reveal spectrotemporal organization of gerbil vocalizations in an uninterrupted social setting, and that manipulating social context changes vocalization frequency and type.

Neurodegenerative Changes in Vestibular Neurons After Unilateral Labyrinthectomy (UL) and Their Protection With Exo- And Endogenous Hormones

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Background: Vestibular compensation after unilateral labyrintectomy (UL) is a convenient model for studying the neurophysiological, neurochemical and cellular mechanisms of plasticity. Recent studies have shown that after UL, changes in the internal membrane excitability, and reorganization of synaptic connections in the vestibular nuclei of both sides are involved in the vestibular compensation We tested the reactions of Deiters' nucleus neurons to bilateral high frequency stimulation of hypothalamic paraventricualar and supraoptic nuclei (PVN and SON) in norm and following unilateral labyrinthectomy (UL). The analysis of spike activity was carried out by mean of on-line selection and special program. The complex averaged peri-event time and frequency histograms shows the increase of inhibitory and excitatory reactions of Deiters' neurons at early stage of vestibular compensation following proline-rich peptide (PRP-1) and cobra venom Naja Naja Oxiana (NOX) injection, reaching the norm at the end of tests. In histochemical study the changes in Ca2+-dependent acidic phosphatase (AP) activity in neurons was discovered. It was shown that in UL animals the total disappearance or delay of decolorizing of Deiters' neurons lead to neurodegenerative pattern as cellular "shade". AP activity after UL and PRP-1 injection exerts more effective recovery of neurons in comparison with events, observed after the administration of NOX. The behavioral observations in "open fieald" indicate that PRP-1 and NOX are protectors, which may successfully recover the disturbed vestibular functions.

Methods: All in Background

Results: All in Background

Conclusions: All in Background

The Rhesus Monkey as an Animal Model to Study Eye Movement-Related Eardrum Oscillations (EMREOs)

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Background: The auditory, visual, and oculomotor systems work together to ensure surrounding stimuli are perceived correctly. We have recently reported an oscillation of the eardrum that is time-locked to the onset or offset of an eye movement in the absence of sounds or visual stimuli. These eye movement-related eardrum oscillations (EMREOs) covary in phase and amplitude with the direction and magnitude of the synchronous eye movement (Gruters, Murphy et al. PNAS 2018). This finding suggests that interactions between auditory, visual, and oculomotor systems may begin as early as the ear itself.

However, much is still unknown about this phenomenon. Open questions include: 1) determining the acting anatomical features of the inner and middle ear and their joint contributions to this eardrum oscillation-possibly the stapedius muscle, tensor tympani muscle, and/or outer hair cells, 2) determining the neural circuits in the brain that drive this oscillation all the way to ear, 3) psychoacoustic experiments to determine any cognitive effect of this oscillation, especially with respect to sound localization.

Methods: In order to study the anatomical and neural circuits in controlled experiments, we propose the use of the rhesus macaque monkey as an important animal model in which we can perform controlled invasive surgical and pharmacological experiments that we could not otherwise perform in humans. The rhesus monkey is able to perform saccadic eye movements on similar time scales to human participants and we are able to record ear canal changes in the same manner as with human participants.

Results: Rhesus monkeys have a highly-reproducible oscillation within both ears, comparable to humans, including alternating phase of the oscillation between the ears and separable horizontal and vertical components related to the horizontal and vertical components of the simultaneous eye movement.

Conclusions: Rhesus monkeys allow for a single, specific surgical or pharmacological intervention after baseline data collection, data collection almost immediately after procedure, control experiments to determine how the procedures affect the ears and auditory system, and data collection on the order of thousands of trials to reach significance in each subject. This is especially important because human subjects with hearing loss do not typically have baseline data for comparison, the nature of their auditory dysfunction is not always known, and many participants have had dysfunction for years, making it difficult to distinguish what effects are due to the original dysfunction and which may be compensatory in nature.

Monosynaptic Auditory Input to the Rodent Paraflocculus

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Background: Based on its connectivity, the cerebellum is divided into the spino- cerebellum, cortico-cerebellum, and vestibulo-cerebellum. Each of these functional divisions is characterized by a well-known input and output connectivity whose main anatomical details have been known for decades. Within the past twenty years, however, it has become clear that the structure of the cerebellar cortex is more complex than the classical basic diagram composed of granule cells, Purkinje cells, and several types of inhibitory interneurons. Growing evidence suggests that the cerebellum plays an integral part in perceptual processing and integration of sensory and motor signals, and responses to auditory stimuli were described in vermal and hemispheral regions of the cerebellar cortex, but evidence for monosynaptic auditory inputs to the cerebellum is lacking. This work investigated the hypothesis that the cerebellar flocculus and paraflocculus (FL/PFL) receive primary auditory inputs.
Methods: We recorded from 74 FL/PFL neurons in anesthetized normal hearing gerbils. The bulla was surgically exposed and opened. A silver electrode was hooked onto the round window to determine baseline cochlear function. The FL/PFL was accessed through the anterior semicircular canal. With sharp glass electrodes, the paraflocculus was penetrated in an anterior trajectory towards the flocculus. From stable neurons, spontaneous discharge rate and the best frequency of the neuron were determined. Firing properties to acoustic stimulation were characterized. The analysis included the calculation of the inter-spike-interval histograms (ISTHs), post-stimulus-interval histograms (PSTHs), and spike and successive spike interval statistics. At the end of the recording, the cells were iontophoretically filled with biocytin. At the end of the experiment, the animals' brains were processed using routine histological techniques to confirm the sites of recording.
Results: Based on histology we recorded from the ventral PFL. Responses were tuned with the best frequency (BF) between 1 and 20 kHz. Most neurons' stimulation thresholds at BF were between 30 and 60 dB sound pressure level (SPL). Spontaneous rates were between 0 and 60 pulses per second (pps), with most neurons below 10pps. The driven rates at about 90dB SPL were 32 to 400pps, with most neurons above 100pps. The average latency for the first peak in the PSTH of the neurons is 10 ms. Four different response patterns were observed, fast onset, broad single onset, ringing onset, where the ringing frequency was well below the BF of the neuron, and a switch at BF from a ringing onset (stimulus frequency below BF) to fast onset followed by an inhibition at stimulus frequency above BF.
Conclusions: The experiments confirm a monosynaptic connection from the cochlea to the cerebellum with a wide frequency range.

Shedding Light on SARS-CoV2 and Audio-Vestibular Symptoms: Causality or Spurious Conjunction?

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Background: Systematic reviews indicate a possible association between COVID-19 and audio-vestibular symptoms. However, the quality of evidence is generally low due to limitations in study methodology that include no control groups and recall bias. More recently, there have been similar reports of audio-vestibular symptoms following COVID-19 vaccination, but again high-quality evidence is lacking. In this study, we explored these issues by collecting self-reported data on auditory and non-auditory symptoms over time. We compared baseline data that were fortuitously collected in March 2019 with data collected in September 2021.
Methods: The 10,401 individuals age 18+ years who completed a YouGov survey in March 2019 were re-contacted in September 2021 with a request to complete the current survey about health and symptoms during the COVID-19 pandemic. No reference was made to our specific interest in audio-vestibular symptoms. The survey

assessed reported onset and duration of several symptoms relative to COVID-19 and COVID-19 vaccination. Some symptoms examined have a known association with COVID-19: Persistent fatigue, Loss of smell, Problems with memory and concentration; some have an indeterminate association with COVID-19: Hearing loss, Tinnitus; and some have no known association with COVID-19: Toothache. Questions were also asked about the extent to which participants attributed symptoms to COVID-19/COVID-19 vaccination.

Results: Responses from 6701 of the original participants were obtained. A greater proportion of individuals reported baseline difficulties with hearing than at follow-up: Hearing difficulties: 25.7% vs 22.1%. The converse was true for tinnitus: 20.8% vs. 26.1%.

Of the 1154 participants (17.2%) who had confirmed or suspected COVID-19, 1.5% reported the onset of hearing difficulties and 1.6% reported onset of tinnitus within a few months of contracting the illness. Over 60% of these individuals said they thought COVID-19 had affected their hearing loss/tinnitus. Notably, however, over 60% of individuals who had had confirmed or suspected COVID-19 and who reported toothache following infection, attributed their toothache to COVID-19. Similar trends were seen for onset of hearing loss, tinnitus, and toothache following COVID-19 vaccination.

Conclusions: There was no increase in hearing loss relative to the pre-pandemic baseline, but there was an increase in tinnitus. However, caution is required when attributing new reports of hearing loss/tinnitus to COVID-19 because many participants also attributed to COVID-19 symptoms that are not known to be associated with infection. These preliminary analyses highlight the need for investigation to disentangle the direct impacts of COVID-19 on the audio-vestibular system from indirect factors (including recall bias, anxiety and stress, and other psychosocial factors) on the perception of symptoms. In our presentation, we will further describe our findings and discuss the implications.

EEG Markers of Implicit Learning of a New Musical Culture Through Passive Exposure

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Background: Music displays great structural variability from one culture to the other and holds a central place in our everyday lives but it remains unclear how individuals learn the statistics of their own musical system. It is nonetheless known that listeners from different cultures react differently to familiar or unfamiliar music (Demorest, 2008). Specifically, listeners, even as young as five years old (Politimou, 2021), predict upcoming notes consistently with an internal model of the statistics of their musical corpora (Krumhansl, 1999). Evidence also suggests that these statistics are acquired through passive exposure in both adults and infants (Loui, 2010, Hanon and Trehub, 2005a, 2005b). To model the brain of such encultured listeners (Pearce, 2005), statistical frameworks have been developed and trained on large corpora of Western musical excerpts, and then used to demonstrate that the expectation of musical notes can be predicted and decoded from EEG and SEEG recordings from Western listeners (Di Liberto, 2020; Marion, 2021).

Methods: This study aims to track the development of these expectations in brain data through passive exposure to traditional Chinese music on naive Western listeners. EEG recordings from 38 participants were collected before, immediately after, and 2 months after a 2-weeks exposure phase in which participants listened to 10 hours of traditional Chinese music. A control group followed the same procedure but was exposed to 10 hours of Bach chorals. A Temporal Response Function analysis was conducted to correlate the neural signals with the musical expectations estimated from a statistical model of music (IDyOMpy) trained on only traditional Chinese music.

Results: Our results demonstrate that the statistical model trained on traditional Chinese music correlates better with the EEG recordings after exposure than before and that this improvement is significantly greater for the participants who listened to the Chinese music relative to the controls who were exposed to Bach Music. This is strong evidence that the participants' internal model of music has been updated to send predictions more consistent with the exposed traditional Chinese music.

Conclusions: Our results demonstrate that passive exposure to an unfamiliar corpus of music induces neural plasticity allowing better predictions of its particular statistics. This finding fulfills the current gap on the application of the predictive processing framework in the case of music listening - namely that predictions are generated during music listening and that an error signal is computed by comparing the bottom-up incoming auditory signal with the top-down predictive signal, and is presumably used to update the inner model (Di Liberto, 2021). Our study shows that this model is updated through passive listening and, therefore, bridges this gap between predictive processing and music cognition.

Antisense Oligonucleotide-Based Degradation of c.151C>T Mutant Coch Transcripts Associated With Dominantly Inherited Hearing Impairment DFNA9

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Background: The c.151C>T founder mutation in COCH is a frequent cause of late onset, dominantly inherited hearing impairment and vestibular dysfunction (DFNA9) in the Dutch/Belgian population. The initial clinical symptoms only manifest between the 3rd and 5th decade of life, which leaves ample time for therapeutic intervention. The dominant inheritance pattern and established non-haploinsufficiency disease mechanism indicate that suppressing translation of mutant COCH transcripts has high therapeutic potential.
Methods: We used Single-Molecule Real-Time (SMRT) sequencing to annotate all allele-discriminating variants on the c.151C>T mutant COCH haplotype that can be exploited to develop highly specific knock-down therapies. We next designed and ordered RNase-H1 dependent antisense oligonucleotides (ASOs) directed agains the c.151C>T variant, or a mutant allele-specific intronic variant. ASO were transfected into our transgenic mutant and wildtype COCH-expressing cell models to assess their knockdown efficiency and specificity.
Results: Single-Molecule Real-Time (SMRT) sequencing resulted in the identification of 11 variants with a low population-frequency ($< 10\%$), that are specific to the c.151C>T mutant COCH allele. Proof of concept was obtained that gapmer antisense oligonucleotides (ASOs), directed against the c.151C>T mutation or mutant allele-specific intronic variants, are able to induce mutant COCH transcript degradation when delivered to transgenic cells expressing COCH minigenes. The most potent AON, directed against the c.151C>T mutation, was able to reduce mutant COCH transcript levels by >80%. Near-perfect allele-discrimination was obtained at a lower dose, which lead to a 60% decrease in mutant COCH transcripts. Chemical optimization studies and preclinical evolution of molecular efficacy in DFNA9 patient-specific iPSC-derived otic progenitor cells are underway.
Conclusions: In view of the adult-onset of DFNA9, it can be argued that a complete knockdown of mutant COCH transcripts is not needed to alleviate the burden of mutant COCH proteins. With the proven safety of ASOs in humans, and rapid advancements in inner ear drug delivery, our in-vitro studies indicate that ASOs offer a promising treatment modality for DFNA9.

Auditory Temporal Integration in Rhesus Macaques: Central Neural Correlates and Dysfunction After Noise Exposure Designed to Cause Cochlear Synaptopathy

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Background: Various models of temporal integration have been used across sensory systems to characterize accumulation of noisy sensory evidence to guide behavior. Temporal integration in the auditory system has been characterized based on the effects of stimulus duration on detection threshold. Studies of the neural correlates of temporal integration have differed substantially in describing how integration evolves along the auditory pathway. Moreover, it's unclear if existing models apply to temporal integration in noisy environments. For these reasons, we characterized the effects of noise on temporal integration in the cochlear nucleus (CN) and inferior colliculus (IC) of macaques. Temporal integration has also been considered as a noninvasive assay of cochlear synaptopathy (CS). Given that our recent model suggests that tone detection at short durations requires integration over many auditory nerve fibers, we tested temporal integration as a behavioral biomarker of noise-induced CS.
Methods: Rhesus macaques (Macacca mulatta) performed a Go/No-Go task used to measure reaction-time detection performance at different tone durations (3.25 – 200 ms) and frequencies (500 Hz – 32 kHz) in quiet, and in continuous, 76-dB SPL broadband noise. To induce CS, anesthetized macaques were exposed to 120 dB SPL, 2-4 kHz noise for four hours, then behavioral data were recollected. Electrophysiological data are reported from tungsten microelectrode recordings, where electrodes were lowered into the IC or CN for acute recordings of single neurons in normal hearing macaques detecting 200 ms tones. Responses were then segmented into time windows that matched the behavioral experiments (latency + 3.25, … 200 ms) to model the effects of duration. Behavioral data was analyzed using signal detection theory. Receiver operating characteristic analysis was

conducted on firing rate distributions to generate neurometric functions, from which neurometric threshold and slope were extracted. Threshold vs. duration trends were fit with exponential functions to estimate time constants (rate of threshold change), a measure of temporal integration.

Results: IC and CN temporal integration rates in quiet were not significantly different, but were faster than behavior. In noise, a subset of IC neurons integrated more slowly, approximating behavior, while the CN rates did not change. This suggests a subcortical transformation in temporal integration in noisy backgrounds. The noise-exposed monkeys showed reduced slopes of the psychometric function for short duration tones, consistent with integration from fewer auditory nerve fibers, and with the prior model. Ongoing analyses will assess the degree to which the effects of duration on accuracy and speed can be modeled as changes in drift rate in a hierarchical drift diffusion model.

Conclusions: These data provide baseline information on the central neural basis of temporal integration. These data also suggest the use of temporal integration measures as CS biomarkers, and form the baseline for studies investigating central neural changes following CS.

Developing System, Method and Program for Fitting of Hearing Aid in Korea at the Era of COVID-19

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Background: After term teleaudiology was first used by Dr. Gregg Givens in 1999 numerous new works have appeared in various field of audiology. In the situation of worldwide fear of the COVID-19, advantage of teleaudiology have become more prominent. Especially for hearing aid (HA) users, who need frequent visits to clinic for their hearing aid fitting, the new untact world could be much more frustrating than others. Therefore, the telefitting, became necessary for alternative of conventional face-to-face situation for the HA users.

Methods: First, an asynchronous accumulation-and-delivery method that stores information such as the subject's hearing-related problems and the characteristics of hearing aids and transmits it to a hearing professional via e-mail or the internet was investigated and tested. Then the subject's information is streamed and stored to the cloud on a computer or mobile device, and the expert begins the study of re-streaming to the expert's computer or mobile device for confirmation, establishing a voice connection, and completing the preparatory phase. Through this, when the remote fitting session is ready, the expert performs and streams it to the cloud, accumulates it, and then re-streams it to the subject. If the problem is not resolved, the expert proceeds with re-fitting and repeats the subsequent steps to confirm the satisfaction of the subject.

Results: As the system was investigate, patent "System, method and program for fitting of hearing aid" was applied (Korean Intellectual Property Office, R.O.K. applied number: 10-2021-0101412). This was about remote fitting of HAs including system that can provide real-time remote fitting through network with several additional functions including video chat, real-time fitting, feedback management and in-situ hearing test. Initiated by push notification, the HA user can start a remote fitting session wherever he/she can use smartphone. Specific sequence of the fitting in the applied patent can be described as fallows: push notification to mobile devices of hearing aid client, cloud connecting between professional and client including data reading, hearing check and fitting HA, streaming and storing data of current fitting, feedback confirming of client with video chat. Overall, the system would provide HA users with live feedback of themselves and adequate and customized services from the HA professionals.

Conclusions: Advantages of telefitting of HAs system are highlighted in the context of transition to non-face-to-face in almost all areas. It is necessary to research and develop the telefitting system in Korea to meet the global trend. In Korea, as with global needs, an untact service is needed by HA users in the ear of COVID-19. This development will be an essential technology for the HA users not only for today but also for future.69

Behavioral and Physiological Approaches for Indexing Auditory Learning in Rats With Cochlear Implants.

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Background: Cochlear implants (CIs) are auditory neuroprostheses that restore hearing by bypassing damaged parts of the sensory transduction epithelium of the inner ear and directly stimulates primary afferent neurons via pulses of electrical current. The present configurations of electrical stimulation leads to significant distortion in the

encoding of sound, yet human cochlear implant users can fully identify spoken words. Peak speech comprehension outcomes are highly variable; our hypothesis is variability in CI outcomes are in part caused by central plasticity.

Methods: Our lab has developed physiologically and behaviorally-validated cochlear implants for rats (King et al. 2016, Glennon et al. 2021). An 8-channel array is implanted through a basal-turn cochleostomy, and its location and functionality is confirmed via perioperative and postoperative ECAPS, post-operative x-ray imaging, and behavioral training.

To examine perceptual learning in a more complex version of our previous go/no-go task, we trained rats on a self-initiated 2-alternative forced-choice (2AFC) task. First, rats were food deprived and maintained at 80% of their original body weight. Next, they were trained to self-initiate and nosepoke the left port 4 kHz tones (center) and the right port for all other stimuli (off-center). Correct responses were rewarded (sugar pellet) and incorrect with a short time-out. After reaching 80% hit-rate across all stimuli, trained rats were bilaterally deafened and implanted with CIs. For CI-training, stimuli delivered by electrode at the middle of the 8-channel array was chosen as center stimulus

Our lab also developed hardware and procedures for chronic 60-channel micro-electrocorticography (µ-ECoG) recordings of auditory cortex in freely moving rodents (Insanally et al. 2016). Tone (0.5-32 kHz, 70 dB SPL) or CI (1-8 channels, -30 to +20 re. ECAP) evoked responses are filtered, 2-100 Hz for event-related potentials (ERPs) and 70-140 Hz for high-gamma (20 ms averaging window). Magnitudes of evoked activity are determined from the absolute values of stimulus averaged responses (20 trails). To determine wither evoked responses could predict stimulus conditions, we implemented a supervised linear classifier.

Results: All rats eventually reached high discrimination performance on our acoustic 2AFC task (n=12, overall hit rate > 80%). Of rats implanted with CIs, high discrimination performances were attained within 2-3 weeks (n=4, overall hit rate >75%).

µ-ECoG arrays and CIs were implanted acutely (n=7) or chronically (n=4). Trial-by-trial evoked metrics revealed robust responses to tones and CI-stimuli (d-prime >1, evoked vs baseline activity). CI-evoked responses did not reveal clear cochleotopical organization across recording sites, whereas a linear classifier achieved above chance performance on predicting stimuli.

Conclusions: CIs in deafened rats effectively restore behaviorally relevant auditory cues and evoke neural activity in the contralateral auditory cortex. Our next steps include indexing auditory learning in CI implanted and behaviorally performing rats, leveraging both 2AFC behavior and µ-ECoG measurements.

Differential Effects of Synaptic Zinc on Sound-Evoked Responses of Long-Range Cortical Projection Neurons in the Mouse Auditory Cortex

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Background: The primary auditory cortex is crucial for the perception of sound. This structure integrates ascending auditory information with a variety of corticocortical and thalamocortical input and sends descending long-range axonal projections to subcortical auditory processing areas such as the striatum, thalamus, inferior colliculus, and brainstem. As a monosynaptic connection from the auditory cortex, these long-range axonal projections allow cortical input to directly influence the activity of neurons in subcortical auditory structures. In layer 5 of the auditory cortex, there are two major classes of long-range projection neurons: pyramidal tract (PT)-type neurons and intratelencephalic (IT)-type neurons. Although it is known that these long-range projection neurons are important for dynamic tuning, auditory learning, and auditory plasticity, much less is understood about the intracortical synaptic mechanisms that shape their activity.

The auditory cortex is highly enriched with synaptic zinc. Zinc (as Zn^{2+}) is loaded into presynaptic vesicles by the membrane zinc transporter protein ZnT3 and is coreleased with glutamate during synaptic transmission. Synaptic zinc is a potent modulator of synaptic signaling and refines the sound-encoding properties of layer 2/3 auditory cortical neurons in a cell-type specific manner. Since long-range projection neurons integrate information from a variety of local sources (including layer 2/3 neurons) and convey these computations to multiple targets, they are the key link between cortical processing and subcortical signaling. However, the effect of synaptic zinc signaling on the sound-evoked activity of these long-range projection neurons remains unknown.

Methods: Here, we performed widefield and 2-photon calcium imaging from PT and IT-type neurons in layer 5 of the auditory cortex in awake mice.

Results: We find that synaptic zinc signaling differentially shapes the sound-evoked properties of these layer 5 long-range projection neurons. Synaptic zinc increases the sound frequency tuning bandwidth and suppresses the response gain of PT-type neurons while it has the opposite effects on layer 5 IT-type neurons. By differentially supporting the sound frequency tuning and the response gain of these neurons, synaptic zinc serves to support the diverse sound-evoked properties of these neuronal populations.

Conclusions: Our experiments reveal a novel role for synaptic zinc in shaping the output of long-range projection neurons in the auditory cortex and suggest a novel intracortical mechanism capable of modulating auditory processing.

Auditory Evoked Potential Recordings Across Species Using High-Density Soft ECoG Arrays
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Background: Auditory evoked potentials (AEPs) are an electrical manifestation of the brain response to an auditory stimulus. It is known that it is very sensitive to the level of consciousness of the subject. In particular, anesthetics agents may affect the electrophysiology of AEPs in their waveform, delay, and amplitude. We have systematically compared AEP across mammalian species and anesthetic conditions. We recorded AEP using customized high-density microelectrocorticography grids (μECoG) in rats, pigs and non-human primates (NHP). We report here on typical subdural AEP recordings highlighting observed differences.

Methods: Technology
We designed and fabricated μECoG arrays, specifically scaled to the auditory cortex anatomy of three animal models. We used microfabrication to embed stretchable interconnects (polyimide / platinum / polyimide) in a thin silicone matrix (150μm thick), allowing for high conformability to the curvilinear cortical surface. Electrode contacts (⌀ 100μm, 25 contacts in rats, 32 for pigs and NHP) are coated with a soft platinum-silicone composite to ensure low electrode impedance.

Recordings
All animal experiments were performed following all relevant ethical regulations and approvals.
We performed recording of auditory evoked potentials in 3 species rats, pigs and macaques under anesthesia, sedation or awake. ECoG placement was as followed: After anesthesia and craniotomy a soft μECoG was slid subdurally at the surface of the auditory cortex. Tone bursts at different frequencies were played in close field - and μECoG recordings were performed using a MCS wireless system.

Analysis
Raw data were averaged over the stimulation period, and ON and OFF responses were compared in different conditions.

Results: To allow for comparable measurements, electrochemical characteristics of customized soft μECoG electrodes were evaluated in-vitro and in-vivo through systematic impedance spectroscopy and measurement of voltage transient (VT).
Auditory function was assessed prior to surgery (ABR recording).
We observed that across all three species, AEPs are modified in amplitude under strong anaesthetic regimen (such as high level of Isoflurane). However, unlike in rats or pigs, AEPs in non-human primate were highly affected and sometimes inexistant even under lower regimen of anaesthesia (isoflurane, propofol, ketamine). In the same rat, in a given channel, AEP amplitude under 1% isoflurane reached 55 μV peak to peak, 18 μV under Ketamine and less than 10 μV when awake. In minipig (8kg), AEP amplitude could reach 60 μV under 11 mL/h propofol. At the same ratio weight/flow rate of propofol, no AEP could be detected in NHP.

Conclusions: Herein, we implemented the soft μECOG neurotechnology to conduct an in-depth review of AEPs recording protocols completed in 3 distinct species that are extensively used as animal model for auditory research. Our conclusions should serve as guidelines to future studies involving cortical recording of auditory perception in both animal models and clinical studies.

Auditory Temporal Processing Throughout Development in Two Different Mouse Models of Autism
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Background: Autism spectrum disorder (ASD) is currently diagnosed in approximately 1 in 54 children in the United States, making it one of the fastest growing developmental disorders. ASD encompasses a wide array of

debilitating symptoms, including sensory dysfunction. This dysfunction ranges from hypo- or hypersensitivity to varying stimuli and may account for numerous behavioral phenotypes identified in ASD, including abnormal anxiety, language development and social dysfunction. Auditory temporal processing is crucial for speech recognition and language development, including production and interpretation, and abnormalities could account for the language impairments associated with ASD. How and when these auditory temporal processing deficits develop remains unclear.

Methods: Here we tested resting EEG and association of power spectral density with mouse movement, response magnitude of sound evoked event related potentials (ERP), and auditory temporal processing with a novel gap-ASSR (auditory steady state response) stimulus in young and adult mice. This stimulus uses gaps strategically placed 25 milliseconds apart in continuous noise to elicit an ASSR at 40 Hz. The experimental manipulation of gap duration and modulation depth within the stimuli allows us to measure the cortical response to rapid and brief fluctuations in sound using EEG recordings. We quantify this using inter-trial phase clustering (ITPC) values that account for phase consistency of the recorded signal across multiple trials.

Results: In the current study, we quantify auditory temporal processing throughout development using two translationally relevant mouse models of ASD that display sensory abnormalities: the Fmr1 knock-out (KO) mouse model of fragile X syndrome and a PTEN-deletion model of autism. Preliminary results from young mice at postnatal day 21 (p21) using the PTEN-deletion model suggest a genotype difference in sound evoked ERP magnitude, with PTEN heterozygous and KO mice showing increased amplitudes in the auditory and frontal cortex (Control – n=11; PTEN heterozygous – n=5; PTEN KO – n=7). However, both PTEN-deleted genotypes show diminished ITPC in their gap-ASSR responses in both brain regions compared to control mice. Moreover, Fmr1 KO mice at the same age show similarly decreased ITPC compared to wild-type (WT) mice at the same age and older KO mice at p30 (Fmr1 KO at p21 – n=5; WT at p21 – n=13; KO at p30 – n=5).

Conclusions: These data suggest that auditory temporal processing deficits are present at an early age and may contribute to sensory processing abnormalities seen in ASD.

Deficits in Vocalization-In-Noise Perception After Noise-Induced Temporary Threshold Shifts in Guinea Pigs
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Background: Exposure to moderate-to-intense sounds can produce temporary threshold shifts (TTS) in the audiogram. Such TTS is hypothesized to contribute to lasting speech perception deficits in noisy listening conditions but not in clean conditions. In normal hearing animals, cortical neurons show selectivity for specific sound features. Preliminary data from our lab suggest that neurons retain this selectivity across different listening conditions, and that independent cortical mechanisms may underlie selectivity for acoustic features and invariance to noisy conditions. Because TTS may be associated with speech perception deficits in noise and since high-threshold auditory nerve fibers required for encoding sounds at loud levels are damaged in TTS, we hypothesized that at loud sound levels, only the invariance circuitry will be affected by TTS.

Methods: We induced TTS in guinea pigs, a highly vocal rodent, using 4–8 kHz or 2–8 kHz noise at 106 dB SPL for 2 hours, and verified TTS using ABRs. We estimated thresholds for vocalization (call) categorization at low and high sound levels in quiet and noise before and after TTS using pupillometry.

Results: Consistent with the loss of high threshold auditory nerve fibers, we found that call-in-noise perception was affected at only loud sound levels. Call categorization was impaired only for calls with frequency content within or above the noise range used for TTS induction.

Conclusions: These results demonstrate a behavioral deficit in call in noise perception after TTS. In ongoing experiments, we are characterizing how TTS impacts feature selectivity and invariance in different laminae of primary auditory cortex using multichannel electrophysiological recordings combined with decoding models.

Characterizing Changes to Event-Related Potentials and Neural Oscillations in the Auditory Cortex Following Inactivation of Parvalbumin Interneurons
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Background: The synchronization of neural activity depends largely on inhibitory interneuron activity, and PV neurons in particular play a crucial role in synchronizing cortical activity (Merker, 2013). In the visual cortex, PV

neurons promote de-synchronization of neural activity (Chen et al., 2017). PV neurons may also regulate synchronization in the auditory cortex (AC). An example of a synchronized cortical response in the AC is the auditory event-related potential (ERP). The ERP is one of the most widely recorded sensory responses in humans, and it is used as an electrophysiological marker to characterize pathophysiology in neurodevelopmental and neurodegenerative disorders. Additionally, the auditory steady-state response (ASSR) is a phase-locking response to a sound modulation rate of 40Hz, and it has been well studied because of the robust phase-locked response that the AC produces. The ASSR is a useful stimulus for identifying the extent to which phase locking in the AC is impacted by conditions that involve alteration in excitation and inhibition levels (Brenner et al., 2009) or aging-related effects on gap detection (Ross et al., 2010). Here, we seek to identify how PV neurons regulate synchronized activity in the AC in response to noise bursts and to 40Hz amplitude modulations of varying gap width in noise presentation. Understanding the impact of altered PV neuron function on the ERP and ASSR will increase the utility of these measurements beyond a descriptive marker to generate specific hypotheses of underlying synchronized circuit level abnormalities in various brain disorders.

Methods: We integrated electrophysiology and chemogenetic techniques in order to directly manipulate the activity of PV neurons in the AC. We injected an adeno-associated virus (AAV) carrying Cre-dependent designer receptors exclusively activated by designer drugs (DREADD) into the AC of C57 Bl6/J PV-Cre mice and implanted recording screw electrodes above right and left AC and a reference electrode above the occipital lobe. Following recovery, mice received systemic injections of the DREADD ligand clozaping-n-oxide or vehicle, and AC activity was subsequently recorded in awake mice as they were freely moving in a sound-insulated arena. Three recording protocols were performed: recording in the absence of sound presentation (resting EEG), repeated presentation of sound bursts (ERP), presentation of an ongoing noise interspersed by amplitude-modulation gaps of varying widths presented at a 40Hz rate (gap ASSR).

Results: Preliminary results suggest that inactivating PV neurons in the AC alters resting EEG by reducing high gamma (60-100Hz) and high frequency oscillation (>100Hz) power and increasing beta (12-25Hz) power in the PSD. In addition, PV neuron inactivation enhanced auditory ERP P1 and N1 amplitudes, suggesting that PV neuron activity reduces highly-synchronous states in cortical activity.

Conclusions: Future experiments will test the effect of PV neuron inactivation on gap ASSR as a measure of temporal processing.

The Contributions of Spatial Congruence and Temporal Coherence to Audiovisual Binding of Moving Objects

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Background: By combining information from different sensory modalities, we form a picture of complex scenes. The present study examines a specific form of cross-modal integration known as audiovisual (AV) binding, in which features from auditory and visual stimuli link to form a single AV object (Bizley et al. 2018). Neurally, temporal coherence between stimuli enhances the representation in sensory cortices (Atilgan et al. 2018). Behaviorally, temporal coherence improves the ability to detect orthogonal stimulus features, i.e., features that are independent of the temporal coherence that drive binding (Maddox et al., 2015). Here, we test whether dynamic spatial congruence contributes to AV binding.

Methods: Subjects in a soundproof booth saw visual spheres rendered in a virtual room using an HTC Vive Proeye VR headset and heard harmonic complexes played from a loudspeaker array (-108° to 108° azimuth) at eye level. At any one time, there were three spheres of different colors, as well as three tone complexes with different fundamental frequencies. Over time, the spheres and tone complexes changed size and level, respectively, and moved in random paths on the horizontal plane. At the start of each trial, one sphere and tone complex were cued as the target object. Subjects were instructed to press a button when a brief luminance change or frequency change occurred in the target object (ignoring discontinuities in the other visual and auditory streams). In each trial, spheres and tone complexes were paired. Within each pair on a given trial, the sphere size and tone complex level were either modulated with the same envelope (temporally coherent) or not, and their azimuthal paths either matched (spatially congruent) or did not. We tested three experimental conditions, which were randomly intermingled across trials: 1) temporally and spatially congruent, 2) temporally and spatially incongruent, and 3) temporally incoherent and spatially congruent. We calculated sensitivity (d') to the orthogonal events for each condition.

Results: Preliminary results (n=5) indicate that even when there is AV temporal coherence, spatial incongruence decreases a subject's ability to detect orthogonal features of the AV object. However, when stimuli are spatially congruent, temporal coherence has little effect on performance. These trends held even though subjects differed greatly in overall accuracy.

Conclusions: When objects are stationary, AV spatial incongruence has little effect and spatial perception is dominated by visual inputs (e.g., the ventriloquist illusion); however, such paradigms do not provide the rich structure present in natural scenes. These results suggest that when stimuli are moving, AV spatial congruence is a stronger binding feature than temporal coherence. It is still unknown what neural circuitry underlies these processes. Future studies are needed to understand the underlying neural coding, and the interaction of binding with top-down attention networks to provide increased salience to higher processing centers.

Spectral Invariance in Pitch Perception

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Background: Information in speech and music is often conveyed through changes in fundamental frequency (f0). One challenge of extracting such information is that the sounds to be compared often have different spectral shapes due to the filtering imposed by a vocal tract or instrument body. Pitch is traditionally defined as the perceptual correlate of a sound's f0, and is envisioned as invariant to spectral shape, potentially providing a solution to this challenge. We examined the extent to which listeners are invariant to spectral changes heard in natural sounds, and whether any such invariance is mediated by representations of f0.

Methods: To assess spectral invariance, we measured up/down and interval discrimination of sounds with different spectra: different instruments or spoken vowels, as well as synthetic tones. To assess the dependence of any observed spectral invariance on f0-based pitch, we compared discrimination for harmonic and inharmonic stimulus variants. Dependence on f0-based pitch should result in impaired performance for inharmonic stimuli, which permit frequency comparisons, but lack a well-defined f0.

Results: Listeners were overall worse at discriminating pitch across different vowel and instrument sounds compared to when vowels/instruments were the same. However, there was no interaction between this effect and harmonicity: performance was matched for harmonic and inharmonic stimuli. The deficit induced by note-to-note changes in the spectrum appears to partly reflect bias: pitch judgments were consistently biased in the direction of the spectral centroid shift. This bias decreased when notes were separated by short delays, suggesting that the underlying pitch representation was itself unbiased, but that listeners' decisions were influenced by changes in the spectral centroid to an extent that lessened over time.

Conclusions: Together the results suggest that 1) robustness to naturally-occurring spectral variation is not mediated by representations of f0, and 2) pitch judgments can be biased by spectral shape imposed by sound source filtering, but listeners encode pitch separately from (and largely unbiased by) spectral shape.

Analysis of Complex Sounds at High Frequencies

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Background: Performance in some basic psychophysical tasks, such as level discrimination, varies little over the audible frequency range. In contrast, performance in some tasks, such as pitch perception, is worse at high frequencies where phase locking is assumed to be absent (i.e., > 4 kHz), although the extent of the deficit depends on the details of the task and stimulus. For example, at high frequencies, listeners can accurately discriminate the F0 of resolved complex tones, differentiate consonant and dissonant two-tone dyads, and identify musical intervals, but cannot detect mistuning of individual components in a complex tone or differentiate major and minor triads or arpeggios. Decreased availability of phase locking at high frequencies --- and varying reliance on that weak phase locking among tasks --- may explain these results. However, it is possible that other factors contribute as well. For example, it may be that listeners apply less efficient or simpler listening strategies at high frequencies due to a lack of experience (e.g., listening to a single auditory filter when integrating information from multiple auditory filters is optimal).

Methods: To further explore these possibilities, we measured performance of young normal-hearing listeners in several psychophysical tasks using bandpass-filtered complex tones at both low and high frequencies in young

normal-hearing listeners. First, we measured performance in level discrimination, profile analysis, spectrotemporal ripple detection, and spectrotemporal ripple orientation discrimination (analysis tasks). Next, we measured the ability of listeners to "hear out" single or several components in a complex tone and make judgments about their pitch on the basis of onset asynchrony or amplitude modulation segregation cues (segregation tasks). Where possible, we analyzed simulated peripheral responses to our stimuli to determine whether any trends in our data could be attributed to differences in information content at the level of the auditory nerve.

Results: Performance was similar at low and high frequencies for analysis tasks that could be readily performed based on cues from individual auditory filters (level discrimination, spectrotemporal ripple detection). In contrast, for analysis tasks that included roving to rule out good performance based on cues from individual auditory filters (profile analysis, spectrotemporal ripple orientation discrimination), performance was worse at high frequencies than at low frequencies. Performance was consistently poorer for segregation tasks at high frequencies, even after accounting for differences in baseline pitch discrimination at low and high frequencies.

Conclusions: Our results reveal that perceptual deficits at high frequencies extend beyond pitch-related tasks to include other types of tasks involving analyzing and segregating complex sounds. These results suggest that perceptual deficits at high frequencies may not be exclusively the result of weak phase locking in the auditory nerve.

Perceptual Segregation of Modulation Components Under Modulation Informational Masking

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Background: The "multiple-bursts same, multiple-bursts different" paradigm [Kidd et al., J. Acoust. Soc. Am. 95, 3475-3480 (1994)] has been used in numerous studies to examine perceptual segregation of frequency components under conditions of masker-frequency uncertainty (informational masking). A core finding from those studies is that the relative coherence of competing frequency components can be an important factor influencing their segregation: coherent components tend to group and thus to be perceived as segregated from incoherent components. In this study, we adapted the multiple-bursts paradigm to the amplitude modulation (AM) domain in an attempt to assess perceptual segregation of competing AM components (i.e., individual components of a complex envelope) under conditions of masker-AM-rate uncertainty [modulation informational masking; Conroy and Kidd, J. Acoust. Soc. Am., 149, 3665-3673 (2021)]. Of primary interest was whether the principle of relative coherence, as demonstrated in the frequency domain, applies in the AM domain.

Methods: Observers were tasked with detecting a target comprising four sequential "bursts" of equal-rate, sinusoidal AM (i.e., a coherent sequence of AM) in the presence of a multiple-bursts, multiple-component masker-modulator applied to the same high-frequency pure-tone or broadband-noise carrier. The target- and/or masker-AM components associated with each burst of AM were gated on and off simultaneously. Conditions differed in terms of the relative coherence of the target- and masker-AM components. In the "multiple-bursts same" (MBS) condition, the multiple-bursts masker-modulator was created by randomly selecting two AM rates from a wide range prior to each stimulus presentation (excluding those within a "protected region" surrounding the AM rate of the target) and using those same two rates to generate a two-component masker-modulator for each burst in the sequence. In the "multiple-bursts different" (MBD) condition, by contrast, two random rates were selected for each burst independently. In both conditions, the randomization of the masker rates across stimulus presentations was intended to produce a high degree of masker-AM-rate uncertainty and thus large amounts of modulation informational masking.

Results: The detectability of the target was greater in the MBD condition than in the MBS condition for both carrier types, consistent with the expectation based on the principle of relative coherence. That is, according to the principle of relative coherence, the detectability of the target should have been greater in the MBD condition than in the MBS condition because, in the MBD condition, the target comprised the only coherent sequence of AM and thus should have been perceived as segregated from the incoherent masker.

Conclusions: The relative coherence of competing AM components can be an important factor influencing perceptual segregation, particularly under conditions of high, masker-AM-rate uncertainty. Other interpretations of the results, however, are plausible (e.g., those based on temporal pattern analysis, multiple looks, etc.) and will be considered.

An Interactive Hearing-Aid Fitting Interface Powered by the Open Master Hearing Aid

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Background: Hearing-aid fitting traditionally begins with audiometric testing. The obtained hearing thresholds are then fed into standard prescription formulae such as NAL-NL2 and DSL, which determine the target gain as a function of frequency, i.e. the amplification profile, according to the hearing thresholds. The emergence of over-the-counter hearing aids enables the possibility for user-directed adjustment of amplification profiles in their own acoustic environments without the requirement of audiometric testing. However, hearing-aid users may lack the sophistication in audio signal processing and hearing-aid technology, hence performing self-adjustment on a large number of configuration parameters for a hearing aid may be challenging for many.

Methods: To address this issue, an intuitive interface was developed that enables user-directed adjustment of amplification profiles in six octave-frequency bands from 250 to 8000 Hz. The interface includes (1) a two-dimensional display on a tablet computer or a smartphone with touchscreen capability and (2) a simulated hearing aid powered by the Open Master Hearing Aid (openMHA). Dragging a cursor to various locations of the two-dimensional display changes the amplification profile for the simulated hearing aid in real time. During each trial of the self-adjustment procedure, the user is presented with speech stimuli in background noise and is tasked to identify a location in the display that corresponds to the most preferable sound quality. The user is able to bookmark candidate locations with drop-pins to revisit and compare later. The map from the two-dimensional cursor location on the display to the gains in the six frequency bands is iteratively determined before each trial.

Results: A few iterative algorithms to update a linear R2-to-R6 map between the control and amplification profile were initially examined using Monte-Carlo simulations. The listener's identified locations over a number of trials resembled a random-walk toward the most preferable amplification profile in the six-dimensional gain space. The efficiency and reliability of the self-adjustment procedure depended on the iterative algorithm.

Conclusions: The interactive user interface may provide a viable means for efficient exploration of a relatively large number of hearing-aid settings and allow preference-based self-fitting of hearing aids.

Role of Chromatin Remodeling Protein CHD4 in Establishing Cellular States during Neuronal Differentiation

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Background: Spiral ganglion neurons (SGNs) in the cochlea are the primary auditory neurons that relay neural signals from sensory hair cells to the cochlear nucleus. SGN loss due to developmental defects or ototoxic insults lead to hearing loss. Progenitor cell replacement therapy to supplant lost SGNs is a viable option, but requires molecular understanding to ensure safety of engrafting progenitors and efficiency of neuronal differentiation. Immortalized otic progenitor cells (iMOPs) can be cultured as proliferating progenitors or differentiated into iMOP-derived neurons. Our previous work has shown that changes in the epigenomic landscape influences iMOP proliferation and differentiation. How these modifications are regulated in iMOPs is not well known. Here we use iMOP cells to identify, study and determine the molecular changes caused by epigenetic regulators such as chromodomain-helicase-DNA-binding protein 4 (CHD4) during neuronal differentiation.

Methods: Stable scrambled shRNA and Chd4 shRNA knockdown (KD) iMOP stable cell lines were generated by lentiviral transduction and drug selection. Stable cell lines were used for cellular and molecular analysis. Cells were subjected to scRNA-seq and initial processing was performed using CellRanger. Secondary analysis was performed using Scanpy and trajectory inference accomplished using scVelo. Single molecule fluorescence in situ hybridization (smFISH) and immunocytochemistry were used to validate results from scRNA-seq data.

Results: Chd4 KD cells displayed decreased proliferative rates and increased cell death. Differentiation of Chd4 KD cells resulted in decreased numbers of TUBB3+ iMOP-derived neurons that have significantly shorter neurites relative to controls. These results implicate Chd4 in neuronal differentiation and neurite outgrowth. scRNA-seq of control iMOP cultures during neuronal differentiation identified distinct cell states. Proliferating iMOP cells existed in a naïve or primed progenitor state. From trajectory inference, progenitors pass through a previously undefined transitional state before becoming neuroblasts. Finally, neuroblasts developed into four putative subtypes of neurons. smFISH and immunocytochemistry using well-defined markers confirmed proliferating and neuronal cell states. scRNA-seq results from Chd4 KD cells showed alterations in both progenitor and neurons. CUT and Tag determined CHD4 binding sites and proximity of CHD4 binding was used to identify target genes.

Gene ontology analysis of target genes suggest that CHD4 may regulate biological processes such as neuron fate commitment and neuron projection extension.

Conclusions: Single cell RNA-seq analysis identified progenitor to neuronal cell states, while velocity analysis predicted the neuronal differentiation trajectory of iMOPs. Identified target genes regulated by CHD4, obtained from CUT and Tag, suggest that altered target gene expression may impair neuronal differentiation in the knockdown cells. iMOPs are a useful cellular system to understand SGN differentiation for future replacement therapies.

Hippo Signaling and p27Kip1 Synergize to Enforce the Postmitotic State in the Organ of Corti
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Background: One of the main ways in which non-mammalian vertebrates restore sensory hair cells is through the division and differentiation of the residual population of supporting cells. In contrast, supporting cells lose the capacity to proliferate postnatally in mammals, and the molecular machinery preventing cell cycle reentry remains poorly understood.
Our work has established that Hippo signaling serves as a major repressive mechanism that controls progenitor cell proliferation in both vestibular and auditory systems to define sensory organ size. Mechanistically, the core kinase cascade in the pathway phosphorylates transcriptional co-activator protein Yap, preventing its nuclear translocation and transcription of the pro-proliferative target genes. We also showed that Hippo signaling is maintained postnatally to suppress Yap activity and prevent regeneration in the adult inner ear. While Hippo inhibition is both necessary and sufficient to drive proliferative response in the adult utricle, the same manipulation is far less effective in the organ of Corti.
Methods: To allow for precise temporal control of Hippo signaling, we have developed a small molecule that inhibits Lats1/2 kinase in the pathway. Using this new tool, we further interrogated Hippo's role in regulating the postmitotic state with the focus on the differences between the auditory and vestibular sensory epithelia. We assayed cell proliferation via EdU incorporation, changes in Yap phosphorylation and its cellular localization via western blotting and immunohistochemistry. Lfng-GFP transgenic mice were utilized to purify cochlear and utricular supporting cells via FACS to compare transcriptional changes in response to Hippo inhibition in the two sensory organs via RNA-seq. Differentially expressed protein-coding genes were identified by DESeq2 and ggplot2 was used for data analysis and visualization.
Results: Our analyses revealed that the Hippo inhibition elicits similar cellular and molecular response in the utricle and the cochlea. In both systems inhibition of the pathway activated the downstream Yap signaling, inducing expression of many pro-proliferative target genes, such as Cdk4 and 6, Ccnd1, and Aurkb. However, while these transcriptional changes potently drove proliferation in the utricle, the organ of Corti supporting cells resisted S-phase reentry. Further analysis revealed that the expression of cyclin kinase inhibitor p27kip1, expressed at a particularly high level in the organ of Corti, remained unchanged in the Yap-activated cells. Strikingly, reducing the levels of p27kip1 in the cochlear supporting cells made them amenable to Yap-induced proliferation.
Conclusions: Collectively our data show that promoting the initial stages of hair cell regeneration through supporting cell division – a process thought to be permanently suppressed in the adult mammalian inner ear – can be achieved through manipulation of the Hippo and p27kip1 pathways.

Mesenchymal Stromal Cells-Activated Repair Pathways Protect Mice From Noise-Induced Hearing Loss
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Background: Mesenchymal stromal cells (MSC) can be derived from different tissue types and have been shown to mediate repair in several degenerative disorders. Modulation of the diseased environment and the immune system are thought to be main processes induced in a paracrine manner via cell-released protective factors and cytokines. The protective effect of MSC in the inner ear has been shown in models of sound- and ototoxin-induced damage. The aim of the present study was to unravel potential molecular mechanisms of action of MSC.

Methods: Mice were exposed to narrow band noise. After exposure, MSC derived from human umbilical cord Wharton's jelly were injected into the perilymph. Controls consisted of mice exposed to sound trauma only. Forty-eight hours after cell transplantation, total RNA was extracted from the cochlea and RNAseq performed to determine changes in gene expression induced by MSC. For functional analysis, a separate cohort of animals was treated in a similar fashion and allowed to survive for 2 weeks after cell therapy.

Results: Treatment with MSC was able to significantly protect mice from severe sound trauma. Based on gene ontology classification, changes in gene expression were grouped. MSC treatment resulted in an up-regulation of genes related to immune modulation, hypoxia response, mitochondrial function and regulation of apoptosis. In addition, a down-regulation of genes related to synaptic remodelling, calcium homeostasis and the extracellular matrix was observed after MSC treatment.

Conclusions: Application of MSC may provide a novel approach to treating hearing loss resulting from sound trauma. Using cutting edge molecular analysis, protective pathways can be identified in the inner ear allowing the development of novel treatment strategies for hearing protection and even regeneration.

Prdm1 Drives a Fate Switch Between Inner Ear and Lateral Line Hair Cells in Zebrafish

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Background: The lack of regenerative ability in the cochlea after the death or damage to mechanosensory hair cells is at the core of permanent hearing loss in mammals. Driving the regeneration of hair cells is a central strategy for restoring hearing in mammals, but triggering proliferative regeneration and maturation of functional hair cells remains elusive. Recently, the co-manipulation of Atoh1 with other genes or reprogramming with a cocktail of transcription factors has produced promising results, including the detection of proliferation and hair cell regeneration after hair cell killing. However, the generation of fully mature and functional hair cells has not been achieved. The zebrafish Danio rerio has an array of mechanosensory hair cell-containing neuromasts along the trunk, called the lateral line. Both the hair cells and surrounding support cells in zebrafish share genetic, functional, and structural similarity with mammalian inner ear hair cells and support cells, but the hair cells of zebrafish readily and rapidly regenerate following death to restore full function. Through our use of scRNA-seq during homeostasis and in a fine time series during hair cell regeneration in zebrafish, we identified sets of genes with both spatially and temporally regulated expression. One such gene is the transcription factor prdm1a, which is expressed increasingly in the maturing hair cell lineage, and in support cells shortly following hair cell killing. prdm1a is not expressed in the hair cells of the zebrafish inner ear. Previously, prdm1 has been shown to control a fate switch in various cell types, including maturing B lymphocytes and maturing photoreceptors in the retina. We mutated prdm1a in zebrafish and found a drastically reduced number of hair cells during development and during regeneration, accompanied by a reduction in the proliferation of support cells during regeneration. We performed scRNA-seq on prdm1a mutants and siblings and discovered a cell type fate switch between lateral line and inner ear hair cells, with many specific inner ear hair cell genes being ectopically expressed in lateral line hair cells of the mutants. We performed ATAC-seq and ChIP-seq to characterize the enhancers of hair cell genes and identified that Prdm1a binding sites are enriched in the promoters and enhancers of these ectopically expressed genes. These findings show that prdm1a plays a crucial role in repressing an inner ear hair cell fate in lateral line organs. Prdm genes might also be central drivers in hair cell type specification and regeneration in other vertebrates. Combined our data show that Prdm1 or other family members are important genes to consider in future regeneration attempts in the mammalian cochlea.

Methods: N/A
Results: N/A
Conclusions: N/A

Single Cell RNAseq Reveals Unique Cell Population Following Hair Cell Injury

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Background: The mature mammalian inner ear lacks the ability to regenerate mechanosensitive hair cells. The neonatal mouse utricle, in contrast, harbors hair cell progenitors. Histological and lineage tracing experiments have demonstrated the capacity of the postnatal utricle to proliferate and regenerate following in vivo ablation of hair cells. However, the mechanisms of proliferation and regeneration post-injury remain unknown.

Methods: We used the Pou4f3-DTR mouse model to ablate hair cells postnatally and determined the most proliferate time points. To capture the cellular dynamics of the utricle post-injury, we focused on the time points with maximum proliferation. We used single-cell RNA sequencing (scRNAseq) to probe the postnatal utricle at two time points, along with wild-type controls. The damaged sensory epithelium was isolated, cells purified via flow cytometry, and the Smartseq2 platform was used to generate scRNAseq data.

Results: We obtained over 2,000 high-quality cells from damaged and wild-type utricles at postnatal days (P) 4 and P6. Our data shows a median read count of 499,422 with a median of 3,105 genes per cell. Dimension reduction and clustering analysis identified hair cells, supporting cells, and transitional epithelial cells using previously described markers. We further identified two unique cell states not present within the wild-type cells that we hypothesize to be damaged hair cell states or activated supporting cells.

Conclusions: We have used an in vivo hair cell damage model to determine the time course of maximum proliferation. Using scRNAseq, we show that the damaged utricular sensory epithelium responds with at least two unique cell clusters. Our current efforts aim to validate these findings and focus on the proliferative response to ultimately determine mechanisms of mitotic regeneration.

Regenerated Hair Cells in the Neonatal Cochlea Are Innervated and Can Differentiate Into Both Inner and Outer Hair Cells

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Background: Previous studies have demonstrated spontaneous hair cell (HC) regeneration in neonatal mice. The degree of terminal differentiation and innervation, and whether both inner hair cells (IHC) and outer hair cells (OHC) form has not been examined.

Methods: Prox1CreERT2 and Plp-CreERT2 mice were paired with Rosa26CAG-loxP-stop-loxP-tdTomato (Rosa26tdTomato) reporter mice in order to fate map pillar and Dieter's cells or inner phalangeal and border cells, respectively. Each CreER::reporter line was then crossed with Pou4f3DTR mice to allow selective HC death by injection of diphtheria toxin. CreER::reporter::Pou4f3DTR-negative mice served as controls. Tamoxifen was administered on postnatal day (P) 0 to induce tdTomato expression in supporting cells (SC), and diphtheria toxin on P1 to induce hair cell damage. Cochleae were harvested on P7-P10 and immunostaining performed using the general HC marker, myosin 7a (myo7A), along with specific markers of IHCs and OHCs. Oncomodulin and prestin were used as immature and mature OHC markers, respectively, and vesicular glutamate transporter 3 (VGlut3) as a mature IHC marker. Synaptic ribbons were identified with CtBP2 and spiral ganglion neurites with Tuj1. Confocal microscopy was used to quantify tdTomato-positive (regenerated) HCs and those cells co-expressing terminal HC markers, synaptic buttons, and neuronal markers.

Results: Prestin, oncomodulin, and VGlut3 were each expressed in a similar proportion of SC-derived HCs targeted by each CreER line. Prestin expression was more robust at P10 and was seen in a significantly higher proportion of regenerated HC derived from the Prox1CreERT2-labeled SCs. Unexpectedly, a majority of regenerated HC were found to co-express both oncomodulin and VGlut3, regardless of the SC subset of origin. Upon further analysis, it appears that VGlut3 was expressed in SCs located in both compartments while oncomodulin expression was constrained to only the lateral SCs located near OHCs. The majority of regenerated HC contained CtBP+ synapses and were adjacent Tuj1-positive neurites.

Conclusions: These findings suggest that spontaneously regenerated achieve terminal differentiation of both IHC and OHC phenotypes, and that the SC subtypes targeted by each CreER line can produce HCs of either type. Different SC subtypes do not appear to have a higher propensity to produce either IHCs or OHCs. Given that co-expression of IHC and OHC markers was seen in the same cells, it appears that spontaneously regenerated HCs deviate from the normal developmental pathways seen in embryogenesis. In spite of potential aberrations in phenotype, nearly all regenerated cells were found to have synaptic buttons and were innervated by Tuj1-positive neurites, suggesting these regenerated cells could become functional.

Hearing Difficulties With Normal Audiograms: Insights from the Auditory Processing Disorder Test Battery

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Background: Increasing evidence suggests that a normal audiogram does not guarantee robust suprathreshold communication in challenging listening environments. An estimated 10% of patients seeking help for hearing difficulties have no abnormal clinical indicators. A small number of these patients are referred for further testing for Auditory Processing Disorders (APD). APD is a phenomenon in which patients have difficulty understanding spoken language in noise, localizing sounds, and deficits in understanding verbal directions and employing auditory memory. There are currently no established norms for APD testing referral, especially in adults. The APD test battery typically consists of three domains of behavioral testing which include binaural integration, dichotic listening, and temporal processing. The purpose of this study was to analyze results from the APD test battery to identify tests that could provide a rapid screener that can inform APD referrals, as well as identify patterns of deficits that may be used to design the next generation of objective diagnostic tests for APD.
Methods: We analyzed data from 47 patients from the past five years in the University of Pittsburgh Medical Center database who had clinically normal audiograms and underwent the APD test battery. The battery consisted of Random Dichotic Digits Test (RDDT) and dichotic words task in the Dichotic listening domain, QuickSIN and Words-in-noise (WIN) in the Speech-in-noise domain, and Gaps in noise and Frequency patterns in the temporal domain. We identified tests that proved most challenging for these patients, as well as relationships between the tests within and across domains. Statistical analyses were used to identify tests that captured the most variance in the data, and a stepwise analysis was performed to identify the smallest number of tests that captured the greatest variability.
Results: Patients experienced most difficulties within the dichotic domain, specifically as the task difficulty increased with competing stimuli. Performance on these tests were not correlated with the patient's age or hearing thresholds. Among the tests, RDDT 3-paired test and WIN were significantly correlated. QuickSIN had the most normalized variance while RDDT 1-pair test had the least. Finally, the stepwise selection model revealed that RDDT 3-paired test followed by Words in Noise and QuickSIN showed greatest promise as a rapid screener to identify patients with APD.
Conclusions: These results support the idea that the use of dichotic listening tasks that incorporate hearing in multi-talker babble may prove most useful when creating objective measures of testing for APD. Audiologists also may choose to include WIN or RDDT into the standard audiometric test battery as a quick screener to inform referral for further APD testing. Ongoing work developing an objective electrophysiological test for APD that targets these domains may help with more efficient diagnoses and provide information related to underlying neural deficits.

Cortical Tracking of Semantic Dissimilarity for Features Derived Using Static and Contextualized Embeddings in Primary Progressive Aphasia

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Background: Primary progressive aphasia (PPA) is a neurodegenerative disorder characterized by the progressive loss of speech/language. There are three PPA subtypes with unique deficits and patterns of underlying brain atrophy affecting the language dominant hemisphere. The semantic variant (svPPA) is characterized by a loss of core semantic knowledge due to anterior temporal lobe atrophy; the logopenic variant (lvPPA) is characterized by a phonological processing deficit due to temporo-parietal atrophy; the nonfluent variant (nfvPPA) is characterized by apraxia of speech and/or agrammatism due to fronto-insular atrophy. Recently, researchers have examined functional changes in neural processing in PPA at rest and in response to continuous speech, shedding light on the mechanistic underpinnings of PPA. The current study expands on previous work by examining neural mechanisms supporting semantic processing of a continuous narrative across PPA subtypes.
Methods: EEG responses were collected while participants listened to an audiobook. For each word in the audiobook, feature vectors were derived using the natural language processing (NLP) models word2vec and GPT2; word2vec uses static embeddings whereas GPT2 uses contextualized embeddings, accounting for polysemy and potentially providing a better approximation of a word's semantic features. For each model, we derived semantic dissimilarity values for the current word given its context by calculating one minus the Pearson correlation coefficient between the current word's feature vector and the mean of the previous words' feature vectors. Cortical tracking of semantic dissimilarity was estimated using temporal response function (TRF) modelling across PPA subtypes and age-matched controls (n = 10 per group). TRF modelling is a multivariate,

time-dependent regression approach that maps acoustic and/or linguistic features of a continuous stimulus to continuously collected neurophysiological data. The prediction accuracy of the TRF reflects the integrity of the cortical representation of the stimulus feature being modelled. Potential differences in TRF prediction accuracy by group and NLP model were analyzed using mixed ANOVA.

Results: Despite differences in the embeddings (static vs. contextualized) and core deficits across PPA subtypes, no significant differences were observed across groups, NLP models, or their interaction. Our choice of how to represent semantics (i.e., the selected NLP models and/or the use of semantic dissimilarity) may not be sensitive enough to distinguish changes in semantic processing as a consequence of neurodegenerative disease from changes in semantic processing that occur in typical aging.

Conclusions: Ongoing work seeks to disambiguate the similarity in prediction accuracy for cortical tracking across these models. One advantage of the TRF approach is the ability to utilize the same data to assess different levels of processing. Thus, there is potential for using the TRF approach to identify biomarkers for differential diagnosis of deficits impacting auditory and/or linguistic processing. Future research will investigate the utility of TRF modelling in differential diagnosis of PPA subtype.

How Pre-Lingually Deafened Cochlear Implant Users Deal With Unreliable Speech

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Background: Pre-lingually deafened cochlear implant (CI) users show good speech perception, language and reading outcomes (Niparko et al, 2010). However, they show more variability in challenging listening conditions like a noisy classroom (Vermeulen et al., 2012), which is not entirely due to peripheral auditory system differences (Shearer et al., 2017). This variability may derive from differences in cognitive processes like spoken word recognition. For NH listeners, multiple words are immediately activated and compete as the word unfolds, until one ultimately "wins" (Luce and Pisoni, 1998). The Visual World Paradigm (VWP) uses eye-movements to track the unfolding timecourse of this competition at the millisecond level. Previous work suggests that pre-lingually deafened cochlear implant users exhibit a "wait-and-see" approach to lexical activation, with significantly delayed fixations to the target and less competition from onset competitors (e.g., wizard and whistle) (e.g., McMurray, Farris-Trimble, and Rigler, 2017). This "wait-and-see" profile could potentially be adaptive as delayed commitment enhances flexibility.

One way to determine the adaptiveness of a "wait-and-see" profile is to examine situations in which the auditory input is unreliable. Typically, these unreliable situations involve background noise, which might invoke other challenging perceptual processes (e.g., grouping or streaming) for CI users. In contrast, unreliable input such as mispronounced words (e.g., dog pronounced as tog) do not require such processes. Apfelbaum, Ellis and McMurray (2019) found that post-lingually deafened CI users experienced less interferences from a mispronunciation during real-time lexical competition compared to NH listeners. It is unclear if pre-lingually deafened CI users exhibit the same pattern. Their "wait-and-see" approach could lead them to exhibit even less interference.

Methods: Thus, the present study tested pre-lingually deafened CI users and age-matched NH listeners (CI: n=28; NH: n=20; matched by age [9.0 to 19.5 years]) in a VWP study to characterize the dynamics of lexical access in the face of unreliable input. Listeners heard a target word in either its correct form, or with single- or multi-feature mispronunciations at word onset or offset. Participants selected the target word from a screen containing pictures of the target word and three unrelated images. Eye-movements were monitored over time to determine how quickly and how often the target word was activated.

Results: Relative to their NH peers, CI users were slower to activate the target (p's<.001) in all conditions except offset multi-feature and looked less to the target in all mispronunciation conditions (p's<.05). However, CI users' fixations did not exhibit as much of a difference between correct and mispronounced conditions in the first 300 msec of processing compared to NH listeners.

Conclusions: These results suggest that a "wait-and-see" lexical access profile creates flexibility for pre-lingually deafened CI users in the face of uncertain input.

The Familiarity of Background Music Modulates the Cortical Tracking of Target Speech at the Cocktail Party

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Background: Cocktail party speech-in-noise listening is often studied using noise or linguistic maskers; few studies investigate speech perception in realistic background music. We recently showed that speech recognition was negatively impacted by the familiarity of concurrent background music. Speech recognition was also modulated by song component; performance was worse when masked by full unprocessed sound moreso than isolated vocals or isolated instrumentals. Here, we expand these findings using EEG and neural decoding techniques in order to measure cortical tracking of continuous speech in these different music maskers.
Methods: While recording multichannel EEG, participants listened to an audiobook (8 min) masked by popular music that was either familiar or unfamiliar, both with and without lyrics. Listeners were instructed to focus on the speech and ignore the background music. Comprehension questions at the end of each block confirmed task engagement. We and computed temporal response functions (TRF) using neural decoding of the target stimulus envelope. Amplitude, latency, and correlation to the speech stimulus envelope were used to index the strength of neural representation of the target speech encoding amidst the different music backdrops.
Results: Speech comprehension was worse during (i) full song vs. instrumental-only music and (ii) but unfamiliar vs. familiar background music, contradicting our previous findings. However, while variable across participants, preliminary EEG data reveal larger speech-TRF amplitudes during unfamiliar music maskers. Additionally, both neural responses and stimulus-to-brain envelope tracking (correlation coefficients) were larger in full song maskers than instrumentals alone. Ongoing data collection aims to clarify the relation between these behavioral and neural results.
Conclusions: Our experimental paradigm creates an ecologically valid cocktail party situation requiring active listening to continuous speech in the presence of popular background music. Implications of this research include gaining further insight into speech-in-noise processing in realistic listening scenarios, as well as the facilitation (or hindrance) of different types of non-speech background sounds (i.e., music) on concurrent learning and cognitive tasks.

A Cross-Linguistic Approach at Investigating Language Learning in Children With Hearing Loss-Preliminary Results
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Background: Fricative sounds /s/ and /z/ hold morpho-syntactic importance in many languages such as English and French, as they signal plurality, for instance. Children with mild-to-moderate sensorineural hearing loss (SNHL) are known to show morpho-syntactic delays such as increased errors in noun and verb morphology. This study assesses whether poor phonetic perception of fricatives relates to poor use of morphology for children with normal hearing (NH) and children with mild-to-moderate hearing loss under noisy conditions. A cross-linguistic approach is taken by testing French and American children in their native languages on how well they perceive fricative speech sounds in quiet and noise, and how well they understand singular and plural word forms signaled by these fricatives.
Methods: Participants included 4- to 7-year-old children in France and in the United States with NH and with SNHL. All children completed two psychophysical tasks. First, participants' accuracy to identify fricative consonants (/s/ vs /z/) in the presence of stationary speech-shaped noise was measured. The signal-to-noise ratio (SNR) was kept constant for 36 trials at either 0, +5, or -5 dB and the consonants were presented in a vowel-consonant-vowel syllable where the vocalic context was counterbalanced between /a/, /u/ and /i/ for each SNR condition.
Second, participants' abilities to identify markers of singularity and plurality, as signalled by fricative sounds, was assessed using an audio-visual task. Children were asked to match sentences with videos based on number agreement information heard. Two videos depicting the same action are shown on a screen; on one side the singular subject is presented and on the other side the plural subject is presented. Children were asked to listen to a sentence embedded in a steady speech-shaped noise at 0, +5, or -5 dB SNR and point to the video depicting the correct action heard.
Finally, vocabulary and language skills with a focus on the acquisition of morphemes were measured using standardized clinical assessments.
Results: Preliminary analyses showed no significant difference in consonant identification performance between SNR conditions (0, and +5, or -5 dB) and no age-related change in performance for NH children in both countries. However, for the audio-visual subject-verb agreement task, children performed worse overall and an age-related

improvement was observed. The current focus of this project is to increase sample size, especially children with SNHL.

Conclusions: Cross-linguistic investigations are crucial in delineating how auditory difficulties experienced by children with SNHL are related to the specific languages they are learning and whether deficits observed are language-specific or universal. The results from this study will advance our understanding of the relationship between what children hear and how it affects perception of the specific language they are learning.

Articulatory Motor Areas and Speech Perception: A 7T fMRI Study

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Background: The association between brain regions involved in speech production and those that play role in the perception of acoustic signals is not yet fully understood. In this study, we compared brain activations during production and perceptual discrimination of speech sounds using ultra-high field 7 Tesla fMRI at 1 mm isotropic voxel resolution.

Methods: fMRI blood oxygen level dependent (BOLD) signal data were obtained by using a simultaneous multi-slice (SMS) echo planar imaging (EPI) acquisition. Fifteen subjects (6 men, mean age 29 ± 9.0 years) completed speech production and speech perception tasks during fMRI. In a phoneme discrimination task, subjects were presented with pairs of syllables (stimulus onset asynchrony 1 s), which were equiprobably identical or separated by three intervals along an 8-step continuum between the prototypic /ba/ and /da/ sounds. The subjects were asked to indicate whether they heard the same or two different syllables in a row. In a speech-sound production task, subjects were asked to produce a silent lip-round vowel /u/ in response to the visual cue ''U'' or to purse their lips in response to seeing the cue "P".

Results: Preliminary univariate fMRI analyses using a parametric modulation modeling approach indicated that BOLD activations related to phoneme category variability in the /ba/–/da/ discrimination task were strongest in the left precentral-premotor and inferior frontal cortex areas. Notably, largely the same regions were also activated in the silent vocalization vs. lip pursing contrast. Conversely, the BOLD signal changes associated with purely acoustic variability of sounds were strongest in the bilateral auditory cortices.

Conclusions: The results support the hypothesis that articulatory-motor networks of left hemisphere play a important role in perceptual categorization of speech signals.

Audiotactile Speech Perception and Neural Mechanisms

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Background: Speech involves a hierarchical structure of information, ranging from phonemes to syllables, words and sentences. These different units of information need to be segmented in order to be processed by the brain. The segmentation presumably relies on oscillations in the delta and in the theta frequency ranges (1-4 Hz and 4-8 Hz) in the auditory cortex, which track incoming speech at the rhythm of syllable and words. The tracking in the theta range plays a functional role in speech processing, as its modulation using transcranial current stimulation has been found to affect speech comprehension. Because these cortical oscillations can also be influenced by somatosensory stimulation, we wondered if such stimulation could impact speech comprehension as well.

Methods: We delivered sparse vibrotactile pulses to the hand of subjects while they listened to speech in background noise. The pulses were aligned to the centre of syllables and shifted in time to study the effect of

different lags between the two sensory streams. We assessed the participants' comprehension of the speech signal. Furthermore, we studied the neural encoding of speech and the vibrotactile pulses through electroencephalographic recordings (EEG).

Results: We found that tactile pulses presented at the rate of syllables can modulate and even improve speech-in-noise comprehension. The enhancement occurred when the pulses were aligned with the perceptual centers of the syllables, without temporal delay. The neural responses to both speech and vibrotactile pulses were modulated by different delays between the two sensory streams, displayed audiotactile integration, and reflected the behavioural modulation of speech comprehension. Finally, we demonstrated that the comfort of subjects in understanding speech could be predicted from the electrophysiological markers of multisensory integration.

Conclusions: Our results provide evidence for the role of cortical oscillations and vibrotactile information for speech processing. The observed enhancement of speech comprehension suggests that such vibrotactile stimulation might be employed in auditory prosthesis to aid people with hearing impairment to better understand others in noisy environments.

Neurophysiological Markers of Central Gain and Their Relationship to Speech-In-Noise Intelligibility

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Background: Optimal speech-in-noise intelligibility requires the successful separation of the target speech stream from multiple competing background streams. The ability to segregate these competing streams of speech depends on the fidelity of bottom-up neural representation of sensory information as well as top-down influences of effortful listening. In this study, we sought to develop objective neurophysiological measures of bottom-up temporal processing and explore their interactions with measures of listening effort, as it relates to speech-in-noise intelligibility. The upper limit for the neural representation of temporal envelope rates decreases along the ascending auditory pathway. This property was exploited to assess temporal processing along the auditory pathway by obtaining envelope following responses (EFRs) to multiple modulation frequencies. The relationship of EFR amplitudes to performance on a speech-in-noise task, and pupil-indexed listening effort were further explored.

Methods: Nineteen English-speaking adult participants with normal hearing sensitivity were recruited to participate in this study. We collected envelope following responses (EFRs) to amplitude modulated (AM) tones with a carrier frequency of 3000 Hz. AM rates consisted of 40, 110, 512, and 1024 Hz, emphasizing neural generators from cortical and sub-cortical regions. Slopes were fit to EFR amplitudes using a growth curve analysis to obtain an objective neurophysiological marker for the ascending transmission of auditory information. Subjects were then assessed for speech-in-noise intelligibility using QuickSIN, a standardized test where listeners are required to repeat target sentences masked in four-talker babble under six signal-to-noise ratio (SNR) levels between 25 and 0 dB. Iso-luminous task related changes in pupil diameter were measured as a marker for listening effort while subjects performed the task, and a similar growth curve analysis was applied to obtain a top-down marker for cognitive load.

Results: Performance on QuickSIN was near ceiling for SNRs of 25 to 5 dB, but listeners showed variability in performance at 0 dB SNR despite having clinically normal hearing thresholds. EFR slopes, but not individual EFR amplitudes, significantly correlated with speech-in-noise intelligibility measured at the most challenging listening condition. Changes in pupil diameter revealed sub-threshold changes in listening effort despite matched performance levels. A multivariate regression model revealed that the combination of EFR slopes and pupil diameters provided the optimal model for speech-in-noise performance.

Conclusions: These results indicate that the EFR slopes are a promising biomarker for the bottom-up neural coding of temporal envelope information. Correlations with speech-in-noise intelligibility for the EFR slope, but not individual EFR amplitudes, suggest that optimal Information transfer along successive nuclei of the auditory pathway is critical for speech-in-noise intelligibility. Ongoing work is aimed at optimizing EFR acquisition parameters to rapidly obtain EFR slopes in young and middle-aged listeners.

Auditory Reverse Correlation on a Phoneme-Discrimination Task: Assessing the Effect of Different Types of Background Noise

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Background: A long-lasted goal in psycholinguistics is to identify the acoustic cues underlying phonetic percepts. The reverse correlation method offers an agnostic approach to explore internal representations of phonemes by relating listeners' responses in a phoneme-in-noise task with the exact time-frequency representation of the tested noises. The outcome is called auditory classification images (ACIs): A fine-grained time-frequency map of the acoustic cues listeners relied upon. Here, we focus on the effect of the statistics of the background noises with respect to the efficiency and robustness of the method. We used three different noise types that have a flat long-term spectrum, but differ in the amount of temporal envelope fluctuations: (1) White noise, (2) white noise low-pass filtered in the modulation power spectrum (MPS) domain, and (3) bump noise.

Methods: We conducted a consonant-in-noise discrimination task using the words /aba/ and /ada/. The speech samples were uttered by a female speaker taken from the French Logatome speech corpus. Each participant performed a total of 5000 /aba/-/ada/ categorizations in each of the three noise conditions. During the task, the signal-to-noise ratio (SNR) was varied to target a score of 70.7%. Subsequently, ACIs were derived using the reverse correlation method, revealing the time-frequency regions where the noises systematically affected the participants' discrimination. Further, we computed ACIs using subsets of trials to evaluate the fidelity and speed of convergence of these "partial ACIs" with respect to the ACI obtained using all trials.

Results: For both participants and for the three test noise types, we succeeded to find stable ACIs which, in line with previous evaluations that used another pair of /aba/-/ada/ utterances, reveal a high-weighting of the speech information between the first and second formants in the consonant-vowel transitions. Our analysis of partial ACIs shows that the three noise types required a different number of conducted trials to converge to a stable ACI, with a faster convergence for the MPS and bump noises compared with the use of white noises.

Conclusions: Our analyses suggest that the reverse correlation method applied to a consonant-in-noise discrimination task converges more quickly to a stable result, the ACI, when the background noises contain dominant components in the modulation frequency range between 0 and 60 Hz, which is the case for MPS and bump noises. The prominent envelope fluctuations in this range lead to more systematic confusion errors compared to white noise and, therefore, to higher prediction accuracy and more robust reverse correlation results.

GPIAS Response in Blast Exposed Chinchillas Using Preyer Reflex

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Background: Though, 'filling-in' interpretation of tinnitus in GPIAS (Gap prepulse inhibition of acoustic startle) paradigm is challenged due to confounding theories on physiology of GPIAS to indicate tinnitus, it has not been ruled out and have been evaluated across species such as rats, mice, guinea pigs, gerbils, hamsters and humans with modifications. However, GPIAS in chinchillas have been probed far less due to long release time or subtle indicators. Further, though, GPIAS cannot substitute traditional psychoacoustic operant conditioning method to probe tinnitus, a pressing need is evolved to establish its validity to link with tinnitus by robust stimulus parameters. Hence in present study, our objective is to investigate the blast induced gap deficits in gap-prepulse inhibition of acoustic startle (GPIAS) in chinchillas using Preyer reflex as an indicator of startle response.

Methods: Healthy adult male chinchillas (n=6) were subjected to blast overpressure at various intensities (172 dB SPL). We have acquired both NBPIAS and GPIAS at various time-points (pre-blast, Post-blast 2 weeks, 1 mo (month), 2 mo and 3 mos). In both NBPIAS and GPIAS, in addition to whole body movement, preyer reflex (pinna movement) will be assessed as a measure of startle response. The pinna movements were analyzed using the motion tracking system (Vicon Motion Systems, Denver, USA). In NBPIAS, we used broadband noise (BBN) as prepulse stimuli (50ms) at 75dB SPL intensity 200ms before the onset of the startle stimuli (20ms at 120dB SPL). In GPIAS, we used the NBN (one third octave bands centered at 1, 2, 4, 6.3, 8 and 10 kHz) as the background noise (75dB SPL) and a gap (of 100ms at 200 ms before the startle onset) was used as the Prepulse inhibition.

Results: The ratio of acoustic startle amplitudes (ASR) of pre-startle and post-startle was used for analysis. In this preliminary analysis, the ASR ratio of GPIAS was analyzed with time points and frequency by repeated measures ANOVA (mixed model) using maximum restricted maximum likelihood estimation. The Geisser-Greenhouse correction (epsilon = 0.7231) was used as there is a violation of sphericity (Mauchly's test). Overall, there is a significant difference between pre-BOP against all other time-points ($F_{(2.892, 940)}=19.51$) at the significance level of 0.05. With multiple comparisons, suppression of GPIAS is significant at all post-Blast time-points at 2 kHz, suppression is significant at post 1,2 and 3 mo at 1 kHz, post 3 mo at 4 kHz.

Conclusions: Results from our study indicates that to study blast induced GPIAS deficits in chinchillas, Preyer reflex is a better indicator of startle response than whole-body movement. Overall, this study will provide a platform to answer uncertainties about GPIAS as a method of tinnitus assessment.

MOC Reflex Measurement in Tinnitus Subjects Compared to Controls

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Background: Tinnitus is a symptom of a variety of health disorders and is often accompanied by emotional exhaustion, leading to a substantial loss in the quality of life. A curative therapy of tinnitus is currently impeded by contradictory views about its neural correlate. This might be related to different sub-entities of tinnitus (with and without hyperacusis). Tinnitus is accompanied by poor temporal resolution as substantiated e.g. by gap detection in noise (Gilani et al. 2013). Especially at the frequency of tinnitus, sensitivity regarding frequency, intensity, and temporal processing duration are affected (Ravirose et al. 2019). It also has been show that tinnitus patients show increased efferent suppression of cochlear activity by the medial olivocochlear reflex (MOCR) system (Knudson et al. 2014). The interpretation of this finding is not straightforward because the MOCR-system is thought to be responsible for improving signal-to-noise ratio of neural signals. As most investigations on MOCR rely on measurements with low time resolution and thus cannot distinguish between fast and slow MOCR effects, we here use paradigms to resolve fast ipsilateral and contralateral MOCR effects.

Methods: Pulsed DPOAE are used here in order to allow for separation of the two major components of DPOAE generation, i.e. the nonlinear distortion and the coherent reflection component (Zelle et al., 2017). We investigated the MOCR by analyzing the time-course of the nonlinear distortion component of pulsed DPOAE responses in 25 subjects (14 healthy controls and 11 tinnitus patients). In addition, we collected Békésy-tracking thresholds on these subjects.

Results: We here present fast MOC-induced ipsilateral and contralateral adaptation of nonlinear distortion component of DPOAE, time-resolved during 160 ms post-elicitor onset in an approach comparable to Dalhoff et al., 2014, for relatively low f2-frequencies (2-4 kHz) and at higher frequencies (6-10 kHz) close to the subjectively determined tinnitus frequency. We will discuss our results in the context of recent concepts of tinnitus pathophysiology.

Conclusions: The temporal adaptation of the time-resolved DPOAE response is a promising approach to the search of a neural correlate for tinnitus.

Effect of Intratympanic Botulinum Toxin Injection on Middle Ear Myoclonic Tinnitus

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Background: Middle ear myoclonic tinnitus (MEMT) is a crackling or buzzing sound in ear caused by involuntary contractive movement of the middle ear muscles, tensor tympani and stapedius muscles. Traditionally, medication including muscle relaxants, anxiolytics and anticonvulsants were used to treat MEMT, and surgical resection of middle ear muscles was done in intractable cases. We propose intratympanic botulinum toxin injection (IT-Botox) as a new treatment modality before surgical treatment in MEMT. In this study, we investigated the effect of IT-Botox on MEMT.

Methods: A total of 24 patients were included in this study. Medical records and tinnitus questionnaire of the patients with MEMT who received IT-Botox at Seoul St. Mary's Hospital by one physician (S.N.P.) from 2019 to 2021 were reviewed retrospectively. Audiologic evaluation and tinnitus questionnaire were conducted before and after IT-Botox.

Results: The average age of the patients was 38.8 ± 12.5 years, and the average duration of follow up after IT-Botox was 5.7 ± 5.2 months. Of 24 patients, 17 patients received IT-Botox once, 5 patients received it twice, and 2 patients received it three times. Mean tinnitus handicap inventory (THI) score before IT-Botox was 45.1 ± 21.1 and

was decreased to 38.0±22.7 (p=0.094) after the first IT-Botox. Visual analogue scales (VAS) for loudness, annoyance and effect on life were all statistically significantly decreased from 4.1±2.3 to 3.0±2.3 (p<0.05), 5.3±2.5 to 3.3±2.7 (p<0.05), 5.1±2.6 to 1.7±1.5 (p<0.05), respectively. No patient showed any complication such as hearing loss, dizziness, facial palsy or swallowing difficulty after IT-Botox. Six patients (25%) showed complete resolution of MEMT after IT-Botox; 4 patients received IT-Botox once, 1 patient received it twice, and 1 patient received it three times. Six patients (25%) had no improvement or showed recurrence of MEMT after IT-Botox and decided to undergo METR; 5 patients are scheduled to receive METR, and 1 patient had received METR and showed complete resolution.

Conclusions: In conclusion, IT-Botox seems to be a good and safe modality to treat intractable MEMT, which could be a new therapeutic option for MEMT after medication and before trying METR.

The Mechanisms of Homeostatic Plasticity in the Inferior Colliculus After Noise-Induced Hearing Loss
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Background: Noise trauma is known to trigger central plasticity from the cochlear nuclei to the auditory cortex : neural activity is enhanced after the noise-induced hearing loss both in terms of spontaneous and evoked activity. It has been suggested that this increase in neural excitability results from homeostatic plasticity, which is supposed to play a role in maintaining the averaged central neural activity around a constant value. A vast repertoire of molecular mechanisms is involed in this plasticity. Neural inhibition is underpinned by the intracellular concentration of chloride ion, that is kept at low level by the KCC2 co-transporters. However, KCC2 density has been shown to be reduced after sensory deprivation. In that case, the intra-cellular concentration of chloride ion should be enhanced and GABA neurotransmitter can depolarize affected neurons. The present study is aimed at providing further insights into the molecular changes of central inhibition after noise induced hearing loss. We first investigated whether KCC2 density in neurons' membrane was reduced in the inferior colliculus (IC) after noise-induced hearing loss. Second, we determined whether the effect of a GABA antagonist Gabazine application, into the IC would defferently affect neuronal activity in the IC of control and sound exposed animals.
Methods: This study was carried out on adult guinea pigs that were controls or exposed to noise trauma (continuous tone of 8 kHz, 115 dB SPL during 4 hours). The KCC2 density was first assessed in 12 animals (controls : n=6 and noise exposed : n=6) using immunohistochemistry and confocal imaging. Another batch of animals (control and sound exposed) were used to test the effect of topical application of Gabazine on neural activity in the inferior colliculus. Both multi-unit activity and local field potentials were recorded from a linear array of micro-electrodes (Neuronexus) in 12 animals (controls : n=6 and noise exposed : n=6).
Results: Our study shows that KCC2 density is significantly reduced at 3 and 30 days after noise trauma in IC. Furthermore, while neural stimulus-induced activity is enhanced after Gabazine application in the IC of control animals, neural activity is unchanged or significantly reduced in the exposed animals. Regarding the spontaneous activity, the spontaneous firing rate is increased after Gabazine infusion in control animals, while neural activity is unchanged or decreased in the exposed animals.
Conclusions: Our study demonstrates that noise-induced hearing loss is accompanied by a down-regulation of KCC2 co-transporters. It further shows that noise-induced hearing loss alters the disinhibitory effect of Gabazine. These results suggest that the hyperpolarizing action of GABA is reduced in IC after noise-induced hearing loss, which could be potentially due to the local down-regulation of KCC2 co-transporters in that nucleus.

Central Auditory Dysfunction Related to Tinnitus and Hyperacusis Caused by Blast Exposure
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Background: Blast exposure is a major cause of tinnitus and hearing loss in veterans. Over one-third veterans develop chronic tinnitus during or after discharge from service, leading to a prevalence of tinnitus that is twice as high in veterans compared to civilians. One potential mechanism linking blast-induced tinnitus is microglia initiated neuroinflammation of the central nervous system. However, the hypothesis has not been confirmed.
Methods: Adult Sprague Dawley rats have been used in the experiment. Operant conditioning test was used to train the rats to perform a sound detection task. Customized blast wave generator was used for blast exposure (two blasts at 193 dB SPL peak amplitude). Sound detection task and reaction time has been used to detect tinnitus and

hyperacusis caused by blast exposure. Errors in sound detection and reaction time to narrow band noise bursts (8 kHz, 40-110 dB SPL, 10 dB step) were used to evaluate tinnitus and hyperacusis. The auditory brainstem response (ABR) was used to monitor hearing threshold. Microglia activation and neuronal excitabilities were identified using immunohistochemistry in the central auditory pathways involved in sound behavioral changes.

Results: ABR test shows that ABR thresholds after blasts showed ~20 dB permanent hearing loss two weeks after blasts. The errors of detecting soft/loud sound increased significantly after blast, suggesting rats may experience tinnitus. The reaction times after blasts were significantly shorter than before blasts, behavior consistent with hyperacusis. Increased c-Fos positive neurons and CD68 positive microglial cells have been detected in the auditory brainstems after blasts.

Conclusions: Our preliminary results suggests that the blast exposure could induce hyperacusis and tinnitus shortly after the blast exposure, consistent with previous reports. We hypothesize that tinnitus and hyperacusis will continue to develop due to the maladaptation of the central auditory system and limbic system after blasts. This model can be used to study the neural mechanisms of tinnitus and hyperacusis after traumatic brain injury caused by blasts.

Aminoglycosides Can Induce Tinnitus and Hyperacusis in Mice

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Background: Aminoglycosides (AG) such as amikacin and tobramycin are commonly used in cystic fibrosis patients with recurrent pulmonary infections and those infected with multi-drug-resistant tuberculosis. AGs are cochleotoxic and have been shown to result in auditory dysfunction including hearing loss, hyperacusis, and tinnitus. Most in vivo AG animal models have limited their hearing assessments to auditory brainstem responses (ABRs) and cochlear histopathology. Recently, we have shown that the acoustic startle reflex (ASR) can assess both tinnitus and hyperacusis in amikacin and tobramycin mouse models of cochleotoxicity. Here we compare results from two longitudinal aminoglycoside studies that demonstrate notable differences between amikacin and tobramycin and their incidence and severity of hyperacusis and tinnitus.

Methods: CBA/CaJ mice three to six months of age were divided into three groups: Group 1 served as a control and did not receive AGs or ebselen; Group 2 received tobramycin (200 mg/kg/s.c.) or amikacin (500 mg/kg/s.c.) daily for 14 continuous days; Group 3 received the same AGs as Group 2 plus ebselen (20 mg/kg/i.p.) following the same dose/schedule. ABR and ASR assessments were performed at baseline and several epochs after the end of treatment. ASR assays included input/output functions to assess general hearing and hyperacusis and gap-induced prepulse inhibition of the acoustic startle (GPIAS) reflex to assess tinnitus. Cochlear histopathology involved hair cell counts/analysis under light and epifluorescence microscopy.

Results: ABR thresholds temporarily increased 10-15 dB at 16 kHz from baseline, primarily between 2-6 weeks, and returned to near baseline by 14-18 weeks after the start of AG treatment. This high-frequency hearing loss was prevented by ebselen cotreatment. Input/output ASR functions revealed exaggerated startle response magnitudes (hyperacusis) following AG treatment. Behavioral evidence of hyperacusis was induced in 83% of mice following amikacin, and 50% of mice following tobramycin. Gap detection deficits, representing behavioral evidence of tinnitus, appeared in 36% of tobramycin mice vs 24% of amikacin mice. Cochlear histology did not reveal significant changes in hair cell counts following treatment of either AG. Ebselen cotreatment was able to ameliorate AG-induced hyperacusis and more significantly in amikacin-treated mice.

Conclusions: These cochleotoxicity models demonstrate that tobramycin and amikacin can result in auditory dysfunctions beyond hearing loss, that are not dependent upon hair cell loss. We have also ascertained that AG-induced hyperacusis is significant and potentially more common than tinnitus. Additional research should elucidate the neural mechanisms underpinning AG-induced hyperacusis and tinnitus and the ability of ebselen to prevent and treat these dysfunctions. These novel results support the ongoing and future testing of ebselen as a treatment for hearing loss, tinnitus, and hyperacusis in AG receiving patients.

Comparison of Afferent and Secondary Neuron Discharge in Response to Electrical Stimulation With a Vestibular Prosthesis.

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Background: Restoration of vestibular function via modulated afferent electrical stimulation from a vestibular neuroprosthesis is currently technically feasible. However, there are several limitations, which must be understood before optimal stimulation can be provided. One such limitation is the degree to which afferents of an individual ampulla can be selectively stimulated. Previous studies in our laboratory have evaluated secondary vestibular neuron activity in response to such stimulation. Here we compare afferent responses to responses of central neurons.
Methods: In rhesus monkeys, we recorded medial vestibular nucleus neuron activity and activity from 8th nerve afferents in response to biphasic pulse electrical stimulation with a human vestibular neuroprosthesis. The pulse rate and current amplitude, as well as the site of stimulation within each of 3 semicircular canals, was controlled by a computer which was interfaced to the implanted neurostimulator via an external processor and RF link. Stimulation was performed in a light tight booth in the dark. Eye movements were recorded with scleral coil.
Results: Vestibular nucleus neurons typically responded at monosynaptic latency to stimulation of a single canal. Increasing current amplitude increased the probability of eliciting a spike from any stimulus pulse. Typically, the probability of eliciting a spike at the highest current amplitude, which failed to elicit a facial nerve response, saturated below a probability of 1.0. Furthermore, at these higher current levels, vestibular nucleus neurons were typically driven from more than stimulation site (i.e., from more than one canal). With increasing pulse rate there was a decrease in the probability of a single pulse eliciting a spike. Trains of such stimuli elicited discharge that aligned on every stimulus pulse, or every second or third stimulus pulse. The typical stochastic nature of the neuron discharge was replaced with discharge that was time locked to the underlying electrical stimulus pulses. 8th nerve vestibular afferents displayed the same discharge properties in response to electrical stimulation, only at shorter latency.
Conclusions: Many discharge properties of secondary vestibular neurons are a direct reflection of the discharge of vestibular afferents during electrical stimulation with a vestibular prosthesis. Phenomena such as saturation of spike probability at intermediate levels and activation of individual neurons from multiple canals do not solely result from convergence of canal input at the level of vestibular nucleus neurons, but rather may be due to current spread across canals and the galvanic sensitivity of individual afferent fibers in response to such stimuli.

How Peripheral Vestibular Damage Affects Velocity Storage

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Background: Velocity storage is a centrally-mediated mechanism that processes peripheral vestibular inputs. One prominent aspect of velocity storage is its effect on dynamic responses to yaw rotation. Specifically, when normal human subjects are accelerated to constant angular yaw velocity, horizontal eye movements and perceived angular velocity decay exponentially with a time constant circa 15-30 s, even though the input from the vestibular periphery decays much faster (~ 6 s). Peripheral vestibular damage causes a time constant reduction, which is useful for clinical diagnoses, but a mechanistic explanation for the relationship between vestibular damage and changes in these behavioral dynamics is lacking. It has been hypothesized that Bayesian optimization determines ideal velocity storage dynamics based on statistics of vestibular noise and experienced motion. Specifically, while a longer time constant would make the central estimate of angular head velocity closer to actual head motion, it may also result in the accumulation of neural noise which simultaneously degrades precision. Thus, the brain may balance these two effects by determining the time constant that optimizes behavior.
Methods: We applied a Bayesian optimal Kalman filter to determine the ideal velocity storage time constant for unilateral damage.
Results: Predicted time constants were substantially lower than normal, similar to patients, and modeled interactions between age-related hair cell loss and peripheral damage.
Conclusions: These results provide a mechanistic explanation for changes in velocity storage after peripheral damage. Results also suggested that even after peripheral damage, ipsilateral noise originating in the periphery or early central processing remains relevant in neurocomputations. Overall, our findings support the hypothesis that the brain optimizes velocity storage based on the vestibular signal-to-noise ratio.

KCNQ4 Plays an Essential Role in the Protection of Vestibular Function Against Excessive Rotational and Gravity Stimulation

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Background: KCNQ4 is a voltage-gated K+ channel and thought to plays an important role in the maintenance of body balance by regulating depolarization and repolarization of vestibular hair cells and corresponding vestibular nerves in the inner ear. However, little is known about the vestibular phenotypes of KCNQ4 dysfunction and definite role of KCNQ4 in the vestibular organ. This study was conducted to identify the role of KCNQ4 in the vestibular system by investigating the vestibular function and histological and molecular changes of vestibular sensory epithelium in p.W276S/p.W276S Kcnq4 transgenic mice after the application of 6G hypergravity stimulation for 24 hours, which is a kind of excessive mechanical stimulation to the sensory epithelium. We also investigated vestibular phenotype of human KCNQ4 mutation.
Methods: 6G hypergravity stimulation for 24 hours was applied to wild type, homo, heterogenous genotype of p.W276S Kcnq4 mutation mouse. Vestibular function changes after the stimulation was compared to the baseline vestibular function of each genotype by measuring vestibulo-ocular reflex. Histological changes of sensory epithelium of vestibular organ in each mouse genotype was evaluated by immunocytochemistry. For comparing the difference of hair cell depolarization duration of each mouse genotype, intracellular [Ca2+] was measured using confocal microscopy. Retigabine (KCNQ4 activator) was treated to wild type mouse to investigate the preventive effect against hypergravity stimulation. Vestibular function test using vHIT and VEMP in patients with KCNQ4 mutation were examined to identify the vestibular phenotype of KCNQ4 mutation in human.
Results: Baseline vestibular function measured by vestibulo-ocular reflex in Kcnq4+/+, Kcnq4+/p.W276S and Kcnq4p.W276S/p.W276S mice was normal and it was significantly decreased after the hypergravity stimulation. Especially, the vestibular function decrease was severer in Kcnq4p.W276S/p.W276S mice than those in Kcnq4+/+ and Kcnq4+/p.W276S mice. Hair cell loss in the sensory epithelium was also more significant in Kcnq4p.W276S/p.W276S mice than those in Kcnq4+/+ and Kcnq4+/p.W276S mice. Hair cells in the sensory epithelium of Kcnq4p.W276S/p.W276S mice showed significantly increased depolarization duration than those in other genotypes. Retigabine prevented the vestibular dysfunction and hair cell loss after the hypergravity stimulation. Most patients (72.7%) with KCNQ4 mutation showed abnormal findings in clinical vestibular function tests.
Conclusions: The findings suggest that KCNQ4 plays an essential role in the protection of vestibular hair cells against excessive mechanical stimulation by contributing the regulation of hair cell repolarization.

Image Based Analysis of the Utricular Macula in the Rat
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Background: The sensory epithelia of the otolith endorgans within the vestibular system present a distinctive cellular organization. Different zones of the maculae exhibit regional diversity in number and type of hair cells and afferent innervations and morphology. Our lab utilizes a rat model to detail the pulsed infrared stimulation sensitivity of vestibular epithelia and the role of different cell types and synaptic inputs. To better understand how the maculae encode spatiotemporal properties of head movement, it is important to characterize this diverse morphology. Spatial characterization of the utricular maculae is typically performed through labor-intensive manual image processing of mounted immunostained samples. Here, we present a semi-automated approach to detail and quantify the morphological map of the utricular maculae in a rat model.
Methods: Utricular specimen were harvested from rat temporal bones for immunohistochemical studies. In whole mount preparations the hair cell bundles were labeled using antibodies against phalloidin and α-tubulin targeting stereocilia and kinocilia respectively. The maculae were visualized on a Leica TCS SP5 Confocal Microscope and Plan Apochromat 40X oil-immersion objective. The images obtained were analyzed via a custom MATLAB script. Image segmentation and thresholding were employed for a semi-automated determination of vestibular hair cell quantity, distribution, and stereociliary bundle orientation.
Results: Image analysis using the semi-automated approach was extended to complete a map of the rat utricular macula including morphometric data and hair cell distribution. In the samples analyzed, vestibular hair cells

present in the neuroepithelia were successfully identified using custom thresholds with image filters based on size, cell boundaries and fluorescence intensity. Details of macular differentiation, including the localization of the line of polarity reversal, were also determined by analysis of cell orientation measured as direction of the vector from the center of the cuticular plate to the center of the kinocilium.

Conclusions: The MATLAB-based approach represents an efficient tool for analyzing regional specializations in otoconial maculae and significantly reduces the time required for quantification. Detailing the cellular and synaptic architecture of the neuroepithelium is important for studies of various vestibular pathologies and for understanding the efficacy of potential therapeutic targets. Semi-automated tools such as those presented here could provide higher throughput for such work.

The Effect of Repetitive Linear Acceleration on Gravity Receptor Function

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Background: The vestibular system is crucial for posture, gait, and the perception of head and body position in space. Damage to this system can manifest as dizziness, imbalance, and poor postural control. Linear acceleration has been reported to result in measurable vestibular short-latency evoked potentials (VsEPs). The precise central neurons that contribute to the production of VsEPs are not well delineated. Manganese acts as a calcium surrogate, accumulating in active neurons. The paramagnetic nature of manganese permits visualization of these active neurons. Therefore, we have combined VsEP and manganese-enhanced magnetic resonance imaging (MEMRI) to assess vestibular function and visualize activity in central neurons responding to varying magnitudes of jerk stimulation (nonuniform linear acceleration).

Methods: Following anesthesia, each male Sprague Dawley rat (n=22) was attached to a mechanical shaker via a ceramic nut that had previously been attached with dental cement and centered on bregma. Each animal was then subjected to a jerk stimulation (either 500 g/s, 2,500 g/s, 3,200g/s or 6,000 g/s). Manganese chloride was administered just prior to stimulation. The stimulation was divided into three blocks. Each block consisted of five trials with 10-minute intervals between each block. For each trial, 200 jerk pair served as stimuli. Responses were recorded (CED power 1401 data acquisition system and Spike2 software) and analyzed using custom MATLAB scripts. Animals were also subjected to MRI (baseline, 24-hour post-, and 2-week post- stimulation) to assess manganese uptake in vestibular nuclei (lateral, medial, superior, and spinal vestibular nuclei).

Results: For each jerk stimulus, the P1 response latency was ~ 1 ms after the stimulus onset. While each jerk stimulus intensity resulted in VsEPs, the 500 g/s stimulus resulted in the least robust signal. The greatest signals were observed after moderate and intense stimulation. At each intensity, the P1 amplitude across the three blocks increased. The VsEP latency at P1 shortened then remained constant after 1000 jerk pairs. The P2 amplitude across the blocks was variable, while the latency was similar to P1. All the vestibular nuclei had elevated manganese uptake following stimulation versus baseline. Manganese uptake was least in animals after 500 g/s stimulation. Greater manganese uptake was observed in vestibular nuclei of animals subjected to jerks of 2500 g/s, 3200 g/s, and 6000 g/s.

Conclusions: Irregular fiber activity (P1) appears to increase over time in response to repetitive linear acceleration. However, neuronal activity in the vestibular nuclear complex (P2) continued to decrease following each block. Our results demonstrate graded increases in manganese uptake in vestibular nuclei corresponding to increases in linear acceleration, particularly in the spinal vestibular nucleus. Manganese and VsEP are promising tools which can be used to non-invasively map vestibular activity.

An Animal Model of Third Window Syndrome: Vestibular-Cochlear Based Explorations of Dehiscence Induced Impairments in Decision Making

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Background: Third window syndrome is a vestibular-cochlear disorder in humans in which a third mobile window of the otic capsule creates changes to the flow of sound pressure level energy through the perilymph/endolymph. The nature and location of this third mobile window can occur at many different sites (or multiple sites); however, the most encountered third window is congenital or acquired superior semi-circular canal dehiscence. The primary physiological symptoms include sound induced vertigo, headaches, pseudo conductive hearing loss, tinnitus, autophony while speaking, and visual problems (nystagmus, oscillopsia). At the same time,

individuals experience measurable deficits in basic decision-making, short-term memory, concentration, spatial cognition, and anxiety. The cause of many of these symptoms have been well-studied; however, without an animal model a fundamental understanding of the central processing changes will not be possible with our current technologies.

Methods: Adult Mongolian gerbils (N=18) received surgical fenestrations to the superior semi-circular canal of the left inner ear. Auditory brain stem responses were carried out prior to surgery and over 10 or more days of recovery. Some animals were trained on a basic decision-making task (amplitude-modulation (AM) rate discrimination) using an appetitive reinforcement operant conditioning procedure, prior to 3rd Window procedure (N=6). A sensitivity measure, d', was computed for each animal across training days. Histological examination of the fenestration was carried out for each animal post-hoc and compared to both ABRs and behavior.

Results: The fenestration created a significant elevation in hearing thresholds of the left ear; especially in the lower frequency domain (1 to 4kHz). In a subset of animals, left (EXP)/right ear(CTL) comparisons via ABR show significant threshold increases at the same frequency representations. For behavior, there was a significant effect on attenuated behavior over 5 additional days of testing. That is, post-surgery animals had lower initial d primes following surgery and took longer to return to pre-surgery levels of performance compared with sham surgery controls, with some animals failing to completely recover behavior.

Conclusions: The central basis of cognitive impairments associated with these kinds of peripheral injuries in humans is unknown. These preliminary results suggest that our animal model will provide a good basis for explorations into the central etiology of some of CNS based symptoms and decision-making impairments associated with 3rd window syndrome in humans.

Galvanic Vestibular Stimulation Activates Parietal, and Temporal Cortex in Humans
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Background: Vestibular Galvanic Stimulation (GVS) has been shown to help stabilize subjects when balance and posture are compromised. In this work we intend to define the cortical regions that are activated by GVS in normal subjects.

Methods: We used the Functional Near Infrared Spectroscopy (fNIRS) to determine the effect of GVS on the cerebral hemodynamic response of the primary somatosensory cortex, associative somatosensory and upper and middle temporal of the left and right cerebral hemisphere. The GVS (cathode in right mastoid and anode in the fronto-polar point) of 2 mA and 10s was used. False GVS (sham), vibration and pasive movement sttimuli were also used. To analyze the cortical response produced by these stimuli in 18 clinically healthy volunteers.

Results: Seventy-two sessions were carried out applying a crossover experimental design. Participants' heart rate, blood pressure, body temperature, head capacitance and resistance were measured before and after each session. The hemodynamics recording of the cerebral cortex was performed with fNIRS over an arrangement of 26 channels, grouped into four regions to perform a ROI-level analysis. The passive movement generated the highest response. A similar significant response was found with GVS stimuli on the right temporal, anterior and posterior parietal regions. Sham and vibrational conditions did not generate significant changes ROI-wise.

Conclusions: Our results show that GVS produce a cortical hemodynamic response which is analogous to the activation induced by passive movement of the subject. This result lend further support to the possibility of using GVS in vestibular prosthetic devices.

Modulation of Response Properties of Vestibular Nerve Afferents by Activation of GABA-B Receptors in the Inner Ear
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Background: We have previously shown that activation of GABA-B receptors results in an excitatory effect on calyx nerve terminals.

Methods: Here, we used in vitro patch clamp recordings from calyx afferent terminals and type II hair cells and vestibular sensory evoked potential recordings (VsEP) from vestibular nerve fibers to further explore the effect of baclofen, a GABA-B agonist. For calyces, we injected a sweep of sines (0.01-100 Hz) or single sines (at different

frequencies from 0.1 – 100 Hz) to the calyx before and after baclofen application. For type II hair cells, we used hyperpolarizing and depolarizing voltage steps to investigate changes in their voltage sensitive currents.
Results: Activation of GABA-B receptors resulted in an increase in the frequency range that calyces responded to (i.e., fired action potentials), an increase in the overall gain of the response, and a decrease in response phase. Overall, the calyces responded faster and at higher rates, with stronger phase locking to the stimulus. In type II hair cells, baclofen increased potassium currents during voltage steps, having a hyperpolarizing effect on these cells. Next, we used VsEP recording and in vivo intralabyrinthine injection of baclofen in anesthetized mice to investigate the net effect of GABA-B activation on the vestibular nerve response. Baclofen injection resulted in an increase in synchronized response of irregular afferents to a fast linear movement, as measured by VsEP recordings. Intracochlear injection of a GABA-B antagonist (CGP 35348) had the opposite effect, suggesting the presence of spontaneous release of GABA in the vestibular periphery.
Conclusions: These results provide evidence GABA-B activation could be used as a modulatory mechanism to increase phasic properties of irregular afferents.

Development and Content Validity of the Bilateral Vestibulopathy Questionnaire
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Background: To date, the burden and severity of the full spectrum of bilateral vestibulopathy (BV) symptoms is not measured in a standardized manner. Since therapeutic interventions focusing on improving BV symptoms are emerging (e.g. the vestibular implant, balance belt and noisy galvanic stimulation), the need for a new standardized assessment tool that embraces all aspects of BV arises. Therefore, the aim of this study was to develop a multi-item Patient Reported Outcome Measure (PROM), that captures all clinically important symptoms of BV and assesses its impact on daily life.
Methods: The development of the Bilateral Vestibulopathy Questionnaire (BVQ) consisted of three phases: (I) initial item generation; (II) cognitive interviews to gain insight in patients' perspectives (face and content validity testing); and (III) an international expert meeting to obtain input from BV experts. Items were derived from a previous literature review and individual semi-structured interviews focusing on the full spectrum of reported BV symptoms (phase I). Subsequently (phase II), individual patient interviews were conducted using thinking aloud and concurrent verbal probing techniques to assess the comprehensibility of the instructions, questions and response options (face validity), and the relevance, missing domains or missing items (content validity). Finally, international experts with extensive experience in the field of the physical, emotional and cognitive symptoms of BV were invited to participate in an online focus group to assess the relevance and comprehensiveness of the BVQ (phase III).
Results: The BVQ that was developed consisted of two sections. The first section included seven constructs with a total of 50 items scored on a six-point Likert scale: three constructs regarding physical symptoms (imbalance, oscillopsia and other physical symptoms), one construct encompassing cognitive symptoms, one construct encompassing emotional symptoms, one construct comprising limitations and behavioral changes and one construct regarding social life. The second part of the BVQ consisted of four scale items, scored on a visual analog scale from zero to 100, to inquire about limitations in daily life, perceived health and expectations regarding future recovery. Face validity tests with BV patients confirmed face and content validity of the developed BVQ (n=8, 50% female, mean age 56 years (range 24-88 years)).
Conclusions: The BVQ was developed to assess the full spectrum of BV symptoms and its impact on daily life. It can be used to characterize the current self-reported symptoms and disability, and for evaluating treatment efficacy for therapeutic interventions such as vestibular implantation. Furthermore, the questionnaire can be used as an addition to the diagnostic BV criteria to portray symptom severity, and for patient counselling and shared clinical decision making in research and clinical settings.

Round Window Assays of Synapse Loss in Aging Gerbil
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Background: Declines in auditory function are common with age and understanding peripheral contributions to such changes is key to informed diagnosis and therapy. Our prior work in animal models of cochlear aging and noise exposure shows that loss of synapses between inner hair cells (IHCs) and afferent neurons is a primary event and suggests that some auditory nerve fibers may be more vulnerable than others. Here, we address this question by studying IHC synapse loss and its electrophysiological consequences in normally aging Mongolian gerbil, a lower-frequency hearer with well-characterized distributions of auditory nerve fibers (ANF) by spontaneous rate (SR) subtype.

Methods: Cochlear function was characterized in an age-graded series of Mongolian gerbils (MF; 14 -144 wks). We employed standard measures of distortion product otoacoustic emission (DPOAE) and auditory nerve compound action potential (CAP) response thresholds and suprathreshold response growth over a frequency range from 2 - 45 kHz. In the same animals, we captured round-window-recorded spontaneous neural noise and sound-evoked post-stimulus time responses (PSTRs), providing insights on the relative contributions of ANFs by SR subtype to these global responses. Immunostained cochlear whole mounts and OCT-embedded sections were studied to quantify hair cells, neurons and the synapses that connect them, and to assess strial condition.

Results: DPOAE and CAP threshold elevations were mild (<15 dB) over the age range of monitoring. They were accompanied by response amplitude declines; for DPOAEs, these were largely restricted to extended basal and apical frequencies where small threshold elevations were evident, whereas for CAPs, declines were present across frequency and averaged about 30% in oldest groups. Sound-driven PSTRs also showed significant changes with age, with 30-40% declines in the magnitude of the high-SR-dominated response peak and up to 50% reduction, depending on frequency, in the steady-state response plateau, which reflects the heterogeneity of ANF distribution in gerbil. In the same ears, spontaneous neural activity showed small declines to 64 wks, but by 96 wks, the high-SR dominated 900 Hz spectral density was little evident in the recorded activity. OHC loss was <20% and restricted to the extreme apex and base. No IHC loss was observed. IHC synapse losses progressed gradually with age across frequency and were modest, reaching about 25% in oldest age groups. Assessment of strial condition is underway.

Conclusions: In animals with well-preserved threshold sensitivity that were assessed to roughly 75% of their expected lifespan in a noise-controlled laboratory environment, age-graded declines in peripheral neural activity were revealed and suggest mixed involvement by SR subtype. Combined, responses captured key properties of the auditory nerve response that should yield important diagnostic information in hearing loss etiologies producing cochlear synaptic and neural loss.

Effects of Aging on In Vivo AVCN Unit Responses in Mice

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Background: Hearing worsens with age, particularly the ability to detect and process speech in noisy environments. Hearing in noise is particularly difficult for individuals with age-related hearing loss, who often report feelings of social isolation and depression. While the major peripheral changes associated with age-related hearing loss are well established, it is not fully understood how changes in the periphery lead to functional changes in aging auditory brainstem neural responses in noise.

Methods: To explore how aging affects coding of sounds in quiet and noise in anteroventral cochlear nucleus neurons (AVCN), we performed in vivo recordings on two mouse strains- CBA/CaJ and C57BL/6J, which show normal and accelerated age-related hearing loss, respectively. Once a chopper or primary-like unit was isolated, tones were played at the characteristic frequency of the cell in the presence and absence of a background noise masker set at either 20 or 30 dB above the cell's noise threshold.

Results: Thresholds increased in the presence of a noise masker for both chopper and primary-like units. The amount of threshold shift, however, was not different between mouse strains and was unaffected by age. The most reliable predictor of a unit's threshold shift in noise was its sensitivity in quiet: units with low threshold demonstrated a larger threshold shift in noise than those with high threshold.

Conclusions: Our experiments indicate that among AVCN neurons that continue to receive input from the periphery, relatively normal function is conserved to enable continued responses to acoustic stimuli, even in noisy acoustic environments. Despite cochlear degeneration in advanced age, the aged AVCN appears ready to receive input from peripheral auditory structures and benefit indirectly from therapies aimed at improving sound detection in the periphery.

Evaluation Effects of Aging on the Central Vestibular System in BXD Mice Strains Using High-Resolution MRI Imaging

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Background: Dizziness, or imbalance, arises from vestibular dysfunction, an elegant and highly conserved neuroanatomical pathway that mediates the ability to perceive linear acceleration, gravity, and angular head motion. Previous research illustrates that the human central vestibular system changes with the aging process, however, little is known about how the aging process affects vestibular structures within the mouse brain. While the mouse model contains less white matter than the human brain, it contains a similar axonal diameter, making it an excellent model for imaging studies. Additionally, neurological and behavioral differences between strains have been identified and multiple strains can be used to create a diverse study population that mimics human diversity. Therefore, the objective of this study was to determine how structures within the vestibular system are affected by the aging process with MRI and to compare these differences between multiple BXD strains.
Methods: Following perfusion fixation, the mouse was sacrificed and the brain and skull were fixed in formalin. The specimens were mounted in a 12 mm diameter radiofrequency (rf) coil composed of silver foil. Magnetic resonance (MR) images were acquired on a 9.4T vertical bore Oxford magnet with Resonance Research gradients providing ~2000 mT/m maximum gradient. After imaging, the files were composed into a 4D array ($256 \times 256 \times 420 \times 51$) and passed to a post-processing pipeline that registered the five b0 images together. A 3D label set was registered onto each 4D volume in the reference frame in which the data were acquired. 184 mice imaged with the aforementioned protocol. The mice consisted of 12 different BXD mice strains (24, 29, 34, 43, 44, 48a, 51, 60, 62, 65b, 89, and 101).
Results: Eight structures within the central vestibular pathway were labeled and analyzed. Nearly all of the structures increased with age, with the exception of the Ventral Posterolateral Thalamic Nucleus (VPL). The Medial Longitudinal Fasciculus (MLF) showed the largest percent increase of all structures.
Conclusions: These findings highlight distinct strain differences in the central vestibular structures. Patterns of aging were seen within the individual structures as well. The large percentage increase in the MLF highlights the dynamic role it plays in visual and vestibular stability. The cause of these changes remains unclear, but it may be related to an increase in immune-related cells, such as glial cells, or synaptic pruning. In contrast, the VPL, essential in maintaining postural stability, was the only structure to decrease with aging. This may be related to an increased risk of falling in the elderly population, but warrants future investigation.

Age-Related Ultrastructural Changes Across the Central Nucleus of the Inferior Colliculus in Fischer Brown Norway Rats

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Background: Downregulation of GABAergic neurotransmission in the inferior colliculus (IC) leads to impairments characteristic of presbycusis. It has been demonstrated that GABAergic and excitatory synapses in the central IC (ICc) decline with age. We sought to determine whether synaptic decline occurs uniformly across the tonotopic ICc.
Methods: We assessed 3-, 24-, and 28–month-old Fischer Brown Norway (FBN) rats, which develop low frequency presbycusis at ~24 months of age. We divided the ICc into three equal regions across the dorsomedial-ventrolateral axis to represent low, middle and high frequencies. We used transmission electron microscopy to characterize GABAergic and excitatory synapses, their post-synaptic targets and presynaptic mitochondria. Ultrathin sections (~50 nm) were reacted for anti-GABA immunochemistry and stained with uranyl acetate and lead citrate. GABAergic synapses were identified as having pleomorphic vesicles, symmetric synaptic junctions, and GABA-positive presynaptic boutons. Postsynaptic targets comprise somas, dendrites of three calibers (<0.05 µm, between 0.5 and 1.5 µm and >1.5 µm) and spines. A total of 3,185 (1,335 GABAergic, 116 non-GABAergic inhibitory and 1,734 excitatory) synapses were characterized.
Results: We found that age-related changes to synaptic density, bouton area and synaptic redistribution were diminished at low frequency when compared to high and middle frequencies. At 28 months 15% of GABAergic synapses and 14% of excitatory synapses had been lost in low frequency ICc, while 27% of GABAergic and

excitatory synapses were lost in middle frequency ICc, and 37% of GABAergic and 32% of excitatory synapses were lost in high frequency ICc. For each ICc region, GABAergic synaptic loss at 24 months was nearly identical to what was observed at 28 months, while excitatory synaptic loss for each region was greater than the respective loss observed at 28 months. The average area of GABAergic boutons in middle and high frequency ICc increased with age, while GABAergic bouton area in low frequency ICc remained the same. The percentage of GABAergic synapses targeting large-caliber dendrites increased with age only slightly in low frequency IC, but much more dramatically in middle and high-frequency ICc. Percentage of target dendrites that were GABAergic also increased with age, from ~10% to ~30%.

Conclusions: It is interesting that in our model, which acquires low frequency presbycusis, the most dramatic changes occurred in middle and high frequency ICc. Perhaps these greater changes reflect frequency specific subcollicular changes and/or a greater need for synaptic compensation at these frequencies during aging. The larger average GABAergic bouton area observed at old age underscores that the lost GABAergic synapses were from smaller boutons, which may indicate that a specific source of GABAergic input is lost during aging. Taken together, the ICc undergoes a non-uniform age-related loss of both GABAergic and excitatory synapses at middle and old age.

Synaptic Release Potentiation at Aging Auditory Ribbon Synapses
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Background: Age-related hidden hearing loss is often described as a cochlear synaptopathy that results from a progressive degeneration of the inner hair cell (IHC) ribbon synapses. The progressive functional changes occurring at these synapses during aging are not fully understood. Here, we characterized this aging process in IHCs of C57BL/6J mice, a strain which is known to carry a cadherin23 mutation and experiences early hearing loss with age.

Methods: ABRs and DPOAEs in mice were measured using a TDT RZ6/BioSigRZ system. Acoustic startle reflex were measured with a SR-LAB-Startle Response system. Fluorescent Ca2+ microdomains at IHC ribbon active zones were measured in ex-vivo organ of Corti with a C2 confocal system and NIS-element imaging software (Nikon) coupled to the FN1 upright microscope. Measurements of Ca2+ currents, BK currents and membrane capacitance of IHCs were performed under whole-cell patch clamp recording in IHCs from P30 and P365 mice by using an HEKA EPC10 amplifier controlled by Patchmaster software. The structural changes of the presynaptic ribbons and postsynaptic AMPARs were characterized under high resolution immuno-confocal microscopy.

Results: C57BL/6J mice, while displaying a large increase in Auditory Brainstem thresholds due to 50 % loss of IHC synaptic ribbons at middle age (postnatal day 365), paradoxically showed enhanced acoustic startle reflex at low sound intensity under white noise stimulation, suggesting a hyperacusis-like response. The auditory defect during aging was associated with a large shrinkage of the IHCs' cell body and a drastic enlargement of their remaining presynaptic ribbons which were facing enlarged postsynaptic AMPAR clusters. Old P365 C57BL/6J IHCs preferentially loose modiolar ribbons (known to be associated with high threshold low spontaneous fibers), therefore mimicking what is happening during noise-induced hearing loss (Fernandez et al., 2015; Liberman and Kujawa, 2017; Hickman et al., 2020). Remarkably, presynaptic Ca2+ microdomains and the capacity of IHCs to sustain high rates of exocytosis were largely increased with aging. In these old IHCs, we also found a disruption of the BK channel clusters and a drastic positive shift in their voltage-dependence, decreasing their potential negative feedback on neurotransmission.

Conclusions: Our study shows that aging IHCs ribbon synapses undergo important structural and functional plasticity that leads to synaptic potentiation. These changes could explain the paradoxical hyperacusis-like exaggeration of the acoustic startle reflex observed in aging C57BL/6J mice.

Peripheral and Central Auditory Function and Dysfunction in APP/PS1 Mice
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Background: In the elderly, hearing loss partially predicts the rate of subsequent cognitive decline, suggesting that audiologic testing may serve as a diagnostic or biomarker for Alzheimer's disease (AD). Factors underlying the correlation between hearing loss and dementia are not clear. Peripheral hearing loss may reduce sensory input to the brain and/or increase cognitive load, thereby leading to CNS dysfunction and suggesting that aural rehabilitation may mitigate cognitive decline. Understanding the associations between hearing loss and various forms of dementia is essential for developing diagnostics and interventions. In mice, we have identified the protein precursors and enzymes required for AD-like pathology in the cochlea and vestibular organs, including elevated levels of Aβ42 and Aβ40 in APP/PS1 mice. This raised the possibility that AD-like pathology in the inner ear may parallel AD pathology in the brain and that genetic associations between age-related hearing loss and dementia underly a common cause of neuronal pathology affecting audition and cognition.

Methods: To determine whether mutations in App and Psen1 that are associated with familial Alzheimer's disease also cause sensory degeneration in the cochlea or affect auditory behaviors, we measured the auditory brainstem response (ABR) and acoustic startle reflex in aging APP/PS1 mice (APPswe/PSEN1ΔE9; C57BL/6J:C3H background). Transgenic mice (Tg) were heterozygous littermates of control mice (WT). Sanger sequencing and PCR confirmed absence of Cdh23 ARHL mutation. Aβ42 and Aβ40 ¬levels were measured with ELISA. Protein immunohistofluorescence was imaged with confocal microscopy.

Results: Using APPKO mice for control, we found higher levels of Aβ42 and Aβ40 in temporal bones from adult Tg mice relative to WT. Amyloid precursor protein (APP), Tau protein (MAPT), and β-secretase (BACE1) were expressed in the cochlea and vestibular organs of WT and Tg mice. Published datasets (e.g., umgear.org) further indicate that β-secretase and gamma-secretase, the proteases responsible for cleaving APP into Aβ, are present in the sensory epithelia of the ear, suggesting that amyloid or Tau deposition and subsequent pathology may occur in these tissues. We observed aggregates of Aβ near inner hair cell synapses, but we did not observe amyloid plaques in the inner ear. ABR thresholds as well as ABR waves I-V and DPOAE I/O functions were similar between Tg and WT mice at at 6, 12, and 16 months. Acoustic startle magnitude was increased, and pre-pulse inhibition of acoustic startle was reduced in Tg relative to WT mice.

Conclusions: APP/PS1 mice have altered acoustic startle behaviors that are not readily explained by peripheral or central auditory field potentials, suggesting involvement of extra-auditory brain regions. Although the inner ears of APP/PS1 mice have elevated levels of Aβ, cochlear output appears unaffected, suggesting that APP/PS1 mutations in familial AD are not sufficient to cause peripheral auditory dysfunction.

Increasing Cochlear Ntf3 Expression at Mid-Life Slows the Rate of Age-Related ABR Peak 1 Amplitude Decrease and Reduces Age-Related Cochlear Synaptopathy.

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Background: Age-related hearing loss (ARHL) is one of the most common health disorders, affecting a third of people over age 65 and half of those over 85 in the US. Histopathology of mouse and human inner ears shows that inner hair cell (IHC) synapse loss (cochlear synaptopathy) is an early event in AHRL, preceding loss of hair cells and spiral ganglion neurons. Although loss of a portion of IHC synapses may not result in threshold elevation, the reduced neural transmission likely contributes to difficulties in understanding speech in the presence of background noise.

Neurotrophin-3 (Ntf3) is a critical trophic factor in sensory neuron survival and establishment of neuronal projections in the inner ear. We previously showed that Ntf3 produced by supporting cells of the organ of Corti plays a critical role in the formation of IHC synapses and that increasing Ntf3 levels induces synapse regeneration and cochlear functional recovery after noise-induced cochlear synaptopathy in young adult mice.

Methods: Here, we investigated whether increasing Ntf3 expression levels in aging mice prevents or slows synapse loss and alters the course of ARHL using a tamoxifen-inducible transgenic mouse line (Ntf3Stop:Slc1a3/CreERT) that drives Ntf3 overexpression in inner border and inner phalangeal supporting cells. Ntf3Stop littermates were used as controls.

Ntf3 overexpression was induced by intraperitoneal tamoxifen injection at 60 weeks of age, and auditory brains stem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs) were measured at multiple time

points over the following additional 35 wks. After the final test, we evaluated Ntf3 expression by RT-qPCR, as well as HC numbers and IHC synapse counts by immunostaining.

Results: Ntf3 overexpression increased suprathreshold ABR peak 1 amplitude without threshold changes 1 week after induction. Furthermore, despite decreasing with age, ABR peak 1 amplitudes remained larger in the Ntf3 over-expressing mice than in controls for the duration of the test period. Quantitative RT-PCR demonstrated that in controls, cochlear Ntf3 mRNA levels at 95 weeks are 40% lower than at 10 weeks, but they remain at the same levels as the young in over-expressers. Furthermore, at 95 weeks, over-expressers exhibited 14-17% more IHC synapses relative to controls. There is a strong correlation between Ntf3 mRNA levels, synapse count and ABR peak 1 amplitudes ($p < 0.03$ and $r > 0.7$), indicating that these variables are co-regulated.

Conclusions: These results indicate that Ntf3 supplementation can rapidly improve cochlear function in middle-aged mice and slow the progression of age-related hearing loss. Furthermore, the effects of Ntf3 appear to be related to the maintenance of IHC synapses, suggesting that supplemental Ntf3 reduces age-related synaptopathy.

Neural Activation and Representations During Auditory Selective Attention in fMRI

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Background: Spatial and non-spatial auditory attention recruit different networks of brain regions, as measured by the degree of BOLD signal activation in those networks. However, no prior work has used fMRI data to understand what information is represented during auditory selective attention, and where in the brain these representations are produced. In this study, we explored both BOLD activation and representational patterns during a challenging listening task where listeners direct attention to a target syllable played among distractors.

Methods: We designed an auditory experiment that required spatial, non-spatial or no attention from listeners (n=19). The experiment had many (21) conditions, so that we could build rich representational dissimilarity matrices (RDMs). First, a general linear model (GLM) identified regions of increased brain activation during spatial and non-spatial attention, as well as regions that differed between them. We then defined a searchlight (radius = 4 voxels) around each voxel. For each voxel and each pair of conditions, we estimated their difference within that searchlight as the Euclidean distance between their activation patterns. A representational dissimilarity matrix (RDM) summarized these pair-wise differences at each voxel. Finally, we characterized patterns in these RDMs using multidimensional scaling and comparison to (ideal) conceptual models, particularly models where the information differentiates target location and target talker pitch/gender.

Results: The GLM analysis revealed that when listeners engage attention, they recruit a distributed brain network. Specifically, the superior precentral sulcus, superior parietal lobule and intraparietal sulcus are bilaterally more active in spatial than in non-spatial attention, whereas the superior temporal gyrus shows the opposite. The inferior frontal sulcus is similarly engaged in spatial and non-spatial attention. The representational analysis identified the medial occipital cortex, including the calcarine sulcus and lingual sulcus, as a brain region where information about the attended location is represented, while information about the gender of the target talker is represented in the right inferior frontal sulcus.

Conclusions: We identified a distributed brain network comprising regions in frontal, parietal, and temporal lobe that are differentially recruited during different forms of auditory attention to similar stimuli. Frontal and parietal regions were more strongly recruited for spatial attention, and temporal regions for non-spatial. We also identified two regions where specific information about the target of attention is represented. These results are the first to show that we can use fMRI to measure where and how the brain represents information during auditory selective attention.

Acoustic- Versus Category-Encoding of Vowel Sounds During Natural Speech in Human Heschl's Gyrus

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Background: During natural speech perception, the human brain transforms sound into meaning through mechanisms which remain largely unknown. The predominant view posits that preceding lexical or semantic interpretation, speech is first encoded in acoustic space and then parsed into a sequence of phonemes. While intracranial studies have shown differentiation of phonetic properties in lateral superior temporal gyrus (STG), it is

unclear whether this region mediates an acoustic-to-categorical transformation or merely inherits it from lower-order regions. We hypothesized that encoding of phoneme coding takes place in the STP, as opposed to acoustic properties alone.

Methods: Here we use sEEG to record from the bilateral supratemporal plane (STP), including Heschl's gyrus, to interrogate the pattern of neural encoding of vowel sounds during natural speech. Intracranial records were acquired for 6 patients while listening to approximately 60-minutes of natural, continuous speech. Stimuli were segmented and labeled for phoneme identities, onsets, and offsets. Neural responses were extracted for all vowel instances, excluding diphthongs. Pairwise decoding was performed for each vowel category pair. Vowel pairs with significant formant overlap were used (e.g. i vs. ɪ, ɛ vs. ʌ) to test the representation of acoustic versus category features in the STP. To measure decoding ability of acoustic versus category-based encoding models, we compared vowel categories with formant dimension held constant (mismatched category + matched acoustics) to same-category vowels distant [separated] in formant space (mismatched acoustics + matched category).

Results: Distance in decoding accuracy between all vowel pairs was well correlated with the pairwise distance in acoustic space. Neural encoding accuracies were not significantly higher between mismatched vowel category compared to mismatched acoustics within Heschl's gyrus.

Conclusions: These results suggest that ensemble neural representations across channels are closely matched to Formant space, or acoustic relatedness. Taken together, these results suggest that neural representation of vowels, when present in natural speech, is better differentiated based on the acoustic information of the vowel sound, compared to the vowel category. In other words Heschl's gyrus in particular, appears to be more engaged in encoding acoustic vowel representations than transforming them into categorical identities.

Functional Geometry of Auditory Cortical Resting State Networks Derived From Intracranial Electrophysiology

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Background: Introduction: Intracranial electroencephalographic (iEEG) recordings from neurosurgical epilepsy patients provide an opportunity to characterize cortical resting state networks (RSNs) with high spatio-temporal precision. Outstanding questions on the organization of the auditory cortical hierarchy include (1) the position in the cortical hierarchy and role in each stream of the planum polare (PP), a region of the superior temporal plane (STP) that is immediately anterior to Heschl's gyrus but demonstrates functionally distinct response properties; (2) the functional roles and connectivity of the upper versus lower bank of the superior temporal sulcus (STSU, STSL), long known to be an important structure in speech and language processing; (3) the degree to which hierarchical organization differs in the language dominant versus non-dominant hemisphere; (4) the details of the organization of connectivity between auditory cortex and limbic structures which likely play critical roles in memory and emotional processing. Here, we apply diffusion map embedding (DME) to a large dataset to map the hierarchical structure of the auditory cortical network and its relationship to prefrontal and limbic structures.

Methods: Methods: Resting state iEEG data were obtained from 46 adult neurosurgical patients awake, daytime recordings. Electrodes were implanted in temporal, parietal, and frontal cortex, with extensive coverage in auditory and other lateral temporal, sensorimotor, prefrontal, limbic structures, and more limited coverage in parietal cortex. Data were denoised and segmented into 1-minute epochs, and functional connectivity was measured as pairwise orthogonalized band power envelope correlations. To combine across subjects, recording sites were assigned to regions of interest (ROIs) and connectivity averaged within ROI, then across subjects. Channel-by-channel or ROI-by-ROI adjacency matrices were thresholded and normalized before DME analysis. To evaluate the effects of sparse, non-random sampling in electrophysiological experiments, functional connectivity was also estimated from pre-surgical resting state fMRI data obtained in 10 subjects and compared to iEEG.

Results: The data cloud in embedding space exhibited hierarchical organization, with early auditory cortical ROIs on STP, (middle/psoterior) superior temporal gyrus (STG), and STSU clustered together, a cluster of prefrontal cortical ROIs located maximally distant, and a cluster of limbic structures also remotely positioned. STSL and PP were distant from the early auditory cluster. No significant differences in functional organization were observed for left versus right hemispheres.

Conclusions: The hierarchical organization of auditory cortical and related structures is evident in iEEG data mapped into embedding space. We observe evidence for both dorsal and ventral streams, as well as a third auditory stream, a limbic stream fconnecting early auditory structures iwth hippocampus and amygdala. The

remote locations of STSL and PP suggests that these regions are at considerably higher level of the auditory hierarchy compared to other superior temporal structures. The absence of functional lateralization suggests that such specializations may only emerge during auditory processing.

Evidence of Direct and Indirect White Matter Pathways From Auditory Cortex to Dorsal Premotor Cortex

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Background: Numerous recent studies have found activation in a region of the dorsal premotor cortex (dPM) during listening to speech. This region appears to respond generally to speech but also specifically to vocal pitch cues within the speech signal; crucially, it also appears to overlap with the dorsal laryngeal motor cortex, which plays a role in voluntary laryngeal control.

Methods: We conjectured that the bimodal response profile of dPM corresponds to transmission of speech cues along two different auditory-motor white matter pathways: a direct pathway from the auditory cortex (auditory-pitch mode) and an indirect pathway through inferior parietal or inferior frontal speech production regions (articulatory-speech mode). Here, we use diffusion-weighted imaging (DWI) to map the pathways between auditory cortex (AC) and dPM. DWI with 64-directional encoding and multiple b-values (1000, 2500) was performed on 37 Veterans (<= 60 years). Fiber orientation distribution functions (FODs) were obtained using multi-shell constrained spherical deconvolution and merged to a common FOD template using diffeomorphic warping. Probabilistic whole-brain tractography (20M streamlines) was performed on the template using iFOD2; streamline weights corresponding to fiber cross-sectional area were obtained using SIFT2. Seeds in pitch-responsive AC and dPM were defined from fMRI data acquired on a subset of the subjects (N=24). Streamlines were then filtered to retain only those that terminate in either AC or dPM. To find intermediate areas connecting these regions, we generated an 'endpoint interaction map' showing brain areas with substantial streamline terminations originating from both AC and dPM.

Results: Two regions with strong interactions were identified: a posterior parietal cortex (PPC) region and an inferior frontal gyrus region (IFG). To map the white matter pathways connecting these regions (streamline geometry) and their connection strengths (SIFT2-weighted streamline counts), we then generated a 'mini-connectome' with AC, dPM, PPC, and IFG as the connectome nodes. The connectome analysis revealed three pathways: (i) a weak direct pathway from AC to dPM; (ii) a stronger indirect pathway from AC to IFG to dPM; and (iii) a very strong indirect pathway from AC to PPC to dPM. Of the indirect pathways, (ii) was consistent with the known architecture of sensorimotor speech networks, but (iii) was unexpected. Examination of all streamline terminations from PPC revealed connections to AC, intraparietal sulcus, frontal eye field, and dorsal motor areas controlling larynx, eye, head and hand movements.

Conclusions: We suggest that pathway (i) implies laryngeal control in an auditory-pitch mode; pathway (ii) supports laryngeal control in an articulatory-speech mode; and pathway (iii) performs effector selection within a dorsal, auditory-motor orienting circuit that includes the larynx.

Deviant Selectivity Within Auditory Cortical Neurons for Stimuli With Varying Contrast

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Background: Stimulus Specific Adaptation (SSA) is the adaptation of neural responses to a repeated Standard stimulus. Further, a different and rare stimulus (Deviant) in the stream of Standards evokes an enhanced response, the basis of deviant detection. Both deviant detection and SSA are some of the characteristic features of the auditory cortical neurons. Previous studies have characterised SSA based on parameters like the distance between Standard and Deviant (octaves), tuning width and relative occurrence probabilities of the two frequencies. Auditory studies so far have characterised SSA and deviant detection primarily using pure tones. Computational models have also been made to explain deviant detection and SSA of pure tones and all of these models rely on principles such as feed-forward excitation, depressing synapse and narrow frequency channel hypothesis. The narrow frequency channel hypothesis considers independent narrow channels for every frequency running along the hierarchy starting from the cochlea to the auditory thalamus and finally to the auditory cortex, forming separate synaptic inputs in the auditory cortical neurons with their independent neurotransmitter pools. Contrary to

this hypothesis, we performed electrophysiology and 2-photon imaging experiments in the mouse primary auditory cortex using Noise-Tone oddball stimulus (Broad-band noise as standard and Tone as deviant) and got significantly higher responses on deviant. These observations do not fall in line with the narrow channel hypothesis and give rise to the possibility that the auditory cortical neurons might be encoding some other parameter rather than a linear combination of the frequency components present in the stimuli.

Methods: We used spectral contrast (2nd-order moment) as a spectral shape parameter to probe deviant detection in A1 based on spectral shapes. We performed our experiments in anaesthetized mice using random spectral shape (RSS) stimuli with different contrasts as Standard and Deviant and found robust responses along with SSA. RSS stimuli essentially consist of 25 frequency bins starting from 6kHz to 48kHz with a bin width of 1/8th octave, each bin containing 8 tones 1/64th octave apart. Amplitudes of tones in each bin were drawn from a Gaussian distribution with 0 dB as mean, and the STD as the contrast of the set of stimuli. Each tone was started at a random phase drawn uniformly from 0 to 2π.

Results: We observed that contrast selectivity operates independently of deviant selectivity and neurons within the auditory cortex showing a high preference for stimuli with specific contrasts. From the imaging experiments, we evaluated the specific roles of inhibitory interneurons like parvalbumin (PV) and somatostatin (SOM) in modulating the contrast selectivity within the auditory cortical neurons. We found that the modulating effects of PV neurons are more network-driven as compared to the SOM and excitatory neurons.

Conclusions: Auditory cortical neurons are selective to the contrast of the stimuli.

The Role of VIP Signaling From the Inferior Colliculus in Modulating the Excitability of Medial Geniculate Neurons

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Background: Many neurons in the medial geniculate (MG), the thalamic relay nucleus of the ascending auditory pathway, express receptors for vasoactive intestinal peptide (VIP), a neuropeptide known to play important signaling roles in several brain regions. We recently uncovered that VIP neurons in the inferior colliculus (IC), a class of glutamatergic principal neurons, project to the MG and express VIP mRNA, pointing to these neurons as a potential source of VIP signaling to MG neurons. VIP signaling has been shown to potently influence neuronal excitability in the somatosensory thalamus, but whether VIP signaling plays a similar role in the MG remains unknown. Based on these data, we hypothesized that VIP signaling modulates the excitability of MG neurons and that IC VIP neurons are an important source of VIP signaling in the MG.

Methods: To test this hypothesis, we used brain slice electrophysiology, pharmacology, immunofluorescence, and anterograde tracing in MG slices prepared from VIP-IRES-Cre x Ai14 mice of both sexes.

Results: We found that puff application of 2 μM VIP near the cell soma elicited depolarization in most MG neurons. This depolarization was potent enough to reach action potential threshold when MG neurons were held at -61 mV during current clamp recordings. We are currently using [D-p-Cl-Phe6, Leu17]-VIP, a VIP receptor antagonist, to determine if the depolarization elicited by VIP application is mediated by VIP receptors. In addition, we are using a Cre-dependent adeno-associated virus to fluorescently label VIP neurons in combination with anti-VIP immunofluorescence to determine whether the terminals of IC VIP neurons express VIP peptide in the MG. Our preliminary data indicate that the MG terminals of VIP neurons express VIP peptide, suggesting that IC VIP neurons may be a source of VIP signaling in the MG.

Conclusions: Our data shows that VIP elicits depolarization and firing in many MG neurons, indicating that VIP is a potent neuromodulator in the MG. Furthermore, our preliminary results suggest that IC VIP neurons may be a major source of VIP signaling in the MG. Thus, VIP signaling from the IC to the MG may play an important and previously unappreciated role in modulating auditory processing in the tectothalamic pathway.

Neural Correlates of Perceptual Invariance in the Ferret Auditory Cortex

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Background: Perceptual invariance, the act of recognising auditory objects across identity-preserving variation and in the presence of other auditory stimuli, is critical to everyday listening. In this study, we tested whether perceptual invariance for an auditory object can be established in the ferret auditory cortex (AC) and whether the perception of an auditory object is encoded within the AC.

Methods: To test perceptual invariance, we trained four ferrets in a Go/No-Go water reward task where ferrets identified a target word ("instruments") from a stream drawn from 54 other British English words (distractors). We then manipulated the mean fundamental frequency (F0) within and across trials.

We recorded neural activity from the auditory cortex using an Omnetics WARP32 chronic implant in one ferret (F1702) and considered sites with a sound-onset response for Euclidean distance decoding. We computed a decoding score for pairwise discrimination of the target word from seven high-occurrence distractors, the target word reversed, and pink noise equal in duration and spectrally matched to the target word. We also computed a decoding score for pairwise discrimination of the target word from a corresponding behavioural response of a correct Go response or an incorrect No-Go response for within-trial F0 manipulated trials and control F0 trials.

Results: The ferrets identified the target word (chance=33% hit rate) when the F0 was roved within a trial with hit rates (Female/Male speaker) of 60%/40% for F1702, 68%/38% for F2002, 43%/38% for F1803, and 48%/52% for F1815. For whole trial modified F0, the hit rate was 55%/40% (F1702), 61%/48% (F2002), 48%/47% (F1803), and 56%/39% (F1815).

An analysis of auditory cortical responses based only on correct trials, from animal F1702, revealed neural responses that discriminated target from distractor responses across variation in F0. Decoder discrimination performance was significantly higher when classifying between F0-manipulated distractor and target stimuli for both F0 manipulations across and within trials. In most cases, these responses did not carry F0 information either in target or distractor responses.

To elucidate neural correlates of perception we considered both correct Go responses (hits) and incorrect No-Go responses (misses) to the target. We then asked whether we could discriminate the neural response to the target on these trials. Several sites showed weak, but significant information about whether the trial was a hit or a miss.

Conclusions: Our preliminary results suggest that auditory objects are represented in the AC and that these responses are resistant to F0 change. Moreover, our findings suggest the AC neural representations of auditory objects align with the behavioural perception of auditory stimuli rather than the ground-truth classification of the stimuli itself. Future work will incorporate hippocampal recordings to determine whether temporal coherence between the hippocampus and AC is required for auditory object recognition.

The Time Course of Auditory Nerve and Brainstem Temporal Envelope Responses to Gated Noise

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Background: Understanding speech in noisy environments continues to prove challenging for individuals with cochlear hearing loss. This challenge may originate, in part, from impaired encoding of the slowly-fluctuating amplitude components of speech and noise, known as the temporal envelope. In laboratory animals with normal hearing, auditory nerve encoding of acoustic transients improves in noisy backgrounds due to mechanisms that control the gain of the auditory system such as the medial olivocochlear reflex, the middle ear muscle reflex, and dynamic range adaptation. We expect that a similar release from masking will be observed for encoding of the temporal envelope in noise measured non-invasively in humans using auditory evoked potentials. This experiment seeks to assess the time course of auditory nerve and brainstem temporal envelope processing in the presence of background noise by comparing measurements of the compound action potential (CAP) measured with a tympanic membrane (TM) electrode and the envelope following response (EFR) measured from a high-forehead electrode. Specifically, this experiment seeks to test whether a release from masking is observed for auditory nerve and brainstem responses to the temporal envelope. Our measurement technique of simultaneously measuring the ear canal sound pressure level (SPL), CAP, and EFR will help us interpret which mechanisms may be responsible for the release from masking.

Methods: We measured auditory evoked potentials in normal-hearing adults in response to a 75 dB SPL, 2250 ms square-wave-modulated tonal probe using a two-channel recording montage, to emphasize brainstem/cortical (high-forehead electrode) or cochlear (tympanic membrane electrode) generators. CAPs measured in response to the temporal envelope (CAPENV) were then compared with the EFR. The amplitude modulation (AM) frequency applied to the 1000-Hz carrier was either 40 or 160 Hz. Broadband noise (BBN) was 750 ms, and was presented ipsilaterally, contralaterally, or bilaterally in the temporal center of the probe.

Results: For the 40 Hz AM ipsilateral BBN condition, we observed a release from masking in the EFR and saw little-to-no release from masking in the CAPENV. We also observed suppression without release from masking in the EFR and no suppression was seen CAPENV for the 40 Hz AM with contralateral BBN condition. For the 160 Hz AM, little-to-no suppression or release from masking was seen for CAPENV and EFR.

Conclusions: These findings suggest that we are able to see a release from masking suggesting that the adaptive mechanisms responsible for enhancing transient sounds may also help improve temporal envelope coding in noise.

Refining Virus Mediated Optogenetic Manipulation of Spiral Ganglion Neurons in Adult Rodents

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Background: Optogenetic stimulation of type I spiral ganglion neurons (SGNs) promise an alternative to the electrical stimulation employed by current cochlear implants (CIs). As light can be spatially confined, the future optical cochlear implant (oCI) promises a significant increase of independently excitable channels (Dieter et al, 2019). To date, the optogenetic modification of adult SGNs is achieved by injection of a viral vector containing the opsin of interest, expressed under human synapsin, directly into the modiolus (e.g., Wrobel, Dieter et al, 2018; Huet, Dombrowski et al, 2021). Identifying optimal virus delivery routes and viral vectors, allowing high, reliable, and safe SGN transduction, is critical en route to clinical translation of optogenetic hearing restoration.
Methods: Here, we evaluated i) administration routes of the viral suspension (modiolus injection, slow administration using a round window catheter alone or with evacuation vent, slow administration from the posterior semi-circular canal and RW vent); and ii) AAV capsids (AAV2.6, AAV2.9, AAV-PHP.eB, AAV-PHP.S) for their utility in expressing Using the ultrafast, red-light activated ChR f-Chrimson in adult gerbil SGNs.
Results: The efficiency of gene transfer and f-Chrimson expression in SGNs was evaluated by recordings of optically evoked auditory brainstem response and fluorescent immunohistochemistry of mid-modiollar cryosectioned cochleas.
Conclusions: Additionally, we established light sheet imaging of intact cleared cochlea and (semi-)automatic segmentation of the SGN for fast and unbiased evaluation of the SGN transduction.

Effects of Synaptopathy on Temporal Coding in the Auditory Nerve: A Computational Modeling and Information Theory Approach

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Background: The present study theoretically investigated to what extent the loss of cochlear synapses (or synaptopathy) affects the encoding in the auditory nerve of the envelope (ENV) and temporal fine structure (TFS) of sounds.
Methods: A well-reported computational model was used to simulate auditory-nerve spike trains evoked by sinusoidally amplitude-modulated (AM) tones at 10 Hz with various carrier frequencies and levels. The model included 16 nonlinear cochlear filters with characteristic frequencies (CFs) from 250 Hz to 8 kHz. Each filter was innervated by 3, 4 and 10 fibers with low (LSR), medium (MSR) and high spontaneous rates (HSR) respectively. For each frequency channel, the simulated spike trains were collapsed into three separate 'population' post-stimulus time histograms (PSTH), one per fiber type. Information theory was applied to reconstruct the stimulus waveform, ENV, and TFS from the obtained PSTHs in a mathematically optimal way. Various synaptopathy scenarios were simulated by removing spike trains from specific fiber types and/or cochlear regions before stimulus reconstruction. The quality of the reconstruction was assumed to reflect the information present in the PSTHs.
Results: We found that the TFS was maximally encoded in the PSTHs from HSR fibers at all stimulus carrier frequencies and levels. The encoding of the ENV was more complex. At low levels, the ENV was maximally encoded in the PSTHs of HSR fibers with CFs near the stimulus carrier frequency. At high levels, the ENV equally well encoded in PSTHs of HSR fibers with CFs remote from the stimulus frequency as in those of LSR fibers with CFs closer to the stimulus carrier frequency.
Conclusions: Altogether, these findings suggest that a healthy population of HSR fibers (i.e., including fibers with CFs around and remote from the AM carrier frequency) might be sufficient to encode the ENV and TFS over a wide range of stimulus levels. On the other hand, however, at high levels, LSR fibers still contributed to encoding the ENV when the reconstruction was based on combining the PSTHs for all fiber types. The implications of these findings for diagnosing synaptopathy and understanding its relevance on auditory perception will be discussed. (Work supported by Oticon Medical and the William Demant Foundation).

A Comprehensive Approach to Postlingual Auditory Neuropathy Spectrum Disorder Through Audiogram Configuration, Genomic Information and Cochlear Implantation Outcomes

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Background: The etiologies and prevalence of sporadic postlingual auditory neuropathy spectrum disorder (ANSD) are rarely documented. In this study, we aimed to explore the diverse molecular etiologies of postlingual ANSD. We report on the outcome of cochlear implantees among our cohort of postlingual ANSD patients.
Methods: We enrolled patients with late-onset, progressive hearing loss from a registry of 799 unrelated Korean families at Seoul National University Bundang Hospital. Type of sensorineural hearing loss (SNHL) was classified as flat type hearing loss, low-frequency hearing loss, mid-frequency hearing loss, downsloping hearing loss or ski-slope hearing loss. We identified patients with postlingual ANSD. Through whole exome sequencing and subsequent bioinformatics analysis, the genetic cause was identified. Individual results of intraoperative neural response telemetry (NRT) and postoperative speech perception abilities of those who underwent cochlear implantation (CI) were reviewed.
Results: The detection rate of ANSD without overt peripheral neuropathy was 5.21% (11/211 probands) among those with postlingual mild to moderate SNHL, and diverse genetic etiologies without substantial overlap were identified in 6 (54.5%) of the 11 ANSD subjects. More than one-fourth of bilateral low-frequency SNHL cases were classified as ANSD. Conversely, nearly two-thirds of postlingual ANSD patients were initially diagnosed with low-frequency hearing loss. A genetic cause that could explain ANSD was found in approximately 85% of ANSD subjects with low-frequency SNHL. The type of electrically evoked compound action potential (ECAP) response obtained during CI surgery in ANSD patients was also diverse—to a similar extent as the molecular genetic causes—and showed some degree of correlation with the etiology. Despite the diverse genetic etiology and ECAP responses, CI in the postlingual ANSD patients in our cohort, including those with unknown etiologies, yielded significant improvements in speech discrimination.
Conclusions: Our study shows significant molecular etiologic heterogeneity in postlingual ANSD. A close association was observed between postlingual ANSD and low-frequency hearing loss, as supported by both audiological and molecular genetic evidence. Our results also preclude the hasty conclusion that CI should be reserved only for patients with nonsyndromic postlingual ANSD with a documented presynaptic etiology.

Perceived Duration of Electrical Chirp Stimuli in Cochlear Implants

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Background: While a click is physically the shortest-duration sound, its peripheral neural representation is temporally smeared by the filtering performed by the cochlea in such a way that apical auditory nerve (AN) fibers are excited several milliseconds after basal AN fibers. By progressively delaying the high- relative to the low-frequency components, it is possible to produce a stimulus that yields more discharge synchrony across AN fibers. Although this "optimized chirp" stimulus elicits larger ABR responses than a click, it is surprisingly not perceived as shorter in duration. This has led to the assumption that the across-fiber delays generated at the periphery may be compensated more centrally. This hypothesis is difficult to test in normal-hearing listeners because of confounding effects arising from cochlear filtering. Here we evaluate it in a group of 8 Med-EL cochlear implant subjects.
Methods: Stimuli were presented through direct stimulation and consisted of "electrical chirps" spanning the whole electrode array of the subjects in the apex-to-base (A->B) or base-to-apex (B->A) direction. Chirps consisted of sequences of pulses presented at a rate of 8196 pps on different channels. The duration was manipulated by adding virtual channels whilst keeping the same pulse rate.
Results: Experiment 1 was a duration ranking task and used 12 loudness-balanced chirps differing in duration (from 1.25 to 40 ms) and in direction (A->B and B->A). It was hypothesized that the B->A chirps (those mimicking the natural cochlear delay) should sound shorter than the A->B when presented at the same duration. This hypothesis was confirmed for overall durations of 10 ms and longer while no effect was observed at shorter durations.
In Experiment 2, we measured electrically-evoked late-latency cortical potentials for a subset of four chirps. The stimuli included two short chirps (1.3 ms) for which there was no difference in perceived duration and two longer chirps (20 ms) for which there was a difference. We assumed that the central compensation should yield more

synchrony at the cortical level, and therefore produce a larger response for the 20-ms B->A chirp than for the A->B. Contrary to this hypothesis, the 20-ms B->A chirp produced a significantly smaller response while no difference was observed between the short chirps.

In Experiment 3, we verified that the effect of chirp direction observed in Experiments 1 and 2 was not due to the end of the B->A chirps being inaudible, e.g. due to forward masking. This was tested using a duration discrimination paradigm. It was hypothesized that if masking was the explanation, duration discrimination should be much worse for the B->A chirp. However, this was not the case.

Conclusions: These results provide strong evidence of the existence of a central process that compensates for cochlear delays and provide information on its possible locus.

Open-CI: Open Cochlear Implant

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Background: Advances in auditory prostheses are hampered by the high costs of iterative hardware development, software (algorithm) revisions or updates, and the necessary but lengthy regulatory approval process. At present, the few established companies producing cochlear implants have limited research and development budgets, slowing innovation in the industry. In order to provide an alternative platform to encourage new developments in implantable hardware, electrode arrays, and advanced algorithms, a research-grade openly-developed cochlear implant would enable start-ups, small companies, and other research groups to work directly on advancing cochlear technologies spurring new innovation in the field.

Methods: We propose a framework for a fully Open-source Cochlear Implant (Open-CI) including hardware description, manufacturing methods, and an integrated software development environment (IDE) for reconfiguring the fundamental operation of the cochlear implant. This system will be designed, from the ground up, using our Quality System (QS) for eventual submission to the FDA for regulatory approval. Similar approaches have been successful in the Deep Brain Stimulator (DBS) industry; here, we will develop the hardware openly, with community input and involvement. The system is designed for expandability and broad compatibility including, for example, open standards for stimulation coding and near field communication protocol for a generalized number of channels. Since the system is openly-developed, we seek the input of researchers, academics, clinicians, and patients in the field to ensure Open-CI will meet current and future research and development goals. Upon regulatory submission, all software and hardware design documentation will be made available to the community.

Results: The Lawrence Livermore National Laboratory (LLNL) Biomedical Foundry has developed and fabricated several different neuroprosthetic devices for both animal and human use, including electrode arrays and implantable hermetically-sealed packages. Neural implants developed at LLNL have been used with an FDA Non-Significant Risk (NSR) in intraoperative studies. The Open-CI concept will leverage these previous ground-breaking technologies. Here, we present the initial prototype concept for community input.

Conclusions: Our proposed openly-developed cochlear implant, Open-CI, will facilitate an accelerated roadmap to innovation through a framework for developing advanced algorithms, access to more stimulation channels, enhanced power and communication protocols, safe generalized stimulation coding, and reduced financial and regulatory hurdles. Additionally, open-source reconfigurable hardware will enable new clinical experiments currently not possible with commercial cochlear implants, including development of hybrid modality stimulation. Funded by NIH NIDCD under grant ADC15001001.

Effects of Electrode Location on Measures of Neural Health in Cochlear Implant Users

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Background: Previous work in cochlear implanted animals has identified measures of the electrically-evoked compound action potential (ECAP) that relate to the density of surviving spiral ganglion neurons (SGNs). Related work in humans has suggested that these same ECAP measures are related to speech recognition outcomes, but ECAPs are also affected by the positioning of the electrode array within the cochlea. Previously, we found that the distance between the electrode and the mid-modiolar axis ('medial-lateral distance') influenced some, but not all, ECAP measures. However, that study did not control for potential confounding variables related to insertion angle or scalar location (scala tympani vs scala vestibuli). Those variables are held constant in the current study.

Methods: Participants included adult (>18 years old), peri- or post-lingually deafened cochlear implant recipients. All were users of Cochlear ™ implant systems, and had at least 3 months' cochlear implant experience. We measured ECAP amplitude-growth functions (AGFs) on each electrode using interphase gaps (IPGs) of 7 and 30 µs, and calculated the slope of the AGF for each fixed IPG as well as the difference in slope between the two IPGs (IPG effect) for each electrode. Post-operative CT images were analyzed and provided estimates of electrode medial-lateral distance, scalar location, and insertion angle. In order to better parse contributions of electrode location on ECAP measures, we examined how ECAP slopes change for 1) fixed insertion angles across a range of medial lateral distance and 2) fixed medial lateral distance across a range of insertion angles.

Results: Preliminary results show that, consistent with previous findings, the slope of the ECAP AGF for a fixed IPG decreased with increasing medial lateral distance, but the ECAP IPG Effect was not systematically affected by medial lateral distance. For a fixed insertion angle, the ECAP AGF slope for a fixed IPG tended to increase with medial lateral distance at each point along the length of the array. For a fixed medial lateral distance, results varied depending on the specific medial lateral distance, but not in a systematic manner. Scalar location did not seem to significantly affect these results, but further data are needed to better examine this factor.

Conclusions: Results agree with previous findings that suggest electrode location, and specifically medial-lateral distance influence ECAP measures using a fixed IPG, when measured across the entire electrode array within each subject. Assuming that the surviving neural fibers increase from base to apex, then results support the hypothesis that increasing medial lateral distance increases the slope of the ECAP AGF because it increases the width of the current field at the modiolus and stimulates more fibers.

Effects of Hearing Aid Directional Processing on Spatial-Change Tuning of Speech in Background Babble
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Background: Hearing aid directional processing (HADP) improves signal-to-noise ratio (SNR) when maskers are spatially separated from target speech, and presumably improves speech understanding. Benefit, however, is typically gauged by self-report, listening effort, or sentence recognition in noise, and not all listeners report advantages over omnidirectional processing. The present study links HADP manipulation and evaluation to a strong and established theoretical model of auditory object-based attention. Because hearing-impaired listeners localize speech more poorly in a mixture than in quiet, auditory streaming is also consequently impaired. It is unclear, however, whether improved SNR (via HADP) improves spatial tuning to auditory objects, and if so, whether indices of auditory streaming are enhanced. We hypothesized that HADP narrowly fixed to the front would enhance auditory streaming for targets at or near the front relative to omnidirectional processing, but lead to reduced indices of auditory streaming when targets were off-of-center.

Methods: Cortical event-related potentials were measured using electroencephalography (EEG) to investigate spatial tuning of moving speech in quiet or background babble (+6 dB SNR) and the subsequent effects of HADP. In active attention conditions, listeners responded in the affirmative to the speech stream when it occurred at a single location (30 or 60° off-of-center) or in the negative at any other location. Two HADP settings, omnidirectional and a fixed, narrow beamforming array, were tested in older, hearing-impaired adults (n = 20).

Results: In quiet, hearing impaired-listeners show comparable responses to older, normal-hearing listeners shown in previous studies. Unaided, the same listeners show considerable disadvantages in background babble compared to past studies of younger, normal-hearing listeners. Hearing aid intervention appears to affect early cortical measures that are associated with bottom-up processing; however, later-latency potentials (e.g., P2) were either smaller or had no change relative to the unaided listening conditions.

Conclusions: Though HADP is assumed to provide valuable SNR advantages, objective measures of its benefit are mixed, especially in diffuse backgrounds and for speech that is not static in space. It is also possible that SNR improvements come at the cost of poorer localization cues, a trade-off that yields little to no measurable benefit. Future studies should investigate how poorer auditory streaming impacts speech recognition and what if any compensatory mechanisms are used by aided listeners.

The Opto-Electrical Cochlear Implant
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Background: In contemporary cochlear implants, the physical properties of the tissue result in a wide spread of the electrical current and limit the number of independent channels for sound processing. Psychophysical tests have shown that increasing the number of independent channels to encode acoustic information can improve performance. Preclinical research suggests that stimulation of the cochlear neurons using focused light provides spectral selectivity that near acoustical stimulation and could be the stimulation modality to generate more independent channels. Light can be delivered by placing optical sources into the scala tympany along the cochlea spiral or by inserting bundles of optical waveguides. Both methods are novel and need exploration. We tested a polymer as a possible candidate for such a bundle of waveguides with the present study.
Methods: Waveguides of a given diameter were fabricated via injection molding and coated using either dip-coating or thermal reflow. Optical coupling, transmission, and bending losses were quantified for radiation at 405, 534, 680 1375, 1460, 1550, and 1860 nm. Bending stiffness for each waveguide and the waveguide's insertion force into an acrylic human-size cochlear model were measured. In a guinea pig animal model, we tested the ability to evoke auditory responses.
Results: The initial results show coupling losses of about 6dB. The propagation losses are wavelength-dependent. At 534, 680, 1375, and 1550 nm, they are between 0.43 and 0.97 dB/cm and 3.78 and 3.55 dB/cm at 405 and 1460 nm, respectively. Bending losses for 360 degrees at 1375 nm are 5.0, 2.4, and 0.46 for a bending radius of 2.5, 3, and 4 mm. Insertion forces for 300 μm diameter waveguides are in the order of 100 mN. The bending stiffness of a 200 μm segment of those waveguides is 1-1.5 mN. Compound action potentials could be evoked with the waveguides inserted into the scala tympani of the guinea pig cochlea.
Conclusions: Coupling was not optimized in our measurements, and losses can be reduced. Propagation losses are more difficult to tackle because they depend on the waveguide's material properties and cladding. As expected, radiation transmitted to the ear of the animal evokes auditory responses.

Does Impedance Reflect Intrascalar Tissue in the Implanted Cochlea?

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Background: The development of intrascalar tissue following cochlear implantation is a potential threat to the long-term preservation of both electrical and acoustical hearing in implanted patients. Impedance measures, which are a common non-invasive test of implant integrity, might also enable monitoring of changes in the cochlear environment. Impedance, in a general sense, is a measure of how much a system resists the flow of electrical current; therefore, increases in impedance might reflect growth of intrascalar tissue. Here, we monitored impedance over time and assessed its relationship to intrascalar tissue, evaluated histologically at endpoints after long-term cochlear implantation.
Methods: Forty-seven adult male guinea pigs were chronically implanted with a banded cochlear implant and impedances for a 1 kHz, 1 μA rms sinusoid were measured for up to 21 months. Histology (mid-modiolar sections) was performed near one electrode location, and the intrascalar tissue between the implant footprint (if one was present) and the spiral ganglion neurons was ranked as: low, medium, or high following the classification procedure reported by Swiderski et al., JARO, 2020. Impedance trends over time were assessed, and tissue rankings and final impedance levels (average of the final 10 measures) were compared.
Results: Histology revealed there were 19 ears with low (including 2 ears with no tissue in the scalar location housing the electrode), 10 ears with medium, and 18 ears with high intrascalar tissue (including 7 ears in which bone filled the scala and surrounded the electrode). Final impedance levels for individuals ranged from 4.6 to 20.6 kOhms. ANOVA found the effect of tissue group on impedance was significant ($p<0.01$). Mean impedances (± standard error) were 7.8 (±0.7) for the low intrascalar tissue group and 7.1 (±0.5) for the medium group, which were not significantly different ($p=0.68$, TukeyHSD). Mean impedance for the high group (13.1±1.2) was significantly higher than that for both of the other groups ($p<0.01$). This high intrascalar tissue group had the greatest variability in impedance including 6 ears with low impedance comparable to the other two tissue groups. Ears with implants completely surrounded by bone had impedances ≥12.7, as did 3 others with high and 3 with low intrascalar tissue. Individual impedance trends over time were variable, sometimes fluctuating, and did not necessarily reflect the intrascalar tissue group. E.g., the two animals with no intrascalar tissue had increasing impedances over time.

Conclusions: While impedances were higher on average in ears with the most intrascalar tissue and new bone, the data did not show consistent relationship between impedance and intrascalar tissue. Impedance as a measure of the implanted cochlear environment seems to be complex and possibly dependent on multiple factors that require further analysis. Measures of individual components of complex impedance might be helpful.
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Anodic Stimulation Increases Facial Nerve Stimulation Thresholds in CI-Stimulation
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Background: Facial nerve stimulation (FNS) in human cochlea implant (CI)-users is a rare but clinically relevant side effect of intra-cochlear electrical stimulation. While post-operative FNS may be reduced by focused-triphasic stimulation, also more severe approaches, like deactivation of affected electrode contacts, or even revision surgery are sometimes necessary. During acute CI-stimulation in guinea pigs, we observed changes in FNS thresholds based on the stimulation mode, including the polarity of the activating phase and the intra-cochlear position. These relations were statistically assessed in 392 electrically evoked compound action potential (eCAP) recordings from 33 ears of 26 guinea pigs. Facial nerve stimulation was compared between anodic- and cathodic-dominant stimulation in broad-monopolar and focused (bipolar/ tripolar) stimulation for biphasic and charged-balanced monophasic stimulation with 50μs-activating phases, each.
Methods: We determined the FNS threshold and analyzed the peak latency at 1dB above the threshold. FNS thresholds were detected in 184 of 392 cases, with higher percentages for monopolar (biphasic, monophasic: 66 and 54 %) than focused stimulation (biphasic, monophasic: 20 and 2 %). The median FNS threshold ranged from 794 μA (monopolar, monophasic, cathodic stimulation) to 6310 μA (focused, monophasic stimulation; both polarities). The polarity effect of the FNS (PE-FNS) was calculated as the difference between the cathodic- and anodic FNS thresholds in dB. Thereby, positive values indicate a lower (i.e. more disruptive) cathodic than anodic FNS threshold. In cases, where only one polarity led to a detectable FNS, the FNS threshold for the second polarity was approximated as 1dB above the highest current level used during the experiment. This approach may underestimate the actual polarity effect on FNS.
Results: The PE-FNS was significantly positive for monopolar stimulation (Wilcoxon test: biphasic: p=0.0001, monophasic: p<0.0001). For focused stimulation, the PE-FNS indicated similar FNS thresholds for anodic and cathodic stimulation (p=0.081). For monopolar stimulation, the anodic stimulation led to 2 dB higher FNS thresholds, with a standard deviation of 3 dB. In 49 pairs of anodic and cathodic broad, biphasic stimulation, we could assess the relation to eCAP thresholds. Although the eCAP thresholds were not significantly different (median: 343 μA, t-test: p=0.242), the offset between FNS threshold and eCAP threshold was significantly (Wilcoxon test: p=0.004) and substantially higher for anodic (median: 7 dB) than cathodic stimulation (median: 5 dB).
Conclusions: Based on these results, we propose that changing the stimulation polarity from the commonly used monopolar cathodic-leading, biphasic pulses to anodic-leading stimulation might be a viable and simple approach to reduce FNS in clinical CI-stimulation while keeping the available number of CI-channels.
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Assessing the Relationship Between Focused Behavioral Thresholds and Vowel Space Errors in Cochlear Implant Listeners
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Background: Speech recognition scores are highly variable across cochlear implant (CI) listeners. The fidelity of information transfer between the CI electrodes and the auditory neurons (the electrode-neuron interface or ENI) may explain some of this variability. CI listeners with poor ENI quality, either from degeneration of neural populations near electrodes or poorly placed electrodes, would be expected to have poor spectral resolution. This poor spectral resolution can negatively impact perception of speech sounds, particularly vowels. Vowel identification relies heavily on faithful transmission of spectral information, particularly the first and second formant frequencies. Focused behavioral thresholds are one promising psychophysical measure that is sensitive to

ENI quality. This work aims to determine how focused threshold profiles may relate to errors in vowel space perception in CI listeners.

Methods: Thirty-five CI listeners (19 adults and 16 children) with Advanced Bionics devices HiRes90k participated in this study. Steered quadrupolar thresholds were measured for 14 electrodes (E2-E14) in the implanted ears (σ= 0.9) through a sweep procedure. The threshold profiles were characterized according to their shape, leading to five categories of "Apical Up", "Flat", "Basal Up", "Smile", and "Frown". Vowel identification was measured in the /hVd/ context for ten vowels, presented at 60 dB SPL in two conditions of quiet and with a +10 SNR of 4-talker babble. Listeners' performance in vowel identification was represented in vowel space based on their errors in two dimensions: vowel height (first formant) and vowel advancement (second formant). To quantify these errors, each vowel was assigned a height value between 1 and 5 (1 = lowest, 5= highest) and an advancement value between 1 and 5 (1=back, 5 =front) to represent the general phonological feature distribution of the vowel space. Error was calculated as the difference between each target and responded vowel for these two dimensions. The relationship between threshold profiles and vowel space errors was assessed.

Results: Visual analysis of subjects with similar threshold profiles does not consistently reveal predictable errors in vowel space perception. However, average thresholds showed a statistically significant negative correlation to overall vowel identification in quiet and noise. Further refinement of threshold profiling and quantification of vowel space analysis is forthcoming.

Conclusions: These findings suggest that certain threshold profiles may result in predictable vowel space errors, but further refinement of profiling is necessary. If true, this information could be used to modify an individual's CI programming to account for this profile.

Deep Neural Network Algorithms for Noise Reduction in Cochlear Implants

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Background: Despite excellent performance in quiet, cochlear implants (CIs) only partially restore normal levels of intelligibility in noisy environments. Current state-of-the-art signal processing strategies in CIs provide limited benefits in terms of noise reduction or masking release. Recent developments in the field of machine learning have resulted in deep neural network (DNN) models achieving high levels of performance in both speech enhancement and separation tasks. However, there are no commercially available CI audio processors that make use of DNN models.

Methods: We implemented two DNN models for CIs: a recurrent neural network (RNN)—a lightweight template model, and the SepFormer—the current state-of-the-art, top-performing speech-separation model in the literature. The models were trained with a custom, 30-hour dataset that included 4 conditions: speech in non-speech noise (environmental sounds), speech in 1-talker, 2-talker, and 4-talker babble backgrounds. An equal number of unique speech-noise mixtures were generated for each condition at signal-to-noise ratios (SNRs) ranging from 1 to 10 dB in 1 dB steps. The enhancement of the target speech (or the suppression of the noise) by the models was evaluated using commonly used acoustic evaluation metrics of quality and intelligibility such as (1) signal-to-distortion ratio (SDR), (2) "perceptual" evaluation of speech quality (PESQ), and (3) short-time objective intelligibility (STOI). We also tested the models behaviorally with CI users listening to the mixtures via their clinical CI processor and direct audio input.

Results: Both models introduced significant improvements in all acoustic metrics we tested (SDR, PESQ, and STOI). Preliminary data on 2 CI subjects also showed improvements in speech-in-noise intelligibility scores when the noisy speech was processed by the models, as compared to the unprocessed conditions.

Conclusions: In conclusion, the evaluated DNN models were able to significantly enhance the target speech within a noisy mixture while suppressing the background noise. This work serves as a proof of concept that DNN technology has the potential to be integrated into CIs to improve the user's listening experience in noisy environments. We hypothesize that the ongoing data collection from more CI subjects will corroborate the promising preliminary results, and guide the next steps towards developing machine-learning-based signal processing algorithms for noise reduction in CIs.

Using Single-Channel Focused Thresholds to Predict Vowel Identification Errors in Cochlear Implant Listeners

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Background: Despite remarkable advancement in cochlear implant (CI) technology, speech recognition scores are highly variable and difficult to predict among CI listeners. The effectiveness of individual electrode channels in activating nearby auditory neurons, the quality of electrode-neuron interfaces (ENIs), is an important factor that may contribute to this variability. Single-channel auditory detection thresholds in response to spatially-focused electrical fields (i.e., focused thresholds) are sensitive to factors contributing to ENI quality; electrode-neuron distance and neural health/density. While focused thresholds can be measured reliably and swiftly and has the potential to be used as a clinical tool, the link between focused thresholds and speech recognition ability is not yet clear, particularly in vowel identification, which relies heavily on the precise transmission of spectral information. This study aims to determine how single-channel focused thresholds may relate to vowel identification errors after accounting for how each vowel is represented across channels in the clinical CI speech processors.

Methods: Eleven adult CI listeners with Advanced Bionics devices participated in this study. Single-channel thresholds were measured for active electrodes in response to a focused electrode configuration (steered quadrupolar, sQP), with a focusing coefficient (σ) of 0.9. Thresholds were weighted by the relative importance of each electrode in representing the vowel sound. Weighting factors were determined from electrodograms of vowel stimuli created with individual listeners' everyday processing strategy and the energy of the current amplitude in each channel for each vowel. The threshold profiles were also characterized by fitting a second degree polynomial, leading to three categories of "flat", "frown", and "smile" for these 11 listeners. Participants also performed a closed-set vowel identification task for ten vowels in /hVd/ context presented at 60 dBA in quiet and +10 SNR of 4-talker babble noise.

Results: Our preliminary results showed that the elevated focused thresholds were negatively associated with vowel identification scores, more so after weighting was applied. Listeners with high, "flat" thresholds performed poorly for all vowels (less than 80% correct), suggesting that almost all electrodes were functioning poorly. For listeners with "frown" profiles, a group of mostly "back vowels" (vowel is produced with a backward shift of the tongue from its neutral position) were most affected by the elevated thresholds in mid-frequency channels. For listeners with "smile" profiles with relatively high focused thresholds in apical and basal electrodes, errors mainly occurred for "low vowels" (the tongue is in a relatively low position in the mouth).

Conclusions: The findings suggest that channels with high focused thresholds could contribute to lost or distorted spectral information and thus poor performance in vowel identification. Further investigation about whether elevated focused thresholds in specific frequency regions (i.e., channels) predict erroneous patterns in height and advancement of specific vowels is warranted.

Age-Related Changes in Adult Postlingually Deaf Cochlear Implant Users' Utilization of Acoustic Cues for Vocal Emotion Identification

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Background: Adult postlingually deaf cochlear implant (CI) users show age-related declines in their ability to identify emotions based on prosodic cues (Cannon and Chatterjee, 2021, Ear and Hearing, DOI: 10.1097/aud.0000000000001095). Here, we investigate possible mechanisms underlying this aging effect. We hypothesize that CI users' access to voice pitch cues declines with age, and that this alters their ability to map voice pitch changes in emotional prosody to the correct emotions.

Methods: Adult postlingually deaf users' ability to identify happy and sad emotions based on voice pitch contours, duration cues, and intensity cues was measured using a set of stimuli resynthesized from recordings by an adult female talker saying the sentence "Time to go" in a happy and a sad way. A continuum of resynthesized stimuli was created by modifying the pitch values for the voice pitch contour from the happy to the sad version in logarithmic steps. A total of five contours was achieved, evenly distributed in log space. A set of 125 sentences was thus created, each with one of the five contours, five durations (from happy (short) to sad (long)), and five intensity levels. These stimuli were blocked by intensity and presented in randomized order within blocks (block order was also randomized across participants). Participants were asked to indicate which of the two emotions (happy or sad) each stimulus seemed best associated with in a single-interval, 2-alternative forced choice task.

Results: Preliminary results in an online experiment using the Gorilla Experiment Builder with 10 adult postlingually deaf CI users suggest an age-related decline in adult postlingually deaf CI users' ability to utilize F0

contour cues to distinguish happy and sad emotions. Regression analysis of the proportion of "Happy" responses plotted against the continuum of F0 contours, provided a coefficient indicating the extent to which each individual relied on the F0 contour to distinguish happy from sad emotions. In preliminary analyses of data with 10 CI users, these cue-coefficients were found to be negatively correlated with the participants' age (correlation coefficient = -0.415), but the correlation was not statistically significant.

Conclusions: Preliminary analyses suggest that age-related declines occur in F0 contour utilization for emotion identification. The extent to which these declines can account for age-related decline in CI users' emotion identification in natural stimuli, remains to be confirmed. In addition, we plan to present results of analyses of confusion matrices from Cannon and Chatterjee (2021) alongside acoustic features of the stimuli to test the hypothesis that emotional contrasts that are most strongly represented by changes in voice pitch cues are the ones most affected by age.

Objective Assessment of Binaural Fusion in Normal Hearing Listeners With Simulated Interaural Asymmetries

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Background: Individuals with bilateral cochlear implants (BiCIs) experience reduced binaural unmasking compared to normal hearing (NH) listeners, which likely stems in part from the significant interaural asymmetries demonstrated by many BiCI users. Specifically, differences in temporal resolution across ears can result in asymmetric speech performance and poor encoding of binaural cues. To improve binaural outcomes in BiCI users, it is important to understand whether binaural performance in listeners with asymmetries is limited more by the poorer ear, or by the degree of asymmetry across ears. To help answer this question, our lab is investigating the effect of asymmetric temporal resolution on binaural unmasking of speech. We manipulated temporal resolution in NH listeners by compressing the dynamic range (DR) of vocoded speech symmetrically and asymmetrically across ears. Compressing DR reduces amplitude modulations in the speech envelope, thus reducing the fidelity of temporal information (i.e., temporal resolution). Results suggest that binaural unmasking of speech is limited more by asymmetry than performance of the poorer ear. However, the physiological underpinnings remain elusive.
To better understand mechanisms underlying these behavioral results, the present study investigated the effect of interaural asymmetries on binaural fusion, a prerequisite for binaural unmaking, in normal hearing listeners using the cortically generated acoustic change complex (ACC). The ACC is elicited by a change in an ongoing auditory stimulus, such as a change in the degree of binaural fusion. We hypothesized that greater similarity in stimuli across ears would result in better fusion and a larger ACC.

Methods: Binaurally presented uncorrelated speech shaped noise (SSN), which is perceptually diffuse in the head, was changed to 40 Hz amplitude modulated (AM) SSN, resulting in better fusion of the sounds. AM was imposed to simulate modulations in speech, and DR was varied symmetrically and asymmetrically to create differences in temporal resolution across ears. To help discern effects of AM on fusion, an additional un-modulated condition in which the uncorrelated SSN was changed to correlated SSN without AM was included.

Results: Preliminary results are consistent with our hypothesis: the correlated SSN condition elicits the largest ACC amplitude, followed by the symmetric AM condition, and finally the asymmetric AM condition.

Conclusions: Results from this study will help elucidate whether reduced binaural unmasking in BiCI users with interaural asymmetries is related to poor binaural fusion.

Interactions Between Slopes of Residual Hearing and Frequency Maps in Simulated Bimodal Hearing

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Background: Bimodal hearing (a hearing aid in one ear and a cochlear implant in the opposite ear) requires integrating spectro-temporal information across ears. Our pilot study suggests that different slopes of residual hearing in hearing aid ear affect bimodal integration significantly. We hypothesized that the effects of slopes of residual hearing depend on frequency overlap between the acoustic and electric stimulations. In this study, using bimodal simulation with normal hearing (NH) listeners, we aimed to test the hypothesis with three different slopes and three different frequency overlaps in bimodal hearing.

Methods: Adults with NH listening to simulation of bimodal hearing were recruited for sentence perception in noise. For the acoustic simulation, three different slopes of simulated high-frequency hearing loss typically found

in bimodal patients were created using low-pass filters with a fixed cutoff frequency of 500 Hz: steep (96 dB/octave), medium (48 dB/octave), and shallow (24 dB/octave). For the electric simulation, 8-channel sinewave vocoder was used with a fixed output frequency ranges (1000-7938 Hz) with three different input frequency ranges to create typical frequency maps found in bimodal patients: overlap (188-7938 Hz), meet (500-7938 Hz), and gap (750-7938 Hz), relative to the cutoff frequency (500 Hz) in the acoustic ear. Sentence perception was measured with acoustic stimulation alone, electric stimulation alone, and combined acoustic and electric stimulations as a function of filter slope and signal-to-noise ratios (SNRs) for each frequency map.

Results: Bimodal benefit was significantly improved as the slopes of the simulated hearing loss decreased and SNRs improved. Significant interactions were found between the slopes and frequency maps.
The effects of the slope on bimodal benefit were marginal for the overlap map. For the medium slope, the bimodal benefit was the greatest with the meet map but the worst with the overlap map. For the shallow slope, the bimodal benefit was the greatest with the meet map but the worst with the overlap map.

Conclusions: Inverse relationship between the slopes and bimodal benefits suggests that spectral information resided under the slope plays a significant role for bimodal integration. The optimal frequency map differed with different slopes, suggesting that the slopes of residual hearing in acoustic ear should be carefully considered in fitting bimodal hearing.

Prepulse Inhibition of the Acoustic Startle Response as a Behavioral Assay for Sound Localization Abilities of the Mongolian Gerbil

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Background: The Mongolian gerbil (Meriones unguiculatus) has commonly been used to investigate the mechanisms underlying binaural hearing and sound localization. However, there are few behavior assays available to rapidly test the spatial hearing ability of gerbils. Traditional behavior paradigms utilize operant conditioning, which can take months of training followed by months of experimentation to obtain meaningful data. Here, we examine whether pre-pulse inhibition (PPI) of the acoustic startle response can be used to assess spatial hearing in gerbils.

Methods: In contrast to operant conditioning paradigms, PPI of the startle reflex requires no training and also offers a high-throughput measure such that a large number of animals can be tested in a relatively short timeframe. Over the course of three months, we were able to concurrently run a cohort of 8 animals through a number of sound localization behavior tasks.

Results: In our first set of experiments, we presented a continuous broadband noise that swapped speaker locations, acting as a prepulse, prior to presenting a startle stimulus. PPI of the startle response increased monotonically with wider angles of speaker swaps, with swap angles at 30 degrees (± 15 deg re: midline) or higher showing significantly higher PPI compared to baseline. These results are wholly consistent with those produced by operant conditioning paradigms. Next, the speaker swap was confined to a given hemifield, instead of swapping across the midline. While the results were not as robust as the midline swap, PPI increased monotonically with larger speaker swaps, indicating that gerbils are able to discriminate sound sources within a hemifield. Next, spatial acuity across the midline was assessed for low- (0.5 kHz) and high-pass (4 kHz) noise, effectively isolating ongoing interaural time differences (ITDs) and interaural level differences (ILDs), respectively. Similar to broadband noise, wider swap angles elicited higher PPI responses, with significant PPI observed at angles greater than 30 degrees for low pass noise and 15 degrees for high pass noise. This shows that gerbils can independently use both low-frequency ITDs as well as high-frequency ILDs to localize sound. Lastly, spatial release from masking ability was assessed, where a broadband chirp was presented from the midline in the presences of broadband masking noise at different angles. PPI was largest, indicating best target detectability, when the maskers were at sources farther from the target and decreased as the masker intensity increased and were moved spatially closer to the target.

Conclusions: Collectively, these data demonstrate that gerbils are proficient in a number of sound localization tasks, and that PPI of the acoustic startle response paradigm is capable of testing a large number of gerbils in a multitude of tasks in a short timeframe. [Supported by R01-DC017924]

Early Life Experience of Cue Reliability Drives Frequency Tuning in the Barn Owl Midbrain

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Background: To create an efficient representation of the world, the brain has to encode informative stimuli while ignoring other stimuli. This has been found in the barn owl's midbrain, which uses the interaural time difference (ITD) to compute sound location in azimuth. Previous work found that ITD-selective neurons in the midbrain's external nucleus of the inferior colliculus are tuned to the frequency range that is most reliable at each location. This effect is predicted by the acoustical properties of the head, causing higher frequencies to convey ITD information more reliably in frontal space and lower frequencies in peripheral space. If the frequency tuning is directly driven by stimulus statistics, then changing the acoustical properties of the head would affect the correlation between ITD and frequency tuning.

In the barn owl, the facial ruff surrounding the face acts as a receiver, similar to the mammalian pinnae. Analysis of acoustical recordings of owls with and without this facial ruff reveals that the removal of the facial ruff decreases the reliability of high frequency sounds originating from frontal locations. While previous reports have measured other effects from facial ruff removal, frequency tuning has not yet been reported.

Methods: In this study, we removed the facial ruff from two hand-reared juvenile owls as it developed. Once the owls reached adulthood, we recorded from neurons in the external nucleus of the inferior colliculus. Tuning curves for ITD and frequency, as well as interaural level difference, were collected consecutively.

Results: Preliminary results indicate a broadening of frequency tuning, as well as a shift toward lower frequency for frontally tuned neurons. Tunings to other stimulus properties are consistent with previous reports of ruff-removed owls.

Conclusions: These results suggest that early experience of cue reliability drives frequency tuning of midbrain space-specific neurons, supporting the role of predictive coding of sensory statistics in spatial perception.

Simulating the Effects of Leakage and Gap Junction Conductances on Bushy Cell Excitability
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Background: Bushy cells within the ventral cochlear nucleus exhibit enhanced synchronized firing to a periodic input signal when compared to the auditory nerve fibers that provide their primary input. However, bushy cells form clusters connected to each other via soma-somatic connections, including gap junctions (electrical synapses). This motivates the study of how excitation may spread through clusters of bushy cells via gap junctions.

Methods: In this study, we implemented Xie-Manis (2013) type bushy cell models in MATLAB. These are modified Hodgkin–Huxley models that consist of multiple sodium and potassium channels, and a leakage current. In our implementation, gap junctions are also included, with the gap junction conductance as well as the leakage conductance being free parameters. The effects of both conductivity parameters g_leak and g_gap on cells' spiking behavior are inspected while also varying the respective current injection levels. In particular, the interaction between supra and subthreshold current injections in a pair of connected cells is explored. In the first simulated experiments, only the first cell is injected with varying current levels. A second experiment is done with an additional subthreshold input to the second cell. Both experiments are repeated with a reduced cell model more similar to a traditional Hodgkin–Huxley model.

Results: The results of both simulated experiments indicate that as g_gap increases, at some point both model bushy cells fire an action potential (AP) with a sufficiently high current injected into the first cell, but for higher g_gap the excitability decreases and both cells stop firing. High values of g_leak also tend to reduce the models' excitability. This is evident in the first experiment even when the cell is provided with an input that would cause an AP for lower g_leak and g_gap values, an AP is not created if g_gap and/or g_leak is too high. In this experiment, there are a narrow range of values of g_gap that allow for an AP to spread across to the second cell. In experiment 2, when a subthreshold input is introduced to the second cell, APs from the first cell spread through the gap junction and cause a spike in the second cell at lower values of g_gap, but at the cost of reducing the amplitude of AP in the first cell. Similar behavior is observed with the reduced cell model, while the patterns of the AP amplitude as a function of g_gap and g_leak change slightly.

Conclusions: Gap junctions and leakage currents cause drastic changes in both detailed bushy cell models' and reduced cell models' behavior. These findings may shed light on how gap junctions might be a mechanism for excitation to propagate between connected bushy cells and contribute to synchrony enhancement in bushy cell networks.

An Information Theoretic Analysis of the Frequency Following Response Without Amplitude Partitioning

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Background: The Frequency Following Response (FFR) is a steady-state auditory evoked potential that is sensitive to sustained features within a stimulus and is dependent on the integrity of phase-locked neural activity in the auditory brainstem. The FFR reflects the fine structure and the temporal envelope of the stimulus. In humans, the FFR has been used to study subcortical representations using many different stimuli such as sinusoids, tonal sweeps, two-tone approximations of vowels, synthetic steady-state vowels, pitch encoding of lexical tones, and naturally-produced vowels. Many methods have been used to analyze the FFR such as pitch tracking, autocorrelation, and phase coherence. These methods provide quantitative metrics that primarily relate to the fundamental frequency of the stimulus. Zen et al [J Neurophysiol 122: 2372–2387, 2019] have introduced the use of information theory to quantify the mutual information between a stimulus and the evoked FFR. Whilst this method can quantify transmission over the whole bandwidth of the stimulus, rather than at just at the fundamental frequency, it requires a somewhat arbitrary partitioning of the FFR amplitudes into bins. In this study, we introduce an alternative method for calculating information transmission between a stimulus and the FFR that does not require partitioning. The method is therefore easier to apply and removes potential sources of bias in an analysis.

Methods: For each stimulus we calculate the ensemble average of the responses. We then calculate the information transmission in bits between each ensemble average and the corresponding stimulus using methods that Stein et al. [Biophysical Journal, 12, 295-322, 1972] introduced for neural modelling. These are based on the coherence between the two signals - a function of frequency that indicates how well the input corresponds to the output at each frequency. The coherence function is given by the cross power spectral density of the input and the output that is normalized by the power spectral densities of both the input and the output. We calculate the spectral densities using Welch's averaged modified periodogram method. From the coherence function, we then estimate the lower bound of the total information transmission over the whole bandwidth of the signal and the information transmission in particular frequency bands.

Results: We show that the method can be used to analyze information transmission between vowel stimuli and the evoked FFR and that artificial degradation of the FFR in different frequency bands leads to congruous changes to information transmission. By way of example, we show statistically significant differences in information transmission between younger and older listeners with normal hearing for their age.

Conclusions: We conclude that calculation of information transmission using the coherence function provides a useful tool for FFR analysis and may extend understanding of auditory processing in the auditory brainstem.

Release of Urocortin 3 Strengthens Synaptic Transmission at the Calyx of Held, Reduces Transmission Failures and Defines the Timing of MNTB Output

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Background: Urocortin 3 (UCN3) is a neuropeptide that is released independently of the body stress axis and is part of an evolutionarily-conserved complex of reactions, whose patterns and brain circuit involvement have little inter-species but high inter-individual variability. The latter is due to the necessary flexibility of coping strategies in response to internal or external stressors. The auditory system plays a dominant role in the remote sensing of external stressors like predators, making the interaction of stress peptides and auditory processing key for the animals' survival. Here we investigate the expression and function of the stress peptide UCN3 and its receptor CRFR2 at the calyx of Held synapse in the medial nucleus of the trapezoid body (MNTB) in the auditory brainstem.

Methods: Patch-clamp recordings were performed on coronal brainstem slices of C57Bl6 mice at 36±1°C. Only calyces and neurons located laterally within the MNTB were selected. Afferent fibers were activated by electrical midline stimulation. Internal solutions contained fluorescent dyes to confirm calyceal or postsynaptic recordings. Postsynaptic data were collected from P14-P30. Presynaptic data were recorded from P9-P12 (young) and from P13-P25 (mature). Single-unit MNTB recordings in vivo were obtained from mature C57Bl6 mice under MMF anesthesia. Extracellular recordings from MNTB neurons exhibited a typical complex waveform, comprised of a presynaptic and a postsynaptic component. The time between the peak and trough of extracellular APs were used as markers for AP half-width.

Results: Our results show that frequency of mEPSCs is significantly reduced during pharmacological blockade of the CRFR2 receptor, suggesting that UCN3 is constitutively released from the calyces of Held in the lateral subdivision of MNTB. Presynaptic patch-clamp recordings from the calyx of Held show that the application of UCN3 broadens the presynaptic action potential, while the absence of UCN3 explored using a UCN3 KO model, reduces the presynaptic half-width. Interestingly, the lack of UCN3 also leads to a shorter synaptic delay, faster first spike latencies in response to sound stimulation and to more transmission failures during high-frequency stimulation.

Conclusions: In conclusion, our data suggest that UCN3 is endogenously released at the calyx of Held-MNTB synapse. UCN3 binding to the CRFR2 receptor leads to increased synaptic efficacy and a correctly timed MNTB output. Therefore, the presence of UCN3 signaling seems to be important for the coincidence of inhibitory input from the MNTB and excitatory input from the cochlear nucleus at the lateral superior olive and aid sound localization accuracy during stressful situations.

Endbulb of Held Synapses With Calretinin Expression Show Improved Synaptic Efficacy With Reduced Asynchronous Release During High-Rate Activity

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Background: Calretinin (CR) is a major calcium-binding protein widely expressed in the central nervous system. However, its synaptic function remains largely elusive. At the ventral cochlear nucleus, principal bushy neurons receive auditory nerve inputs via large endbulb of Held synapses. Our previous study showed that endbulb of Held synapses selectively express calretinin (CR). These synapses transmit auditory information from different subtypes of spiral ganglion neurons with diverse levels of spike activities. We hypothesize that endbulb of Held synapses with and without CR expression differ in synaptic properties to accommodate such different activities.

Methods: Combining electrophysiology with immunohistochemistry, we investigated the synaptic transmission at the endbulb of Held synapses with and without endogenous CR expression in mature CBA/CAJ mice of either sex.

Results: Both synapse subtypes had similar spontaneous and single evoked release under quiescence, except a larger quantal size in CR-expressing synapses. During high-rate stimulus trains, CR-expressing synapses showed improved synaptic efficacy with significantly less depression and lower asynchronous release, suggesting more efficient exocytosis than non-CR-expressing synapses. Conversely, CR-expressing synapses had smaller readily releasable pool sizes, which were countered by higher release probability and faster synaptic recovery to support sustained release during high-rate activity. EGTA-AM treatment did not change the synaptic transmission of CR-expressing synapses, but reduced synaptic depression and decreased asynchronous release at non-CR-expressing synapses, suggesting that CR decreased calcium accumulation during high-rate activity.

Conclusions: These findings suggest that CR may play a key role in promoting synaptic efficacy during sustained activity at the endbulb of Held synapses.

Developmental Shift to Mitochondrial Respiration for Energetic Support of Sustained Transmission During Maturation at the Calyx of Held

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Background: A considerable amount of energy is expended following presynaptic activity to regenerate electrical polarization and maintain efficient release and recycling of neurotransmitter, especially at synapses in the auditory system that fire continuously at relatively high frequency. Mitochondria are the major suppliers of neuronal energy in the brain, generating ATP via oxidative phosphorylation. However, the specific utilization of energy from cytosolic glycolysis at the presynaptic terminal during synaptic activity remains unclear and controversial. We use the calyx of Held, an auditory synapse optimized for high-frequency transmission in mice, to test sources of energy used to maintain transmission during short activity bursts (<1 s) and sustained activity (30–150 s). We acutely and selectively blocked glycolysis and mitochondrial respiration in a synaptic preparation where mitochondria and synaptic vesicles are prolific, under near-physiological conditions.

Methods: Acute brainstem slices were generated from C57bl6 mice of both sexes at P8-10 or P16-18, and used for postsynaptic whole cell voltage clamp recording of MNTB principal cells. Synaptic activity at the calyx of Held was driven by midline extracellular stimulation of the auditory stria near midline. Glycolysis or

mitochondrial ATP production were acutely inhibited during recordings by bath application of inhibitors and removal of metabolic substrates. Recordings were made at near physiological temperature.

Results: On the whole, we observed that selective inhibition of either ATP production pathway did not affect synaptic depression or recovery during short bursts of high-frequency activity. In slices from young animals before the onset of hearing, where the synapse is not yet fully specialized, both glycolytic and mitochondrial ATP production are required to support sustained, high-frequency neurotransmission. Mature synapses had no glycolytic requirement if pyruvate was present, but lost transmission if mitochondrial respiration was inhibited. Only when driving transmission at high frequency for extended periods, several minutes in mature animals, do we consistently observe defects in synaptic transmission. During sustained high-frequency activity, APs failed first, followed by dysregulation in SV recycling and release. Modeling of synaptic failures showed ATP demand increased supralinearly with sustained stimulation.

Conclusions: Although most neuronal ATP is produced by mitochondria, the role of glycolysis is less well appreciated. This study examined the relative contribution of glycolytic- versus mitochondrially derived ATP to support presynaptic function during high-frequency neurotransmission, in a synapse specialized for sustained high frequency firing. To our surprise, there was little activity dependent effect of inhibiting either pathway during brief stimulation trains beyond a previously described effect on initial SV release, and failures were only apparent in response to sustained high-frequency stimulation.

Can't Touch This: The Ventral Nucleus of the Trapezoid Body is Spared in an Animal Model of Autism Spectrum Disorder

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Background: Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by repetitive behaviors, poor social skills, and difficulties with communication and hearing. The hearing deficits in ASD range from deafness to extreme sensitivity to routine environmental sounds. Previous research from our lab has shown drastic hypoplasia in the superior olivary complex (SOC) in both human cases of ASD and in an animal model of autism. However, in our study of the human SOC, we failed to find any changes in the total number of neurons in the ventral nucleus of the trapezoid body (VNTB) or any changes in cell body size or shape. Similarly, in animals prenatally exposed to the antiepileptic valproic acid (VPA), we failed to find any changes in the total number, size or shape of VNTB neurons. Based on these findings, we hypothesized that the neurotransmitter profiles, ascending and descending axonal projections of the VNTB are also preserved in these neurodevelopmental conditions.

Methods: VPA exposure in dams was completed via oral doses of the drug in peanut butter on embryonic days 10 and 12. Pups were weaned on postnatal day 28 and only male pups were included in this study. To investigate neurotransmitter profiles of the VNTB, we utilized immunohistochemistry for acetylcholine using choline acetyltransferase (ChAT) and for GABA using glutamate decarboxylase (GAD). Projection patterns were investigated via stereotaxic injections of retrograde tracers Fast Blue or Flourogold in the cochlea, central nucleus of the inferior colliculus (CNIC) and medial geniculate (MG).

Results: We found no difference between control and VPA-exposed animals in the number of VNTB neurons immunoreactive for ChAT or GAD. Our results indicate no significant differences in the ascending and descending projections from the VNTB between control and VPA-exposed animals despite drastic changes in these projections from surrounding nuclei.

Conclusions: These findings provide evidence that certain neuronal populations and circuits may be protected against the effects of neurodevelopmental disorders.

Inferior Colliculus Morphology is Associated With Subcortical Envelope Responses in Older and Younger Adult Humans

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Background: The inferior colliculus (IC) is an auditory brainstem region that is crucial for temporal processing. Prior work in our group investigated subcortical envelope processing in younger and older adults using electroencephalography and found no age differences in the representation of sustained temporal envelopes. These findings are consistent with literature showing that age differences in subcortical envelope processing are

generally limited to onset responses and stimuli with high modulation rates, and may be driven primarily by peripheral hearing loss rather than age per se. However, the structural underpinning of these normative differences in envelope processing are less well understood in humans. The current study characterizes the extent to which structural morphology of the IC, particularly white matter architecture, potentially changes with age in humans and relates to temporal envelope processing.

Methods: Subcortical temporal envelope processing (via envelope following responses, EFRs) and structure of the inferior colliculus (R1 or longitudinal relaxation rate) was assessed in 27 older (56-82 years, Mean = 65.6 years, SD = 6.67 years, 19 females) and 16 younger adults (19-30 years, Mean = 24.7 years, SD = 3.52 years, 10 females). EFRs were measured in response to amplitude modulated tones with carrier frequencies of 3000 Hz, modulation frequencies of 80 Hz, and three modulations depths. At each modulation depth, EFR metrics of phase locking value (PLV), response amplitude at the modulation frequency (SNR), and stimulus-to-response correlation coefficients (SRcorr) quantified the synchrony, strength, and regularity of the neural response. Cross-correlation coefficients were calculated between EFRs at full and at the shallowest modulation depths to quantify robustness to degradation (Xcorr). An MP2RAGE sequence (Siemens Prisma 3T scanner: TE = 2.98 ms, TR = 5000 ms, TI1 = 700 ms, and TI2 = 2500 ms) was used to collect quantitative R1 images that are sensitive to myeloarchitecture. The average R1 was collected from within bilateral inferior colliculus probabilistic ROIs that were derived from IC histologic data. Linear regression was used to assess the degree to which differences in IC R1 predicted EFR metrics and changed with age.

Results: Results show that IC morphology (R1) significantly predicted all EFR metrics after controlling for pure-tone thresholds (β = 0.3-0.45, $p < .049$, 95% Confidence intervals based on 10,000 bootstrap iterations). That is, younger and older adults with higher R1 exhibited EFRs with higher SNR, better temporal regularity, and higher synchrony. Consistent with prior results, there were no age differences in the EFR metrics or in the IC R1 values, suggesting that these results were strongly influenced by normative individual differences.

Conclusions: Taken together, the results suggest that normative variation in inferior colliculus white matter can explain, at least in part, envelope processing in the brainstem, an important feature of temporal processing.

Multiple Sources of Cholinergic Input to the Superior Olivary Complex
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Background: The superior olivary complex (SOC) exhibits high expression levels of cholinergic receptors but few studies have addressed the functional role of acetylcholine in the region. The source of the cholinergic innervation is unknown for all but one of the nuclei of the SOC, limiting our understanding of cholinergic modulation. The medial nucleus of the trapezoid body, a key inhibitory link in monaural and binaural circuits, receives cholinergic input from the pontomesencephalic tegmentum (PMT) and also from other SOC nuclei (Zhang et al., 2021 J. Neurosci. 41:674-688). Here, we investigate whether these same regions are sources of cholinergic input to other SOC nuclei. We also investigate whether individual cholinergic cells send collateral projections bilaterally (i.e., into both SOCs), as has been shown at other levels of the auditory system.

Methods: We injected fluorescent retrograde tracers into the SOC in adult gerbils, then identified retrogradely-labeled cells that were also immunolabeled for choline acetyltransferase (ChAT), a marker for cholinergic cells. By injecting different tracers into the left and right SOC we were able to identify cholinergic cells that send branching axonal projections to both targets.

Results: Following tracer injections, we observed ChAT+ (cholinergic) and ChAT-negative tracer-labeled cells in multiple regions. Tracer-labeled ChAT+ cells were numerous in the SOC and the PMT ipsilateral and contralateral to the tracer deposit. These cholinergic projections appear to innervate all major nuclei within the SOC. We also observed a small cholinergic projection into the SOC from the lateral paragigantocellular nucleus (LPGi), a small nucleus in the reticular formation that has numerous ties to the auditory pathways.

Conclusions: The SOC receives cholinergic input from three sources: the PMT, the LPGI, and cholinergic cells within the SOC itself. The various sources likely serve different functions. The PMT has been associated with arousal, sleep-wake cycle, sensory gating, reward and cortically-driven plasticity. The SOC is likely to provide feedback that is more narrowly tuned to specific auditory stimuli. The LPGi, like the SOC, receives ascending auditory input from the cochlear nucleus and descending input from the midbrain and cortex, so it could also contribute to bottom-up and top-down modulation. Finally, individual cholinergic neurons in each of these regions – SOC, PMT and LPGi – can send axonal branches into both left and right SOCs. Such projections present an opportunity for cholinergic modulation to be coordinated across the auditory brainstem.

Synaptic Plasticity of Inhibitory Synapses Onto Medial Olivocochlear Efferent Neurons

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Background: The synaptic plasticity of inhibitory synapses onto medial olivocochlear (MOC) efferent neurons in the brainstem is poorly understood. Inhibitory inputs onto MOC neurons from neurons of the medial nucleus of the trapezoid body (MNTB) have been identified but the effect of synaptic stimulation rate and calcium concentration have not been fully characterized.

Methods: MOC neurons in the ventral nucleus of the trapezoid body (VNTB) were genetically identified for recordings in brainstem slices from P14-P23 mice using a ChAT-IRES-Cre mouse line crossed with a tdTomato reporter mouse line to label cholinergic neurons. To investigate plasticity at these synapses we performed whole-cell patch-clamp recordings of evoked post-synaptic currents (ePSCs) from MOC somata while electrically stimulating presynaptic MNTB axons to release neurotransmitter. AMPA type glutamate receptors were blocked with CNQX to isolate inhibitory neurotransmission. Different extracellular calcium concentrations from 0.5 to 2.0 mM were used to test the dependence of synaptic plasticity on extracellular calcium. Electrical stimulation was delivered at rates from 10 to 500 Hz. In some experiments MNTB axons were stimulated at rates of 10-100 Hz to mimic spontaneous background action potential rates, followed by faster stimulation rates to mimic sound-evoked activity.

Results: Evoked PSCs depressed at stimulation rates from 50 to 100 Hz in physiological (1.2 mM) and high (2.0 mM) calcium. In low (0.8 mM) calcium MNTB synaptic responses facilitated with 100 Hz stimulation. ePSCs were rare at the lowest calcium concentration (0.5 mM). At the fastest rates of 200 and 500 Hz (in 1.2 mM calcium) responses strongly depressed and distinct PSCs could not be observed, but a tonic inhibition remained despite synaptic depression. When low rates of stimulation were applied to mimic spontaneous MNTB activity, responses to subsequent faster synaptic activity were reduced in amplitude relative to control conditions. To determine the kinetics of recovery from depression at 1.2 mM and 2.0 mM calcium, ePSCs were evoked from a single pulse applied to MNTB axons following 100 Hz stimulus train at different intervals ranging from 20 ms to 15 s following the stimulus train. ePSC amplitudes recovered to baseline faster in the higher calcium condition.

Conclusions: Overall these results suggest that MNTB suppression of MOC activity is reduced over the course of a sustained stimulus to reach a tonic inhibition of MOC neurons. In addition, because MNTB neurons have a high spontaneous rate of activity in vivo, MNTB-MOC synapses may be tonically depressed even in quiet. However, faster rates of MNTB-MOC synaptic activity that mimic sound-evoked activity are able to evoke significantly larger ePSCs in MOC neurons, suggesting that the MNTB will inhibit MOC neurons even on a background of tonic synaptic depression.

Olfactory Function in Classical (Infratentorial) Superficial Siderosis

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Background: Infratentorial (classical) superficial siderosis (iSS) is a rare but potentially disabling and progressive neurological disorder that commonly involves auditory and vestibular neural pathways. Olfactory nerve function may also be affected in iSS but dedicated studies are lacking. The aim of this study was to assess olfactory function in individuals with iSS.

Methods: Ethics approval was granted. Eleven participants with a known diagnosis of iSS and no prior symptoms or history of COVID-19, were provided with the 40-item (British version) University of Pennsylvania Smell Identification Test (UPSIT) kits by post. The scores (40 as maximum best) were calculated and compared with age- and gender-matched norms; percentile values were obtained. Olfactory function was graded as normal, microsmia (mild/moderate/severe) and anosmia. Smoking status was determined; pack-years, years since smoking-cessation and disease duration (from presumed causative event) were calculated.

Results: Ten participants completed the test. The mean (±standard deviation, SD) age was 52.5 (±14.5) years; 2 (20%) were females; 6 (60%) never smoked; 1 participant (10%) was a current smoker. The mean (±SD) disease

duration (n=9) was 23.2 (±11.4) years. The mean (±SD) UPSIT score was 25.5 (±7.8), with no difference between males/females (t=-1.528, p=0.165). The UPSIT scores were statistically significantly lower than age-/gender-matched norms (t=4.016; p=0.003), and below the 30th centile for all participants (<10th percentile for 4 (40%); in 10-20th percentile for 5 (50%); in 20-30th percentile for 1 (10%)). Six (60%) had anosmia or moderate microsmia and 4 (40%) had mild microsmia. There was no correlation (assessed by Kendall's tau-b, Tb) (p>0.05) between the UPSIT scores and: age (Tb(10)=-.114); years since smoking-cessation (Tb(4)=.333), pack-years (Tb(4)=-.333), or disease duration (Tb(9)=.310).

Conclusions: We report a novel quantitative assessment of the prevalence of olfactory dysfunction in iSS. Given its high prevalence, olfactory dysfunction may be another key feature of the iSS clinical syndrome that is currently under-investigated but should routinely be assessed.

Assessing the Clinical Prevalence and Management of Auditory Neuropathy Spectrum Disorder (ANSD) and Cochlear Nerve Deficiency (CND) in a Pediatric Cohort

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Background: Disorders of the auditory nerve such as Auditory Neuropathy Spectrum Disorder (ANSD) and Cochlear Nerve Deficiency (CND) can profoundly impact children's ability to hear and develop language. These two disorders can present similarly during auditory brainstem response (ABR) testing, which results in delayed differential diagnosis. Furthermore, appropriate management options, including candidacy for cochlear implantation, cannot be established until nerve status is determined via imaging. Due to limitations in timeline and modality of imaging, the prevalence of ANSD and/or CND in children remains poorly understood, which may contribute to variability in outcomes. This study aimed to examine the clinical prevalence of ANSD and CND in a cohort of pediatric patients at Stanford Children's Health. Additionally, this study evaluated trends in management for children with ANSD and/or CND.

Methods: A retrospective chart review was conducted for 85 children between 0 and 21 years of age who were diagnosed with ANSD, CND, or both disorders. Diagnostic criteria for ANSD included the presence of a cochlear microphonic with absent or grossly abnormal neural ABR waveforms or present distortion product otoacoustic emissions (DPOAEs) with behaviorally obtained sensorineural hearing loss, both suggesting normal outer hair cell function with abnormal auditory nerve function. Diagnostic criteria for CND were defined by pediatric radiology reports noting an "absent or hypoplastic nerve" on MRI or a "narrow internal auditory canal and/or stenotic cochlear aperture" on CT. Although MRI is the preferred imaging modality for neural structures, CT has historically been more common for hearing loss workup. Only recently has MRI gained traction in this diagnostic algorithm. The prevalence of ANSD and/or CND as well as intervention options were assessed.

Results: Of the 85 children in this cohort, imaging results were unavailable for 23 children with ANSD (16 unilateral; 7 bilateral). Of the 62 children for whom imaging results were available, the prevalence of concomitant ANSD and CND in the same ear was about 20%, the majority of which (approximately 70%) were unilateral. Of the ears with ANSD for which imaging results were available, approximately 25% had concomitant diagnoses of CND. Of ears with CND, approximately 30% presented with ANSD. Among children with bilateral ANSD and/or CND, the most common intervention was cochlear implantation followed by hearing aid use. Only a small subset of children with bilateral ANSD and/or CND did not use hearing devices. In contrast, the majority of children with unilateral ANSD and/or CND did not use any device. Of those who did, the most commonly used devices were those that routed sound to the better-hearing ear.

Conclusions: These results suggest concomitant diagnoses of ANSD and CND are common in children. The finding that the majority of cooccurrences are unilateral underscores the importance of early imaging to determine all possible intervention options.

Exploring MEMR and Natural Sounds in Connection With Hyperacusis

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Background: The Middle Ear Muscle Reflex (MEMR) is an involuntary contraction of the stapedius muscle in response to loud sounds to protect the middle ear from damage. A study from Saxena et al. (2020) showed abnormal responses in the MEMR intensity for participants suffering from oversensitivity to sounds (hyperacusis). Hyperacusis is usually assessed with questionnaires (Hyperacusis Questionnaire (HQ); Khalfa, 2002) or LDL. Enzler at al. (2021) developed the "Core Discriminant Sounds (CDS)", using natural sounds instead of artificial sounds for hyperacusis assessment. These results indicate a connection between hyperacusis and MEMR using artificial stimuli, and that hyperacusis can be assessed using natural sounds. It remains unclear how MEMR and hyperacusis are connected when using natural sounds. We tested MEMR with artificial and natural sounds to assess how the perception of pleasantness affects MEMR in participants with and without hyperacusis.

Methods: Normal hearing subjects participated in the experiment. The presence of hyperacusis and tinnitus was assessed using HQ, CDS, and a question on tinnitus presence (yes or no). The MEMR was measured using a research platform interfaced with clinical equipment (Titan, Interacoustics). The wide band tympanometry was measured. The relative tympanic peak pressure was extracted and used to measure the MEMR from 60 to 100 dB, in 5 dB steps (2 natural and 3 artificial sounds for experiment 1 and 11 natural and 3 artificial for experiment 2). For each sound at each level of the stimulus, the participant was asked to rate through a Visual Analogue Scale both the pleasantness and loudness of the sound.

Results: The results show that is possible to measure the MEMR with natural sounds. The response varies across sounds and listeners. In experiment 1 the results showed the Wide-Band Noise being the most effective in generating a response, while the Pure Tone at 500 Hz the weakest. Pleasantness or loudness perception were not found to be suitable predictors for the strength of the MEMR response. Given that equal MEMR strength can be elicited by sounds with different perceived pleasantness, the results indicate that this might be used to customize the MEMR test and make it less bothering for people suffering from hyperacusis. Our data could not confirm the correlation between hyperacusis and the MEMR strength.

Conclusions: The reason why some sounds are more effective than others is still uncertain. One reason could be the differences in spectro-temporal properties of the stimuli used. Data for longer stimulus intervals to allow for identification of the sound show that the method used to assess MEMR for transient sounds is not directly transferrable for longer stimulation. It needs to be clarified if this is a technical limitation or due to physiological changes in the response when the stimulus is less and less transient.

Can Hidden Hearing Loss Be Predicted Using Outer Hair Cell Normative Data?
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Background: Persons with hidden hearing loss (HHL), defined as simultaneously exhibiting auditory thresholds within normal limits accompanied by an underlying otopathology, may report difficulty hearing in the presence of background noise. Our previous studies have shown that outer hair cell (OHC) dysfunction is one otopathology that can occur in persons hearing within normal limits, OHC dysfunction is highly correlated with both an increase in auditory thresholds, and a decrease in hearing-in-noise performance in persons exhibiting HHL and Mild-Moderate sensorineural hearing loss. The aim of this current study is to determine whether HHL can be predicted by normative OHC function data in a clinical population.

Methods: A pilot study of 20 adults hearing within normal limits (all thresholds less than 25 dB HL) were asked whether they experienced difficulty hearing in noise or experienced tinnitus and then Distortion-Product Otoacoustic Emissions (DPOAEs dB SNR) were measured. These frequency specific dB SNRs were compared to the dB SNRs (lower-bound 95% confidence intervals) obtained from our previous study (N=285) to determine whether these normative values could be used to predict hearing in noise difficulty persons hearing within normal limits. Persons with dB SNRs below the lower bound 95% confidence intervals were designated as the patient, and those persons exhibiting dB SNRs above this value were designated as healthy. A True Positive (TP) was defines as a person with dB SNRs below the lower bound 95% confidence intervals and who also reported difficulty hearing in noise, while a True Negative (TN) was defined as someone with robust dB SNRs and no complaints of hearing in noise. A False Positive (FP) was defined as someone with low dB SNRs and who didn't report difficulty hearing in noise, while a False Negative (FN) was defined as someone with low dB SNRs and who reported difficulty hearing in noise. The following statistical metrics were derived from this rubric; Accuracy (TP+TN/TP+TN+FP+FN), Specificity (TN/TN+FP), Sensitivity (TP/TP+FN), Positive Predictive Value (TP/(TP+FP), and Negative Predictive Value (TN/(FN+TN).

Results: DOAPE dB SNR measurements exhibited 100% Sensitivity, 43% Specificity, a 43% Positive Predictive value, and 100% Negative Predictive Value, and an Accuracy of 60% in predicting hearing in noise difficulty.
Conclusions: Based on the statistical characteristics of this small pilot study, DPOAE dB SNR may suited for screening purposes but may not be suitable for the final confirmation of difficulty hearing in noise. These results are expected to change as the sample size is increased.

Chromatin Remodeling Protein CHD4 is Required for Spiral Ganglion Neuron Development
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Background: In the mammalian cochlea, spiral ganglion neurons (SGN) innervate hair cells (HC) in a stereotypic pattern during development. Improper wiring and abnormal function of SGNs impairs audition. The chromodomain helicase binding protein 4 (CHD4) is an ATP dependent chromatin remodeler and a component of the NuRD (nucleosome remodeling and deacetylase) complex that alters chromatin accessibility and inhibits transcription. De novo missense mutations in CHD4 causes Sifrim-Hitz-Weiss syndrome (SIHIWES). Patients with SIHIWES show delayed development, intellectual disability, facial dysmorphism, and ear abnormalities including hearing loss. The molecular and cellular mechanisms of how CHD4 mutations leads to hearing loss are not known.
Methods: To study whether epigenetic activity of Chd4 is required for SGN development, we generated a mouse model that ablates exons coding the ATPase domain of Chd4 in SGNs and marks mutant cells with tdTomato fluorescence. Cochlea tissues were collected and subjected to either immunostaining or single molecule fluorescence in situ hybridization (smFISH) to determine changes in protein or mRNA levels after Chd4 deletion. Using neurons derived from immortalized multipotent otic progenitors (iMOP), direct target genes of CHD4 were identified using CUT and Tag. Cellular functions for CHD4 were inferred by gene ontology analysis. To explore if gene expression levels of target genes are altered in Chd4 cKO, quantitative reverse transcription PCR (RT-qPCR) was performed.
Results: Neurogenin1(Ngn1) CreERT2 tdTomato (control) and a Ngn1 CreERT2 Chd4 flox/flox tdTomato (cKO) mice were generated. The cKO animals were viable after tamoxifen induction at E8.5-10.5. Harvested E18.5 cochlea from cKO and control animals showed no macroscopic differences. However, cKO displayed an increased number of tdTomato+Tuj1- cells in the cochlea compared to control. Dual labeling with Tuj1 and peripherin was used to identify radial and spiral peripheral axons originating from spiral ganglion neurons. Chd4 cKO showed disorganized innervation of outer HC (OHC) and increased prevalence of fibers entering the OHC region. 3D-rendered images displayed extension of outer spiral fibers under OHCs in the cKO. The pattern of inner radial bundles projecting to inner HCs were also altered. High magnification images revealed disorganized radial bundles in Chd4 cKO that significantly decreased spacing between fiber bundles. Using iMOP-derived neurons, CUT and Tag was employed to identify CHD4 target genes. Gene ontology analysis of CHD4 target genes implicated CHD4 in regulating axonogenesis and axon guidance. Changes in target gene expression that correlate with SGN phenotypes were validated in Chd4 cKO cochlea by RT-qPCR or smFISH.
Conclusions: Taken together, the results of the current study suggest that CHD4 is required for SGN development. Ablation of CHD4 chromatin remodeling activity alters gene expression and changes the stereotypic pattern of peripheral projections from spiral ganglion neurons.

Rapid Tissue Dynamics During the Formation of the Calyx of Held
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Background: The largest mammalian CNS nerve terminal, the calyx of Held (CH), provides strong and temporally precise excitation of target principal cells of the medial nucleus of the trapezoid body (MNTB). The CH exhibits hallmark developmental features of strengthening and pruning necessary to yield monoinnervation of MNTB neurons. Using serial block-face scanning electron microscopy (SBEM), we previously found that between postnatal (P) days 2 and 4, most of the 10-20 small pioneering inputs on each cell are pruned while the remaining 1-3 terminals expand as rapidly as 200 μm2 per day. By P6, 75% of principal cells are monoinnervated suggesting

most competition is resolved between P3-6. During CH formation, collateral processes of variable length extend from the edges of the developing terminal and permit local influx of Ca2+. Although we found no evidence of direct interaction between CHs along the principal cell soma, collaterals largely occupy shared territories as revealed by overlapping convex hull volumes. However, little is known about the dynamics of the calyceal collaterals during terminal formation.

Methods: Here, we sought to examine the temporal changes in synaptic organization in the MNTB by employing a rapid and high-resolution image acquisition technique, lattice light-sheet (LLS) microscopy. Acute coronal brainstem slices (300-600 μm thickness) were produced from neonatal mice ranging from P0-14. Following the 4D image acquisition, data were imported into syGlass ™, a custom software package designed in-house, for immersive virtual reality (VR) aided-analysis.

Results: We found filopodia and growth cone-tipped collaterals collectively form a dynamic field around each CH. Moreover, the extent and motility of the collateral network exhibited an age-dependent relationship wherein peak growth rates rival or exceed rates of axonal extension described elsewhere in the CNS. Growth cones, perhaps the most dynamic structure in this system, extend fastest during the ages of CH expansion yet slow after monoinnervation has been established. Moreover, live imaging and ultrastructural analysis demonstrate that CH arbors frequently form transient associations between developing terminals and that such contacts may serve a communicative role in circuit formation.

Conclusions: These data indicate significant interaction among growing calyces in locations off of the somatic surface which may underlie the organization of the developing neuropil. Examples of such interactions can lead to attractive or repulsive dynamics which persist only during only the period of early postnatal development.

Neuroligin-1 Identified by Mice Genome-Wide Association Study is the Novel Organizing Protein of the Cochlear Ribbon Synapse

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Background: The cochlear ribbon synapse is a key apparatus for hearing perception between mechanosensitive hair cells and the cochlear nerve. Cochlear synaptopathy is a common mediator of acquired hearing loss, such as that from presbycusis or noise-induced hearing loss; however, little is known about the underlying molecular mechanism or synaptic organizing proteins in the cochlea. Our previous genome wide association study utilizing suprathreshold auditory brainstem response wave 1 intensity identified Neuroligin-1 (Nlgn1) as a candidate genetic marker for age-related and noise-exposure related hearing loss. Nlgn1 is a post-synaptic cell adhesion protein involved in synaptic transmission. Mutations in Nlgn1 have been associated with various neurological diseases such as autism and Alzheimer's disease. However, the role of Nlgn1 in the cochlear synapse has not been reported.

Methods: We generated inner ear specific Nlgn1 conditional knock-out mice (Nlgn1-cKO) controlled by Pax2-Cre. Wild type (WT) and Nlgn1-cKO mice underwent auditory brainstem response (ABR) testing at 8 weeks of age. Wave 1 of the ABR was subsequently analyzed. Immunostaining of Nlgn1 and the synaptic marker CtBP2 was performed in WT and Nlgn1-cKO mice at 8 weeks of age.

Results: 8-week-old Nlgn1-cKO mice showed a statistically significantly increased wave 1 latency at the vast majority of noise levels tested (4, 8, 16, and 24kHz) when compared to WT mice. This difference was not observed for response thresholds and wave 1 amplitude. Immunostaining revealed that Nlgn1 localized at the postsynaptic neuron. WT and Nlgn1-cKO mice had a similar number and distribution of CtBP2.

Conclusions: This is the first study investigating the effects of Nlgn1 in the inner ear. Nlgn1 localizes at the post synapses and wave 1 on ABR was significantly delayed in Nlgn1-cKO mice, indicating that Nlgn1 plays a pivotal role in the functional maturation of cochlear synapses. These findings may represent a unique phenotype of Nlgn1 distinct from our GWAS utilizing suprathreshold wave 1 intensity. Our findings may provide new therapeutic targets for the prevention of cochlear synaptopathy and acquired hearing loss.

Transcriptomic Differences Between Adult Cochlear and Vestibular Sensory Epithelia Revealed by Single-Cell RNA-Seq

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Background: The sensory epithelia of the mammalian inner ear's auditory and vestibular end organs contain mechanosensitive hair cells (HCs) and supporting cells (SCs). HCs transduce mechanical stimuli, i.e., movement in their environment, into electrical activity. SCs, on the other hand, maintain homeostasis of ionic and chemical environment surround HCs. Although HCs and SCs in the adult cochlear and vestibular end organs are similar, they have substantially different morphology and physiology. Moreover, adult vestibular HCs (VHCs) are able to repair themselves spontaneously after damage while adult cochlear HCs (CHCs) no longer retain the capability. Adult vestibular sensory epithelial SCs (VSCs) have some capacity to spontaneously transdifferentiate to HCs while the cochlear sensory epithelial SCs (CSCs) lose that capability. The molecular mechanisms underlying the differences between adult cochlear and vestibular sensory epithelia are still poorly understood. We used single-cell RNA-seq (scRNA-seq) to examine the transcriptomes of cells dissociated from adult cochlear and vestibular sensory epithelia.

Methods: Sensory epithelium from cochleae and vestibular end organs (sacculus, utricles and crista) were dissected from 6 – 8 CBA/J mice (10 week-old, both sexes). Cells were then dissociated and ran through 10x Genomics single-cell RNA sequencing platform for preparing the cDNA Libraries. Libraries were sequenced on an Illumina NextSeq and scRNA-seq data was analyzed and clustered using Seurat. A minimum of three biological replicates were included for each group. RNAscope was used for validation for gene expression in the two sensory epithelia.

Results: We collected and sequenced in excess of 20282 single cells from cochlea and 11444 single cells from vestibular end organs. After mapping the heterogeneous landscape of all cochlear and vestibular sensory epithelia cells, 21 and 19 distinct cell types were identified from the cochlear and vestibular sensory epithelium, respectively. There are 413 outer hair cells (OHCs), 394 inner hair cells (IHC), 1180 CSCs in the sequencing data from the cochlea, and 231 type I VHCs, 257 type II VHCs and 192 VSCs in the sequencing data from the vestibular end organs. The analysis identified both common and unique genes in CHCs, VHCs, CSCs, and VSCs. In addition, the representative top 50 differentially expressed genes (DEG) between CHCs and VSCs include Ocm, Gata3, Slc17a8, Lbh, Dnm3, Kcnj13. We generated the Kcnj13 conditional knock out and the mice show severe hearing loss and mild balance disorder at adult age.

Conclusions: We profiled the transcriptomes of HCs and SCs in the adult cochlear and vestibular sensory epithelia at the single-cell level. This analysis provides insights into the transcriptomic characteristics and molecular pathways underlying differences between adult cochlear and vestibular sensory epithelia.

Neural Maturation in the Murine Superior Olivary Complex

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Background: Development in the mouse auditory brainstem is believed to be consolidated and nearly mature around thirty days post-natal (P30). However, mice at P30 are still juvenile and the auditory system is probably fully adult-like only at 3-6 months.

Methods: In this study we observed, through electrophysiological recordings, neuronal passive properties and neurotransmission in two of the main nuclei in the mammalian auditory brainstem: the medial nucleus of the trapezoid body (MNTB) and lateral superior olive (LSO) at P30-40 and in young adults (3-6 months old – 3MO). To avoid the early high-frequency hearing loss seen in C57 mice, we used the CBA strain.

Results: Initially in MNTB development, neurons undergo morphological changes, affecting membrane passive properties, reducing input resistance and making them less excitable. However, our data show no difference in the average input resistance in both MNTB and LSO after P30 ([in $M\Omega$] MNTB: P30=129, n=2; 3MO= 129, n=5 / LSO: P30= 63, n=9; 3MO= 59, n=20) nor in their holding membrane potential ([in mV – same n as the previous dataset] MNTB: P30 =-72; 3 MO=-72 / LSO: P30=-67; 3 MO=-70). Since these nuclei are subjected to intense synaptic input, we verified how they cope with afferent fiber high frequency stimulation (HFS – 100 Hz). HFS produced short term plasticity (STP), leading to depression of the postsynaptic currents. In LSO, it reduced the EPSC amplitudes to $58\pm5.5\%$ (n=9) of the initial amplitude in P30, and $52\pm5.1\%$ (n=12) in 3 MO. In the MNTB, EPSCs were reduced to $46\pm8.0\%$ (n=5) at P30 compared to $68.1\pm3.6\%$ (n=8) in 3 MO. LSO IPSCs also underwent STP, with their amplitudes reduced to $55.5\pm0.6\%$ in 3MO. Data in P30 is yet to be gathered for comparison. STP data also allows us to estimate the vesicle releasable pool (RRP) size. LSO neurons did not show differences in their EPSC RRP size (P30: 2.42 ± 0.7 nA, n=9; 3MO: 2.81 ± 1.0 nA, n=6). LSO IPSCs in 3MO had an RRP size of 1.31 ± 0.23 nA (n=6). MNTB neurons displayed a reduced RRP at P30 (95.3 ± 18.2 nA, n=5) compared to 3 MO (144.1 ± 8.7 nA, n=8). Membrane properties and neurotransmission characteristics determine action potential (AP)

firing. In MNTB for example, HFS protocols at 100 Hz yielded 100% success in both ages (P30, n=6; 3MO, n=7). However, if challenged at higher frequencies (500 Hz), the younger cells tended to fail more often (P30: 2 AP failures out of 6 cells; 3MO: no failures in 7 cells).

Conclusions: These results clearly point towards a yet not fully developed superior olivary complex at P30. Although neurotransmission is functional, neurons fail to fully deliver information when excitatory input interval is short (i.e. high frequency), which is fundamental for a system that operates within the sub millisecond realm.

The Role of Eph/Ephrin Signaling in Type II SGN Turning

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Background: To understand normal development of the auditory system, the molecular mechanisms dictating spiral ganglion (SGN) neuron growth and cochlear innervation must be resolved. SGNs are bipolar neurons that receive glutamatergic input from mechanosensitive receptor hair cells and relay that input to the cochlear nuclei. Type II SGNs represent an interesting subdivision of neurons in the inner ear and have characteristics similar to nociceptors. Type II SGNs have a highly stereotyped projection pattern whereby they shoot past the IHCs, make a 90° turn toward the cochlear base, then extend an extra 100-300 microns while synapsing 10-15 outer hair cells. Previously, it was shown that core planar cell polarity proteins mediate type II SGN turning events (Ghimire et al., 2018, 2019), but it has remained unclear whether additional axon guidance mechanisms are involved. In this research, we are investigating axon guidance mechanisms that facilitate type II SGN guidance and OHC innervation.

Methods: To determine the involvement of Eph/Ephrin signaling in mediating type II SGN turning events, we generated Efna3 null, heterozygous and wildtype mice that possess Neurog1CreERT2 and R26RtdTomato, which together permit SGN sparse labeling. Using immunostaining and confocal imaging we imaged hundreds of type II SGNs. Imaris software and 3D rendering was used to quantify type II SGN turning, branching and other growth and navigation characteristics.

Results: As shown by immunostaining experiments using knockout tissue to control for specificity, Ephrin-A3 appears to be expressed on the membranes of outer pillar and Deiters' cells of the cochlear epithelium. Compared to controls, Efna3 null mice showed a significant increase in type II SGNs incorrectly turning toward the apex. Both Efna3 null and heterozygous mice showed increased proportions of type II SGNs with odd navigation behaviors (e.g., aberrant forking, misrouting). Efna3 nulls also showed increased branch lengths and thicker branch diameters. Interestingly, Efna3 het mice displayed branches with the greatest complexity. In ongoing experiments, type II SGNs from Epha7 null cochleae are being analyzed to determine if EphA7 acts as an Ephrin-A3 receptor in this process. In addition, Efna3; Vangl2 double knockout mice are being generated to determine possible interactions between Eph/Ephrin and planar cell polarity signaling.

Conclusions: Taken together our findings suggest that Eph/Ephrin signaling accompanies planar cell polarity signaling to mediate type II SGN guidance during development.

The Early Whispers of Jagged1: Insights Into Jagged1-Mediated Notch Activation in Inner Ear Patterning From the Nodder Mouse Model for Alagille Syndrome

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Background: Proper auditory function requires a precisely ordered mosaic of mechanosensitive inner hair cells (IHCs), outer hair cells (OHCs) and associated supporting cells within the Organ of Corti. The development of this mosaic necessitates a series of cell fate decisions mediated, in part, by Notch signalling, starting from early pro-sensory domain induction followed by inhibitory cues specifying hair cells versus supporting cells. Jagged1 (Jag1), a Notch ligand, is suggested to be involved in pro-sensory domain development as its expression co-occurs with pro-sensory domain establishment and Notch target gene activation. However, late embryonic lethality of homozygous Jag1 loss of function animals has precluded postnatal analysis of the role of Jag1 in Organ of Corti development.

The Notch1 signalling-defective Jag1 mutated Nodder (Jag1Ndr/Ndr) mouse model is viable in the homozygous condition and recapitulates Alagille syndrome, a multisystem disorder that includes hearing loss. At ARO 2021 we reported hearing impairment and altered hair cell patterning in Nodder mice, including: supernumerary IHCs,

fewer OHCs and atypical cells in the inner hair cell compartment that share morphological and molecular characteristics with OHCs, referred to as OHC – like cells.

To resolve whether the phenotypes described above could be a consequence of aberrant Organ of Corti patterning, we investigated early pro-sensory domain formation in the presence and absence of functional Jag1 in wild type and Nodder mice, and analysed scRNAseq data to investigate Jag1-Notch-mediated cell fate decisions in hair cell development.

Methods: Cellular patterning and expression of Notch target genes was investigated using immunohistochemistry and RNAscope. Stereocilia bundle arrangement was visualized by scanning electron microscopy (SEM). Hearing function analysis was performed by Auditory Brainstem Response (ABR) and Distortion Product Otoacoustic Emission (DPOAE) measurements. In silico modelling of Notch signalling using scRNAseq was done by constructing developmental trajectories using Monocle3/Slingshot in R.

Results: Homozygous Nodder animals show altered inner ear patterning including supernumerary IHCs, fewer OHCs and OHC-like cells in the inner hair cell compartment with morphological and molecular characteristics of outer hair cells, including W-shaped stereocilia bundles and expression of OHC-specific Prestin. Additionally, Nodder animals showed severe hearing loss across all frequencies. Preliminary analysis of pro-sensory domain establishment in Jag1Ndr/Ndr animals indicates minor differences in pro-sensory domain size, but lower expression of specific Notch target genes.

Conclusions: The Nodder mouse model shows impaired hearing function and altered inner ear patterning. Preliminary analysis of pro-sensory domain formation suggests a link between early Notch activation and the later post-mitotic appearance of OHC-like cells in the inner hair cell compartment. Further research is ongoing to understand mechanisms of Notch-mediated patterning of the inner ear.

Investigating the Specification of Spiral Ganglion Neuron Subgroups Using Single Cell RNA-Seq
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Background: The afferent innervation to the cochlea is composed of the spiral ganglion neurons (SGNs), which transmit mechanosensory input from the hair cells centrally to the cochlear nucleus. Based on their morphology, two populations of SGNs can be distinguished in the mammalian cochlea: Type 1 SGNs which constitute 90-95% of the total population, and form contacts with inner hair cells (IHCs); and Type 2 SGNs which constitute the remaining 5-10% of the total population and form contacts with outer hair cells (OHCs). Recent studies using single cell RNA-Sequencing (scRNAseq) have found three molecularly distinct subgroups within the Type 1 population, termed 1A, 1B and 1C. However, despite the importance of the SGNs, relatively little is known about when this cellular diversity arises, and what genes are important in specifying and maintaining the different SGN subtypes.

Methods: We generated a profile of SGN development using scRNAseq to transcriptionally profile 5,441 SGNs across the timepoints E14, E16, E18 and P1. Lineage analysis of this dataset indicates that specification of the SGN subgroups occurs in two phases: an initial split at E14 where SGNs are specified into two lineages which will become either Type 1A/Type 2 or Type 1B/C, followed by later splits at E16 where all 4 groups become distinct.

Results: We were interested in understanding the transcriptional networks which could underlie the specification of SGNs into their respective subgroups. We therefore combined differential gene expression, pseudotime and regulon analyses to identify candidate genes, in particular transcription factors, which could be involved in this process. Among the identified candidates, Pou4f1 appears to be transcriptionally active in the early and Type 1C SGN lineages, suggesting it could be involved in specifying this subgroup. To further investigate this we are assessing the SGNs in a Pou4f1 knockout mouse to understand if deletion of Pou4f1 affects specification of Type 1C SGNs. In addition we are carrying out Bulk RNA-Seq on E14 SG from both wild type and knock out animals to better understand the transcriptional pathways that are regulated by Pou4f1.

Further work is focusing on other gene candidates, for example Tle4 which is specifically expressed in the Type 1A/Type 2 lineage, and Etv4 which is highly expressed as SGNs undergo their initial lineage split at E14, and as Type 2 SGNs become specified. This will involve obtaining knockout mice to assess defects in SGN specification, and/or an in vitro culture system where the effect of gene knockdown can be assessed in a higher throughput manner.

Conclusions: These analyses will aid in our understanding of SGN development and the role of SGN subgroups in normal hearing, and may provide targets for regenerative therapies for hearing loss in the future.

Assessing the Function of Novel Genes Expressed in Developing Outer Hair Cells of the Murine Auditory System

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Background: The organ of Corti (OC) is a complex cellular mosaic of a few thousands of specialized cell types necessary for sound transduction. The inner hair cells (IHC) are the main transducer of auditory information, while the three rows of outer hair cells (OHCs) are necessary for sound tuning and amplification. The high degree of structural complexity and cell heterogeneity in the OC has made it difficult to identify the unique genes responsible for the divergent development of OHCs from the transducing IHCs. To address this barrier, our lab has leveraged the use of single-cell RNA sequencing (scRNA-seq) which can yield valuable insights about the unique temporal genetic expression profile of different cell types. We hypothesize that genes that are uniquely expressed in OHCs are necessary for their proper development and function. We investigated how genes not previously known to be expressed in OHCs contribute to cochlear development, anatomy, and function.
Methods: We used RNA fluorescent in situ hybridization (RNA-FISH) to validate the temporal and topographical expression of OHC candidate genes from the results of scRNA-seq studies. Next, we used knockout (KO) and conditional knockout (cKO) mice to assess the function of those genes. In animals where the KO resulted in viable and fertile offspring, we used auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAE) to assess auditory function. When viable offspring were not available, we used embryonic dissections and ex-vivo cochlear explants to assess anatomy and development of the OHCs.
Results: Despite the robust and unique expression of some genes in OHC we found some mutants to have no substantial disruption in hearing function or anatomy. These results do not negate the need of these genes for proper OHC development. Our results might indicate genetic compensatory effects or an inability to identify the true function with the techniques used.
Conclusions: While the results of scRNA-seq studies can identify intriguing new cell-specific candidate genes, the effects of knocking out those genes during development might result in mild deficits that cannot be observed by our traditional physiological assessments such as ABR and DPOAE. These results suggest that the ability to fully understand the role of some genes may require additional tests and protocols.

The Effects of Age on Outcomes of Cochlear Implant Use in Children and Adolescents With Short Durations of Single-Sided Deafness

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Background: The aim of the present investigation was to identify effects of age at cochlear implantation in a unique cohort of children/adolescents with limited duration of SSD. Past research shows children with SSD provided with CI use their devices consistently and that among children with hearing loss, longer hearing experience is associated with consistent CI use (Easwar et al, 2016; Polonenko et al, 2017, Ganek et al, 2020). In addition, early implantation is found to promote auditory development to supplement hearing outcomes (Polonenko et al, 2017).
Methods: 57 children/adolescents with SSD received a CI after limited durations of deafness (1.94 years ± 1.56 SD). Of these, 40 had prelingual SSD (AgeCI = 2.47 years ± 1.58 SD) and 17 had later onset of SSD (AgeCI at 11.67 years ± 3.91 SD). Outcome measures were CI use (datalogging in 37 pre-lingual; 16 late-onset), SRTs to measure spatial release of masking (SRM) [9 prelingual; 7 late-onset], speech perception accuracy (RAU) [17 prelingual; 8 late onset] in age-appropriate tests (PBK, MLNT, ESP, GASP and WIPI), self-reported hearing using Speech, Spatial and Qualities of Hearing Scale (SSQ) [35 prelingual; 14 late onset], and localization of stationary and moving sound (band-pass filtered white noise presented within 120° arc in frontal azimuthal/horizontal plane. Linear mixed effect models assessed effects of group on each outcome.
Results: Datalogs revealed slight decreases in daily CI use with ongoing CI experience [Estimate (SE) = -0.039 (0.014) daily hours/months, p = 0.003] but no significant difference in cumulative CI dose (hours) between hearing loss onset groups [F(1, 51) = 1.73, p = 0.19]. Of note, daily CI use across groups decreased during the present pandemic compared to pre-pandemic levels [Estimate (SE) = -1.47 (0.70) daily hours, p = 0.04]. Speech perception (RAU) on the side of the implanted ear was better in children with pre-lingual than later onset SSD

[Estimate (SE) = 23.9 (8.9) RAU, p = 0.008]. Location of stationary sound showed high RMSE (CI on: 26.6°-30.4°; CI off: 25.8°-40.0°) which improved slightly with CI especially on the CI side [Estimate (SE) = -10.4° (5.55), p = 0.06]. SSQ revealed hearing challenges [SSQ total = 6.4 (out of 10)] which increased significantly as sound localization error increased.

Conclusions: Children and adolescents with short duration of SSD can have challenges maintaining consistent daily CI use. These challenges were exacerbated during the pandemic period with associated school closures. Those with later onset of SSD hearing loss experienced more asymmetrical hearing than children with pre-lingual SSD. This is consistent with declining cortical representation from the deaf ear in this group (Lee et al. 2020). Subtle improvements in spatial hearing with CI use could be valuable to these children given increased self-reported hearing challenges with poor sound localization.

A Humanized Murine Model, Demonstrating Dominant Progressive Hearing Loss Caused by a Novel KCNQ4 p.G228D From a Large Chinese Family

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Background: DFNA2 is genetically associated with the pathogenic variants in KCNQ4, a voltage-gated potassium channel. In order to understand the genotype-phenotype correlations of DFNA2 and the role of KCNQ4 in hearing, this work was to report a novel KCNQ4 pathogenic mutation (p.G228D) in patients and establish a murine model with homologous mutation (p.G229D) to recapitulate DFNA2 patients.

Methods: Exome sequencing was performed to identify the novel KCNQ4 mutation in a large Chinese deaf pedigree of patients. CRIPSR/Cas9 technology was used to construct the mouse model, the genotype of which was verified by PCR and Sanger sequencing. ABR and DPOAE were carried out to evaluate the auditory function. Histological analysis was illustrated by immunohistochemistry and scanning electron microscopy.

Results: We identified a novel KCNQ4 mutation (p.G228D) in a large Chinese deaf pedigree of patients, including heterozygotes characterized by high-frequency hearing loss that is progressive across all frequencies and homozygotes with more severe hearing loss. After generating the mouse model with the pathogenic variant, we analyzed the phenotypes of the mice. The results show that 1) heterozygotes have mid-frequency and high-frequency hearing loss at 4 weeks, and move toward all frequency hearing loss at 12 weeks, while homozygotes had profound deafness by 8 weeks, which are consistent with the hearing characteristics of the patients; 2) loss of outer hair cells was revealed from basal turn to apical turn of basilar membrane; 3) the stereocilia morphology does not show obvious change in surviving hair cells.

Conclusions: We have successfully established a mouse model with a novel pathogenic mutation (p.G229D) in Kcnq4, which simulates the patient characteristics and provides a murine model for exploring the pathological process and treatment of DFNA2.

Development of Novel Helper Dependent Adenoviral Vectors for Inner Ear Gene Therapy

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Background: Hearing loss is the most common sensory disorder worldwide and most often occurs from dysfunction within the inner ear sensory organ. Currently, no FDA approved biological therapeutics are available. Viral vector mediated gene therapy has emerged as a promising strategy to target underlying molecular mechanisms of hearing loss. Adeno-associated virus (AAV) is the most commonly studied vector for inner ear gene therapy. However, the packaging capacity of AAV is small (~4.8 kb) which limits its potential use in therapeutic applications that require expression of large or multiple transgenes. Helper-dependent adenovirus (HdAd) is a safe, non-toxic viral vector that has a large packaging capacity (~37 kb). We have found that HdAd Type 5 serotype transduces multiple cell types in the inner ear in guinea pig and mouse models but with a low efficiency. The adenovirus fiber knob protein is used for attachment of the virus to specific receptors on the cell surface. One strategy to improve efficiency and/or target subpopulations of cells is to engineer vectors with alternative fiber knob proteins which can alter cell surface receptor interactions and tropism. We sought to develop chimeric HdAd and first-generation Ad vectors and evaluate their ability to transduce cochlear tissues in adult guinea pig and mouse models .

Methods: HdAd 5, HdAd 5/35 and first-generation Ad 5/50 reporter vectors with CAG promoters were developed. Vector was injected in mouse and guinea pig models using three approaches: 1) round window 2) round window with canal fenestration and 3) direct injection into scala media. Cochleae were harvested 7 days after injection. Immunohistochemical staining and confocal microscopy were performed to study transduction patterns.

Results: Transduction in multiple cell types was achieved using HdAd5 and chimeric vectors. Transduction was most notably seen in the spiral ligament, peri-lymphatic lining, modiolar region and supporting cells.

Conclusions: Transduction of various cell types of the inner ear is feasible with HdAd-based vectors at 7-days post injection. Studies are ongoing to further characterize the transduction patterns, safety, and stability of HdAd in the inner ear.

Contribution of Ultrarare Missense Variants in Connexin Genes to Sporadic Meniere Disease

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Background: Gap junctions consist of connexin proteins forming hemichannels in the sensory epithelia. Mutations in GJB2 cause approximately half of genetic deafness, besides GJB6 and GJB3 mutations lead to non-syndromic hearing loss. Meniere Disease (MD) is an inner ear disorder characterized by episodic vertigo and associated with sensorineural hearing loss (SNHL), tinnitus and/or aural fullness. Here, we carried out a Gene Burden Analysis (GBA) in sporadic MD patients and found an enrichment of rare variants in certain connexin genes.

Methods: Single Nucleotide Variants (SNV) in connexins were obtained performing Whole Exome Sequencing (WES) in 314 patients with sporadic MD. Variant calling was carried out following GATK best practices for SNV. GBA was done filtering SNV by a Minor Allele Frequency (MAF) < 0.05 and using gnomAD database as reference population. Connexin genes expressed in the mammalian inner ear were retrieved for further analyses. The pathogenicity of each variant was estimated according to American College Medical Genetics (ACMG) guidelines and Combined Annotation Dependent Depletion (CADD) score.

Results: We found an enrichment of rare missense variants in 3 genes expressed in the inner ear: GJB5 (OR = 29.44, FDR = 4.77E-9), GJB1 (OR = 80.38, FDR = 3.67E-2) and GJD2 (OR = 13.73, FDR = 9.62-3). GJB5 is expressed in non-sensory cells of the organ of Corti and it is related with a disease characterized by congenital SNHL among other symptoms. Three ultrarare missense variants in four patients were found in the GJB5 gene: rs753359366 and rs142643584 (not described in non-finnish european population in gnomAD), that were classified as likely pathogenic (CADD > 22); and rs112931204 which was considered as variant of uncertain significance (Allele Frequency (AF) in non-finnish europeans in gnomAD = 2.17E-4, CADD = 25.1). Functional analysis revealed biological processes involved in gap junction, cell communication or transmembrane transport. Those genes encode different members of the gap junction protein family and are related with Bart-Pumphrey Syndrome, X-linked Charcot-Marie-Tooth disease and several eye diseases, respectively.

Conclusions: We have found a burden of ultrarare missense variants in patients with sporadic MD in 3 genes: GJB5 (CX31.1), GJB1 (CX32) and GJD2 (CX36). Further studies including segregation analysis are needed to confirm the role of these variants in sporadic MD.

Understanding Parents Perceptions of Genetic Testing for Children With Hearing Loss

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Background: Over 50% of congenital and childhood hearing loss is genetic; however, genetic testing is not routinely conducted as part of otolaryngology/audiology practices. The reasons and barriers why remain largely unknown. The current study examined parents' beliefs, knowledge, and experiences with genetic testing.

Methods: Thirty-seven parents were recruited from the University of Miami Ear Institute to complete a survey assessing genetic knowledge. A subset of parents also completed an open-ended interview (n = 10) to obtain more qualitative data related to their experiences with genetic testing and/or reasons for their decision to pursue testing.

Audio recordings from the open-ended interviews were transcribed and coded using NVivo to identify common themes. The majority of parents were mothers (78.4%), Hispanic (59.5%), and 92% identified as white. Most parents completed college or earned a graduate degree (56.7%) and 75.6% were between the ages of 30-49 years. Children had a mean age of 9.62 (SD = 4.52) and the majority had congenital hearing loss (63.6%). Forty-three percent of the children wore cochlear implants and 51% wore hearing aids.

Results: Results from the survey revealed that most parents were unsure of the cause of their child's hearing loss (59.5%), but 54.1% had their child undergo genetic testing. Overall, parents reported positive feelings when asked how they felt about new discoveries in the genetics of hearing (e.g., excited, hopeful enthusiastic). However, six parents also reported mixed feelings.

Reasons for not undergoing genetic testing included: unaware, not interested, cost, time, and never offered. In addition, during open ended interviews, insurance was frequently mentioned as a barrier to obtaining genetic testing (n = 4). For those who did get genetic testing for their child, 38.9% reported that the cause of their child's hearing loss was identified, 55.6% reported it was not identified, and 5.6% were unsure of the results. Most parents reported that they did not receive counseling before (75%) or after (89%) the testing, however, those that did reported that they found it effective (100%). Most parents indicated that the best method to help them understand the information was face-to-face (75%), but they also reported wanting the results in written format. The majority of families reported using the results to make decisions about family planning and were willing to share results of genetic testing with their child (74.1%); 7.4% did not plan on sharing, and 18.5% were unsure.

Conclusions: Parents of children with hearing loss reported being open to genetic testing to aide in their decision making and treatment options. However, there continues to be significant barriers to obtaining genetic testing. Future research is warranted to help ameliorate these barriers and improve access to genetic testing for patients with hearing loss.

Evidence of a Deafness-Related Genetic Interaction Between Clic5 and Radixin in Mice

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Background: Chloride intracellular channel protein 5 (Clic5) and radixin (Rdx) are deafness-associated genes in mice and humans. The products of these genes are structural proteins that interact with the filamentous actin cytoskeleton and are located at the base of stereocilia in mouse mechanosensory hair cells. When either gene is mutated, a similar phenotype is observed: the plasma membrane lifts away from the F-actin core of stereocilia. Clic5 and Rdx have been proposed to be part of a larger protein complex that also includes myosinVI, protein tyrosine phosphatase receptor type Q, and taperin. Here, we report that recombinant Clic5 and Rdx interact directly via the N-terminal FERM domain of Rdx using an in vitro affinity pull-down assay. In addition to the Clic5/Rdx protein-protein interaction, we investigated the relationship between Clic5 and Rdx through assessment of physiological function and hair cell loss in mouse models by altering their gene dosages.

Methods: Auditory brainstem responses (ABRs) were measured at 8, 16, and 32 kHz in wild-type (WT), Clic5-deficient (Clic5KO/KO), Rdx-reduced (Rdx+/KO), and combined Clic5KO/KO Rdx+/KO mice. By postnatal day 60 (P60), Clic5KO/KO mice were profoundly deaf, but at half that age, the effects of these genetic manipulations were apparent. At approximately P30, ABRs were recorded under anesthesia, then ears were examined for hair cell loss.

Results: When compared to ABR thresholds in WT and Rdx+/KO mice, ABR thresholds of Clic5KO/KO mice were elevated to moderate hearing loss at all three tone frequency locations. Reduction of Rdx gene dosage in the Clic5KO/KO background further increased hearing loss at all frequency locations. We quantified outer hair cell (OHC) and inner hair cell (IHC) loss by whole mount staining of cochleae with hair cell-specific antibodies using confocal microscopy. IHC loss across genotypes and frequency locations was less than 2%. However, for OHCs, Rdx+/KO and WT mice showed no significant difference whereas loss of Clic5 function (Clic5KO/KO) led to elevated OHC loss at all three frequencies. When Rdx function was reduced in the Clic5KO/KO background, we did not observe a change in the 8-kHz region, but there was a significant increase at 16 kHz and 32 kHz. These results correlated with respective ABR threshold elevations.

Conclusions: In summary, we found: 1) Clic5 interacts directly with the FERM domain of Rdx; 2) the reduction of Rdx function in the Clic5KO/KO background substantially raises the ABR threshold of hearing beyond the elevation from the loss of Clic5 function alone; and 3) OHC loss increases in the Clic5KO/KO background and further increases when combined with a reduction in Rdx function. Together, these data support the proposed Clic5-Rdx relationship at the base of hair cell stereocilia.

Contribution of Rare Variants to Hearing Loss

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Background: Hearing loss is one the most common sensory deficits with the prevalence of 2.5 in 1000 individuals. Causes of hearing loss is most frequently genetic; more than half of early-onset hearing loss is due to monogenic form of pathogenic variants. To date, more than 224 genes are linked to syndromic or non-syndromic hearing loss (NSHL). Although genetic heterogeneity of the disease is large, current genetic diagnosis testing explains only about 40% of cases, leaving a significant portion of hearing loss still genetically unexplained. This study focused on rare variants found in undiagnosed patients and its potential contribution to hearing loss in case-control study.
Methods: We examined the burden of rare variants of hearing loss genes using gene-level association study. Rare variants in 121 NSHL-causing genes were extracted from WES data of 211 genetically undiagnosed patients and 739 independent controls after stringent variant quality control.
Results: In rare variant collapsing analysis, the proportion of patients with qualifying variants (rare missense or loss-of-function variants) was statistically larger than that of control individuals. Genes with significant burden of rare variants include OTOG, USH1C, and PTPRQ. When aggregated in the function of genes, NSHL patients are more likely to carry rare variants in genes specifically related to hair bundle development as well as in cochlea ion homeostasis. Furthermore, gene-gene pair analysis suggested case-enriched potential oligogenic candidates of carrier variants that were absent in controls.
Conclusions: Overall, we concluded that rare variation may contribute to currently unexplained hearing loss cases and the effect might be multifactorial.

hiPSC-Derived Model System-Based Analysis of Hearing Loss-Causative GJB2 Variants

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Background: Hearing loss (HL) is the most common sensory disorder. There are 120+ genes that have been implicated in genetic HL with GJB2 being the most commonly mutated HL gene. GJB2 codes for the gap junction protein Connexin26 (Cx26) that is expressed by the supporting cells of the inner ear and is vital for nutrient and signaling molecule distribution. A common GJB2 variant is c.109G>A which causes a missense mutation and a single amino acid change (p.V37I), leading to mild-to-moderate hearing loss. As human cochlear tissue is hard to obtain, previous in vitro studies to characterize GJB2 variants have been carried out in non-hearing relevant expression systems, such as HeLa cells. These systems have greatly aided our understanding of Cx26, however do not recapitulate the genomic environment of individuals with variants and factors native to the otic epithelium.
Methods: To better account for the genomic environment of individuals with variants and factors native to the otic epithelium, we have used an established model of Cx26-espressing otic epithelium (Cx26-OE) differentiated from human induced pluripotent stem cells (hiPSCs). We reprogrammed an hiPSC line from a GJB2 c.109G>A+/+ individual using Sendai virus reprogramming. qPCR and immunocytochemistry (ICC) for pluripotency markers Oct4, NANOG, Tra1-80, and Sox2, and directed trilineage differentiation with ICC for markers of each germ layer were used to assess pluripotency. The c.109G>A+/+ line was differentiated into early otic epithelial aggregates along with a GJB2WT/WT control line. Relative levels of GJB2 mRNA transcripts were compared between the mutant and wildtype line by qPCR. Further analysis to be completed prior to the meeting will determine the relative Cx26 protein levels between the lines by Western blotting and subcellular localization by ICC with organelle specific markers.
Results: The c.109G>A+/+ line was shown to be pluripotent by qPCR and ICC for pluripotency markers Oct4, NANOG, Tra1-80, and Sox2, and by directed trilineage differentiation with ICC for markers of each germ layer. The c.109G>A+/+ and GJB2WT/W lines differentiated into otic epithelial progenitor aggregates were shown to

express equivalent levels of GJB2 transcripts by qPCR (n=3, p=0.39) and Cx26 by ICC. Results for the protein levels, ICC co-localization, and Cx26 functional analysis will be completed by the presentation date.
Conclusions: Variants in GJB2 are the most common cause of genetic hearing loss. There are several proposed mechanisms of dysfunction, but a precise understanding of these factors in human tissue is still elusive. Adequate characterization of this hiPSC-derived, Cx26-expressing otic epithelial model will allow future studies into the mechanism of dysfunction for various GJB2 variants, affording a better understanding of Cx26 functionality for therapeutic development.

Identification of Genes and Variants Associated With Adult-Onset Hearing Loss

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Background: Adult-onset hearing loss is a common, heterogeneous disease with a strong genetic component. However, although to date over 150 genes have been identified as contributing to human hearing loss, many more remain to be discovered, as does most of the underlying genetic variation. Many individual variants have been found to underlie adult-onset hearing loss, but they tend to be rare variants with a high impact upon the gene product. It is likely that combinations of more common, lower impact variants also play a role in the prevalence of the disease.
Methods: We have adopted a variety of approaches to identifying genes and variants associated with adult-onset hearing loss. We have extensive phenotypic data from 532 older adult volunteers, including 78 older adults with normal hearing, which is a very important control set. The audiograms of the 454 people with adult-onset hearing loss have been scored according to audiometric phenotype (Dubno et al., 2013), which may indicate differences in underlying mechanisms of adult-onset hearing loss; these include gradually sloping and steeply sloping audiograms, which indicate metabolic (strial) and sensory components of adult-onset hearing loss, respectively. Whole exome sequencing data from these volunteers was processed and filtered stringently, resulting in a list of high quality variants, a selection of which have been confirmed by Sanger sequencing, with an accuracy rate of 97.5%.
Results: We have carried out an outlier analysis and identified 167 genes with a high variant load in older adults with hearing loss compared to those with normal hearing. Six of these genes are known deafness genes (GLI3, PLIN4, PKD1, ENPP1, CBLN3, HCN2), and several more are promising candidates which we are investigating further (eg SCN7A, MUC5B, HSPG2). We have used a burden test to explore the association of variants with phenotypic score, and identified multiple candidate genes, most of which were associated with the phenotypic score for a gradually sloping audiogram (eg RIPPLY2, DSG2, SNX17) as opposed to a steeply sloping audiogram. Based on these data, we have chosen genes for further investigation in silico and in vivo, to confirm their link to hearing loss.
Conclusions: From these analyses we have identified some known deafness genes with a high variant load, but most of our candidate genes have not previously been associated with hearing loss. While our results support the theory that genes responsible for severe deafness may also be involved in milder hearing loss, they also suggest that there are many more genes involved in hearing which remain to be identified.

ACF7 Deficiency Does Not Impair Hearing in Young Mice

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Background: To enable hearing, the sensory hair cell contains specialized subcellular structures at its apical region, including the actin-rich cuticular plate and circumferential band. ACF7 (actin crosslinking family protein 7), encoded by the gene Macf1 (microtubule and actin crosslinking factor 1), is a large cytoskeletal crosslinking protein that interacts with microtubules and filamentous actin to shape cells. ACF7 localizes to the cuticular plate and the circumferential band in the hair cells of vertebrates. The compelling expression pattern of ACF7 in hair cells, combined with conserved roles of this protein in the cytoskeleton of various cell types in invertebrates and vertebrates, led to the hypothesis that ACF7 performs a key function in the subcellular architecture of hair cells.
Methods: To test this hypothesis, we conditionally target Macf1 in the inner ears of mice.

Results: Surprisingly, our data show that in young, but mature, conditional knockout mice, cochlear hair cell survival, planar cell polarity, organization of the hair cells within the organ of Corti, and capacity to hear were not significantly impacted.

Conclusions: Overall, these results fail to support the hypothesis that ACF7 is an essential hair cell protein in young mice, and the purpose of ACF7 expression in the hair cell remains to be understood.

Dynamic Changes in Stereocilia Cytoskeleton Alignment Driven by Mechanotransduction in Auditory Mammalian Hair Cells

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Background: Recent publications have shown that the activity of mechano-electrical transduction (MET) channels influences auditory stereocilia height, thickness and tip shape (Vélez-Ortega et al. 2017, Krey et al. 2020). In the presence of a normal resting calcium influx through the MET channels, actin remodeling is limited to an active zone at the tips of stereocilia (Zhang et al. 2012, Narayanan et al. 2015). However, when the calcium concentration inside the stereocilia decreases or increases significantly, stereocilia start to retract or re-grow respectively (Vélez-Ortega et al. 2017), which would affect actin organization at the tips of stereocilia. We routinely image these stereocilia with Focused Ion Beam Scanning Electron Microscopy (FIB-SEM), and have noticed qualitative differences in the alignment patterns of the actin core along the length of stereocilia. We sought out to determine if decreases in the MET activity will result in more disorganization of actin filaments by quantifying alignment patterns in FIB-SEM data.

Methods: Murine cochlea tissue samples were cultured in normal conditions (control) or with soluble MET channel blockers (treated). Serial FIB-SEM was used to view the 3D architecture and visualize the organizational changes of the filamentous actin core. Regions within the stereocilia core were selected and quantified for their degree of alignment along the stereocilia length using ImageJ.

Results: For inner hair cells (IHCs), the stereocilia tips had consistently less actin alignment than the shaft region while outer hair cell (OHC) stereocilia showed more uniform alignment throughout the entire stereocilia. After MET channel blockage, no significant changes were seen in IHC stereocilia, while a lower degree of alignment was observed at the tip region of OHC stereocilia.

Conclusions: Stereocilia actin structure showed significantly more alignment in the shaft region compared to tips, and blocking MET channel activity seemed to exacerbate the tip vs shaft differences. The changes observed in the actin cytoskeleton alignment by FIB-SEM match our previous observations that the MET-driven stereocilia morphological changes are more pronounced in OHCs than IHCs. These observations are consistent with the prevailing theory that the active remodeling zones for actin filaments exist primarily within the stereocilia tip. Supported by NIDCD R01DC019054 to G.I.F and R21DC017247 to A.C.V.

Functional Significance of Cuticular Plate Defects

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Background: The cuticular plate is an F-actin-based structure into which hair cell stereocilia are anchored, but there is little known about its formation or function. However, as the foundation of the hair bundle, its maintenance is likely critical for proper mechanotransduction (MET) function. We previously reported that in Lmo7 knockout mice, where the cuticular plate F-actin density is reduced, stereocilia rootlets are shorter and disorganized and stereocilia become fused, leading to loss of MET. In the present study, we further investigated the impact of noise exposure and other genetic mutations on the integrity of the cuticular plate and hearing function.

Methods: Confocal microscopy and immunofluorescence were used to observe the cuticular plate before and after noise exposure and to detect the localization of proteins of interest. Auditory brainstem response testing was used to measure hearing function.

Results: We show here that the cuticular plate can be damaged by loud noise exposure. Large holes in phalloidin staining of the cuticular plate are evident within 1 hour after noise and are not repaired within 1 week. We expect that if these holes are not repaired, effects similar to the phenotype of the Lmo7 knockout mice will be seen. Additionally, we have identified another protein that may be necessary for cuticular plate maintenance. SMPX (small muscle protein, X-linked) has been linked to non-syndromic progressive sensorineural hearing loss

(DFNX4). It is expressed beginning during development in murine auditory and vestibular hair cells and, in zebrafish, was shown to colocalize with the cuticular plate. We found that SMPX is enriched in the cuticular plate in murine auditory and vestibular hair cells, but is localized to a lesser degree throughout the whole cell, including the stereocilia. Smpx knockout mice develop holes in the cuticular plate, similar to those observed following noise exposure, and develop progressive hearing loss.

Conclusions: Our results further implicate the cuticular plate in the maintenance of hearing function and suggest a role for SMPX in maintaining cuticular plate integrity.

Use-Dependent Loss of Synaptic Transmission and Auditory Nerve Fiber Spiking Upon Deletion of Calcium Binding Proteins CaBP1 and 2

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Background: Inner hair cell (IHC) synaptic transmission is mediated by influx of calcium ions through voltage-gated calcium channels CaV1.3. Greatly reduced inactivation of IHC CaV1.3 channels supports their continuous opening and closing upon ongoing sound stimulation. Next to distal autoregulatory channel's domain and auxiliary beta subunits, CaBPs seem to play a central role to attenuate calcium channel inactivation. The loss of CaBP2 function results in moderate to severe hearing deficit in the patients as well as mouse models. However, in the absence of Cabp2, IHC CaV1.3 channels in isolated organs of Corti still show relatively modest (and mostly voltage-dependent) inactivation (at least at room temperature), suggesting other factors are at play. Furthermore, the predicted impairment of synaptic transmission is surprisingly not apparent in vitro. To better understand how combined action of the two predominant Cabp-isoforms in IHCs, Cabp1 and 2, may together modulate IHC synaptic function and support hearing, we investigated the phenotype of Cabp1/2 double knock-out mice.
Methods: We employed the patch-clamp technique, immunehistochemistry and systems physiology (ABR, Single-Unit recordings) and AAV-mediated gene-transfer to asses the hearing phenotype in those mice.
Results: Using the patch-clamp technique we found a pronounced calcium- and voltage-dependent inactivation of IHCs CaV1.3 channels with slowed recovery from inactivation. This resulted in impaired exocytosis, particularly when the IHCs were challenged by longer or persistent mild stimulation. In line with these observations, the auditory fibers of Cabp1/2 double knockout mice showed a remarkable and use-dependent reduction of sound-evoked spiking, which resulted in very high hearing thresholds and small amplitudes of ABR (auditory brainstem response) waves. Upon viral transduction leading to recovery of Cabp2 expression in the organs of Corti of otherwise Cabp1/2-deficient mice, partial recovery of a "non-inactivating" nature of calcium channels is observed, which is most apparent when IHCs are exposed to prolonged mild stimulation. Furthermore, restoration of Cabp2 expression supports very good recovery of IHC exocytosis. This in turn leads to mostly restored ABR wave amplitudes and significantly improved sensitivity of hearing.
Conclusions: Our results are important in the light of our recent attempts to develop gene therapy for hearing impairment DFNB93, caused by pathological mutations in CaBP2. Furthermore, it suggests synergistic functions of Cabp1 and 2 in modulating calcium channel function and IHC exocytosis and show cases how molecules that enable persistent activation of calcium channels and thus continuous exocytosis are critical for indefatigable encoding of sound information.

CIB2 and CIB3 Resemble KChIP Proteins and Regulate the Function of the Mechanotransduction Channel of Cochlear Hair Cells

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Background: Mechanoelectrical transduction (MET), the conversion of mechanical stimuli into electrochemical signals, is of fundamental importance for organisms to sense a range of external and internal stimuli. Studies of genes linked to deafness have identified components of the MET channel complex. These include the integral membrane proteins TMC1/2, TMIE, LHFPL5, and CIB2. CIB2 is localized to stereocilia of murine cochlear hair

cells, binds to TMC1 and TMC2, and is essential for MET by cochlear hair cells. However, the atomic structures of CIB2 and CIB3 are not known, and how CIB2 and CIB3 interact with TMC1 remains to be elucidated.
Methods: We used Co-immunoprecipitation and western blotting to identify a domain of TMC1 that strongly binds to CIB2 and CIB3. We applied injectoporation combined with electrophysiology to demonstrate that CIB2 and CIB3 are functionally interchangeable in cochlear hair cells. We then purified CIB3 and CIB3/TMC1 peptide complexes and determined by X-ray crystallography the atomic structures of CIB3 and of the CIB3/TMC1 peptide complex. Mutation and Co-IP were applied to confirm the structural studies. Additionally, we used CRISPR-Cas9 to generate mice with mutations in the CIB2 gene and analyzed them by ABR and DPOAE measurements.
Results: Using X-ray crystallography, we show here that CIB2 and CIB3 are structurally similar to KChIP1 and other NCS proteins. A hydrophobic groove in CIB2/3 mediates interactions of CIB2/3 with TMC1, mirroring the interaction of KChIP proteins with K v 4 channels. We also tested 6 CIB2 deafness mutations and showed that all of them affected TMC1 binding. ABR and DPOAE showed that all the CIB2 deafness mutations disrupt hearing in mice by affecting MET channels.
Conclusions: We conclude that CIB2 and CIB3 are regulatory subunits of the MET channel with structural homology to KChIP proteins.

Role of Ferritin Light Chain in the Cochlea

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Background: Hearing relies on two major kind of sensory auditory hair cells in the cochlea: the outer hair cells (OHCs), which amplify sound stimulation, and the inner hair cells (IHCs), which transduce sound stimulation into release of neurotransmitter. Thus, cochlear activity sets high demands on the cellular metabolism. Ferritin, which belongs to the iron-binding protein family, stores the intracellular iron, require for several metabolic processes.
Methods: Here, we probed the role of ferritin in the cochlea by phenotyping the ferritin light-chain (Ftl) subunit knock-out mouse (Li et al., Plos One, 2015).
Results: Wild-type and heterozygous mice showed comparable ABR and auditory thresholds. In contrast, deletion of Ftl leads to threshold shifts in 40% of the homozygous mice, leaving the other fraction of mouse unaffected. In the hearing-impaired Ftl KO mice, the distortion products of otoacoustic emissions were reduced, indicating the functional loss of OHCs, which normally amplify sound-stimulation within the cochlea. Using electron microscopy, OHCs harbored splayed or absent stereocilia together with mitochondria alteration. In addition, we observed a progressive alteration of the hair bundle in the IHCs.
Conclusions: Our data show that the light-chain of ferritin is critical for the maintenance of the hair cells. The fraction of Ftl KO mice with normal hearing might be explained by a compensation by the heavy chain of the ferritin.

Acute Recreational Noise-Induced Cochlear Synaptic Dysfunction in Humans With Normal Hearing: A Prospective Cohort Study

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Background: Noise exposure is the most common environmental factor causing hearing loss in adults. The objective of the study was to identify the acute high-intensity recreational noise-induced effects on auditory function, especially the cochlear synaptopathy-related audiological metrics, in humans with normal hearing.
Methods: This prospective cohort study enrolled 32 young adults (14 males and 18 females); the mean age was 24.1 ± 2.4 years (ranging from 20 to 29). All participants with normal hearing (audiometric thresholds ≤ 25 dB HL at frequencies of 0.25, 0.5, 1, 2, 3, 4, 6, and 8 kHz for both ears) had already decided to participate in the outdoor music festival. Participants were asked to measure the noise exposure dose and complete auditory examinations, including the air-conduction pure-tone audiometry (PTA), distortion product otoacoustic emission (DPOAE), contralateral suppression (CS) on transient evoked otoacoustic emission (TEOAE), auditory brainstem response (ABR) test and Mandarin Hearing in Noise Test (MHINT), at baseline and 1 day and 14 days after music festival noise exposure.

Results: The mean time of attending the music festival was 7.34 ± 0.63 h (ranging from 6.4 to 9.5), the mean time-weighted average (TWA) of noise exposure dose was 93.2 ± 2.39 dB(A) (ranging from 87.9 to 97.7). At neither 1 day nor 14 days post exposure, there were no statistically significant effects on PTA thresholds, DPOAE amplitudes, CS on TEOAEs, or MHINT signal-to-noise ratios (SNRs) of acute outdoor music festival noise exposure, regardless of sex. While the ABR wave I amplitudes significantly decreased at 1 day after exposure and recovered at 14 days after exposure, the exposed/unexposed ABR wave I amplitude ratio was significantly correlated with MHINT SNR change at 1 day after exposure, although it was not correlated with the noise exposure dose.

Conclusions: In young adults with normal hearing, we found the self-compared decrement of ABR wave I amplitudes at 1 day post acute recreational noise exposure at high intensity, which also contributes to the change in speech perceptual ability in noisy backgrounds. This study indicated that auditory electrophysiological metric changes might be a more sensitive and efficient indicator of noise-induced cochlear synaptic dysfunction in humans. More attention should be paid to the recreational noise-induced cochlear synaptopathy and auditory perceptual disorder.

Neural Correlates of Auditory Working Memory in Cochlear Implant Users Are Related to Speech Perception in Noise Ability

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Background: A common concern for individuals with severe-to-profound hearing loss fitted with cochlear implants (CIs) is difficulty following conversations in noisy environments. Previous work has shown that clinically measured speech perception in noise scores yields high degrees of variability in CI users where 10-22% of the variance can be explained by age and duration of hearing and even less can be explained by surgical factors leaving a majority of the variance unaccounted for. A possible factor contributing to the wide range of speech perception scores is individual differences in the recruitment of cognitive resources including working memory and attention.

Methods: In this study, we investigated behavioral and neural correlates of auditory working memory in 13 CI users using a high-density electroencephalogram (EEG) while participants completed an N-back task consisting of two conditions, 0-back and 2-back. The behavioral outcomes and neural activations from this task were then correlated with speech perception in noise scores. The auditory stimuli presented in each trial were ten double-digit numbers (DDN). In the 0-back control condition, the participants were primed with one DDN and were instructed to indicate with a button press when the double numbers were heard. The 2-back experimental condition was similar to 0-back except that the task was to identify DDN matches from two pairs in the sequence.

Results: Behavioral results yielded significant correlations between the 2-back task performance and speech in noise perception in noise. CI users with higher speech perception in noise scores perform more accurately on the 2-back condition compared to CI users with lower speech perception scores suggesting higher degrees of working memory ability in this group. Electrophysiology showed that auditory encoding (P1/N1) was significantly related to speech in noise perception. Significant correlations between neural oscillations (alpha, 8-12 Hz and theta, 4-6 Hz) and speech perception in noise ability were observed.

Conclusions: These results suggest that both bottom-up (encoding) and top-down (attention and working memory) processes contribute to the observed variability in speech in noise perception in CI users.

Differentiating Inner Hair Cell Dysfunction From Cochlear Synaptopathy Using Non-Invasive Measures of Temporal Envelope Coding in Chinchilla

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Background: Identifying the interplay of cochlear synapse, stereocilia, and hair cell body damage and how they impact neural coding remains elusive. Animal studies that use carboplatin to induce inner hair cell loss in chinchillas demonstrate that selective damage to inner hair cells reduces ABR amplitudes for high level stimuli and disrupts neural envelope coding, similar to the effects of cochlear synaptopathy (CS). These findings, however, are confounded by other cochlear deficits (e.g. OHC/IHC dysfunction/loss, drops in endocochlear

potential). The use of rectangularly amplitude-modulated (RAM) stimuli has been highlighted as a way to bypass OHC-mediated adaptation and therefore selectively assess the IHC/AN junction; however, IHC death and stereocilia damage must be considered when aiming to diagnose CS. This pilot study further investigated EFRs to RAM stimuli, finding that it appears to be more sensitive to IHC dysfunction than CS.

Methods: Chinchillas (n=8; 4 female; aged between 32-48 weeks) were used in this experiment. Animals were randomly assigned to evenly balanced, sex-matched TTS and CA exposed cohorts. Baseline OAE, wide-band MEMR, and EFR (to both sinusoidally (SAM) and rectangularly (RAM) modulated tones) data were collected from all animals prior to exposure. The TTS group was exposed to band-limited noise (1 kHz center frequency, 100 dB SPL, 2hrs). For the CA group, a previously described 38mg/kg injection protocol was used, which results in roughly 10-15% IHC loss and significant IHC stereocilia damage.

Results: A consistent and prominent drop in phase-locking value (PLV) at higher harmonics of the modulation frequency was noted post-carboplatin exposure in response to RAM, and to a less consistent degree, to SAM stimuli. A less marked decrease was observed in the TTS cohort. The results are more salient when a ratio is taken between the upper harmonics to the lower harmonics. Two weeks post-exposure, MEMR was most strongly reduced in the TTS group, and less-reduced in the CA group for high-level noise elicitors (i.e., >80 dB SPL).

Conclusions: These data indicate that while MEMR is sensitive in detecting both noise-induced CS and IHC dysfunction, EFR responses to RAM tones exhibit stark deficits in modulation coding in the presence of IHC stereocilia damage but not CS. It must be considered that this observation may not be limited to cytotoxic CA damage to IHCs; it is likely present in subjects with IHC dysfunction due to PTS noise exposure, aging or reduced EP. These conditions all result in decreases in spontaneous/driven rate, and flattened rate level functions, which may be in part due to changes in the IHC transduction non-linearity. Therefore, the role of IHC dysfunction should not be overlooked by attempts to design targeted assays for CS (e.g., in noise-induced and age-related models of SNHL).

Mutation of Spermine Synthase Leads to Hearing Loss

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Background: Polyamines interact with nucleic acids, phospholipids, and proteins for essential roles in cell survival and proliferation. Polyamine content is regulated by the biosynthesis of spermidine and spermine from putrescine. Studies have demonstrated that Snyder-Robinson syndrome is an x-linked disease associated with spermine synthase mutation. We hypothesize that decreased spermine synthase activity would alter cochlear functions.

Methods: We used a humanized mouse model with G56S mutation in spermine synthase to test the hypothesis. ABR and DPOAE were tested at different ages (6,10,12,16 weeks old) of hemizygous mice compared with their littermate wild-type (WT). Immunostaining of MelEM, Kir4,1, NaKATPase, Myo7a, Tuj1, and Peripherin (PRP) was performed. Tunnel assay and Telomere length quantification assay were performed.

Results: The bodyweight of mutant mice was significantly lower than WT. Hemizygous mice showed profound hearing loss mice. Hearing thresholds were increased for click and tone pips. By 6-weeks, mutant mice who were deaf showed high-frequency (32k) hearing loss, and the overall hearing phenotype declined with age. The mutant mice have shortened telomere length compared with WT mice. There was progressive outer hair cell loss and type II spiral ganglion neurons, and their neurite degeneration. MelEM and Kir1.4 expression in mutant mice was weak. Finally, we show substantial inner ear cellular apoptosis of the mutant mice.

Conclusions: We demonstrate profound cochlear disruption in the G56S hemizygous mice and show that these changes suffice to produce progressive hearing loss.

The Role of Inflammation in Sensory Hair Cell Aging in a Zebrafish Model

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Background: Thirty-six million Americans suffer from hearing impairment, resulting in significant decreases in quality of life for those affected. Over 50% of individuals above the age of 75 experience age-related hearing loss (ARHL), and as the Baby Boomer cohort ages an increasing portion of the population will require assistive listening devices. While assistive technologies improve hearing capabilities in some, it is of great interest to

prevent ARHL and preserve hearing. We proposed that two likely primary causes of ARHL are localized inflammation and mitochondrial dysfunction; here we focus on the role of inflammation in a zebrafish model. Zebrafish are an excellent model for auditory research, particularly studies of sensory hair cell development and physiology, due in part to the presence of exterior lateral line hair cells which are analogous to the hair cells in the human inner ear. Additionally, zebrafish development occurs rapidly and lateral line hair cells are in a constant state of turnover, resulting in lateral line organs containing cells of different ages. Lateral line hair cells are relatively mature in five days-post fertilization (dpf) animals, allowing us to study hair cell aging in a period of days, rather than waiting years for an animal to age.

Methods: We hypothesize that inhibiting inflammation will prolong the functional lifespan of lateral line hair cells, and thus we would expect reduced hair cell addition and decreased longevity under inflammatory conditions. Using genetically modified zebrafish that express green fluorescent protein (GFP) in hair cells, we pharmacologically manipulated either a key inflammatory cytokine, TNF-alpha, or global inflammation using dexamethasone or lipopolysaccharide, then examined hair cell longevity in vivo. Fish were incubated in 4',6-diamidino-2-phenylindole (DAPI) at 4 dpf to label existing hair cells, then incubated in one of our inflammatory modulators.

Results: We can discriminate between cells that were present at 4 dpf and newly added cells, as older cells are GFP+ and DAPI+, while newer cells are only GFP+. Our preliminary data demonstrates that inhibiting TNF-alpha and other pro-inflammatory signaling increases hair cell longevity, consistent with our hypothesis.

Conclusions: Future work will include genetic manipulation of inflammatory signaling and examine the role of mitochondrial oxidative stress in the hair cell aging process. This work will increase understanding of inflammatory signaling in ARHL and may yield molecular targets for therapeutic development.

LOXHD1 Mutations Cause Mechanotransduction Defects in Inner Hair Cells

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Background: Here we investigated the molecular function of the LOXHD1 (Lipoxygenase Homomoly Domain 1) gene that we previously linked to an autosomal-recessive form of hearing loss in mice and humans (DFNB77) (Grillet N., AJHG, 2009). LOXHD1 encodes a protein made of 15 PLAT (Polycystin/Lipoxygenase/Alpha-Toxin) domains, known in other proteins to bind lipid and proteins. The molecular function of LOXHD1 is unknown. LOXHD1 is expressed selectively by hair cells and is required for their operation as assessed by auditory brain stem responses (ABR) and distortion products of otoacoustic emissions (DPOAE). As outer hair cells of a Loxhd1PLAT10 mutant were able to mechanotransduce at postnatal day (P) 7, we hypothesized that the onset of a mechanotransduction phenotype could occur later.

Methods: To test this hypothesis, we studied two mouse strains carrying mutations in the LOXHD1-PLAT10. We analyzed the auditory phenotype (ABR, DPOAE, cochlear microphonics), the hair bundle morphology (scanning electron microscopy), the IHC mechanosensitivity (patch-clamp), and the stereociliary membrane dynamics (Fluorescent Recovery after photobleaching).

Results: We found that, while inner hair cell mehanotransduction currents of Loxhd1PLAT10 mutant animals are comparable to control at P7, the amplitude of the MET current is reduced by 95% at P11. Despite this strong MET phenotype, the hair bundle organization is not affected, and the number of tip-links has not decreased. Both the upper tip-link protein Harmonin and the lower tip-link protein LHFPL5 were still properly localized at P11 (Trouillet et al., 2021). Since it appears that the mechanotransduction machinery is present but inactive, we investigated the lipid diffusivity of the stereociliary membrane. While the lipid diffusivity was stable in control animals between P7 and P11, in both Loxhd1PLAT10 mutants, the lipid diffusivity increased during this period. At P11, the lipid diffusivity increased more in the tallest row (55.6 ± 43 % increase compared to control) than the second row (23.5 ± 12 % increase compared to control). These results indicate that LOXHD1 regulates at least some stereociliary membrane properties. Due to the repeated structure of LOXHD1 (15 repeats) and many exons (42), we wondered if additional / earlier functions of LOXHD1 could have been masked by splicing compensation. To address this possibility, we recently have generated a novel mouse allele consisting of the deletion of the entire gene (160 kb) using CRISPR/Cas9 genome editing. Our preliminary results suggest an earlier requirement of LOXHD1 for the hair cell development, as assessed by a change of the hair bundle structure.

Conclusions: We have identified a new pathway required for hair cell mechanotransduction activity which affects also the stereociliary membrane properties. Understanding this mode of mechanotransduction regulation is critical, as it underlies both congenital and age-related forms of hearing loss in humans.

Auditory Brainstem Responses to Masked Clicks and Chirps in Macaque Monkeys: Exploring Potential Biomarkers for Auditory Nerve Loss

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Background: The search for biomarkers that are both sensitive and specific to neural damage independent of outer hair cell (OHC) pathology has been ongoing in several animal models. In rodents, cochlear synaptopathy (CS) causes reduction in the amplitude of compound action potential (CAP) of the auditory brainstem response (ABR) to high level tone-pips. These reductions are correlated with the location and extent of synapse loss. In larger, genetically more heterogeneous species, including macaques and humans, tone-pip CAP responses are small and variable and may not be sensitive to CS. Here, we measured ABRs in normal hearing macaque monkeys using suprathreshold clicks and chirps in with masking paradigms to i) target evoked potentials from large populations of high threshold neurons and ii) control the frequency region of responding auditory nerve fibers. This normative data set will serve as a baseline to investigate frequency specific changes in auditory nerve density after noise exposure.

Methods: ABRs (vertex-to-mastoid) were measured in anesthetized young adult, normal hearing macaque monkeys (Macaca mulatta, 6-10 years old, n= 16 male, 4 female). Clicks (100µs) and chirps (derived from normal-hearing macaque ABR Wave I latencies in response to 90 dB SPL tones) were used as stimuli. Stimulus paradigms included unmasked clicks and chirps (30-90 dB SPL in 10 dB steps), clicks (70-90 dB SPL) in broadband noise (30-60 dB SPL spectrum level), and chirps (80-90 dB SPL) in broadband noise with varying high-pass cut-off frequencies (0.4 - 32 kHz) at different signal-to-noise ratios (SNRs; chirp dB SPL/noise dB SPL spectrum level: 90/50, 90/40, 80/45). All ABR Waves (I, II, and IV) were analyzed for amplitudes and latencies.

Results: Click and chirp ABR wave amplitudes increased monotonically with stimulus level; Wave II amplitudes were largest, followed by Waves IV and I. Click and chirp ABR wave latencies decreased monotonically with increasing stimulus level. Compared to clicks, chirps evoked larger amplitudes and comparable latencies for all waves, across all suprathreshold stimulus levels. Masking reduced click ABR amplitudes and increased latencies, and these changes scaled with masker level. Raw masked click amplitudes, amplitudes normalized to unmasked click amplitudes, and slopes of input-output functions were highly variable across subjects. Masked chirp amplitudes increased and latencies decreased for all waves and SNRs with increasing high-pass cut-off frequency. Masked chirp latencies were not different across SNRs and were less variable across subjects compared to masked amplitudes.

Conclusions: Chirps could have greater diagnostic power compared to clicks, but similar to clicks, the utility of raw CAP amplitude may be limited by large between-subject variability, combined with potentially small within subject changes. Within-subject comparisons in genetically heterogenous species such as macaque monkeys will help assess the diagnostic sensitivity of a variety of masked ABR metrics.

Mechanisms of Estrogen Induced Auditory Plasticity in the Plainfin Midshipman (Porichthys Notatus)

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Background: Estrogen alters hearing in many vertebrates including rats, mice, humans, zebra finches (Taeniopygia guttata), and plainfin midshipman fish (Porichthys notatus). However, the exact mechanisms of estrogen-induced auditory plasticity are largely unknown. Here we focus on the plainfin midshipman (midshipman), a species of toadfish native to the Pacific coast of North America. Midshipman are a vocal species that spawn at night in the intertidal zone during the summer. The intertidal zone is a shallow, murky, rocky environment where large type I males establish nests and produce long duration acoustic advertisement calls, called hums, to court reproductive females. In female midshipman, the saccule, which is their primary auditory organ, exhibits a seasonal increase in temporal encoding of the high energy harmonics of male advertisement calls, which likely enhances the female's ability to detect and locate calling males. Previous work has also shown that seasonal change in auditory sensitivity is mediated by estrogen, which acts to lower auditory thresholds (i.e., become more sensitive) and increase hair cell densities in the saccule. Thus, we hypothesize that the observed

auditory plasticity is, in part, modulated by estrogen-induce changes in the cellular pathways that regulate supporting cell proliferation, differentiation, and hair cell survival.

Methods: To test this hypothesis, we first quantified hair bundle densities of estrogen-implanted winter female midshipman and compared them to reproductive females. We then analyzed cell proliferation in reproductive and nonreproductive female midshipman inner ears via BrdU labelling. Last, we performed qRT-PCR to analyze the gene expression of Wnt, Notch, and heat shock protein (Hsp) pathways to understand how estrogen alters the expression of signaling pathways responsible for cell proliferation, differentiation, and survival, respectively.

Results: We found that estrogen implantation in nonreproductive females increased saccular hair bundle density up to levels observed in summer reproductive females. Additionally, we found that saccules from summer females expressed genes involved in Wnt, Notch, and Hsp signaling.

Conclusions: Our research has the potential to provide insight into the mechanisms responsible for estrogen-induced auditory plasticity and could reveal conserved mechanisms shared by other vertebrates, including humans.

Distinguishing Different Cochlear Deficits With Diagnostic Tools

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Background: One of the most common and pressing goals in the hearing research field is to find a way to non-invasively diagnose specific sites of cochlear deficits. An efficient and targeted treatment strategy can be better planned if a non-invasive diagnosis can determine the specific site of abnormality in the cochlea as the underlying cause of hearing impairment in a patient. Age-related hearing loss has been classified by Schuknecht and Gacek (1993) into three main types based on their site of lesion: sensory (hair cells), neural (auditory nerve), and metabolic (strial). Our goal is to put together a battery of electrophysiological tests that we can use to diagnose these specific sites of cochlear deficit.

Methods: For this project, we used four mutant lines each representing a specific cochlear primary defect: 1) S1pr2<stdf> (strial), 2) Slc26a5<tm1(EGFP/Cre/ERT2)Wtsi> (outer hair cell), 3) Klhl18<lowf> (inner hair cell), 4) Wbp2<tm1a(EUCOMM)Wtsi> (synaptic/neural). All the mice are on the same C57BL/6N background and wildtype littermates were used as controls for each mutant. We tested the mice at specific ages when their mutation caused appreciable hearing impairment compared to controls, but the mice still had usable responses for analyses. We performed auditory brainstem response (ABR) tests across 3-42kHz; distortion product otoacoustic emission tests across 6-30kHz; frequency tuning of responses to 12kHz tones; tests for adaptation such as forward masking and increasing click-repetition rate; a 12kHz tone-in-noise test; and 12-24kHz ABR wave I intertrial coherence measures.

Results: Our initial analyses of the responses suggest that there are patterns uniquely determinant of each specific cochlear abnormality. One example is that the strial and synaptic defect mice have normal frequency tuning while there is little sign of any tuning in the mutants with either inner or outer hair cell defects.

Conclusions: We plan to follow up the most promising tests in other mouse mutants with well-characterised defects to determine if the same patterns occur in other exemplars of different sites of lesion.

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Applying Deep Learning to the Counting and 3D Mapping of Human Spiral-Ganglion Urvival in Cochlear Serial Sections

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Background: Quantifying the survival patterns of spiral ganglion cells (SGCs), the cell bodies of auditory nerve fibers, is critical to the study of sensorineural hearing loss. Classically, SGCs have been counted manually in a painstaking process which takes > 30 hrs for a well-trained observer to complete on a "standard" set of human cochlear sections. Although stereology approaches can be faster, in cochlear applications they can only be used to estimate total cell numbers per ear, thus the spatial pattern of surviving SGCs is lost.

Methods: Here, we present a deep-learning algorithm that automatically finds, counts and maps the SGCs in digitized images of semi-serial human temporal-bone sections. The bones (from the collection at the Massachusetts Eye and Ear Infirmary) were fixed, decalcified, embedded in celloidin, serially sectioned at 20µm in the horizontal plane, mounted on slides, and stained with hematoxylin and eosin. The slide sets were digitized

in a scanner (Leica Aperio AT2), with a high- N.A. 20X objective, that stitches the tiled images into a composite that can be seamlessly zoomed from low- to high-power.

Results: The algorithm is built in Python, using a deep neural network, modified from an open-source package (Segmentation Models). The algorithm was trained on a hand-annotated set of 51 slides selected from 13 cases, chosen to include a wide range of overall staining intensities. The code can now successfully identify all SGCs in a new cochlear cross-section from a test set, without any user-driven identification of the regions of interest, with a correlation coefficient of 0.975 when compared to manual counts by a trained observer. Further custom software automatically registers the serial sections from each case, using cross-correlation approaches. This allows mapping of the 3D locations of all surviving SGCs, and relating them to the appropriate regions of the spiraling organ of Corti by assuming radial projections of the peripheral axons.

Conclusions: Preliminary results suggest that, with minimal modifications, and new training sets, the same algorithmic approach can be used to count and map most, if not all, cell types in the inner ear. This level of automation can enable a 'big-data' approach to otopathology, rapidly acquiring morphometric data on hundreds of relevant cases and using rigorous statistical approaches to unpack the relations among multiple histopathologic metrics and the available audiometric data. The rapid creation of 3D maps of surviving cells can also be useful in the creation of cochlear models, for example, in relating the differing spatial patterns of cochlear neural degeneration to the relative success of cochlear implants.

Revisiting the RC Time Constant Problem in Avian and Mammalian Auditory Hair Cells
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Background: Auditory frequencies are extremely high for biological cells, which are subjected to their intrinsic low-pass filter, often referred to as RC filter. This filter is due to the combination of the membrane capacitance and the membrane resistance. It is characterized by the RC time constant, where R is the membrane resistance and C the membrane capacitance. This low-pass filter of biological cells poses a significant challenge for hair cells in the ear.

Methods: The RC time constant problem has been intensely discussed with respect to outer hair cells (OHCs) in the mammalian ear. OHCs are expected to inject power to the oscillation in the inner ear for the sensitivity and frequency specificity of the mammalian ear. Since all hair cells are subjected to this low pass filter, it is of interest to re-visit this issue, comparing mammalian OHCs with avian hair cells.

Results: Avian hair cells depend on electrical resonance for frequency selectivity. The upper bound of the frequency range is limited by the RC time constant of hair cells because the sharpness of tuning requires resonance frequency lower than the RC roll-off frequency. In contrast, tuned mechanical vibration of the inner ear is the basis of frequency selectivity of the mammalian ear. This mechanical vibration is supported by OHC with their electromotility (or piezoelectricity), which is driven by the receptor potential. Thus, it is also subjected to the RC time constant problem. Due to the piezoelectricity of OHCs, mechanical resonance of the organ of Corti leads to piezoelectric resonance. This resonance can nullify the membrane capacitance due to the reciprocal effect of piezoelectricity and solves the RC time constant problem for OHCs.

Conclusions: Therefore, avian and mammalian ears solve the same problem in the opposite way.

Examining the Link Between the Regions of IDP Generation by OHCS and the DPOAE in Ear Canal for a Wide Range of Frequency Ratio
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Background: Distortion product otoacoustic emissions (DPOAEs) are weak sounds in response to a two-tone stimulus that are measured in the ear canal and can be used for hearing loss screening. Intracochlear distortion products (IDPs) are commonly assumed to arise from the nonlinearity of the electrical response of outer hair cells (OHCs). After being generated in the cochlea, IDPs travel back to the ear canal and can be measured as DPOAE. However, whether DPOAEs includes contributions from regions located significantly basal to the f2 tonotopic place has been under debate. Precise understanding of which cochlear regions contribute to DPOAEs is crucial for the diagnosis of frequency-dependent sensorineural hearing loss.

Methods: To study IDP generation by OHCs in the gerbil cochlea, we combined a three-dimensional nonlinear computational model with mechanical-electrical-acoustic coupling with in vivo measurements of the pressure in

scala tympani and of the LCM. The pure tone and IDP responses of the calibrated model were in excellent agreement with experimental voltage and pressure measurement. However, our previous work only focused on the distortion component generated in response to a two-tone stimulus with a relatively wide frequency ratio (f2/f1=1.25).

Results: In this work, we examine the link between the regions of IDP generation by OHCs and the DPOAE in ear canal for a range of frequency ratios which includes narrow frequency ratios where the DPOAEs might be dominated by its reflection component. Cochlear roughness is introduced by adding random perturbations to the electromechanical properties of OHcs, allowing us to model the reflection component of DPOAEs. The calibrated model with cochlear roughness is used to simulate two-tone responses at different frequency ratios to study electrical and mechanical IDPs as well as DPOAE in ear canal. Model simulations are compared to in vivo measurements of eIDPs and fIDPs at the base of the cochlea. To determine how different longitudinal locations give rise to fIDP waves and DPOAE, cochlear damage is mimicked in the model by locally turning off cochlear activity.

Conclusions: This work not only improves our understanding of the role of the OHC electromotility in DP generation, but will also benefit clinical noninvasive hearing assessment based on otoacoustic emissions.

Due to Its Higher Compliance, the Response of the Reticular Lamina, but Not of the Basilar Membrane, Can Be Suppressed by a High Frequency Tone at Locations Basal to Its Peak

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Background: Two-tone suppression refers to the phenomenon whereby the cochlear response to a tone (probe) is reduced in the simultaneous presence of another (suppressor) when compared to the response to a pure tone stimulus. It has been assumed that cochlear nonlinearity due to outer hair cell (OHC) forces is constrained to the peak region (the region of basilar membrane (BM) nonlinearity) of a given stimulus, however recent experiments comparing two-tone suppression of the BM and reticular lamina (RL) indicate that OHCs provide electromotile feedback over a much broader basal region. We previously calibrated a computational model of the cochlea using experimental pure tone responses of the Scala tympani (ST) pressure and ST electrical potential (local cochlear microphonic - LCM). Post-calibration, the model predicts BM nonlinearity only around the BM peak region, but it predicts broader (more basal) LCM, RL and TM nonlinearity.

Methods: In this work, this model is used to analyze two-tone suppression of the mechanical (BM, RL and tectorial membrane (TM) velocities) and electrical (LCM) responses of the cochlea. The model, based on the physiology of the gerbil cochlea, consists of a 3D fluid domain of the cochlear ducts coupled to the BM, in turn coupled to other organ of Corti (OoC) structures. OHC hair bundle deflections are converted into a transduction current due to nonlinear mechanoelectrical transduction. Due to electromotility, the modulation of the OHC receptor potential caused by the transduction current generates a force that acts on the BM and RL.

Results: Computational predictions of two-tone suppression are consistent with the experiments described earlier. Specifically, RL, TM, and LCM probe responses are suppressed due to a high-frequency suppressor that peaks in spatial regions basal to the BM peak region. This suppression in the RL, TM, and LCM probe responses is localized within the peak region of the suppressor, and is absent in the peak region of the probe. In contrast, the BM response is unaffected. The BM only exhibits two-tone suppression at its peak region, and only in response to a suppressor that peaks within or apical to the probe's peak region.

Conclusions: The model provides interpretation of these observations. In response to a pure tone, the OHCs generate a force at locations basal to the BM peak region. In presence of a suppressor, these OHC forces are suppressed. Because the basal OHC force is only large enough to influence the RL and TM vibrations, but not the stiffer BM, suppression of the probe response below the BM peak region of the LCM only causes RL and TM suppression. This indicates that while OHCs provide feedback to the RL and TM basal to the BM peak region, it does not play a role in the amplification of BM traveling waves.

Cochlear Suppression of Tones by Dynamic Stimuli

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Background: Behavioral studies show that when a tone is presented together with a competing sound, the audibility of the tone can be reduced (i.e., masking). The amount of masking depends on the characteristics of the

competing sound. While masking by sounds with static properties is relatively well explained, masking by sounds that change dynamically over time (e.g., frequency chirps) is less understood. For instance, upward linear frequency chirps (i.e., sweeping frequency from low to high) are typically much more effective maskers than downward sweeps (high to low sweep), especially at lower masker levels. This discrepancy in masker effectiveness occurs only in subjects with normal hearing, suggesting that this phenomenon depends on active cochlear processing. Previously, these puzzling results were interpreted in terms of cochlear dispersion: Upward sweeps augment the effects produced by cochlear dispersion, resulting in cochlear responses that ring longer and produce more masking compared to downward sweeps, which partially compensate for cochlear dispersion and produce more temporally localized responses.

Methods: In this study, we investigate "masking" as seen directly in cochlear vibrations (i.e., suppression) when a tone is presented together with a swept suppressor tone. Because the cochlear frequency-place map is roughly exponential, we use exponential frequency chirps instead of linear ones. To explore the effect of cochlear dispersion on the strength of suppression, we manipulate the direction of the frequency sweep, as done in behavioral studies, as well as its rate (or, equivalently, the total sweep duration). Mechanical vibrations in a mouse cochlea are measured using optical coherence tomography.

Results: The suppression produced by slow sweeps is expected to be almost independent of sweep direction. In contrast, the suppression produced by sweeps whose rate is comparable to the instantaneous-frequency trajectory resulting from cochlear dispersion is expected to depend on sweep direction, with upward sweeps suppressing the probe tone more than downward sweeps. Preliminary data obtained in an intact mouse cochlea corroborate these predictions at low but not high tone/suppressor level combinations.

Conclusions: We interpret the observed patterns of suppression caused by swept tones in light of the known characteristics of two-tone and temporal suppression in the cochlea. Specifically, we discuss the relationship between the instantaneous frequency of the suppressor sweep and the amount of probe tone suppression observed at given time.

Material Properties of the Skull and Their Anatomical Distribution: A Combined Experimental and Fem Approach

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Background: The human skull bone is a hierarchical composite. Its macroscopic properties depend not only on the bone geometry and mineral density but also on its microstructure and microscale tissue properties. Therefore, the transfer path of the stimulus to the cochlea is very complex, due to the material properties of bone that varies across the skull's geometry. The aim of this work is to identify the relationships between the structure and physiology of the skull by investigating its micromechanical behavior and its dependence on the local micro-structure at several positions across the skull geometry.

Methods: A CT scan (0.5mm voxel size) is obtained from a human whole head in order to obtain the shape, layer thickness, and curvature. Further, five bone samples were extracted from different sections of the cadaver heads and were scanned using a micro-CT (15µm voxel size) to allow a thorough analysis of the microstructure. The micro-CT data are processed (i.e., cropping, filtering, and segmenting) via a custom MATLAB script, in order to prepare it for meshing. Extracted bone samples were mechanically loaded using both tension/compression and bending test machine recoding the resultant force, while the corresponding displacements were tracked by a digital image correlation stereo-camera setup. The tensile and bending measurements were repeated multiple times, to assess hysteresis, and then continued until failure. The CT and micro-CT geometry and labeled data were used as a basis for the formulation of the finite element modeling (FEM) geometry and material domains, individually for each sample piece. Finally, the mechanical tests were digitally recreated using the FEM, where model parameters (material properties, boundary conditions, etc.) were varied until the predicted deformation matched with the strain field observed in each sample during the mechanical tests. Thus, the bulk material properties are obtained indirectly.

Results: Preliminary results based on CT data showed a large variation of the bulk thickness between different sections of the skull. Also, micro-CT data showed a characteristic 3-layer sandwich structure within each bone

piece, with significantly different porosity between layers. Moreover, preliminary comparisons show a good match between FEM and experimental data, at the individual piece level.

Conclusions: Future work will investigate the correlation between location and dynamical behavior of the individual pieces of the skull in preparation for the formulation of a full skull model.

Dexamethasone Eluting Implants Suppress Infiltration of Lymphocytes in Cochlea Following Cochlear Implantation

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Background: Cochlear implants (CIs) bypass the auditory function of the damaged hair cells and improve the quality of life of profoundly deaf people. Surgical implantation of a CI leads to a foreign body response (FBR) that can negatively impact implant performance and may contribute to loss of residual acoustic hearing. In recent studies we, and others, find that macrophages infiltrate into the cochlea after CI in mice. Additionally, single cell RNA sequencing and immunofluorescence imaging demonstrate that a fraction of these infiltrating macrophages are MCHII+ antigen presenting cells capable to present antigen to CD4+T cells. Moreover, dexamethasone-eluting CI reduce the infiltration of macrophages, and MHCII+ macrophages in the murine model. The presence of MHCII antigen presenting cells raises the possibility of involvement of CD4+T lymphocytes in the FBR. Studies on human temporal bones from CI recipients and guinea pig models have shown that lymphocytes infiltrate into cochlea following cochlear implantation. This study investigates the involvement of T lymphocytes in the FBR post-CI and the impact of the dexamethasone eluting-CI on infiltration of T lymphocytes.

Methods: 10-12-week-old CX3CR1-GFP+, Thy1-YFP+, C57BL6 mice were implanted with either regular or dexamethasone-eluting CIs in the left ear with the right ear acting as controls. After implantation the CIs were stimulated for 7-28 days and all were sacrificed at 56 day post-implantation. The cochleae were fixed with 4% PFA and cryosectioned at 30 micrometer thickness. Images were taken using a Leica STELLARIS 5 confocal microscope. CD45 was used to identify leukocytes in cochleas post implant, and we focused specifically on cells that did NOT express CX3CR1. These included CD3 (T cells), CD4 (helper T cells) and other cells that expressed CD45 and lacked CX3CR1. The cochlear regions of the scala tympani, Rosenthal's canal, lateral wall, and modiolus were manually traced and cells within these regions were counted using IMARIS software.

Results: At 56 days post-CI, leukocytes were present in the inner ear in large numbers. Many of them were macrophages, some of them that were not macrophages were CD3+. A subset of the CD3+ cells were CD4+. Dexamethasone-eluting CI suppressed the infiltration of both macrophage and non macrophage cells throughout the cochlea.

Conclusions: These results suggest that T lymphocytes and specifically CD4+T lymphocytes are involved in the FBR following implantation and can be suppressed by immunosuppressive dexamethasone-eluting CIs.

Rescue of Spiral Ganglion Loss in a Mouse Model of Usher 1B

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Background: Usher disease is one of the most devastating inherited forms of syndromic hearing loss. In its severest form, it affects hearing, balance, and vision leading to significant disability. Usher 1B is inherited recessively and is caused by mutations in the MYO7A gene. The shaker 1 mouse carries a point mutation in MYO7A and homozygous mice present with circling behavior but initially develop hearing. Hearing is then rapidly lost in the third to fourth postnatal week.

Methods: Shaker 1 homozygous mutant mice were evaluated over time with serial ABR and histologic evaluation of spiral ganglion density. To determine if gene therapy approaches could halt the progression of cellular loss, we treated P17 day old shaker 1 homozygous mutant mice with a third-generation lentiviral vector expressing the MYO7A driven by the CAG promoter and evaluated the effects on hearing and spiral ganglion survival

Results: Evaluation of shaker 1 homozygous mutant mice over time demonstrated a profound hearing loss by age 30 days postnatal. A full complement of hair cells appears present for at least the first two months of life followed by a progressive loss of hair cells from base to apex over the next year. There is a subsequent loss of spinal ganglion density that can be detected by six months of age. By 1 1/2 years of age, there is a complete loss of neural content in the basal turn with few surviving apical neurons. The histologic appearance of the cochlea is similar to human temporal bone specimens from Usher type I demonstrating a complete loss of the spiral ganglion. In MYO7A treated mice, ABR testing demonstrated the arrest of progression of hearing loss, maintenance of hair cells, and a stabilized spiral ganglion population.

Conclusions: Despite the lack of significant hearing rescue, gene therapy-based delivery of MYO7A may support the survival of hair cells and a healthier spiral ganglion population, leading to more effective targets for cochlear implantation.

The Potential of the Chemical Chaperone TUDCA to Protect Against Cochlear Trauma

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Background: Commonly used mouse strains differ in their susceptibility to the common causes of hearing loss, genetic factors and environmental noise. Cochlear cells share several conserved stress-signalling pathways that can regulate or mediate the effects of these stressors. A growing number of studies have suggested that endoplasmic reticulum (ER) stress is involved in cochlea pathologies and that it may trigger apoptosis of cochlear cells. We have shown using mutant mouse models that the maintenance of ER homeostasis is critical for the survival of hair cells and for proper hearing function (Herranen et al. 2020). Tauroursodeoxycholic acid (TUDCA) is a naturally occurring bile acid derivative. TUDCA and other chemical chaperones have been suggested to exert otoprotective effects, specifically by antagonizing ER stress (Li et al. 2019; Hu et al. 2019). In this study, we asked if TUDCA can attenuate noise-induced hearing loss and if it can slow down early-onset age-related hearing loss.

Methods: (1) For noise experiments, we used 2-to-3-months-old mice of the C57BL/6J-129S6 hybrid and CBA/Ca strains (total n = 20 mice). Half of the mice received TUDCA by s.c. injection, 250 mg/kg diluted in PBS, and the other half PBS. The compounds were delivered one day before the noise exposure (102 dB SPL, 8-16 kHz for 2 h) and thereafter every third day during the 21-day-long study period. Auditory brainstem responses (ABRs) were recorded at day 21. Outer hair cell (OHC) loss was quantified in whole mount specimens (myosin 6, phalloidin, DAP1). Also inner hair cell (IHC) synaptopathy was quantified (presynaptic ribbons, CtBP2; postsynapse, Homer 1). (2) For age-related studies, we used mice of the CD-1 strain that is a model of very early-onset hearing loss (total n = 20 mice). TUDCA was delivered twice a week between 3 and 9 weeks of age. In the end, the read out parameters were as above.

Results: (1) Noise-exposed TUDCA and control groups (C57BL/6J-129S6 and CBA/Ca strains) did not show significant differences in the extent of ABR threshold elevations, OHC loss or synaptopathy. Inter-animal variability in the noise-exposed groups was high. (2) TUDCA treatment yielded a statistically significant decrease in synaptopathy in CD-1 mice, as analysed at 9 weeks of age.

Conclusions: Our results suggest that the effect of TUDCA against acute noise-induced cochlear trauma is not significant, but our results suggest that TUDCA may have a role in antagonizing age-related hearing loss. Even though more mechanistic data is needed to confirm the possible link between TUDCA and ER stress, the results here are consistent with our previous findings that the cochlear ribbon synapses are vulnerable to chronic ER stress. Together, chemical chaperones might have therapeutic potential in resolving ER stress and slowing down age-related synaptopathy.

Cisplatin Induced Stress Granules Have Reduced Sequestration of Key Signaling Proteins

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Background: Cisplatin is a highly effective, but ototoxic, platinum-based chemotherapeutic. The hearing loss that develops in up to 80% of adults and 50% of children (Frisina et al., 2016, Knight et al., 2005) undergoing cisplatin chemotherapy is bilateral, sensorineural, progressive and irreversible. The mechanisms underlying cisplatin ototoxicity are not fully understood but there are multiple proteins that have been shown to interact with cisplatin, several of which are involved in the formation and regulation of stress granules (SGs), condensations of mRNA and RNA-binding proteins. We previously identified a role for SGs in protecting against aminoglycoside antibiotic-induced ototoxicity (Goncalves et al., 2019). We have shown, in both UB-OC2 and human retinal pigment epithelial RPE-1 cell lines, that cisplatin induces persistent SGs and that cisplatin-treated cells have an impaired response to further stresses (ARO 2021).

Methods: Here, we investigate how cisplatin-induced SGs differ from canonical SGs including in the sequestration of signaling proteins DDX3X and RACK1 and the effect of cisplatin on P-bodies (PBs). PBs are RNA granules that are proposed to act as storage sites for translationally repressed mRNAs and inactive mRNA decay enzymes. PBs interact and share several components with SGs but unlike SGs they are constitutively present within the cytoplasm of cells. However, in response to stress PBs can increase in number and size although the reasons for this remain unclear. SGs can influence cell fate by acting as signaling hubs, modulating pathways by sequestering signaling proteins. Sequestration of DDX3X in SGs prevents the assembly of the NLRP3 inflammasome reducing the production of inflammatory cytokines like IL-1. Recruitment of RACK1 to SGs represses pro-apoptotic MAPK signaling.

Results: We show that cisplatin, unlike other stresses, causes a dose-dependent reduction in the number of PBs (60%) and can double the size of those PBs ($p<0.001$) in UB-OC2 and RPE cells. Additionally, using immunostaining and Western blots we have shown that cisplatin-induced SGs have reduced sequestration of DDX3X and RACK1 ($p<0.001$). The reduced sequestration of DDX3X in cisplatin-induced SGs raises the possibility of a greater pro-inflammatory response to cisplatin. The recruitment of RACK1 to SGs is a critical step in the repression of the intrinsic apoptotic pathway (Arimoto et al., 2008). Hence reduced RACK1 sequestration in SGs after cisplatin may contribute to greater activation of apoptosis compared to the SGs induced by other stresses. Interestingly, we found that in cisplatin treated OC2 cells RACK1 is also localized to PBs.

Conclusions: By identifying two significant changes to the properties of cisplatin induced SGs our work reveals additional possible therapeutic opportunities for prevention of cisplatin ototoxicity.

Impact of MCMV-Neutralizing Antibodies on the Inflammatory Response in the Cochlea and Development of Hearing Loss in MCMV Infected Mice

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Background: Congenital Human Cytomegalovirus (HCMV) infection is a leading cause of non-familial hearing loss. The mechanisms by which HCMV infection of the developing auditory system leads to sensorineural hearing loss (SNHL) are poorly understood and effective treatments are lacking. We developed a murine model that takes advantage of the postnatal development of hearing in rodents. Following peripheral inoculation of newborn mice with murine CMV (MCMV), hematologic viral spread leads to infection of most organ systems, including the CNS. MCMV infected mice present with neurodevelopmental abnormalities and SNHL, providing an informative model to study the mechanisms of SNHL that is associated with cCMV infection. We have shown that MCMV infection leads to production of proinflammatory molecules and immune cell activation in the cochlea which is associated with histopathologic changes and elevated hearing thresholds in infected mice. To begin exploring the potential of adaptive immunity to limit cochlear damage in this model, we have tested the capacity of murine monoclonal antibodies (Mabs) to decrease the viral load in the cochlea. Our results demonstrated that virus neutralizing Mabs can reduce but not eliminate virus replication in the cochlea; however, the decreased viral load following Mab treatment leads to a decrease in inflammatory responses and a decrease in the incidence of hearing loss in infected mice.

Methods: Mice (Balb/c) were inoculated intraperitoneally with 200-400 plaque forming units of MCMV within 24 hours of birth. MCMV neutralizing Mabs were administered intraperitoneally at PND2 and PND5. Temporal bones were collected at PND8, 14, and 32. For RT-qPCR, temporal bones were snap frozen immediately after removal, weighed, and DNA and RNA extracted. For imaging studies, temporal bones were removed and fixed with 4% PFA. Samples were decalcified, frozen in OCT, and sectioned for imaging experiments.

Results: Expression of multiple proinflammatory molecules was elevated in MCMV-infected cochlear samples at each timepoint compared to uninfected controls. MCMV neutralizing Mab treatment reduced but did not eliminate

the viral load in the cochlea. In addition to decreased viral load, we found lower expression of proinflammatory molecules in the cochleae of Mab treated mice. Finally, the occurrence of elevated hearing thresholds was lower in Mab treated mice compared to untreated, infected mice.

Conclusions: Treatment with Mabs early after infection lowered the viral load in the cochlea which in turn decreased the amount of inflammation and immune cell activation induced by infection of the inner ear. These data argue that antiviral antibodies that limit but do not prevent virus infection of the inner ear can modify the virus-induced inflammatory response in the cochlea, and as a result, decrease the incidence of SNHL in infected mice. These data argue that non-sterilizing adaptive immunity can prevent the development of SNHL even in infants with cochlear CMV infection.

Noise-Induced and Age-Related Changes in the Olivocochlear Innervation in Mice
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Background: Noise-exposed and aging mice show loss of afferent synapses between auditory-nerve fibers (ANFs) and inner hair cells (IHCs) before elevation of thresholds or loss of IHCs. Here, we asked whether this afferent cochlear synaptopathy is mirrored by degeneration of the olivocochlear (OC) efferent innervation in the medial (M) or lateral (L) OC systems.

Methods: 6-week-old CBA/CaJ mice were exposed to 8–16 kHz noise band for 2 hrs at 98 dB SPL, allowed to recover for 2 days, 2 wk, 1 mo, 4 mo, or 8 mo, and compared to unexposed age-matched controls, plus an additional 24–28-month unexposed group. This exposure causes a large temporary threshold shift, but minimal permanent shift. It also destroys up to 50% of ANF/IHC synapses throughout the basal half of the cochlea. Cochleas were immunostained for myosin VIIA (hair cells), vesicular acetylcholine transporter (VAT, cholinergic efferents) and tyrosine hydroxylase (TH, dopaminergic efferents). High-power confocal z-stacks (76 nm/pixel) of IHC and OHC areas were acquired at half-octave intervals (4–64 kHz), and medium-power (444 nm/pixel) z-stacks were tiled to cover the entire cochlea. HC survival was quantified, and the density of efferent innervation was estimated in IHC and OHC regions by 1) measuring silhouette area in maximum projections from the VAT and TH channels of the high-power stacks, 2) measuring volume of all VAT or TH-stained boutons in IHC and OHC areas in the tiled stacks as a continuous function of cochlear distance, and 3) manually counting MOC terminals per OHC in the high-power stacks.

Results: Results showed no noise-induced de-efferentation in LOC or MOC areas by any metric. Indeed, VAT staining was enhanced in noise-exposed ears, especially in the OHC area, while the number of terminals per OHC was not significantly altered. The dopaminergic innervation was not altered by noise: dopaminergic fibers were rare, were only in the IHC area, and restricted largely to the apical and basal extremes in all groups.

In the aged group, 30–70% of IHCs and OHCs were lost throughout the apical half of the cochlea, with minimal loss in the base. The cholinergic innervation was diminished in both IHC and OHC areas. LOC terminals were redistributed to contact IHCs closer to the cuticular plates, and IHCs were greatly reduced in volume, as previously reported after surgical de-efferentation.

Conclusions: This study examined noise-induced and age-related changes to the efferent innervation. The MOC upregulation observed after noise exposure might contribute to "toughening," whereby a moderately traumatic noise exposure renders the ear less vulnerable to subsequent overexposure. The loss of VAT-positive innervation in IHC and OHC regions of aged ears may increase vulnerability to noise overexposure, as seen following surgical de-efferentation.

In Vitro and Ex Vivo Cisplatin Ototoxicity Models to Identify New Drug Candidates to Restore Inner Ear Function
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[1]*Sensorion*

Background: Cisplatin is a chemotherapeutic agent widely used to treat a variety of malignant tumors. Serious ototoxic dose-limiting side effects occur with its use, including bilateral and irreversible hearing loss , which is particularly relevant in the pediatric population. Cisplatin-related ototoxicity is linked to the loss of hair cells within the organ of Corti (OoC), of spiral ganglion neurons (SGNs) and of cells within the spiral ligament,

including fibrocytes (FCs). Hence, there is an urgent need for new otoprotective agents to preserve these cellular structures from chemotherapy-induced lesions

Methods: Here, we describe in vitro and ex vivo cultures of a) spiral ligament fibrocytes, b) organ of Corti explants, and c) primary spiral ganglion neurons, allowing us to characterize time-course and dose-response effects of cisplatin in these models developed for the pharmacological screening of otoprotective candidate drugs. Wistar rat pups of postnatal ages P3 (OoC), P4 (FCs) and P5 (SGNs) were used and cultures grown either on 48- or 96-well plates under culture-specific conditions. Cultures were treated with different cisplatin concentrations (10 and 30 µM) for 24h under specific modalities. Live-cell imaging (IncuCyte, Sartorius) was used to assess cell viability in OoC cultures (FM1-43) and cell death in OoC and FC cultures (Annexin V Red). SGN and FC cultures were immunolabeled at specific time points (T24, T48 and T120/144) with DAPI and NF-200 or DAPI, respectively, to assess cell proliferation, cell density, neuron survival and neurite integrity. NF-200 labelling was analyzed using an automated cell imaging system (ImageXpress Pico, Molecular Devices). One of the compounds shown to act on the cellular level as an otoprotective agent, sodium thiosulfate (STS, Sheth et al., 2017), was used as a reference molecule in the models to protect from the cisplatin-induced lesion.

Results: While the effect of 10 µM cisplatin on OoC explant cultures and FC monolayer cultures was moderate but visible at late time points, cisplatin had a stronger ototoxic effect on SGN monolayer cultures visible at early time points. 30 µM cisplatin induced strong ototoxic effects on all three culture models, with early effects observed in monolayer culture models (FCs and SGN) and delayed effects observed on OoC explants. To conclude, these two distinct cisplatin treatment modalities allow to investigate the cell-specific cellular mechanisms (cell proliferation, cell death, cell viability) affected by moderate and strong cisplatin treatment. Otoprotection with STS against cisplatin-induced effects was observed in all three inner ear cell models.

Conclusions: These data demonstrate the feasibility of a screening approach quantifying ototoxic lesions in the three main inner ear structures: spiral ligament, OoC, and spiral ganglion. Therefore, this approach is relevant and applicable to the screening of drug targets and potential therapeutic candidates for otoprotection and repair in the inner ear.

Electrically Evoked Compound Action Potentials After Chronic Micro-Lesions
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Background: Speech perception in CI users is likely influenced by heterogeneous degeneration of spiral ganglion neurons (SGN). However, the interpretation of human psychophysical and electrophysiological results is difficult, as reliable markers of locally restricted SGN degeneration are missing. In our previous study (Konerding et al. 2020, Hear Res) we assessed the effects of acute micro-lesions on the response characteristics of electrically evoked compound action potentials (eCAP). The acute setting had the limitation that the neurons remained excitable at all parts central to the lesion. The recent study therefore used a chronic animal model to also include the effect of progressive SGN-loss at the lesion site.

Methods: We inflicted 15 guinea pigs (7 male, 8 female) with dendritic (n=8) or soma (n=7) lesions and let the SGN degenerate for 8-12 days before the electrophysiological recordings. At the final experiment, the animals were deafened by intrascalar application of Neomycin and implanted with a 6-contact CI-electrode (guinea-pig adjusted, MedEl Comp.). ECAP responses to 50 µs biphasic pulses with alternating polarity were recorded from a ball electrode at the round window niche. The input-output functions, including latency and amplitude at 1dB above threshold were analyzed using custom Matlab programs. In line with our previous publication on acute lesions, we assessed the late N2P2 component for anodic and cathodic stimulation, separately, as well as together. In order to relate the eCAP response characteristics to the site and size of the lesion, we performed anatomical and histological analyses. The micro-lesion site relative to the CI-electrode position was assessed via micro-tomography images and the auto-fluorescence of the cleared cochleae was used for histological evaluation of the SGN degeneration. In 8 animals (3 male, 5 female), we also recorded from the non-lesioned ear as internal control.

Results: The short-term chronic micro-lesion led to spatially restricted regions of reduced SGN density, including degenerated peripheral dendrites, somata, and central axons. These regions were flanked by healthy SGN. The eCAP responses showed high intra-individual variability, which was higher for the lesioned ear than for the control side. We revealed a complex interaction between stimulation electrode (apical-basal), lesion site (distance to the CI electrode) and lesion targets (dendrites/somata). Local changes of the eCAP responses, restricted to

stimulations at electrodes close to the micro-lesion site, were observed for chronic dendritic lesions. Chronic soma lesions led to global changes in eCAP responses across the whole CI-array.

Conclusions: The chronic micro-lesions are a good model to understand the complex response patterns to CI stimulation, resulting from locally restricted SGN degeneration.

Characterization of Extra- and Intracochlear ECochG Recordings in the C57BL/6 Mouse

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Background: Electrocochleography (ECochG) is a promising tool to monitor changes in cochlear function during cochlear implant (CI) surgery and to guide surgery towards more structure/function-preserving outcomes. However, the interpretation of ECochG amplitude and phase changes during CI surgery often remains controversial. To overcome this limitation, experiments directly correlating electrophysiological and structural changes are deemed necessary. In this proof-of-principle study, we evaluated C57BL/6 as a model to correlate ECochG patterns during CI surgery with distinct changes in the cochlear structure.

Methods: ECochG recordings were conducted from an extracochlear location adjacent to the round window (RW recordings) and during stepwise insertion of an electrode from different intracochlear locations (IC recordings). As acoustic stimuli, sinusoid tone bursts at 4, 6, and 8 kHz were used. Those frequencies are represented in apical cochlear regions in the mouse, resembling low-frequency ECochG recordings in human CI patients. The recording electrode for RW as well as IC recordings was a Teflon-coated silver wire with a diameter of 0.125mm. In RW recordings, the neural component of the ongoing ECochG response was eliminated using the neurotoxin kainic acid (KA). Responses were recorded before (pre-KA) and 30 minutes after KA was applied (post-KA) on the RW. The removal of neural components was confirmed by the absence of a compound action potential in post-KA recordings. In control animals, sodium chloride 0.9% instead of KA was applied to the RW. In IC recordings, the recording electrode was inserted in a stepwise manner using a micromanipulator. As soon as an amplitude drop in ECochG recordings was detectable, the electrode was fixed in position. Then, a micro-computed tomography as well as plastic-embedding histological analysis of the specimen was performed.

Results: Post-KA RW recordings revealed amplitude as well as phase changes at all recorded frequencies over a wide range of intensities. Amplitude changes in post-KA recordings were more pronounced at 4 and 6 kHz whereas phase changes were similar at all recorded frequencies. In control animals, no amplitude or phase changes were detectable. Correlation of IC recordings with histological and radiological findings revealed lateral wall trauma and/or displacement or rupture of the basilar membrane in all cases. Phase changes preceded amplitude drops in 4 out of 6 cases.

Conclusions: In C57BL/6 mice ECochG signals at 4, 6, and 8 kHz resemble low-frequency ECochG recordings in the normal human cochlea. Amplitude drops in IC recordings are associated with cochlear trauma in the electrode tip region. Phase changes preceding amplitude drops may represent an early indicator for electrode contact with the lateral wall and/or basilar membrane. Overall, C57BL/6 mice seem to be a suitable model to correlate ECochG patterns with acute cochlear trauma during CI surgery.

Comparison of Actions of Ketamine and Telazol on Cochlear Function in a Rodent Model of Noise-Induced Hearing Loss

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Background: Prior studies have suggested that the anesthesia used in noise-induced hearing loss (NIHL) models play potentially protective roles and may confound the effects of therapeutics under evaluation. Acoustic overexposure negatively affects the inner ear structures through multiple pathways including glutamate excess on postsynaptic cochlear nerve N-Methyl-D-Aspartic (NMDA) receptors. Ketamine, an NMDA receptor antagonist, is widely used in rodent models as an anesthetic and studies have shown potential protective effects of ketamine in NIHL. Tiletamine is chemically related to ketamine and is also an NMDA antagonist. Tiletamine combined with zolazepam (Telazol) may be a substitute for ketamine in NIHL rodent models with less severe side-effects and

long-acting capacity. In the present study, we investigated the actions of effects of ketamine and Telazol on cochlear function in rats.

Methods: Brown Norway rats were exposed to noise between 4-8 kHz at 110 dB for 1 hour. Cochlear function was assessed over multiple time points using either intramuscular ketamine (44mg/kg) and xylazine (5 mg/kg) (n=6) or intraperitoneally injected Telazol (20 mg/kg) and xylazine (5 mg/kg) (n=6). Changes in auditory brainstem response (ABR) threshold and wave 1 and 5 amplitudes were compared to baseline (pre-NIHL) over 28 days. Immunohistochemistry was used to quantify hair cell (HC) and synaptic union counts.

Results: There was no significance difference found between the baseline, post-NIHL day 1, and post-NIHL day 3 ABR thresholds with regards to time, frequency, and anesthesia groups. Based on the results, we anticipate no significant differences in either functional evaluations (ABR thresholds) or cellular analysis (HC and synapse count) between the Ketamine/Xylazine and Telazol/Xylazine groups in the long-term. There was an increased mortality in the Telazol/Xylazine group.

Conclusions: Our findings suggest that Telazol could be an alternative in rodent model experiments for the evaluations of hearing sensitivity following noise or other traumas.

Tamiflu as a Therapeutic Candidate for Noise-Induced Hearing Loss

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Background: Hearing loss caused by noise exposure and aging is a major unmet medical need in our society, affecting over 600 million people worldwide. For military-service personnel noise-induced hearing loss can be a daily threat during training or combat, increases likelihood of posttraumatic stress disorders and is the most common cause of hearing loss. To date, no drugs have been approved by the FDA for treating noise-induced hearing loss.

Methods: In the aim of identifying efficient and safe drugs for noise-induced hearing loss, we recently conducted unbiased high-throughput screens of 1,300 FDA-approved drugs for protection against cisplatin-induced cell death in an inner ear cell line HEI-OC1, and identified as one of the best hits, the drug oseltamivir phosphate (Tamiflu). Tamiflu is an oral, widely used anti-viral drug to treat the flu (Influenza types A and B) in humans two weeks and older, who have had common flu-like symptoms for 48 hours or less. Tamiflu was tested in ex vivo cochlear explants against cisplatin-induced hair cell death, and in adult mice against permanent noise-induced hearing loss, as some of our top screened drugs show protection against both insults. Tamiflu was administered by oral gavage at 100 mg/kg body weight twice daily for three days, 45 minutes before, and 24 hours after noise exposure. Auditory Brainstem Response (ABR) and Distortion Product Otoacoustic Emissions (DPOAE) threshold shifts were measured in adult FVB mice 14 days after noise exposure of 100 dB, 8-16 kHz octave band, for two hours. Protection of the cochleae synaptic ribbons connecting to the neuron fibers by Tamiflu treatment was evaluated by synaptic ribbon scaffolding protein Ctbp2 co-staining.

Results: Tamiflu protected from cisplatin-induced hair cell death in mouse P3 cochlear explants with an IC50 of 450 nM and therapeutic index larger than 220. Importantly, Tamiflu protected adult mice by oral delivery against permanent noise-induced hearing loss. Significant protection of 24-28 dB was observed with ABR and DPOAE measurements when the drug was administered orally 45 minutes before noise exposure and when administered 24 hours after noise exposure to mice for three consecutive days in equivalent doses to those approved for human use. Cochleae were co-stained for synaptic ribbon scaffolding protein, Ctbp2, to measure loss of synaptic ribbon integrity in inner hair cells at the 16 kHz. Treatment with Tamiflu partially rescued loss of the Ctbp2-punta in the mice cochleae compared to mice exposed with noise alone.

Conclusions: Tamiflu is an excellent candidate for treating noise-induced hearing loss, being orally bioavailable and FDA-approved drug since 1999. Our studies aim to characterize Tamiflu's properties and measure the lowest in vivo dose required for protection against various levels of noise-exposure in the aim of repurposing and fast-tracking the drug for use in humans.

Deletion of the KSR1 Gene Validates the Role of the MAPK Pathway in the Mechanism of Cisplatin Induced Hearing Loss

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Background: Cisplatin is a commonly used chemotherapeutic agent for treatment of many types of malignant tumors. Unfortunately, cisplatin treatment leads to hearing loss in 40-60% of patients, severely impacting patients' overall quality of life and creating a debilitating barrier to language development. Currently, there is no FDA-approved compound for treatment of cisplatin ototoxicity, creating a clear need for further scientific study of potential therapeutic agents. Recently, BRAF inhibitor dabrafenib was found to protect from both noise and cisplatin ototoxicity in vivo when administered at clinically relevant doses. Dabrafenib is FDA-approved for treatment of multiple cancers and may be repurposed to preserve patient hearing during cisplatin treatment. In addition, dabrafenib does not interfere with cisplatin's tumor killing ability in cell lines. Moreover, Kinase suppressor of Ras (KSR1) is a scaffolding protein whose role is to bring MAPK proteins in proximity to one another and allow for cascading phosphorylation signal transduction. In this study, we seek to further validate the role of the MAPK pathway in the mechanism of cisplatin ototoxicity by employing a genetic KSR1 knockout (KO) model.
Methods: Initially, single-cell RNA sequence was conducted to determine the expression pattern of KSR1 in adult mouse cochlear tissues. Next, C57BL/6 KSR1 wildtype (WT) and KO mice were IP injected with a single dose of 18 mg/kg cisplatin then allowed to recover for 14 days before assessing permanent hearing loss. The experiment was repeated with twice daily oral dabrafenib treatment for three days, beginning 45 minutes prior to cisplatin injection. Mouse hearing ability was measured by auditory brainstem response (ABR) at 8, 16, and 32 kHz both before and after experiments to determine hearing threshold shift. Cochleae from these mice were then fixed and stained with myosin VI to determine loss of outer hair cells (OHCs) at the basal, middle, and apical regions.
Results: Single-cell RNA sequence reveal high levels of KSR1 expression in cochlear supporting cells, spiral ganglion neurons, and stria vascularis which correlates with previously observed MAPK activation upon cisplatin treatment. KO of KSR1 conferred significant protection against cisplatin ototoxicity when compared to wildtype (WT) mice as measured by reduced ABR threshold shift at all frequencies tested and reduced loss of OHCs at the middle and basal regions. Moreover, cisplatin treated WT mice administered oral dabrafenib display significant auditory protection, while cisplatin treated KO mice display no additional protection when administered dabrafenib.
Conclusions: This data confirms dabrafenib confers hearing protection through inhibition of the MAPK pathway using a genetic KO model and further validates the role of the MAPK pathway in the mechanism of cisplatin induced hearing loss. Future work will investigate the combination of dabrafenib and other MAPK inhibitors, such as trametinib, for enhanced auditory protection.

Gaussian Models and Fourier Analysis of Human ABRs: The Search for Biomarkers of Cochlear Nerve Degeneration

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Background: In mouse studies, cochlear nerve degeneration (CND) can be diagnosed by measuring the reduction of suprathreshold amplitude of ABR wave 1 (or AP), so long as cochlear thresholds remain normal. In humans, the diagnosis of CND is challenging, as AP amplitude is smaller and more variable across subjects when measured via conventional ABR electrode configurations. Despite efforts to enhance AP amplitudes with intra-meatal electrodes and/or by varying electrode montages, AP amplitudes remain highly variable, presumably due, in part, to differences in head size, electrode contact, etc. In our quest for reliable CND markers in humans, we hoped to reduce the variability of AP amplitude by normalizing it to the summating potential (or SP), a low-frequency component classically thought to comprise hair cell receptor potentials. As it turned out, SP itself has proven to be a better predictor of performance on word identification tasks, which, in turn, may be a biomarker of CND.
Methods: SP is traditionally extracted by visual inspection, a technique prone to subjectivity and error. Here, we assess the utility of a fitting algorithm (Kamerer et al., 2020) in a large cohort of normal-threshold subjects and analyze the nature of the discrepancies with measurements by visual inspection. In the same cohort, we also investigate the generators of the early ABR waves using FFT and by comparing these data to those obtained from patients with mutations of the otoferlin gene that disrupt synaptic-vesicle release from the IHC ribbon synapses, leaving hair cell receptor potentials intact.
Results: Results show that identification of SP and AP peaks by a two-Gaussian model is slightly improved by constraining one of the Gaussians to a user-supplied AP latency and significantly improved by high-pass filtering. However, the latter comes at the expense of attenuating the SP amplitude, which is an unacceptable outcome. We also found that SP extracted by visual inspection correlated better with speech in noise tests than those from the

model fits when measured by comparing unfiltered ABR waveforms acquired under different repetition rate and masking conditions designed to have a differential effect on SP and AP amplitudes. FFT analysis suggests that SP generators also dominate a spectral peak at 310 Hz, which, in turn, may include a strong contribution from excitatory post-synaptic potentials in auditory nerve terminals as well as from hair cell receptor potentials.
Conclusions: Research supported by a grant from the NIDCD (P50 DC015857)

Pharmaceutical Intervention to Reduce Hearing Threshold Shift in a Preclinical Animal Model of Cochlear Implantation

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Background: There is a growing need to develop prophylactic and therapeutic interventions to prevent loss of residual hearing post-cochlear implantation. During cochlear implantation, the initiation of the electrode insertion trauma (EIT) triggers the activation of oxidative stress, apoptosis and inflammatory pathways that can damage sensory cells and consequently lead to the loss of residual hearing. Preserving these sensory cells by blocking the activation of these host pathways can improve hearing preservation and allows implanted individuals to benefit from better hearing outcomes. The aim of this study was to investigate the effect of a new molecule on the preservation of the residual hearing in a preclinical animal model of cochlear implantation.
Methods: Animals were divided into various groups. In first group, animals were implanted unilaterally. In second group, the compound was applied on the round window membrane before cochlear implantation followed by insertion of the electrode. The animals in third group served as vehicle control whereas naïve animals that were not subjected to treatment with compound and cochlear implantation served as control group. Contralateral ear from each group also served as control group. Hearing thresholds of animals in each group were determined by auditory brainstem recordings (ABRs). Cochleae harvested from animals in each group was subjected to histopathological examination to determine pathological manifestations. The organ of Corti was dissected and stained with FITC phalloidin to visualize and count the number of hair cells.
Results: Hearing thresholds were significantly lower after drug application than in the EIT group. The organ of Corti harvested from cochlea of implanted and treated animals subject showed a significantly higher number of hair cells after immunostaining compared to implanted animal alone. The molecular mechanisms behind otoprotection involved abrogation of activation of oxidative stress and caspase pathways.
Conclusions: The result of the present study suggests that identified compound provides protection against electrode insertion trauma and can be explored for developing effective interventions. The availability of new interventions to prevent electrode insertion trauma holds great potential to promote hearing preservation and expanding indications of cochlear implantation.

Characterizing Hearing Function Loss in Populations With Hazardous Noise Exposure

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Background: An estimated 40 million United States adults have noise-induced hearing loss (NIHL), with approximately 22 million workers exposed to occupational noise in the last year (Carroll et al., 2017; Tak et al., 2009). With proper use of hearing protection, NIHL is preventable, yet adoption remains inconsistent. Furthermore, conventional monitoring techniques are insensitive to pre-clinical structural and physiological changes. Reductions in Distortion Product Otoacoustic Emissions (DPOAEs) occur in occupationally noise exposed populations, despite normal audiometric thresholds (Attias et al., 2001). Additionally, in animals, thresholds and DPOAEs recover after acute noise exposure; however, reductions in wave I of the Auditory Brainstem Response (ABR) persist and are accompanied by disruption of auditory nerve synapses (Kujawa and Liberman, 2009). The identification of early clinical markers of NIHL could inform policy changes, guide prevention strategies, inform therapeutic development, and allow for earlier detection of auditory damage. The first aim of this study is to ascertain the effects of routine noise exposure in an occupationally noise exposed

population, firefighters, using audiological functional assessment. The second aim is to examine the efficacy of DPOAE and ABR wave I amplitudes as early clinical markers for noise-induced auditory impairment

Methods: This study employed a cross-sectional prospective design. We recruited pre-career firefighters (0 years of service), mid-career firefighters (10-19 years of service), and late-career/retired firefighters (20+ years of service) from south Florida fire departments and age-matched controls from the general population. Only controls with minimal noise exposure were included, as determined by the screening procedure. Primary outcome measures included standard pure-tone thresholds (250-8,000 Hz), extended high-frequency pure-tone thresholds (9,000-16,000 Hz), DPOAEs, and supra-threshold ABRs. DPOAEs were assessed 1,500-10,000 Hz using $L1 = 65$ dB SPL, $L2 = 55$ dB SPL, and f2/f1 primary ratio = 1.22. ABRs were recorded for rarefaction click and 4000 Hz tone burst 113 dB peak SPL stimuli using a tiptrode electrode.

Results: Normal audiometric pure-tone thresholds at standard test frequencies were observed in the majority of firefighters tested and did not differ significantly from controls with minimal noise exposure. Late career firefighters showed increased extended high-frequency pure-tone thresholds and decreased DPOAE amplitudes when compared to both pre-career firefighters and age-matched controls. The amplitude of Wave I of the ABR varied within the 3 service year groups and between the firefighters and controls.

Conclusions: Firefighters exhibit deficits in DPOAEs and wave I of the ABR when compared with age- and sex-matched controls. These findings suggest physiological differences in outer hair cell function and auditory neural response between firefighters and controls with minimal noise exposure history. Firefighters exhibit these physiological differences despite having near-normal audiometric thresholds. These findings suggest that audiometric thresholds, the most common method used in hearing conservation programs, is not sensitive to pre-clinical changes in hearing function.

Hair Cell Death After Exposure to Noise or Aminoglycosides is Mediated by Activation of CaMKK2
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Background: The Ca2+/calmodulin-dependent protein kinase kinases (CaMKKs) are serine/threonine-directed protein kinases that are activated following increases in intracellular calcium, playing a critical role in neuronal signaling. Inner-ear-trauma-induced calcium overload in sensory hair cells has been well-documented in the pathogenesis of noise- and aminoglycoside-induced hair cell death and hearing loss, but there are no established pharmaceutical therapies available due to our incomplete knowledge of potential molecular targets and the optimal delivery route of agents for prevention of hair cell death. In this study, we investigated the activation of CaMKK2 in the inner ear after acute inner ear trauma by exposure to noise or kanamycin-furosemide (KM-FU) treatment in vivo for addressing its relationship to outer hair cell (OHC) damage and noise-induced loss of inner hair cell synapses.

Methods: Both CBA/J and FVB/NJ mouse strains were exposed to traumatic noise that induces OHC loss and permanent hearing loss. A model of KM-FU-induced massive OHC loss was also employed in CBA/J mice. Knockdown of CaMKK2 by short hairpin RNA (shRNA) via adeno-associated virus vector transduction and small interfering RNA (siRNA) via posterior semicircular canal (PSC) delivery were used to evaluate noise-induced hearing loss (NIHL) and KM-FU-induced hearing loss. Hair cell loss was counted from whole mount surface preparations along the entire cochlear spiral. Auditory function was assessed by auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE).

Results: Traumatic noise exposure activates CaMKK2 as demonstrated by immunolabeling for p-CaMKI in damaged OHCs in both CBA/J and FVB/NJ mice. Knockdown of CaMKK2 expression significantly attenuates noise-induced OHC loss and NIHL. Additionally, pretreatment with CaMKK2 siRNA attenuates noise-induced losses of inner hair cell synapses and OHCs and NIHL. Furthermore, delivery of CaMKKβ-siRNA via the PSC nearly completely prevents the massive OHC loss induced by KM-FU, without disrupting OHC mechanotransduction channels.

Conclusions: These findings demonstrate that acute-inner-ear-trauma-induced OHC loss occurs primarily via activation of CaMKK2. Targeting CaMKK2 is therefore a key strategy for prevention of acute inner-ear-trauma-induced hearing loss.

Small-Molecule KCNQ4 Agonist Protects Against Outer Hair Cell Loss in the SAMP8 Mouse Model of Age-Related Hearing Loss

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Background: Age-related hearing loss (ARHL) is the most common sensory impairment and with no causal medical treatment available. KCNQ4 is a voltage-gated potassium channel expressed in the basal pole of outer hair cells (OHCs) responsible for potassium efflux and OHC resting potential. It thus plays an essential role in cochlear function, contributing to potassium recycling and homeostasis. Impaired surface expression or reduced activity of KCNQ4 has been associated with age- and noise-related hearing loss. The aim of this study was to investigate the effect of a novel small-molecule KCNQ4 agonist ACOU085 in a sustained release formulation (Acousia Therapeutics, Tübingen, Germany) against ARHL in the senescence-accelerated mouse prone 8 (SAMP8) mouse model.

Methods: In a pharmacokinetic study, drug concentrations in cochlear perilymph and tissue were sampled from SAMP8 mice at different timepoints after a single administration of ACOU085 via transtympanic injection. Sampling timepoints were 0.25 (6 h), 7, 14, 21, and 28 days. ACOU085 concentrations were analyzed using liquid chromatography and mass spectrometry. In an electrophysiology study, two groups of SAMP8 mice received unilateral transtympanic injections of either 0.6% w/v (n = 10) or 6.0% w/v (n = 8) ACOU085 and vehicle control in the contralateral ear at the age of 45, 75, and 105 days, with final auditory function assessment and extraction of cochleae at 135 days of age. Before each administration, click- and toneburst-evoked auditory brainstem response (ABR) thresholds were determined. ABR threshold shifts were evaluated vs. pre-treatment thresholds for each ear and timepoint. Cochleae extracted at 3-months post-treatment were analyzed using immunolabelled whole-mount preparations to determine hair cell loss along the cochlear length using cytocochleograms.

Results: The pharmacokinetic study demonstrated dose-dependent ACOU085 diffusion into the cochlear perilymph and tissue from the formulation injected into the middle ear cavity. The therapeutic window was estimated to be between 7 and 14 days after a single administration. In the group treated with 6.0% w/v ACOU085, the electrophysiology study showed significantly reduced click-evoked ABR threshold shifts at 3-months post-treatment, with a mean threshold shift of 26 ± 17 dB SPL for ACOU085-treated ears compared with 40 ± 8 dB SPL for vehicle-treated ears ($p < 0.05$). Correspondingly, OHC loss was significantly reduced in the high-frequency range (> 8 kHz) of the ACOU085-treated compared with vehicle-treated ears ($p < 0.05$).

Conclusions: In the present study, we demonstrated that the novel small-molecule KCNQ4 agonist ACOU085 readily diffuses into the cochlea after a single transtympanic injection. Repeated administrations in the SAMP8 model of ARHL were shown to significantly reduce age-related ABR threshold shifts as well as OHC loss in the higher concentration group. Pharmaceutical targeting of KCNQ4 or other potassium channels affected by aging could, therefore, be promising approaches to decelerate or prevent ARHL.

VEGFA165 Gene Therapy Ameliorates Blood-Labyrinth Barrier Breakdown and Hearing Loss

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Background: Millions of people are affected by hearing loss. Hearing loss is frequently caused by noise or aging, which is often associated with loss of pericytes. Pericytes populate small vessels in the adult cochlea. However, their role in different types of hearing loss is largely unknown.

Methods: To evaluate the role of pericytes in hearing loss, we established an inducible and conditional pericyte depletion mouse model and noise-exposed mouse model in vivo. In vitro, advanced tissue explants from pericyte fluorescence reporter models were cultured in combination with exogenous donor pericytes to investigate the role of pericytes in angiogenesis.

Results: We found that loss of pericytes leads to marked changes in vascular structure, leading to vascular degeneration and hearing loss. Our in vitro data showed that pericytes, signaled by endothelial growth factor isoform A165 (VEGF-A165), vigorously drives new vessel growth in both adult and neonatal mouse inner ear tissue. In addition, the delivery of an AAV1-mediated VEGF-A165 viral vector to pericyte depleted or noise-exposed animals prevented and regenerated lost pericytes, improved blood supply, and attenuated hearing loss.
Conclusions: These studies provide the first clear-cut evidence pericytes are critical for vascular regeneration, vascular stability, and hearing in adults. The restoration of vascular function in the pericyte damaged cochlea, including in the noise-exposed mouse model suggests that VEGF-A165 gene therapy could be a new strategy for ameliorating vascular associated hearing disorders.

A Neurogenic Small Molecule Provides Otoprotection for Noise Induced Trauma in a Rat Model

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Background: Noise induced hearing loss (NIHL) is one of the most common causes of hearing loss worldwide. Due to ever increasing levels of noise, NIHL is becoming more prominent in the modern world. There are no Food and Drug Administration (FDA)–approved drugs available for NIHL. There is a continued need to develop effective treatment modalities for NIHL. The objective of this study was to determine the efficacy of a small neurogenic molecule to provide otoprotection for noise induced trauma using a rat model.
Methods: Adult Sprague Dawley rats were divided into various groups. Group 1 served as control; Group 2 served as the vehicle control; Group 3 were subjected to noise trauma (NT); Group 4 received the drug subcutaneously 24h before NT followed by six more doses after acoustic trauma; Group 5 received the drug subcutaneously immediately after NT followed by six more doses. The baseline hearing thresholds were determined by auditory brainstem recordings (ABRs) and distortion product otoacoustic emissions (DPOAEs) followed by subjecting the animals to NT. ABRs and DPOAEs were performed at different days post-NT followed by harvesting the cochlea at day 30. The cochlea was sectioned and subjected to immunostaining to determine the molecular pathways involved in otoprotection provided by the drug.
Results: Our results suggest that this neurogenic small molecule provides otoprotection for NIHL. Hearing thresholds were significantly lower in animals subjected to NT and pre or post-treated with drug compared to untreated or vehicle control rats. Immunostaining of the cochlea revealed that drug provides otoprotection through multiple protective mechanisms including targeting apoptotic and oxidative stress pathways.
Conclusions: The results of the present study suggest that the identified compound have the potential to provide otoprotection for NIHL and should be explored for developing pharmaceutical interventions for NT. The availability of novel effective treatment modalities for NIHL will lead to improved quality of life of many affected individuals and their families.

An Exosome-Mediated HSP70–TLR4 Pathway in Hair Cells That Reduces Ototoxicity

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Background: Millions of people worldwide suffer annually from permanent hearing loss caused by lifesaving yet ototoxic medications, such as the anti-cancer drug cisplatin and aminoglycoside antibiotics. These drugs kill mechanosensory hair cells (HCs) that convert sound energy into neural input to the brain. HC death and consequent hearing loss caused by ototoxic drugs are reduced by the non-cell-autonomous induction of heat shock proteins (HSPs), particularly by upregulation of HSP70 in glia-like supporting cells (SCs) that surround HCs. We recently discovered that heat shock causes the release of small secretory vesicles from SCs called exosomes. These exosomes deliver HSP70 to HCs, where it interacts with Toll-like Receptor 4 (TLR4) to trigger a pro-survival response. However, the signaling downstream of this HSP70-TLR4 interaction remains elusive. TLR4 is commonly activated by bacterial lipopolysaccharides (LPS) to generate a potent pro-inflammatory response. Additionally, TLR4 signaling can be robustly cytoprotective, such as the exosome-stimulated HSP70-TLR4 non-inflammatory pathway in cardiomyocytes that activates HSP27 to protect against ischemia-reperfusion injury. Here we investigated if release of protective exosomes by the heat shock response activates components of either a canonical, pro-inflammatory, or a non-inflammatory TLR4 pathway in HCs.

Methods: To examine if the exosomal HSP70-TLR4 interaction activates components of either a pro-inflammatory pathway (e.g., MyD88, Traf6, IRAKs, NF-kB), or a cytoprotective, non-inflammatory TLR4 pathway (e.g., HSP27), we performed Western blot and immunohistochemistry analyses of mouse utricle explants that model the adult hearing organ. Given the limited biomass obtained from whole-organ cultures of utricles, we expanded our studies to cell lines, using RAW 264.7 macrophages as model for the pro-inflammatory TLR4 pathway activated by LPS, and auditory HEI-OC1 cells to evaluate and validate expression of TLR4 signaling components in inner ear-derived cells. Cell lines and utricle explants were tested for TLR4 pathway activation upon heat stress and this response was compared to the canonical pro-inflammatory response triggered by LPS treatment.

Results: RAW 264.7 macrophages and auditory HEI-OC1 cells express numerous pro-inflammatory TLR4 pathway components (e.g., MyD88, NF-kB) that are upregulated or activated upon LPS stimulation and heat shock. Immunohistochemistry analyses indicate that the pro-survival mechanism in HCs may be distinct from the cardioprotective TLR4 pathway, as activated HSP27 was upregulated in SCs in heat shocked utricle explants.

Conclusions: Information about the protective signaling pathways downstream of TLR4 activation in cell lines and utricles upon heat stress and LPS may enable the development of targeted therapies for HC preservation when patients are undergoing treatment with ototoxic drugs. This work is supported by the NIDCD Division of Intramural Research.

Development of a Method to Determine Small Molecule Drug Distribution in the Inner Ear Using Maldi MSI

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Background: With the advent of new and repurposed drugs targeting the inner ear for protection and repair from damage, the ability to determine their pharmacokinetic and pharmacodynamic (Pk/Pd) properties is of paramount importance. Studying the inner ear is inherently challenging due to its location and difficult access. The current gold standard for determining drug distribution in the inner ear is through sequential perilymph sampling at either the cochlear apex or semicircular canal, and subsequent analysis via liquid chromatography/ mass spectrometry (LC-MS). This indicates drug spatial distribution in the perilymphatic space but lacks granularity in determining target tissues; it also requires invasive surgery. Drug in tissue can be analyzed via tagged drug molecules, but this changes their structure and likely distorts their Pk/Pd properties. These challenges indicate the need to develop strategies for directly determining drug distribution within inner ear tissue in an unlabeled manner. Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging (MALDI MSI) is a tool which allows for untargeted analysis of mass spectra within a 2-dimensional space, which can be later screened to look for the distribution of specific masses. MALDI analysis of the inner ear presents its own unique challenges, due to the combination of its bony encasement necessitating some sort of decalcification process and the particulars of MALDI tissue preparation where the need to not fix tissue due to ionic suppression effects can cause the loss of analytes during the decalcification. Here we present our development of a protocol for tissue preparation and subsequent analysis of small molecule distribution in the inner ear.

Methods: 4–8-week-old CBA/CaJ mice were treated (locally or systemically) with drugs that are known or suspected to target the inner ear. Mice were euthanized according to each drug's known Tmax , and known non-cochlear target tissues were harvested, sectioned, mounted, sprayed with matrix (TM-sprayer, HTX Technologies LLC) for MALDI analysis (Ultraflextreme, Bruker). These served as positive controls for drug presence in tissue. Mice also had cochleae harvested, decalcified, prepared, mounted, sprayed, and analyzed for drug distribution. 300-350g Hartley guinea pigs were also treated with the drugs, at a time according to each drug's Tmax , their perilymph was collected serially. Perilymph samples were analyzed and quantified via LC-MS to determine the drug's distribution within the perilymphatic space and correlated with tissue distribution determined via MALDI.

Results: We were able to obtain images showing drug distribution within the inner ear from our known and candidate drugs.

Conclusions: Here we show the development and proof of concept of a method for determining tissue distribution of small molecule drugs in the inner ear in an unlabeled and unbiased manner via MALDI MSI. This technique opens up the ability to further optimize and refine drug development and delivery to the inner ear.

Dysfunction of One MYO7A Allele Results in Late-Onset Sensorineural Hearing Loss in a Mouse Model

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Background: The shaker 1 mouse is a well-characterized model of Usher1B which is considered a recessive disorder. After identifying the presence of heterozygous pathogenic MYO7A alleles in cochlear implant patients we wanted to evaluate the effect of one pathogenic MYO7A allele on hearing in a mouse model
Methods: Shaker 1 +/- mice were evaluated over time with ABR and DPOAEs. Immunohistochemical evaluation of oxidative stress markers was carried out from P3 to 6 months of age. A subgroup of Shaker +/- mice was treated at P4 with a lentivirus carrying the native MYO7A gene and compared to untreated controls.
Results: Shaker 1 heterozygotes develop hearing loss between three and six months of age and do not demonstrate any of the balance abnormalities that their homozygous mutant littermates show. Evaluation with distortion product otoacoustic emissions demonstrates maintenance of normal outer hair cell function despite a progressive hearing loss documented by ABR. Immunohistochemical evaluation of heterozygotes with markers of oxidative stress show increased labeling in the organ of Corti compared to wild-type littermates. Delivery of native MYO7A using a third-generation lentiviral vector completely prevented the progression of hearing loss.
Conclusions: This demonstrates that in the presence of one functional allele of MYO7A stereociliary function is probably adequate for the function of vestibular and outer hair cells. Loss of one functional allele of MYO7A in inner hair leads to increased oxidative stress and eventual dysfunction of the inner hair cell. Correlation to human data is needed to evaluate if MYO7A mutations where only one pathogenic copy of the gene is present could contribute to adult-onset progressive hearing loss when present along with the normal copy of the gene.

Development of Osmotic Pump Based Chronic Drug Delivery to Normal Hearing Guinea Pigs

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Background: Guinea pigs are a commonly used animal model in hearing research. Amongst others, they are used for pharmacology studies to develop inner ear therapies. To understand a drug's impact on cochlear cell function drug delivery needs to be reliable in reaching steady state concentrations. Local fluid based delivery into the perilymph allows the application of an exactly known drug concentration without the release kinetics being affected by a delivery matrix. Osmotic pumps are well-established for continuous drug delivery in hearing research. They are combined to a cochlear implant or a tube, both inserted into the cochlea via the round window or a cochleostomy and causing massive hearing threshold increase. Surgery related threshold shifts hinder examination of parameters such as noise induced threshold shift for the development of preventive substances after implantation. We developed a method that enables chronic pump-based local drug delivery to the cochlea without causing relevant threshold shifts.
Methods: A small microneedle was bend, shortened and attached to a silicon catheter (Alzet rat jugular catheter) using tissue glue. The modified catheter was attached to an osmotic pump (Alzet 2006), both were filled with artificial perilymph and the pump was transferred into a NaCl filled well of a 6 well plate to induce pumping. The microneedle hook was placed in an Eppendorf tube. Before implantation, fluid filling of the tube was check to verify the pump is working properly. The hook was inserted in the scala tympani via the round window and the osmotic pump was placed between the guinea pig's scapulae. Before and one week after surgery hearing thresholds were determined and CT scans were performed. Finally, the pumping was re-checked after explanting the device.
Results: The self-build device is implantable into the guinea pig inner ear via the round window and is stable within the observation period. The quality control of the fluid delivery in a well plate is an easy and cheap method to determine the functionality of each self-made pump-catheter-hook system before implantation in vivo. One week after surgery the hearing threshold shifts were reduced compared to previously reported shifts after insertion of electrode arrays. The microneedle can be visualized by CT, allowing determining the correct location of the hook as known from cochlear implants.
Conclusions: We developed a method to implant the cochleae of normal hearing guinea pigs with a catheter attached to an osmotic mini pump. The induced threshold shift was lower than the threshold shift caused by the

insertion of an electrode array. Since residual hearing is important for studies concerning the ability of substances to prevent various insults e.g. noise, this catheter construction can be used in a wide field of hearing research.

Anatomic, Physiologic, and Proteomic Consequences of Repeated Microneedle-Mediated Perforations of the Round Window Membrane

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Background: We have developed 3D-printed microneedle technology for diagnostic aspiration of perilymph and intracochlear delivery of therapeutic agents. Single microneedle-mediated round window membrane (RWM) perforation does not cause hearing loss, heals within 48-72 hours, and yields sufficient perilymph for proteomic analysis. In this study, we investigate the anatomic, physiologic, and proteomic consequences of microneedle-mediated perforations of the same RWM at multiple timepoints. By developing this technique, we establish a means of monitoring the response to inner ear interventions over an extended period of time.
Methods: 100-μm-diameter hollow microneedles were synthesized using two-photon polymerization (2PP) lithography. The tympanic bullae of Hartley guinea pigs (n=7) were opened with adequate exposure of the RWM. Distortion product otoacoustic emissions (DPOAE) and compound action potential (CAP) were recorded to assess baseline hearing. The hollow microneedle was introduced into the bulla and the RWM was perforated; 1 μL of perilymph was aspirated from the cochlea over the course of 45 seconds. Following perforation, DPOAE was obtained and the bulla was closed. 72 hours later, the above procedure was repeated with aspiration of an additional 1 μL of perilymph. 72 hours after the second perforation, final CAP and DPOAE were obtained and the animal was euthanized. RWMs were harvested immediately following euthanasia for confocal imaging. Perilymph proteomic analysis was completed using mass spectrometry-liquid chromatography. Two-tailed paired t-tests and repeated measures ANOVA tests were used to evaluate for significance; the Hochberg procedure was used to adjust for multiple comparisons.
Results: CAP, DPOAE, and confocal microscopy were completed for 6/7 animals; proteomic analysis was completed for all 7 animals. Hearing tests demonstrated mild hearing loss at 2-4 kHz most consistent with conductive hearing loss. Hearing at 0.5 kHz – 40 kHz was otherwise intact across all three timepoints. Confocal microscopy demonstrated complete healing of all perforations with full reconstitution of the RWM. Perilymph proteomic analysis identified 1855 proteins across 14 samples (2 aspirations each for 7 animals). The inner ear protein cochlin was observed in all samples, indicating successful aspiration of perilymph. Non-adjusted paired t-tests with $p < 0.01$ revealed significant changes in 13 of 1855 identified proteins (0.7%) between the first and second aspirations. The majority of these proteins were ubiquitous proteins with largely unrelated functions, and none had a role in acute inflammation. Importantly, after adjustment for multiple comparisons using the Hochberg procedure, no proteins had significant changes between the two aspirations.
Conclusions: We demonstrate that repeated microneedle perforation of the RWM is feasible, does not directly cause hearing loss, allows for complete healing of the RWM, and does not change the proteomic expression profile. Thus, microneedle-mediated repeated aspirations in a single animal can be used to monitor the response to inner ear treatments over time.

Application of Genome Editing to a Mouse Model of DFNA20

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Background: Sensorineural hearing loss is a common disorder which affects the world's population. Recent studies of inner ear gene therapy have demonstrated promising results in improving auditory and vestibular functions in mouse models of sensorineural hearing loss. While many studies of gene therapy focus on mouse models of 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 due to the later onset and slower progression of hearing loss that offers a wider window of opportunity for therapeutic interventions. In this pilot study, we applied genome editing to a mouse model of DFNA20, which carries a pathogenic amino acid substitution of the wild type proline at residue 264 with a leucine (Actg1P264L).
Methods: Neonatal (P0-P5) Actg1P264L/P264 mutant mice were used in this study. AAV2.7m8-Cas9 and AAV2.7m8-gRNA-GFP were injected simultaneously into the inner ears of Actg1P264L/P264 mutant mice through the posterior semicircular canal. Auditory brainstem response (ABR) recordings were used to assess auditory function at P30. Scanning electron microscopy (SEM) was performed to examine stereocilia morphology. Immunohistochemistry was used to evaluate cell morphology and viral transduction efficiency.
Results: Actg1P264L/P264 mutant mice develop early onset hearing loss due to outer hair cell stereocilia bundle staircase formation defects and are profoundly deaf by 6-7 weeks. SEM imaging revealed improved outer hair cell stereocilia morphology in Actg1P264L/P264 mutant mice treated with AAV2.7m8-Cas9 and AAV2.7m8-gRNA-GFP (with the gRNA designed to target the mutant P264L allele), compared to non-treated mutants. The treated Actg1P264L/P264 mutant mice also showed improved hearing at P30 compared to non-treated mutant mice.
Conclusions: Our results showed that CRISPR genome editing was able to improve the stereocilia morphology and auditory function in the Actg1P264L/P264 mutant mice.

In Vivo Real-Time Imaging Reveals That Megalin Transports Aminoglycosides Into the Mouse Cochlea and Its Inhibition is Otoprotective

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Background: Aminoglycosides (AGs) are highly effective broad-spectrum antibiotics used worldwide to combat gram negative bacteria. Irreversible cochlear hair cell (HC) loss is a critical side-effect of AGs treatment, yet how AGs enter the cochlea and then target HCs remains unresolved.
Methods: We developed an in vivo imaging method to track AGs transport into adult mouse cochlea in real-time. An imaging window (IW) was created on otic capsule bone via novel chemo-mechanical cochleostomy, that enables us to observe multiple cochlear cells from a live mouse. Auditory brainstem responses (ABR) showed no effects from the surgical approach. Texas Red-labeled gentamicin (GTTR) was systemically administered to the mice to track its pathway of entry into the cochlea in vivo using two-photon imaging. A separate multidose model of ototoxicity was used and evaluated with ABR responses and immunohistochemistry.
Results: GTTR enters the cochlea via the stria vascularis, then selectively enters HCs in the organ of Corti. The GTTR uptake into HCs was completely abolished in transmembrane channel-like protein1 (TMC1) knockout mice where mechanotransducer (MET) channels are not functional, indicating the MET channel is the major pathway of AG transport into HCs. As an initial entry site of AGs into the cochlea, we targeted megalin which is an endocytic transporter found in strial marginal cells and Reissner's membrane, because it tightly binds to AGs, and is the major transporter of AGs in the kidney. Co-administration with cilastatin, a binding competitor of megalin, prevent the GTTR accumulation in HCs, suggesting megalin is a critical route of AG transport into endolymph. Lastly, cilastatin treatment markedly reduced AG-induced HC degeneration and hearing loss in vivo.
Conclusions: Together, our in vivo real-time tracking of megalin-dependent AG transport across the blood-labyrinth barrier identifies new therapeutic targets for preventing AG-induced ototoxicity.

AAV2/7 is a Promising Vector to Transduce Spiral Ligament Fibrocytes in a Save and Efficient Way

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Background: Inner ear gene therapy is a promising approach to restore sensorineural hearing loss, for which several gene therapy applications have been studied and reported in preclinical animal studies. Although gene

delivery by using adeno-associated viral vectors (AAV) is considered as the best option, most animal studies to date have only injected viral vectors into neonatal ears to effectively transduce inner and outer hair cells. Our objective is to select an AAV that can transduce the spiral ligament fibrocytes.

Methods: Six-months old C57BL/6NTac-Cdh23<ahl+em3H>/H mice received an injection with a CMV-eGFP-T2A-FfLuc AAV2/7 vector (4.50E+12 vg/ml) through the posterior semi-circular canal (PSC) approach in the left ear. Hearing assessment involved distortion product otoacoustic emission (DPOAE) and auditory brainstem response (ABR) measurements at baseline and one week after injection. In addition, in vivo bioluminescence imaging (BLI) was performed to follow up transduction efficiency. One and four weeks after injection, mice were euthanized and immunohistochemical staining was performed to visualize macrophages and eGFP-protein in the spiral ligament.

Results: DPOAE and ABR-measurements revealed that injection of AAV2/7 in the inner ear of adult mice has no negative influence on cochlear functioning as hearing function was completely preserved in all injected animals after one week. Furthermore, in all mice, BLI signal was observed in the region of the left ear indicating efficient transduction of inner ear cells. Immunohistochemistry showed no increase in macrophages or activated macrophages when compared to control mice demonstrating that PSC injection does not result in inner ear inflammation. The next step is to assess eGFP expression using an anti-eGFP antibody.

Conclusions: As these preliminary results are highly promising, we will inject more mice with AAV2/7 and assess hearing function, inner ear inflammation and transduction efficiency at different time points, up to three months.

Origins of Peptidergic Inputs to the Inferior Colliculus
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Background: Corticotropin releasing factor (CRF) and its receptors, well known for signaling in the hypothalamic-pituitary-adrenal (HPA) axis, are also expressed in other brain regions and peripherally. We have previously reported that CRF is expressed throughout the central auditory pathway, revealing various neuronal populations and terminal fields. CRFcre-driven tdTomato positive cell bodies are not coincident with calbindin-, calretinin- or parvalbumin-expressing neurons along the central auditory pathway, providing support that CRF-expressing neurons are a population distinct from these calcium-binding neurons. CRF-expressing neurons form a dense terminal field in the inferior colliculus; we have been interested in characterizing this terminal field. Our previous work showed that the CRF tdTomato-positive terminal field is coincident with the highest density of cytochrome oxidase within the inferior colliculus. Despite knowledge that this terminal field lies within a highly metabolically active region, the location of origin of the cells producing this terminal field is not known. We hypothesize that the CRF-positive terminal field in the inferior colliculus might be comprised of descending inputs from auditory cortex or ascending inputs from the auditory brainstem. To address this, we carried out a tract tracing study to assess the location of CRF tdTomato positive neurons retrogradely labeled from the inferior colliculus.

Methods: 22-27gm tdTomato CRFcre male mice were anesthetized with ketamine (70-100mg/kg) and xylazine (10-20 mg/kg). A Midgard CS3 High Voltage Precision Current Source (Transkinetics, Canton, MA) was used to iontophorese biotinylated dextran amine (3,000 MW) at 7µA, 7 seconds on / 7 seconds off for 10 minutes. Using a dorsal approach, two to three injections were made unilaterally into the inferior colliculus. Following a 10-day survival, animals were transcardially perfused with buffered 4% paraformaldehyde. Brains were harvested, cryoprotected, frozen sectioned and processed for immunohistochemistry. Sections were stained with rabbit anti-red fluorescent protein and fluorphore labeled antibodies, goat and rabbit Alexa 594 (to localize CRF tdTomato) and streptavidin Alexa 488 (to localize BDA). Sections were counterstained with DAPI and imaged with a Zeiss LSM880 Confocal Microscope.

Results: Following BDA injection, cells double labeled for tdTomato and BDA were present in the contralateral ventral cochlear nucleus (VCN) and the ipsilateral lateral superior olive. In both of these target areas, in addition to double labeled cells, cells that are either singly labeled with the red or green fluorphores are present. BDA iontophoresis backfilled neurons in auditory cortex, none of which co-expressed CRF tdTomato.

Conclusions: These results show that CRF-positive ascending peptidergic inputs from the ventral cochlear nucleus and the lateral superior olive terminate in a highly metabolically active zone of the inferior colliculus and may modulate glutamatergic and GABAergic signaling (e.g. Bagosi et al., 2012, 2017) at this level of the brainstem for central auditory processing.

Embryonic Medial Ganglionic Eminence Cells Survive and Integrate Into the Inferior Colliculus of Adult Mice

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Background: Acoustic overexposure can lead to decreased inhibition in auditory centers, including the inferior colliculus (IC), and has been implicated in the development of central auditory pathologies. While systemic drugs that increase GABAergic transmission have been shown to provide symptomatic relief, their side effect profiles impose an upper-limit on the dose and duration of use. A treatment that locally increases inhibition in auditory nuclei would mitigate these side effects. One such approach could be transplantation of cells derived from the medial ganglionic eminence (MGE). While naturally destined for the cerebral cortex, these inhibitory precursor cells have been shown to mature and integrate into the a variety of subcortical structures and even the adult spinal cord. The present study tests the hypothesis that transplanted MGE cells will functionally integrate into the IC of adult mice.

Methods: MGE cells were harvested on embryonic days 12-14 from embryonic, homozygous Tg(act-EGFP)Y01Osb (green) mice, which have widespread expression of green fluorescent protein in all cells, or green mice crossed with Vgat-ires-cre-dT (VGAT-dT) mice, in which GABAergic neurons express the fluorescent protein, dtTomato. MGE cells were injected bilaterally into the IC of adult mice. Mice of either sex were randomly selected for bilateral noise exposure. Analysis of migration, differentiation, and c-fos expression was performed using epifluorescence microscopy. The existence of synaptic connections was investigated using electron microscopy.

Results: Transplanted MGE cells migrated from the injection site and survived in the IC of control and noise exposed mice. At one week post transplantation, MGE cells possessed small, elongated soma and bipolar processes, characteristic of migrating cells. By 5 weeks, MGE cells exhibited a more mature morphology, with multiple branching processes and axons with boutons that stain positive for VGAT. The MGE survival rate after 14 weeks post transplantation was not significantly different between control (1.64%) and noise-exposed subjects (1.23%). In both groups the majority of transplanted MGE cells expressed VGAT (control=98.0% and noise-exposed=96.7%). Furthermore, transplanted MGE cells formed synapses with and received synaptic endings from host IC neurons. Acoustic stimulation significantly increased c-fos expression in endogenous inhibitory cells in the IC of non-injected control mice. C-fos expression in transplanted MGE cells was not significantly increased by acoustic stimulation in control or noise-exposed mice. Transplanted cells were observed in the IC up to 22 weeks post transplantation, suggesting that long term survival and integration occurs.

Conclusions: Our data demonstrate that transplanted MGE cells survive and integrate into the adult mouse IC in both control and noise-exposed animals. Ongoing analysis on the effects of noise exposure and MGE injection on the acoustic startle response will help determine whether MGE transplantation is a useful tool to mitigate the pathological loss of neuronal inhibition in central auditory areas following noise exposure.

Postsynaptic Responses of IC Neurons in Unanesthetized Mice Reveals the Importance of Temporal Integration of Excitatory and Inhibitory Inputs for Sound Processing

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Background: Inferior colliculus (IC) is a major integrative center of the central auditory system. It receives and integrates ascending as well as descending information from many auditory as well as from non-auditory brain structures. Deep knowledge about temporal integration of excitatory and inhibitory inputs is critical for our understanding of information processing in IC neurons. Previous in-vivo IC studies mainly utilized extracellular recording techniques often in anesthetized animals. The goal of the present study was to examine postsynaptic responses in IC neurons to pure tones in unanesthetized animals to determine how temporal integration of excitatory and inhibitory inputs contribute to sound-evoked firing.

Methods: Intracellular recordings were conducted with quartz micropipettes filled with 1 M potassium acetate having impedance around 250 MΩ in unanesthetized mice. Electrodes were inserted into the IC via a small opening (≈100 μm) in the skull. Spontaneous and sound evoked activity to pure tones presented at different sound frequencies and at three sound levels (30, 40, and 55 dB SPL) were recorded. The resting membrane potential

(RMP), spontaneous firing rate (SFR), characteristic frequency (CF), and timing of both the postsynaptic potentials and sound evoked spikes contributed to our data analysis.

Results: RMPs and SFRs of IC neurons ranged from -35.07 mV to -79.58 mV (-48.53 mV, mean) and from 0 Hz to 77.4 Hz (11.03 Hz, mean), respectively. We found no correlation between RMPs and SFRs. Three different response types to pure tones were observed at neurons' characteristic frequency: onset (n=17, 50%), sustained (n=14, 41.18%), offset (n=3, 8.82%). These response types had characteristic underlying synaptic mechanisms. Onset response type usually exhibited EPSP/spike. Sustained responses typically showed EPSPs lasting during the sound duration. The offset type showed a long lasting IPSPs followed by a postinhibitory rebound. The onset and sustained response types often showed a complex temporal integration between excitatory and inhibitory inputs on IC neurons.

Conclusions: Our research suggests that a complex temporal integration of excitatory and inhibitory inputs underlines different response types of IC neurons in their responses to pure tones.

Excitatory Commissural Synapses Activate Mg2+-Insensitive NMDA Receptors on VIP Neurons in the Inferior Colliculus

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Background: The inferior colliculus (IC) is a hub of integration for ascending auditory information and plays an important role in sound processing. VIP neurons are glutamatergic stellate neurons found throughout the IC, and they receive both excitatory and inhibitory inputs from the contralateral IC through the commissure. Prior research has shown that excitatory commissural inputs to VIP neurons can elicit EPSPs that include an NMDA receptor (NMDAR) component at resting membrane potential, even though most NMDARs require depolarization to relieve Mg2+ block before they can be activated. We therefore hypothesized that VIP neurons express NMDARs containing NR2C/D or NR3A subunits, which are less susceptible to Mg2+ block than the more common NR2A/B-containing receptors.

Methods: We performed whole-cell patch-clamp electrophysiology recordings combined with electric and optogenetic stimulation to activate excitatory commissural inputs onto VIP neurons, which were targeted using VIP-IRES-Cre x Ai14 mice. The NMDAR-mediated component of EPSPs was isolated using the AMPA receptor antagonist NBQX. We then applied PPDA, an NR2C/D subunit selective antagonist, to determine whether the NMDAR component was mediated by receptors containing these subunits. Additionally, we performed single molecule fluorescent in situ hybridization (RNAscope) to determine whether NR2C, NR2D, and NR3A subunit mRNA is present in VIP neurons.

Results: Using patch-clamp recordings combined with pharmacology, we found that commissurally evoked EPSPs in VIP neurons were often only partially blocked by the AMPA receptor antagonist NBQX, even though VIP neurons were held near their resting membrane potential. The remaining EPSP component was partly sensitive to PPDA, suggesting that VIP neurons express NMDARs containing NR2C/D subunits. Additionally, our RNAscope results show that 99% VIP neurons express NR2D mRNA and 87% express NR2C mRNA, while only 24% of VIP neurons express NR3A mRNA.

Conclusions: Our results demonstrate that VIP neurons express NMDARs that contain NR2C and/or NR2D subunits, which allows these receptors to activate at resting membrane potential. This mechanism could expand the time window for synaptic integration in VIP neurons given the longer temporal dynamics of NMDA receptors, suggesting that VIP neurons may play a computational role that involves integrating auditory information over periods of tens of milliseconds.

Cholinergic Inputs to Ascending, Descending and Commissural Pathways That Terminate in the Inferior Colliculus

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Background: Cholinergic axons terminate throughout the auditory system, modulating neuronal responses from the cochlea to the auditory cortex. Cholinergic receptors are present at very high levels in virtually all the nuclei of the central auditory pathways, indicating that ACh acts at each level of auditory processing. Moreover, ACh

modulates both ascending and descending auditory pathways, suggesting roles in both bottom-up and top-down modulation of sound processing. Despite the widespread distribution of ACh, little is known about which auditory pathways are directly targeted by cholinergic axons. Here, we focus on auditory pathways that converge in the inferior colliculus (IC), a midbrain center that integrates ascending and descending auditory pathways. Specifically, we ask whether cholinergic axons are likely to make direct contact with cells that project to the IC.

Methods: We injected retrograde tract tracers into one IC in adult mice, then identified cholinergic axons with an immunostain against vesicular acetylcholine transporter (VAChT), a marker of cholinergic axons. We then examined retrogradely labeled cells for close apposition with VAChT+ (i.e., cholinergic) boutons.

Results: We observed retrogradely labeled cells in many auditory nuclei, including the cochlear nucleus, nuclei of the superior olivary complex and lateral lemniscus, contralateral IC, nucleus of the brachium of the IC and the auditory thalamus. VAChT+ axons were present in all these regions and often appeared in close apposition to the soma or dendrites of retrogradely labeled cells. For the ascending pathways to the IC, we observed appositions on labeled cells in the dorsal and ventral cochlear nuclei and multiple nuclei of the superior olivary complex. Close appositions were also observed on the cells in the contralateral IC, indicating input to the commissural pathway. Finally, appositions were apparent on cells in the nucleus of the brachium of the IC and on cells in the auditory thalamus, indicating input to descending pathways.

Conclusions: We conclude that cholinergic axons are in a position to directly modulate subcortical auditory neurons that provide ascending, commissural or descending inputs to the IC. Studies in the auditory thalamus indicate that ACh differentially modulates ascending and descending pathways, likely to enhance the extraction of important auditory information from surrounding noise (Richardson, Sottile, Caspary 2020 doi.org/10.1016/j.heares.2020.108003). Our results suggest that ACh can also modulate ascending and descending pathways to the IC. Given the integrative role of the IC and its contributions to auditory perception and behavior, it is likely that cholinergic modulation of inputs to the IC plays a substantial role in many aspects of hearing. Supported by NIH DC004391, the Department of Anatomy and Neurobiology, Northeast Ohio Medical University and the College of Medicine, Northeast Ohio Medical University

Characterizing the Osteologic Effects of Cholesteatoma and Oncolytic Virotherapy

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Background: Cholesteatomas (CHST) are temporal bone lesions that arise from keratinizing squamous epithelial cells. Although benign, CHST erode the structures of the temporal bone which can lead to hearing loss, vestibular weakness, facial paralysis, and meningitis. The current standard of care for CHST is resection surgery; however, the recurrence rate is reported to be as high as 60%. Oncolytic herpes simplex virus (oHSV), a virotherapeutic agent, has shown to target CHST cells while sparing healthy cells (Samy et al., 2019). It may also promote recovery of bone through immunomodulation. The purpose of this study was to quantify the degree of bone erosion in response to CHST, and to measure the recovery of normal bone anatomy and structure post oHSV treatment in a gerbil model.

Methods: CHSTs were induced bilaterally in 10 gerbils (n = 20 ears) via Pseudomonas aeruginosa (PA) inoculation plus double ligation of the cartilaginous external auditory canal. Animals were treated with 1-3 intra-bullar injections of oHSV 2 weeks post CHST induction. High-resolution micro-computed tomography (micro-CT) scans were obtained pretreatment and 2-4 weeks after each oHSV injection. Micro-CT scans of two un-operated gerbils (n = 4 ears) were used as anatomical controls. Regions of interest (ROIs) included a cross-section of the auditory bulla and the ossicular chain. ROIs were manually traced and filtered (based on Hounsfield units, HU). The volume of the ossicles (mm3), perimeter of the auditory bulla (mm; a marker of bullar bone integrity), and bone mineral density (BMD) of the ossicles and bullae (mean HU-per-voxel) were measured.

Results: Post CHST induction, the mean bullar perimeter of experimental animals was significantly lower than the control animals (p = 0.044). There were no significant differences in volume and estimated BMD in the ossicular chain. Following treatment with two injections of oHSV, a significant increase in estimated BMD of the bulla was observed (p < 0.001) and the perimeter significantly increased (p = 0.017) by the third injection. The ossicular chain significantly decreased in volume after each oHSV injection (p ≤ 0.021). The ossicular chain ROIs were refiltered to account for variations in BMD across different areas, and when refiltered at a range of 5000-8000 HU, the volume significantly increased from the second to the third oHSV treatment (p < 0.003).

Conclusions: Findings suggest that 2 weeks post CHST induction, the gerbil auditory bulla is more prone to resorption than the ossicles. The bulla is also more responsive to oHSV treatment with significant increases in perimeter (i.e., reversal of CHST-induced bone erosion) and estimated BMD. However, BMD heterogeneity of the ossicular chain appears to influence the response to oHSV. These results support further investigation to measure these effects over a longer duration to determine if the osteologic responses to oHSV are time-dependent.

Methods for the Calibration of High Frequency Bone Conduction Transducers
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Background: Bone conduction (BC) testing aids in the discrimination of inner-ear hearing deficits from middle-ear and external-ear based deficits. While standard audiometric practices utilize BC for low and mid-frequency threshold testing, limitations in electromagnetic transducer above 4kHz limit BC testing at higher frequencies. Novel magnetostrictive BC headphones have superior high-frequency (HF) output and offer the opportunity for HF BC measurement, but the lack of established BC calibration methods above 10kHz limits interpretation of such measurements. Specifically, neither the impedance nor the sensitivity of standard calibration devices, such as B and K 4930 artificial mastoid (AM), has been defined above 10kHz. We report a new method to determine the impedance and force sensitivity of the B and K 4930 over the 6-20kHz frequency range.
Methods: Using a B and K 4810 minishaker as a drive, force and acceleration were measured from an unloaded B and K 8001 impedance head over the 0.5 to 20kHz range. Acceleration and force from the loaded 8001 were then obtained with the setup coupled to the AM diaphragm under varied contact areas and static forces. AM output voltages were also assessed. We assumed the impedance of the loaded 8001, defined by the ratio of the measured force and acceleration when the 8001 was in contact with the AM (Z_8001_loaded), equaled the sum of the AM impedance (Z_AM) and the self-impedance of the unloaded 8001: $Z\_8001\_loaded = Z\_8001\_unloaded + Z\_AM$. Force sensitivity of the AM was computed as the ratio of the measured AM voltage and 8001 force acting on the AM.
Results: Over the 0.5 to 20kHz range, the ratio of the force and acceleration from the unloaded 8001 was generally consistent with the mass of the 8001 above its force transducer. Measurement noise was less than 1% of the measurement mean. When coupled to the AM, both accelerations and forces from the 8001 differed substantially from the unloaded condition at frequencies below 2kHz but were similar to the unloaded condition at higher frequencies. A contact area of 250mm2 and static force of 4.9N resulted in Z_AM values similar to the factory calibration below 10kHz and a relatively constant Z_AM above 10kHz. The measured force sensitivity of the AM showed large variations in magnitude between 12 and 18kHz.
Conclusions: Our techniques describe the mechanical input impedance of the AM diaphragm at frequencies between 0.5 and 20kHz. We also confirmed the presence of sharp irregularities in the sensitivity of the AM force sensor above 12kHz. It may be that more regular calibration could be based on the acceleration provided by HF vibrators when coupled to a well-defined HF load, such as Z_AM. Defined characteristics of the AM may be then used to calibrate any vibratory transducer in the HF range.

Development of an in Vivo Model for Eustachian Tube Dysfunction
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Background: Eustachian tube dysfunction (ETD) can be one of the reasons for chronic otitis media. Several treatment options are available, but none of them guaranties success. To develop new treatments such as stents for the Eustachian tube (ET), it might be beneficial to have a large animal model of ETD available in order to test functionality of the stents.
Methods: In cadaver experiments with blackface sheep, a method for aseptic deposition of hyaluronic acid in the vicinity of the ET was developed. The pharyngeal orifice of the ET was approached under endoscopic control with a bendable cannula. The tip of the cannula was protected during insertion and can be pushed into the tissue at the desired location. The generated HA reservoir (0.3 to 1 ml) was visualized by addition of Imeron in DVT scans before stents were implanted in the ET. This approach was transferred to in vivo experiments . Injected amounts of HA were between 0.3 ml and about 4 ml and success regarding ETD was evaluated by tympanometry.

Results: The HA reservoir could be reliably established next to the cartilaginous part of the ET and implanted stents kept the ET open in the ex vivo experiments. In vivo, a minimum amount of 2.5 ml cross-linked HA had to be injected to induce an ETD that lasts at least one week. In some cases, fluid was detected in the middle ear. With amounts between 1 ml and 2.5 ml, only ETDs lasting few days were provoked, whereas less HA did not result in any changes in tympanometry. The sheep tolerated the induction of ET and did not show any sign of discomfort. **Conclusions:** An aseptic dysfunction of the ET could be established and the model is now available for further experiments.

Synthesis of LTB4, a Critical Leukocyte Recruitment Factor, is Distributed Across Different Middle Ear Cell Types during Otitis Media

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Background: Background: Leukocyte recruitment is a critical component of both pathogenesis and infection resolution during otitis media (OM). The eicosanoid leukotriene B4 (LTB4), a potent chemoattractant for neutrophils and monocytes, is obesrved in middle ear (ME) effusions of OM patients. Synthesis of LTB4 is initiated when cytosolic phospholipase A2 (PLA2G4A) generates arachidonic acid (AA) from membrane lipids. AA is processed through the lipoxygenase pathway by arachidonate 5-lipoxygenase (ALOX5), with the participation of its activating protein (ALOX5AP) into AA 5-hydroperoxide (5-HpHETE), which can metabolize to leukotriene A4 (LTA4). LTA4 is in turn converted into LTB4 by leukotriene A4 Hydrolase (LTA4H). LTB4 exerts its biological activities through the specific receptor LTB4R1, or via LTB4R2 which also responds to other eicosanoids.
Methods: Methods: We evaluated the expression of genes encoding the enzymes and receptors of the LTB4 pathway in 24 ME cell types, one day after bacterial inoculation of the ME, using single-cell RNA-Seq. We also assessed the functional role of LTB4 in bacterial OM using a specific LTB4 receptor inhibitor.
Results: Results: Twenty-four distinct cell types were present in the infected ME. Pla2g4a mRNA was present in virtually all monocytes, and in subsets of other ME cell types. Alox5 mRNA was observed primarily in a subset of infiltrating neutrophils (PMNs), and a few monocytes. Alox5ap message was expressed by almost all monocytes, as well as the majority of PMNs and small subsets of other cell types. Lta4h mRNA was present in most epithelial cells and monocytes, but also small subsets of other cell types. Ltb4r1 mRNA was produced exclusively by all monocytes and a subset of PMNs. Ltb4r2 mRNA was not observed. Thus expression of elements of the LTB4 synthetic pathway in the ME was extensive, but all were present in only a very small number of cells. In particular, ALOX5 expression did not match that of PLA2G4A, which generates its substrate, or of ALOX5AP, its activating protein. The injection of bacteria into the MEs of rats, with the LTB4R1 inhibitor U75302, essentially eliminated the entry of leukocytes into the ME.
Conclusions: Conclusions: The distribution of LTB4 synthesis across different cell types is not unique to the ME. Eicosanoids can be the result of intercellular interactions involving cell-to-cell signaling and the transfer of intermediates, known as the transcellular pathway of eicosanoid biosynthesis (Sala et al., 2010). This process clearly operates in the ME to generate LTB4. In contrast, the virtual elimination of leukocyte infiltration by LTB4 inhibition is surprising, since many other leukocyte chemotactic factors are generated in during OM. A requirement for LTB4 activation of leukocytes for extravasation into the ME may partially explain this result. (Supported by NIH/NIDCD grants DC000129 and DC012595 and BX001205 from the VA.)

Radix Puerariae Lobatae Attenuates Age-Related and Noise-Induced Hearing Lose in Rats

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Background: Radix Puerariae lobatae (RPL) is a kind of traditional Chinese herb, which has been used in the treatment of cardiovascular diseases, cerebrovascular disorders, cancer, Parkinson's disease (PD), Alzheimer's disease (AD), diabetes or diabetic complications and free radical-mediated injury in various organs for its antioxidant properties [1,2]. Even though, RPL was applied for treatment of hearing loss or deafness as its quite well "Tui Re Sheng Jin" effect in Chinese Traditional Medical Theory[3]. The purpose of the present study was to investigate if RPL has the potential to attenuates age-related and noise-induced hearing lose in rats.

Methods: Rats in the experimental group were administered the water extract of RPL intragastrically. Auditory thresholds were assessed by sound-evoked auditory brainstem response (ABR) at click and tone bursts of the noise-induced hearing lose in rats with 8, 16 and 32 kHz, 72h before and after exposure to impulse noise, and the age-related group induced by D-galactose for 8 weeks. The malondialdehyde (MDA), glutathione (GSH), reactive oxygen species (ROS) and glutamic acid (GA) of blood serum, and superoxide dismutase (SOD) of cochlear tissue were also tested.

Results: The results showed that the sound-evoked ABR at click and tone bursts of RPL treated groups with age-related and noise-induced hearing lose at 8, 16, 32 kHz, were significantly reduced, the level of serum MDA, ROS and GA, and SOD in cochlear tissue of RPL treated groups all were decreased significantly.

Conclusions: It is concluded that showed that RPL could attenuates the hearing lose in age-related and noise-induced guinea pig, through the reduction of the oxidative stress in blood serum and cochlear tissue.

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Children Living With HIV Have Reduced DPOAEs Despite Normal Hearing Thresholds

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Background: Our previous work has shown subclinical auditory deficits in young adults living with HIV (20-30 years) with clinically normal hearing, but little is known about peripheral auditory function in pediatric populations with HIV. Children living with HIV might show early evidence of hearing loss if HIV infection or treatment does affect peripheral function. The goal of this study was to compare peripheral auditory function in two age- and gender-matched groups of pediatric subjects with clinically normal hearing with and without HIV.

Methods: A cross-sectional matched cohort study was conducted at the Infectious Disease Center in Dar es Salaam, Tanzania. Participants included HIV-positive (N = 71) and HIV-negative (N = 71) children ages 3-8 years who had clinically normal hearing, defined as Type A tympanograms, air-conduction thresholds <25 dB HL bilaterally from 0.5-8 kHz, and DPOAEs > 6 dB above the noise floor bilaterally from 1.5-8 kHz. Primary outcome measures included tympanogram static admittance, air-conduction audiograms, DPOAEs, and click-evoked auditory brainstem responses.

Results: HIV-positive and HIV-negative groups did not significantly differ in age, static immittance, or air-conduction thresholds. HIV-positive status was independently associated with approximately 4.1 dB lower DPOAE amplitudes from 4-8 kHz and lower ABR wave I, III, and V amplitudes.

Conclusions: Children living with HIV have slightly, but reliably, smaller DPOAEs and ABR wave amplitudes than matched HIV-negative controls. The magnitude of these differences is small, but these results support measuring auditory function in HIV-positive individuals as they age. More generally, these results reaffirm that a normal audiogram does not guarantee normal hearing. Work is underway to characterize the functional consequences of this subtle hearing deficit, which may include a dynamic efferent brain-to-ear influence in the auditory system.

Dual Vector Gene Therapy Restores Hearing in a Mouse Model of DFNB16 Hearing Loss

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Background: Hearing loss (HL) affects an estimated 430 million people worldwide, with a significant fraction (up to 60%) due to genetic causes. The second most common form of genetic HL is due to mutations in the STRC gene, which encodes the protein stereocilin. STRC mutations cause mild to moderate autosomal recessive HL, known as DFNB16. Based on our genetic analysis of heterozygous carrier frequency in a cohort of ~1,200 normal hearing subjects, we estimate that ~2.3 million patients worldwide may carry biallelic pathogenic STRC mutations and suffer from DFNB16.

Stereocilin is associated with horizontal top connectors, which connect outer hair cell (OHC) stereocilia to one another, and connect the tallest stereocilia to the overlying tectorial membrane. Mice and humans with Strc/STRC mutations lack horizontal top connectors and proper OHC connections with the tectorial membrane. As a result, they lack distortion product otoacoustic emissions (DPOAEs) and have elevated auditory brainstem responses (ABRs). Currently, there are no biological treatments for DFNB16. To address this unmet need, we developed a dual AAV gene therapy strategy for DFNB16 and demonstrate the efficacy of the approach in a mouse model of human STRC HL.

Methods: To model DFNB16 HL, we generated a Strc gene knockout mice (StrcΔ/Δ) using CRISPR/Cas9. Synthetic dual AAV9-PHP.B vectors were used to deliver the transgene into inner ear of StrcΔ/Δ mice by utricle injection (Lee et al. 2020) performed at P1. Immunostaining was performed with an anti-stereocilin antibody and AlexaFluor555 Phalloidin. Measurement of hearing function was performed using ABR and DPOAE.

Results: One of the main challenges for DFNB16 viral-based gene replacement is that the size of STRC gene exceeds the cargo capacity of single adeno-associated viral (AAV) vectors. To drive expression of the full-length STRC protein in OHCs of DFNB16 mice, we overcame the size limitation of AAV vectors using a dual-vector protein-recombination strategy. Immunostaining with an anti-stereocilin antibody performed at 4 weeks for cochleas excised from StrcΔ/Δ mice injected with dual AAVs revealed robust recovery of STRC expression in OHCs, proper STRC localization at the tips of OHC bundles and recovery of OHC bundle morphology. We found that recombination of full-length STRC led to significant hearing recovery at 4 weeks of age, in some cases close to wild-type levels. DPOAEs were improved with average thresholds of 40 dB and ABR thresholds were as low as 30 dB in some cases.

Conclusions: The data demonstrate that our dual AAV protein-recombination strategy is an efficient gene therapy approach for DFNB16 HL in mice, which suggests this therapeutic strategy could potentially benefit ~2.3 million patients worldwide affected by STRC mutations.

Novel Adeno Associated Vector-Based Gene Therapy for the Autosomal Recessive Non-Syndromic Deafness (DFNB9)

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Background: The autosomal recessive deafness 9 (DFNB9) is affecting 2-8% of all cases of congenital genetic deafness. It is due to mutations in the gene encoding Otoferlin (OTOF). Otoferlin is highly expressed in the inner hair cells (IHC) wherein it is critical for the fusion of synaptic vesicles with the presynaptic plasma membrane. OTOF gene defect leads to the failure of synaptic transmission between IHC and the auditory nerve, resulting in profound deafness. There are no approved curative therapies for otoferlin deficiency and cochlear implantation is the only option proposed to young patients (<18 months old). Even though this solution improves the quality of life and language acquisition, hearing recovery is limited and thus a more targeted treatment for DFNB9 is necessary to address this unmet medical need. Due to its recessive genetic origin, DFNB9 is an ideal target for gene therapy.

Methods: Adeno-associated virus (AAV) is the vector of choice for in vivo gene therapy. A major limitation to the development of gene therapy for DFNB9 is the size of the OTOF coding sequence largely exceeding AAV packaging capacity. To overcome this limitation, we adopted a dual AAV vector strategy, with OTOF coding sequence being split into two AAV vectors at the optimal cleavage site. Qualitative and quantitative evaluation of Otoferlin expression and integrity upon reconstitution of the full-length sequence was performed using RT-PCR confirmed by Sanger sequencing and immunoconfocal analyses. Then, various constructs of each of the split expression cassettes were packaged in AAV vectors and injected into the cochlea of DFNB9 mice, which are congenitally deaf. At different timepoints post-injection, we assessed by immunoconfocal microscopy whether the

otoferlin protein was properly targeted to the IHC and investigated hearing restoration using auditory-evoked brainstem response (ABR).

Results: Using the chosen AAV construct, we demonstrate long-term and specific de novo otoferlin expression in IHC, with no sign of toxicity. Stable and long-term efficacy to reverse deafness is achieved (currently more than 180 days) with ABR thresholds ranging from 30 to 80 dB, overlapping with that of WT mice across the 5-32 kHz frequency range. Then, using GFP as a reporter, we demonstrated that the selected AAV vector components are also effective in non-human primates (NHP) to efficiently target IHC, and evaluated the tolerability of our surgical approach.

Conclusions: Our NHP data indicate an effective recovery after surgery and a transduction rate of the targeted IHC at levels compatible with therapeutic intervention of Otoferlin deficiency, which constitute a major step toward future clinical trials in DFNB9 patients using our therapeutic candidate.

A Novel Mouse Model of USH3 Displays Auditory and Balance Deficits

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Background: Usher syndrome type IIIA (USH3A) is an autosomal recessive disorder caused by mutations in clarin-1 (CLRN1) gene resulting in post-lingual, progressive auditory and vision loss with or without vestibular dysfunction. Expressed in sensory cells of the inner ear, CLRN1 is believed to be involved in the maintenance of F-actin cores of hair bundle stereocilia, as well as synaptic organization, potentially providing molecular scaffold, recruiting proteins in cell adhesion at the plasma membrane.

We present here the characterization of a novel mouse model, generated using CRISPR/Cas9 to introduce a previously reported pathogenic point mutation (Y176X) resulting in premature termination of the CLRN1 gene. Mice were generated by electroporation of gRNA and donor vector into FLP C57BL/6 ES cell lines that were then screened for intended mutation and off-target changes before injection. Mice breed without morbidity.

Methods: To determine the auditory phenotype of Y176X knock-in mutant mice, we assessed auditory function recording auditory brainstem responses (ABR) to broadband click and pure-tone signals and distortion-product otoacoustic emissions (DPOAEs). To investigate the vestibular phenotype of the Y176X mutant mice, we assessed linear Vestibular Evoked Potentials (VsEP) at 4, 8 and 12 weeks. Whole-mount immunohistochemistry was performed in tissue harvested from P3 through 12 weeks, stained with antibodies to C-terminal binding protein 2, glutamate receptor, myosin7a, and phalloidin to quantify inner- and outer- hair cells as well as hair bundle morphology and synapse counts in apical, middle, and basal turns of heterozygous and homozygous Y176X mice.

Results: ABRs were recorded in homozygous and heterozygous Y176X knock-in mice from P16 through 12 weeks of age. Homozygous mice show profound hearing loss and absent DPOAEs by P16. while heterozygous mice had normal auditory thresholds. Vestibular assessments via VsEP recordings demonstrates progressive vestibular impairment reflective of impaired vestibular afferent nerve response/central vestibular neuronal response. We are now assessing cochlea and vestibular tissues to determine hair cell survival, hair bundle morphology, and synapse morphology at different times during development and in adult homozygous and heterozygous mice. Preliminary findings show disorganized cochlear hair bundles and disorganized and absent outer hair cells in adult animals. We are also assessing if mechanotransduction is affected by this mutation by performing FM1-43 studies in organotypic cochlear cultures from P3-P5 heterozygous and homozygous mice.

Conclusions: Clarin-1 Y176X homozygous mice present with profound sensorineural hearing loss and progressive vestibular dysfunction, providing a valuable model for studying the function of CLRN1 in the auditory and vestibular systems. The progressive onset of vestibular phenotype especially may provide opportunity to identify a targetable window for treatment of USH3. CLRN1 Y176X is one of the most common pathogenic mutations among USH3 cases in humans and understanding its function is critical for successful development of therapeutic strategies for USH3A patients.

Automated Multi-Class Classification of Otitis Media Using Deep Learning

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Background: We have developed a middle ear diagnostic systems based on artificial intelligence. We will be able to confirm the condition of children in general home through the development of the system, which will be helpful to the cloud-based remote care in the future.

Methods: 6,641 oto-endoscopic images were collected from the patients who visited Asan Medical Center. After classifying the collected images according to the diagnosis name, the label of each image was reviewed by expert otolaryngologists and the images with complete agreement were included in this study. For the deep learning-based diagnosis, the primary class was annotated as one of chronic otitis media (COM, 1,546 images), otitis media with effusion (OME, 1,643 images) and 'none' without COM and OME (3,452 images), while the binary labels were given for the secondary classes of myringitis (1,127 images), attic cholesteatoma (885 images), otomycosis (179 images) and ventilating tube (1,669 images). Deep learning network of EfficientNet-B4 was customized for the classification of tympanic membrane images. RGB images reformatted into 256×256×3 with circular cropping were used as input to the deep network. Data augmentation was performed with random rotation ($-90°$ to $90°$), translation shift (0–20% of image size in horizontal and vertical axes), random downscale (0–50%), random contrast (40%–50%), zoom (0–20%) and horizontal flip. The pre-trained weight from ImageNet was applied for transfer learning. Categorical cross-entropy loss was applied. 5-fold cross-validation was conducted and the fold proportion of training, validation and test sets was fixed at 3:1:1.

Results: Prediction accuracy of the primary class was 93.53%. Dice similarity coefficients (DSCs) were 94.29%, 91.47% and 94.97% for COM, OME and 'none' in the primary class, respectively. Misidentification between COM and OME rarely occurred (11 images). The average AUROC for the primary class was 0.9856. Among the secondary classes, ventilating tube was most accurately diagnosed (DSC = 98.32%), followed by attic cholesteatoma (DSC = 85.11%) and myringitis (DSC = 84.99%). Otomycosis showed poor predictive performance due to the small number of positive cases (DSC = 64.35%).

Conclusions: Deep learning classification of oto-endoscopic images may facilitate the clinical decision support for otitis media by providing high predictive performance even for the tympanic membrane with multiple diseases.

Losartan Prevents Tumor-Induced Hearing Loss and Augments Radiation Efficacy in NF2 Schwannoma Rodent Models

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Background: Hearing loss is one of the most common symptoms of neurofibromatosis type 2 (NF2) caused by vestibular schwannomas (VSs). Fibrosis in the VS tumor microenvironment (TME) is associated with hearing loss in patients with NF2. We hypothesized that reducing fibrosis using losartan, an FDA-approved antihypertensive drug that blocks fibrotic and inflammatory signaling, could improve hearing.

Methods: Using the cerebellopontine angle NF2 mouse model (n= 7-10 mice), we first examined the losartan effects (40 mg/kg) on reducing extracellular matrix, normalizing tumor vasculature, and changing hearing function. Then, we characterized the mechanisms of action by transcriptomic profiling using single-cell RNA-seq on tumor-associated macrophages isolated from control and losartan-treated tumors (n=3) and in vitro molecular studies on peritoneal macrophages from wild-type or AngII receptor 1 knockout mice (n=3). Last, we confirmed the preclinical findings in archived patient samples by histological analysis (n=23); in fresh, surgically obtained VS tumor samples by RNA-seq (n=6), enzyme-linked immunosorbent assay (n=57), and mouse organotypic cochlear cultures (n=35); and by retrospective analysis of hearing outcomes in patients with VS and hypertension (n=45). Animals were randomly assigned to experimental groups such that the mean tumor burden was similar in each group to avoid biased results. For the hearing test, the experimenters were blinded; for the efficacy, molecular mechanism, and clinical patient sample studies, the experimenters were not blinded.

Results: Using NF2 mouse models, we found that losartan treatment normalized the TME by (i) reducing neuroinflammatory IL-6/STAT3 signaling and preventing hearing loss, (ii) normalizing tumor vasculature, and

alleviating neuro-edema, and (iii) increasing oxygen delivery and enhancing the efficacy of radiation therapy. In preparation to translate these exciting findings into the clinic, we used patient samples and data and demonstrated that IL-6/STAT3 signaling inversely associated with hearing function, that elevated production of tumor-derived IL-6 was associated with reduced viability of cochlear sensory cells and neurons in ex vivo organotypic cochlear cultures, and that patients receiving angiotensin receptor blockers have no progression in VS-induced hearing loss compared with patients on other or no antihypertensives.

Conclusions: Our study demonstrates that losartan prevents hearing loss and enhances radiation efficacy in rodents. As one of the most commonly prescribed drugs for hypertension, the safety and low cost of losartan warrant rapid translation of our research to patients with VS-induced sensorineural hearing loss.

Direct SARS-CoV-2 Infection of the Human Inner Ear May Underlie COVID-19-Associated Audiovestibular Dysfunction.

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Background: COVID-19 is a pandemic respiratory and vascular disease caused by SARS-CoV-2 virus. There is a growing number of sensory deficits associated with COVID-19 and molecular mechanisms underlying these deficits are incompletely understood.

Methods: We report a series of ten COVID-19 patients with audiovestibular symptoms such as hearing loss, vestibular dysfunction and tinnitus. To investigate the causal relationship between SARS-CoV-2 and audiovestibular dysfunction, we examine human inner ear tissue, human inner ear in vitro cellular models, and mouse inner ear tissue.

Results: We demonstrate that adult human inner ear tissue co-expresses the angiotensin-converting enzyme 2 (ACE2) receptor for SARS-CoV-2 virus, and the transmembrane protease serine 2 (TMPRSS2) and FURIN cofactors required for virus entry. Furthermore, hair cells and Schwann cells in explanted human vestibular tissue can be infected by SARS-CoV-2, as demonstrated by confocal microscopy. We establish three human induced pluripotent stem cell (hiPSC)-derived in vitro models of the inner ear for infection: two-dimensional otic prosensory cells (OPCs) and Schwann cell precursors (SCPs), and three-dimensional inner ear organoids. Both OPCs and SCPs express ACE2, TMPRSS2, and FURIN, with lower ACE2 and FURIN expression in SCPs. OPCs are permissive to SARS-CoV-2 infection; lower infection rates exist in isogenic SCPs. The inner ear organoids show that hair cells express ACE2 and are targets for SARS-CoV-2.

Conclusions: Our results provide mechanistic explanations of audiovestibular dysfunction in COVID-19 patients and introduce hiPSC-derived systems for studying infectious human otologic disease.

Distinct Synaptic Plasticity Mechanisms Determine the Diversity of Cortical Responses during Behavior

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Background: Heterogeneous neuronal responses are found in many cortical areas including visual, auditory, parietal, and frontal cortex. The extent of this heterogeneity is vast. A fraction of cells exhibit trial averaged responses with obvious task-related features such as pure tone frequency tuning in the auditory cortex (classically responsive). However, a substantial number of cells do not appear to fire in a task-related manner and are often neglected from analysis (non-classically responsive). Our recent study used a novel spike-timing-based analysis to show that both classically and non-classically responsive auditory cortical neurons contain significant task information suggesting that non-classically responsive cells may play an underappreciated role in auditory perception and behavior (Insanally et al. 2019). Where do these diverse response profiles come from and what is their contribution to behavior? What are the synaptic inputs that might drive such diverse responses?

Methods: To address these questions, we trained a novel spiking recurrent neural network model that incorporates spike-timing-dependent plasticity (STDP) mechanisms and FORCE learning to perform the same task as behaving animals. By leveraging excitatory and inhibitory plasticity, this model reproduces neurons with response profiles consistent with published in vivo data, including classically and non-classically responsive neurons.

Results: We found that both classically responsive and non-classically responsive units encode behavioral variables in their spike times as seen in vivo. Using simulated perturbation experiments to assess the functional contribution of these subpopulations, we found that classically responsive cells contribute more to task performance via output connections while non-classically responsive cells contribute more to performance via recurrent connections by increasing the dimensionality of activity during the choice period. These results provide strong evidence for a double dissociative function of classically and non-classically responsive neurons. Both excitatory and inhibitory plasticity played a significant role in shaping response profile distributions. However inhibitory plasticity alone resulted in a large fraction of inactive units, indicating that excitatory plasticity is required to maintain the engagement of all network units. Interestingly, local patterns of connectivity predicted single-unit responsiveness and placed constraints on the synaptic patterns required for non-classically responsive activity to emerge making predictions that we compare to in vivo whole-cell recordings taken from the auditory cortex of behaving animals performing a frequency recognition task. Remarkably, using parameters derived entirely from our model we were able to correctly predict the responsiveness of auditory cortical neurons recorded in vivo using only their average synaptic inputs.

Conclusions: Our approach recapitulates the heterogeneous cortical response profiles found in behaving animals and provides a powerful lens for exploring large-scale neuronal dynamics and the plasticity rules that shape them.

Auditory Cortical Plasticity Associated With Socially Reinforced Complex Sounds

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Background: We often encounter contexts where complex sounds reliably predict rewarding social interactions, like the distinctive footsteps of a friend approaching or the unique ringtone from a partner's phone call. Social rewards may serve as a potent driver for the sensory learning of such arbitrary, communicative sounds, but how they may be encoded in the auditory system and altered by social experience is unknown. By contrast, much research using natural vocalizations, such as in the maternal mouse model of ultrasonic vocalization (USVs) communication, has helped elucidate the auditory cortical processing of socially salient, evolved signals. Yet whether findings from that framework generalize to when novel sounds become associated with rewarding social interactions, or only applies for ethological communication, is unclear. Prior studies with USVs have suggested that motherhood increases both pup call-evoked suppression in frequency bands lateral to the USVs (Galindo-Leon etal, 2009), and the prevalence of call-excited Off-only responses (Chong etal, 2020). Do these changes also occur when an arbitrary sound is socially reinforced?

Methods: We trained mother mice on a T-maze (Dunlap etal, 2020) to use a sinusoidally and linearly frequency modulated target tone with characteristics outside the normal USV range, as a cue to localize and retrieve a pup – an innate behavior that is highly rewarding and stereotyped. We then compared neurons recorded from the auditory cortex of awake, passively listening mothers that were either trained or exposed to the sound in the T-maze.

Results: Akin to the plasticity observed for USVs in mothers versus virgins, the target-evoked average response in frequency bands lateral the target sound was significantly suppressed in trained compared to sound-exposed animals (n=138, trained; n=101, sound-exposed; $p<0.05$ Wilcoxon ranksum). By contrast, lateral band neurons in both trained and exposed mice responded to spectrally similar but behaviorally irrelevant control sounds with overall excitation, indicating that the lateral band suppression was specific to the socially salient sound. Moreover, the prevalence, but not firing rate, of late Off-only responses to the target increased after training (n=59/309, trained; n=28/277, exposed; $p<0.05$ Fisher's Exact), as typically seen for pup USVs that gain relevance for mothers. This was most apparent for layer 5/6 neurons (n=53/104, trained; n=25/89, exposed; $p<0.05$ Fisher's Exact). Finally, for both deep and thalamorecipient cortical layers, response latencies became less concentrated at sound onset and more widely distributed throughout the target after training ($p<0.05$, kruskal-wallis), similar to a reduced Onset firing rate for pup USVs seen in mothers.

Conclusions: Together, our results suggest that lateral band suppression, Off excitation, and reduced Onset firing, are general coding features for socially salient sounds, whether they are natural or not, prompting future questions

into their functional role in coding perceptual qualities and social behavioral actions. Supported by R01DC008343.

Long-Term Auditory Training Prevents Age-Related Changes to Population Activity in the Primary Auditory Cortex

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Background: More than one in three people over the age of sixty-five suffer from age-related hearing loss, presbycusis. Presbycusis can be caused by changes in the peripheral or central auditory system. Changes in the peripheral auditory system have been well studied, but the changes in the central auditory system have been less studied. Changes in the central auditory system are thought to include changes in the primary auditory cortex (A1).

Indeed, our studies of mouse A1 have shown that aging increases the correlation of neuronal activity while decreasing the stimulus sensitivity of neuronal responses. These attributes of the aging A1 combine to decrease the encoding stimuli information compared to young A1 contributing to central hearing loss (Shilling-Scrivo, Mittelstadt, and Kanold in press).

In humans, auditory training of various durations has been shown to improve auditory performance, thereby indicating that sensory plasticity of the central auditory system might be effective in preventing some of the changes with aging. For example, life-long instrumentalists and vocalists maintain better hearing compared to non-musicians in their age group. Despite this, it is not known how long-term auditory training affects the central auditory system of older individuals. Given that one major effect of aging in A1 is the increase in correlated activity in A1, we speculated that long-term auditory training might be effective in preventing this increase in activity correlations.

Methods: To examine this hypothesis, we continuously trained mice on an auditory task for a period of at least six months until they reached old age (>17 months) utilizing automated home-cage training systems. We then compared the sound-evoked responses of large populations of single neurons (>7800 neurons) in A1 of old trained (N=16) and old naive (N=7) mice using in vivo 2-photon Ca2+ imaging.

Results: We found that training prevented many of the age-related functional changes in A1 associated with aging. Specifically, we found that long-term auditory training prevented the rise in correlation of neuronal population activity, some of which we speculate may be responsible for lower auditory performance in older individuals.

Conclusions: Our results suggest that long-term auditory training has the potential to prevent changes to neuronal population dynamics that occur during aging. While numerous therapeutic options exist to treat peripheral hearing loss, no such options exist for central hearing loss. Long-term auditory training is an accessible, simple, and quick tool that can be rapidly deployed to the aging population that may postpone or minimize functional changes to the central auditory system with aging.

Modeling Gain Control Mechanisms for Robust Auditory Categorization in Noise

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Background: To facilitate robust vocal communication in the real-world, the auditory system has adapted sound-processing mechanisms to generalize over production variability in vocalizations (trial-to-trial and subject-to-subject variability) and environmental variability (e.g., noisy backgrounds). These mechanisms are mediated by a hierarchical processing strategy, which, broadly speaking, consists of a dense spectrotemporal representational stage followed by a sparse feature detection stage. We previously implemented this hierarchical architecture in a computational model, and showed that optimal performance in call-categorization tasks can be achieved by detecting a few maximally informative features, thereby generalizing over production variability. Here, we extend the model to generalize over environmental variability.

Methods: To achieve noise invariance in call categorization, we explore the effects of two biologically feasible gain control mechanisms, (1) adaptation to sound statistics in the spectrotemporal representational stage and (2) sensitivity adjustment at the feature detection stage. To compare model performance to behavior, we trained guinea pigs on a call-in-noise categorization task using a go/no-go paradigm.

Results: We found that one or both gain control mechanisms are required for model performance to approach the behavioral performance of guinea pigs engaged in the same call categorization task.

Conclusions: Overall, these results highlight the contributions of gain control mechanisms at both the representational and feature detection stages to achieve noise invariance in auditory categorization tasks. In ongoing experiments, we are recording neurophysiological data from the primary auditory cortex in response to vocalizations presented in clean and noisy conditions to validate these model predictions.

Decoding the Perceptual Consequences of Stimulus Specific Adaptation

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Background: Adaptation to repeated stimuli is a widespread phenomenon in the nervous system. In the auditory system in particular Stimulus Specific Adaptation (SSA) has been studied and widely described across multiple levels in neural processing and species: the repeated presentation of a pure tone stimulus leads to a local reduction in a neuron's tuning, while stimulation at other frequencies in the neuron's tuning curve leads to unadapted or even facilitated responses. As this pattern of neural responses can be seen as a reduction in response to predictable information, SSA has been considered as one of the simplest manifestations of predictive coding and potentially underlying more general phenomena like Mismatch Negativity (MMN). Here, we investigate the properties of SSA on a more general level, by decoding the neurally represented, and thus putative perceived stimuli from population responses.

Methods: We presented various adaptor sequences with randomly occurring changes in frequency to awake mice and urethane anesthetized rats while electrophysiologically recording in the primary auditory cortex. We then trained a neural decoder to predict the represented sounds from the population response.

Results: We find that the repeated presentation leads to a locally restricted adaptation of the tuning curve. As a consequence, decoding the represented (putatively perceived) sound indicates a local shift away from the frequency location of the adaptor tone by 0.5-1 semitones. The level of this shift depends on the included population of cells, and is maximized for cells with not-too-narrow V-shaped tuning, while almost absent for very narrowly tuned cells. This effect can be modelled in a basic multistage adaptation model, which points to a semi-local adaptation as the underlying cause for this shift, which can arise from integrating the adaptation from lower levels up to the level of the auditory cortex.

Conclusions: Adaptation with a local adaptor leads to a repulsive shift around the adaptor, which could be interpreted as a local deviation from accurate representation of the environment, or a local increase in frequency resolution contrast. Depending on the cells representing this information, the change in representation becomes more or less salient.

Using Poisson-Wiener Theory to Estimate the Auditory Temporal Processing of Both Natural and Artificial Sounds

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Background: The time-consuming and labor-intensive nature of psychophysical measurements of temporal modulation transfer functions (tMTFs) limits their use in routine clinical practice. However, in animal models, tMTFs can simply be computed based on electrophysiological responses. Prediction of tMTFs with a mathematical model would further improve the efficiency of tMTF computation. However, many models are only accurate when predicting artificial sounds and cannot be applied to natural sounds. Despite still being uncertain, recent studies suggest that a system identification method, Poisson-Wiener theory, can be applied to biologically relevant sounds which often differ based on their temporal patterns, such as speech and other conspecific communication.

Methods: To assess the predictability of the model in the awake auditory cortex and its application to natural sounds, extracellular recordings of primary auditory cortex (A1) neurons were obtained from chronically implanted cats (n=3) in response to acoustic stimuli: (1) 2-64 Hz periodic clicks trains to construct tMTFs, (2) Poisson-distributed impulse trains to compute the kernels, and (3) cat vocalizations (purring). For each neuron,

Poisson-Wiener kernels were derived and used to estimate peristimulus time histograms (PSTHs), rate modulation transfer functions (rMTFs), and tMTFs specific to that unit.

Results: Consistent with other findings, most units were predicted to have a bandpass tuning shape. Compared to the linear model, the nonlinear model was better able to estimate the modulation tuning and best modulation frequency. The accuracy of both the linear and nonlinear models were similar to results obtained in anesthetized animals. The nonlinear prediction of the neuronal responses to conspecific vocalizations were more accurate than the linear prediction. The improved fit of the nonlinear prediction is likely attributable to the ability of the model to imitate forward suppression by correcting the impulse response for interactions with preceding impulses. This is consistent with studies in other animal models suggesting that compressive nonlinearities inhibit the processing of redundant information when conspecific vocalizations are presented in A1. The tendency of the model to predict a bandpass tuning shape suggests it would be ideal for estimating tMTFs from non-invasive electrophysiological recordings in humans previously found to have a bandpass shape.

Conclusions: Our preliminary results suggest the model has the potential to accurately estimate the auditory temporal processing of both natural and artificial sounds. Following successful application to the prediction of conspecific communication, extension of the model to humans has the potential to provide a more efficient and accurate assessment of auditory temporal processing than currently used clinical measures. Along with its potential clinical applications, the characterization of auditory temporal processing using system identification techniques would provide insights into the neuronal processes underlying modulation tuning as well as cortical responses to biologically relevant sounds in individuals with abnormal hearing, similar to those obtained here in animal models.

Development of Spectral Resolution in Cochlear Implanted School-Aged Children

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Background: Speech perception is one of the most important outcomes in pediatric cochlear implantation (CI). Currently, there are no clinical measures of discrimination to determine the benefit of CIs in children too young to participate in speech perception tests. In CI adults, speech perception correlates with performance on tests of spectral resolution. As such, spectral resolution has been proposed as a proxy measure of CI efficacy. Spectral resolution refers to the ability to distinguish peaks and troughs of energy across frequency and relies on frequency resolution (FR) and spectral modulation sensitivity (SMS). FR matures in infancy while SMS remains immature through adolescence. In the present study, we used spectral-temporally modulated stimuli to study the development of spectral resolution in school-aged CI children. Prior testing of normal hearing listeners using this task showed that children had poorer SMS, but similar FR compared to adults. It was hypothesized that CI children and adults would show a similar pattern of performance, specifically, immature SMS in the younger cohort.

Methods: Participants included 12 CI children (age 5-16; mean 9.7, SD 3.5) and 5 CI adults (age 51-73; mean 59, SD 10); data collection is ongoing. Pediatric listeners were implanted prior to age 2; adults were post-lingually implanted. The better hearing ear was tested. Stimuli were 1-second wide-band noises with spectral-temporally modulated envelopes in which spectral modulation, or "ripple" density varied from 1 – 20 per octave. A 3-alternative forced choice task was used to determine the highest ripple density each listener could discriminate from 20 ripples per octave (RPO) at various modulation depths (3dB, 7 dB, 10 dB, 15 dB). As modulation depth increases, task performance increases as a logarithmic function with slope and x-intercept reflecting FR and SMS respectively; FR and SMS were derived for each listener.

Results: All participants completed the task. Mean spectral ripple densities did not significantly differ between the school-aged and adult CI listeners. Neither mean FR nor mean SMS significantly differed between CI children and adults.

Conclusions: Findings from this study suggest that these school-aged CI children had mature FR and SMS relative to adults. This data should be considered very preliminary due to the small sample sizes. It is possible that CI children might show earlier maturation of spectral resolution than expected compared to CI adults due to peripheral and central auditory plasticity, benefits of early implantation and auditory habilitation. The next stage of the study, currently underway, is to examine whether FR and/or SMS are correlated with speech perception.

Validation of the Adaptive Scan Method in the Quest for More Efficient Methods for Testing Auditory Processes

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Background: Several costs associated with psychophysical testing of central auditory processes prevent their widespread use in clinical settings. Portable Automated Rapid Tests (PART) can circumvent most of these costs as a compliment to standard clinical practice. A major barrier to the clinical application of psychophysical measures is the cost in terms of the time required to obtain accurate and precise estimates of listening abilities.
Methods: In this study, we first validated a novel Adaptive Scan (AS) method of threshold estimation that is designed to adapt on a range of values around threshold rather than on a single threshold value—thus providing the listener more familiarity of the stimulus characteristics near threshold and affording precise measurement with increased efficiency. We then explored the efficiency of AS in comparison to typical adaptive methods. AS was compared with two adaptive algorithms and the method of constant stimuli in two psychophysical tasks: the detection of a gap in noise and the detection of a tone in noise. Seventy undergraduates with hearing thresholds typical for their age were tested using all four methods.
Results: The AS method was similar to other adaptive methods in its accuracy and precision when using trial-by-trial data to estimate the position of a psychometric function, and is thus a valid adaptive method. Further, we conducted a post hoc exploration of possible stopping rules for the adaptive algorithms based on group statistics indicating the potential clinical utility of the AS method when the goal is to obtain data sufficient to guide clinical decisions as quickly as possible.
Conclusions: This work may be used to implement more efficient methods across a variety of psychophysical assessments, further improving the rapid nature of PART.

Preceding Maskers Only Affect Comodulation Masking Release but Not Binaural Masking Level Difference
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Background: Comodulated masking noise and binaural cues can facilitate detecting a target sound from noise. These cues induce a decrease in detection thresholds, quantified as comodulation masking release (CMR) and binaural masking level difference (BMLD), respectively. Previous studies showed that CMR is affected by a preceding masker, possibly due to the adaptation of the auditory system to features of the preceding masker. However, its relevance to speech perception is unclear. Here, we investigated the effect of preceding maskers on CMR and BMLD (Experiment 1) and its ecological validity using sounds with speech-like spectro-temporal dynamics (Experiment 2).
Methods: For the first experiment, we hypothesized that the adaptation results from top-down processing, and both CMR and BMLD would be affected with increased preceding maskers' length. We measured CMR and BMLD when the length of preceding maskers varied from 0 (no preceding masker) to 500 ms. We used four different maskers: the reference condition with uncorrelated masker (RR), and three maskers consist of a comodulated masker preceded by three different maskers: uncorrelated masker (RC), comodulated masker (CC), and the masker with comodulated flanking-band (FC). For BMLD, we used the interaural phase difference (IPD) of pi. In the second experiment, we further evaluated the ecological validity of such grouping effect with stimuli reflecting formant changes in speech. We extracted spectro-temporal dynamics and placed three masker bands at formant frequencies F1, F2, and F3 based on CV combination: /GU/, /FU/, and /PU/. The target was set to match the instantaneous center frequency of F2.
Results: Results from the first experiment showed that CMR was affected with longer preceding masker. In the diotic conditions, with the increased duration of the preceding masker, CMR was further enhanced in the CC condition, while CMR was reduced in the RC and FC condition. In dichotic conditions, however, only the FC condition showed reduced CMR. The BMLD was independent of the length of the preceding masker in all conditions. This indicates that the grouping of frequencies in the preceding masker has an influence on following frequency grouping by comodulation but not by IPD. In the second experiment, while BMLD was comparable to previous findings (~9 dB), the CMR was little (~2 dB), and some listeners showed negative CMR.
Conclusions: Here, we investigated i) the effect of preceding maskers on CMR and BMLD, and ii) the ecological validity of CMR and BMLD with speech-like stimuli. The adaptation to maskers affected CMR but not BMLD,

suggesting an adaptation process may occur at the early stage of the auditory system. Furthermore, based on the results of the second experiment, we suggest that factors other than comodulation play a role in the frequency grouping of speech sounds.

Discontinuous Stochastic Figure Ground Task Predicts Speech-In-Noise Recognition

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Background: In noisy environments, listeners must quickly segregate the target speaker from irrelevant background sounds. Stochastic figure-ground (SFG) tasks (Teki et al., 2011), which involve detecting "figures" of temporally coherent, but inharmonic, pure-tone chords from random background tones, provide insight into auditory stream segregation processes necessary for speech-in-noise recognition. The present study investigated: 1) the effect of coherence —induced by varying the number of tones in a figure chord— on accuracy and response time (RT), and 2) whether individual differences in working memory capacity, musical ability, and speech-in-noise recognition modulated the coherence effect.
Methods: Thirty-two participants completed the study. An SFG task was administered, composed of 50 ms tones sampled from 30 frequencies between 0.10–6.4 kHz on a log-scale. Each stimulus (N=160) lasted six seconds. Half the stimuli contained random background tones only and half contained a figure of 12 repeating chords amongst background tones. Unlike previous SFG tasks, figure chords were non-continuous and randomly separated by 2.5–150 ms, making them more like speech, which is oscillatory. Figure chords contained 4, 6, 8, or 10 tones and participants were instructed to quickly indicate if they detected the figure. The quick speech-in-noise task (QSIN) served as a measure of speech-in-noise recognition and scores were based on the average SNR across four lists. Working memory capacity was measured via number of words recalled in the reading span task, in which participants verbally recalled sentence-final words from sets of short sentences. Participants were separated into musicians (n=16) and non-musicians (n=16) based on self-reported measures of musicianship.
Results: Linear mixed-effects models showed that SFG task accuracy increased with increasing figure coherence, reaching an asymptote between 8 and 10 tones per figure chord. RT decreased as figure coherence increased. Higher overall SFG task accuracy related to better speech-in-noise recognition, but only for self-identified musicians. In easier conditions, faster RTs were associated with better speech-in-noise recognition. Finally, for the highest coherence level, higher working memory related to lower SFG accuracy.
Conclusions: The novel SFG task's relationship to a standardized measure of speech-in-noise recognition positions it as a viable assessment for measuring auditory stream segregation processes necessary for speech recognition, without being contaminated by individual differences in strength or speed of lexical processing. This makes our measure especially suitable for use among individuals with different language backgrounds and in investigating issues with auditory stream segregation.

Effects of Age and Hearing Impairment on Amplitude Modulation Frequency Selectivity

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Background: The accurate perception of the temporal envelope of sounds is crucial to speech intelligibility. For this, modulation frequency selectivity has been argued to be critical, based on results from computational modeling studies employing a modulation filterbank stage in the preprocessing of the speech and interferer signals. Recent neurophysiological studies suggest that modulation frequency selectivity may be adversely affected by age and hearing impairment. However, behavioral studies have reported that modulation frequency selectivity generally seems unaffected in older hearing-impaired (HI) listeners. Furthermore, the effects of age on modulation frequency selectivity have not yet been thoroughly explored in isolation. The present study investigated modulation frequency selectivity in young normal-hearing (NH) as well as older NH and HI listeners in an attempt to separate the effects of age and hearing impairment.

Methods: Temporal envelope processing acuity was probed using amplitude modulation (AM) detection and AM masking tasks in 11 young NH, 10 older NH, and 10 older HI listeners. The stimuli were generated using a 2.8 kHz sinusoidal carrier with an imposed sinusoidal AM at modulation rates of 4, 16, 64, and 128 Hz. AM masking data were obtained using fixed-bandwidth random modulation maskers, centered at modulation frequencies ranging from -5 to 2 octaves relative to the target modulation rate. The modulation maskers had bandwidths corresponding to ½ octave when centered on frequency. The envelope power spectrum model of masking (EPSM) was used to identify the underlying shapes of the hypothesized modulation filters in NH listeners from the AM masking data.

Results: The results showed higher AM detection thresholds as well as an apparent loss of modulation frequency selectivity in the older NH listeners as compared to the young NH group, particularly at a modulation rate of 4 Hz. However, substantial variability was observed in the individual results. The HI group showed substantially lower AM detection thresholds than the NH listeners and did not exhibit a general loss of modulation frequency selectivity, consistent with results from earlier studies. However, at a modulation rate of 4 Hz, the data indicate a reduced modulation tuning with hearing impairment.

Conclusions: AM detection and AM masking data were collected in young and older NH as well as HI listeners to study the effects of age and hearing loss independently on perceptual modulation frequency selectivity. The results suggest a detrimental effect of age but reflect no consistent effect of hearing impairment. The apparent loss of modulation frequency selectivity in older NH listeners and the lack of an effect in older HI listeners suggest an interaction between the effects of age and hearing loss on AM masking. The results provide a valuable basis for studying the relation between modulation frequency selectivity and speech intelligibility as a function of age and hearing loss.

Effects of the Medial Olivocochlear Reflex on Human Cochlear Tuning
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Background: Cochlear tuning can change depending on the state of activation of the medial olivocochlear reflex (MOCR). In humans, cochlear tuning is often assessed using psychoacoustical tuning curves (PTCs), a plot of a pure-tone masker level required to just mask a fixed-level pure-tone probe as a function of masker frequency. For a low-level probe, a PTC is thought to partly reflect the tuning of the cochlear site most sensitivity to the probe frequency. Sometimes, however, the stimuli used to measure a PTC are long enough that they can activate the MOCR by themselves, thus affecting a PTC.

Methods: Here, PTCs are measured in forward masking using short maskers (30 ms) and probes (10 ms) to minimize the activation of the MOCR by the maskers and/or the probes. PTCs are also measured in the presence of long (300 ms) ipsilateral, contralateral, and bilateral noise precursors to investigate the effect of the ipsilateral, contralateral and bilateral MOCR on the PTCs. Probe frequencies were 500 and 4000 Hz to assess the effects of the MOCR activation on tuning at the base and the apex of the cochlea. Masker frequencies ranged from 0.5 to 1.2 (4000 Hz) or 1.6 (500 Hz) times the probe frequency. Four listeners with normal hearing participated in the experiments.

Results: At 500 Hz, ipsilateral and bilateral precursors decreased masking thresholds for maskers with frequencies at or near the probe frequency with minimal effects on thresholds for maskers remote from the probe. At 4000 Hz, by contrast, ipsilateral and bilateral precursors barely affected masking thresholds for maskers near the probe frequency and broadened PTCs by reducing thresholds for maskers far from the probe. Contralateral precursors barely affected PTCs.

Conclusions: The pattern of results at 4000 Hz is consistent with the ipsilateral and bilateral MOCR inhibiting the cochlear gain similarly and more strongly than does the contralateral MOCR. The pattern of results at 500 Hz is, however, hard to interpret. [Work supported by the University of Salamanca and Banco Santander to DLR and the Spanish Ministry Science and Innovation (grant PID2019-108985GB-I00) to EALP].

Characterization of Pou4f3 Transcriptional Activators for Hair Cell Regeneration in Adult Mice
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Background: As opposed to non-vertebrate mammalian vertebrates, mammals are unable to regenerate lost cochlear hair cells following damage and loss, making hearing loss permanent. Direct cellular reprogramming

strategies offer a novel therapeutic approach for regenerating hearing in mammals. The expression of Atoh1 and Pou4f3 coincide with the initial and terminal differentiation of HCs, respectively. Neither, however, are expressed in mature supporting cells. Therefore, they are commonly targeted for hair cell regeneration. Pou4f3 activation has shown to be sufficient to induce supporting cell to hair cell conversion in adult mice (Walters et. al 2017). While genetic overexpression of Pou4f3 and other factors has been demonstrated to promote conversion of SCs to HC-like cells in vivo in adult mice, there have been no reports of HC regeneration in adult wild-type mice using only a single small molecule. Therefore, we sought to discover a small molecule Pou4f3 transcriptional activator to promote hair cell regeneration in adult mice. Previously, we collaborated with Novartis to screen over 45,000 compounds for POU4F3 activation in our human POU4F3 promoter-driven dual-luciferase reporter HeLa cell line. This resulted in a hit- C18 (compound 18) which facilitates Atoh-1 mediated conversion of supporting cells to hair cells. Further testing of C18 in-vivo has shown it promotes conversion of supporting cells to hair cells in adult mice in vivo.

Methods: A Novartis screen was performed to look for Pou4f3 activators using the NanoLuc and Firefly reporters. Adult (P28) FVB mice of both sexes were treated via transtympanic injection into the left middle ear with 500μM C18 in 50% DMSO. Controls were contralateral uninjected ears and vehicle-only injected mice. Mice were euthanized 4 or 6 weeks after injection. The cochleae were then harvested, fixed, decalcified and stained with for Pou4f3, Myosin VI, DAPI, and Sox2. The experiment will be repeated using Sox2-CreER; TdTomato mice for lineage tracing. Cochlear explants were obtained from P2-P3 mixed background mice and placed in HBSS. Explants were cultured overnight in 37oC/5% C02. Culturing was continued for 2 additional days with either C18 (10uM) or vehicle (3% DMSO). Following 48hr treatment with C18 or vehicle, explants were immunolabeled. Fluorescence intensity was quantified using ImageJ. GraphPad Prism 9 was used for all statistical analysis.

Results: Through in vivo in adult mice and ex vivo in neonatal explants, we have shown that a single Pou4f3 activator- C18- is sufficient for SC to hair cell conversion. C18 increases Pou4f3 expression in supporting cells, helping convert the supporting cells to a hair-cell like phenotype.

Conclusions: A small molecule Pou4f3 activator is sufficient to convert supporting cells to hair cells in adult mice.

Characterization of Responsiveness to Vestibular Stimuli During Natural Regeneration of Type II Hair Cells in Adult Mice

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Background: Following destruction of utricular hair cells (HCs) in adult mice, ~20% of HCs are regenerated, all of which are type II (HCII). Regenerated HCs establish mature features, including markers, morphology, electrophysiological response properties, and innervation, but they maintain signs of immaturity. To determine if vestibular stimuli evoke brainstem responses after regeneration, we examined changes in protein levels of the immediate early transcription factor cFos in vestibular nucleus neurons (VNNs) following off-axis centrifugation. cFos levels have been used in rodents to gauge VNN responses to sinusoidal galvanic stimulation (sGVS) at the vestibular periphery.

Methods: We administered diphtheria toxin (DT) to Pou4f3+/DTR (DTR) mice, which kills HCs. We examined two DT doses: 25 ng/g (low dose) and 50 ng/g (high dose). Pou4f3+/+ wildtype (WT) were also injected and served as no-damage controls. At different times post-DT, we counted HCs in utricles and horizontal cristae. We assessed the horizontal vestibulo-ocular reflex (hVOR) from 0.3 to 1.0 Hz in the dark. To assess VNN responses to vestibular stimuli, mice underwent off-axis sinusoidal centrifugation (0.01 Hz, pk-pk 600°/s, r=28 cm) for 10 min; 45 min later, brain sections were labeled with cFos antibodies. We examined if sGVS evokes cFos in VNN in DTR mice. Finally, we assessed survival of vestibular ganglion neurons (VGNs), VNNs, and other neurons in the vestibular ocular reflex (VOR) pathway following high dose DT.

Results: DT killed utricular and ampullary HCs in a dose-dependent manner; at 170 days post-DT, there were more HCs in each organ after low-dose DT compared to high-dose DT. In utricles, half of type I HCs (HCI) survived low-dose DT, while most HCI died after high-dose DT. By contrast, HCII numbers were similar after each dose. We noted comparable HCI:II ratios in horizontal ampullae. In DTR mice, hVOR gains after low-dose DT were reduced to approximately half of WT, and to near zero after high-dose DT. Off-axis centrifugation of WT mice triggered a significant increase in cFos in medial and spinal VNNs relative to non-centrifuged controls.

DTR mice with low-dose DT had approximately half of the cFos-labeled VNNs of WT mice after centrifugation. Centrifuged DTR mice with high-dose DT resembled non-centrifuged WT mice. In both WT and DTR mice, sGVS induced significant increases in cFos-labeled VNNs. We found no evidence for DT-induced neuronal death in any nucleus examined following high-dose DT.

Conclusions: Mice with regenerated HCII and few surviving HCI had little or no centrifugation-evoked VNN cFos or VOR. Both responses were significantly larger in mice with numerous surviving HCI. Peripheral sGVS triggered cFos increases in VNNs of DTR mice, suggesting that signal transduction central to the vestibular organs remained intact after HC damage. Pathology preventing VNN responses to centrifugation likely resides at the sensory organs

Long-Term Survival of LGR5+ Supporting Cells After Ototoxic Trauma in the Adult Mouse Cochlea

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Background: Sensorineural hearing loss is mainly caused by irreversible damage to sensory hair cells (HCs). A subgroup of supporting cells (SCs) in the cochlea express the leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5), a marker for tissue-resident stem cells. These LGR5+ SCs could potentially be used as an endogenous source of stem cells for regeneration of HCs to treat hearing loss and deafness. We have recently described that LGR5+ SCs survive one week after ototoxic trauma (Smith-Cortinez et al., 2021, Front. Mol. Neurosci., 10.3389/fnmol.2021.729625). However, it is still unknown whether LGR5+ SCs from the deafened mouse cochlea retain regenerative potential or if they are present in the long term after deafening. Here, we will evaluate long-term survival of LGR5+ SCs in adult deafened cochleas of Lgr5GFP transgenic mice and determine the regenerative potential of these LGR5+ SCs.

Methods: Adult (postnatal day 30-50) normal-hearing Lgr5-eGFP-IRES-creERT2 heterozygous (Lgr5GFP) and deafened Lgr5GFP mice will be used. Animals will be deafened with a single dose of furosemide (100 mg/kg i.v.) and kanamycin (males: 700 mg/kg s.c. and females: 900 mg/kg). Seven and 28 days after deafening, auditory brainstem responses (ABRs) will be recorded. Cochleas will be harvested to characterize mature hair cells and LGR5+ SCs by immunofluorescence microscopy, quantitative real time PCR (q-RT-PCR), and FACS. In addition, we will sort LGR5+SCs from cochleas of normal-hearing and deafened mice and culture them as 3D cochlear organoids to analyse their regenerative capacity.

Results: As previously described we found survival of LGR5+ SC in the third row of Deiters' cells one week after deafening in adult Lgr5GFP mice (Smith-Cortinez et al., 2021, Front. Mol. Neurosci., 10.3389/fnmol.2021.729625). The q-RT-PCR expression profile showed up-regulation of Lgr5 in the deafened cochlea, compared to the normal-hearing cochlea. Animal experiments evaluating long-term survival are currently ongoing and we will present conclusive results. Preliminary data showed that sorted LGR5+SCs from adult normal-hearing mice develop cochlear organoids. The initial results from the cochlear organoids derived from deafened mice will be presented.

Conclusions: The presence of LGR5+ cells in the adult mouse cochlea demonstrates potential endogenous cochlear stem cells with regenerative capacities in adulthood. This is confirmed by the development of organoids from these cells. Furthermore, these LGR5+ SCs do survive an ototoxic trauma. To our knowledge, this is the first study showing increased Lgr5 expression after deafening in the adult mouse cochlea. This might be a result of ototoxicity-induced LGR5+ cell proliferation, and will be further explored and objectified in a future study.

A Regulatory Network of Sox and Six Transcription Factors Guide Fate Determination During Hearing Regeneration in Adult Zebrafish

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Background: Damage to the mammalian sensory epithelium is irreversible and results in permanent hearing loss. A feature that sets mammals apart from avian and aquatic vertebrates is that mammals are unable to replenish hair cells while the non-mammalian species either continually produce new hair cells or regenerate them in response to trauma. Enhancer regulatory elements are traditionally known to control development. Injury-responsive or regeneration-associated enhancers that direct gene expression in injured tissues have been identified in the regenerating heart and fin of zebrafish. We predict that the adult zebrafish inner ear also contains regeneration

responsive regulatory elements and sought to gain a comprehensive understanding of the epigenomic and transcriptomic landscape during hair cell regeneration.

Methods: On adult zebrafish that have undergone hair cell ablation, we investigated the epigenome and transcriptome of single-cells from the inner ear at consecutive time-points following hair cell ablation. We identified regeneration induced open chromatin locations using scATAC-seq and examined transcription factor dynamics using motif analysis and machine learning. We correlated enhancer activation with transcription (using scRNA-seq) to identify gene regulatory networks and examined cell fate trajectories. Finally, we functionally validated an identified enhancer using CRISPR/Cas9 gene editing to generate enhancer KO alleles and investigated the inner ear hair cell regenerative abilities of animals with enhancer deletions.

Results: Bioinformatics revealed thousands of cell specific putative regeneration responsive elements and several hundred regeneration inducible genes. We determined that overlapping expression of Six- and Sox-family transcription factors activated in different cell types and that chromatin containing Six and Sox family binding motifs simultaneously became accessible in cell-specific patterns. This indicated that an integrated, combinatorial program of key transcription factors and not a simple linear pathway of factors was likely necessary to determining cell fate. Single-cell transcriptomic analyses revealed that a Sox-family TF, sox2, turns on in a switch-like pattern. scATAC-seq analyses identified a 2.6 kb DNA sequence element upstream of the sox2 promoter that acquires dynamic changes in accessibility during hair cell regeneration in a cell type specific manner. Using CRISPR/Cas9 gene editing to knock out the predicted upstream regulator of sox2, we found that deletion of the enhancer singly or in double knockouts resulted in a hair cell regeneration defect in the lateral line and adult inner ear of zebrafish.

Conclusions: By correlating cell-type, enhancer activation, and TF expression, we are beginning to understand the combinatorial "code" of TFs that initiate regeneration and instruct hair cell differentiation in ways that have not been done before. Moreover, we have characterized an enhancer with a bona fide specific function in hair cell regeneration but not in the normal development of the hair cells. This work indicates that deploying regeneration-specific enhancers could provide a therapeutic approach to stimulate repair of a vertebrate inner ear.

Psilocybin and N-Acetyl Cysteine Reduces Behavioral Deficits Following Blast Injury and Stress Trigger in a Rat Model

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Background: Mild traumatic brain injury (mTBI) and post-traumatic stress disorder (PTSD) are significant health issues that often coincide and impact each other. The National Institutes of Health estimates there are 8 million new cases of PTSD a year and that 6.8% of the U.S. population suffer PTSD during their lifetime. At the same time, 5.3 million Americans live with the effects of mTBI, a 53% increase compared to ten years ago. Depending on the circumstances, over 50% of mTBI cases have concurrent PTSD, therefore the combination of these disorders affects millions of individuals. To date, there are no effective pharmaceutical treatments for the combination of these disorders. Prior experimental and clinical studies of drugs working via a single mechanism have failed to address the full range of pathologies present. We focused on two drugs with the potential to benefit multiple pathways implemented in mTBI and PTSD. The first is psilocybin (PS), a naturally occurring tryptamine hypothesized to act by stimulating neurogenesis in parts of the brain responsible for emotion and memory including the hippocampus. The second is N-acetyl cysteine (NAC) which is widely used in over-the-counter medications for its anti-inflammatory properties. We examine a drug therapy that combines N-acetyl cysteine and psilocybin for the treatment of PTSD and mTBI in a preclinical rat model.

Methods: Mild TBI was produced with controlled blast exposure and PTSD was induced via exposure to fox urine for 10 minutes before TBI insult and behavioral testing. The four main exposure groups (n=5-8 rats/group) consisted of blast plus stress trigger (predator scent) and gavage 1) NAC (8 mg) and PS (2 mg), 2) NAC (8 mg), 3) PS (2 mg), and 4) sterile water alone. Outcome measures include auditory startle response, light-dark emergence tasks, and auditory brainstem response (ABR). Comparisons were made between the performance of the rats within each group on each test using statistical methods to assess group mean differences (ANOVA, etc.)

Results: Rats treated with both oral PS and NAC spent significantly more time in the light during light-dark emergence tasks than rats treated with PS alone [$F(1,14)=37.8$, $p <.001$], NAC alone [$F(1,13)=19.92$, $p<.001$] or with vehicle [$F(1,11)=26.53$, $p<.001$]. Auditory startle response and auditory brainstem response results did not yield significant differences amongst the exposure groups.

Conclusions: We found that oral administration of NAC and PS mitigates some of the behavioral deficits induced by mTBI and stress. The therapeutic effects of PS and NAC together have not been fully elicited nor have the exact brain targets that mediate the improvement in the light/dark paradigm. Nevertheless, these findings suggest that the two medicines in combination may have beneficial effects in neurosensory disorders and support their potential as an important treatment worthy of further study.

Effect of Configuration of Hearing Loss on Speech Recognition in Quiet and Noise

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Background: Difficulty with understanding speech in noise (SIN) is the most common complaint in individuals with hearing loss. Despite this widespread concern, SIN abilities are not routinely measured in audiologic practice. We argue that SIN measures should replace traditional measures of word-recognition in quiet (WRQ) in audiologic assessment. Specifically, we have data demonstrating that SIN performance 1) can largely predict WRQ scores, and 2) is more sensitive than WRQ scores at identifying the effects of auditory pathology, age, and perceived patient handicap. However, to fully introduce routine SIN measurement into audiologic practice requires a better understanding of the variables that influence SIN performance. Our data from thousands of patients indicates that the degree of hearing loss is a crucial factor affecting SIN understanding. We also saw considerable variability in SIN performance for different individuals with similar amounts of hearing loss. The degree of hearing loss is often categorized from variants of the pure-tone average such as the high-frequency PTA (HFPTA; average of 1, 2, and 4 kHz). Unfortunately, PTA-based measures do not account for the configuration of hearing loss, raising the possibility that some of the variance in SIN abilities reflects the audiometric configuration as opposed to hearing acuity or non-auditory factors. Here we addressed this issue by examining SIN data in over 3,000 patients and determining the effect of audiometric configuration and degree of hearing loss on speech recognition in quiet and noise.

Methods: Data from over 3,000 patients were included in this study. All patients completed pure-tone audiometry, WRQ, and the QuickSIN. The QuickSIN determines the signal-to-noise ratio required to repeat 50% of key words in low-context sentences. We then categorized patients according to their HFPTA into different degrees of hearing loss (i.e., mild, moderate, moderately-severe, severe, profound), and examined the effect of audiometric configuration within each degree of hearing loss. Sample audiometric configurations were 'flat', 'gradually sloping', 'steeply sloping', and 'rising'. All audiometric configurations were based on air conduction thresholds. Finally, we modeled the influence of HFPTA and audiometric configuration on both WRQ and QuickSIN scores to determine how each variable contributed to speech-recognition performance.

Results: While preliminary, our data suggest that audiometric configuration offers a small, but significant contribution to performance on the QuickSIN beyond that of HFPTA. In contrast, WRQ scores were less affected by degree of hearing loss than QuickSIN scores, largely because performance was more likely to remain as hearing loss increased.

Conclusions: Taken together, these data provide important information for audiologists, physicians and researchers seeking to better understand speech recognition in both quiet and noise in large clinical populations. Such information is crucial for making the subtle, but profound transition from WRQ to SIN measures in routine audiologic practice.

Differences in Neural Encoding of Speech in Noise Between Cochlear Implants With Combined Electric-Acoustic Stimulation and Electric-Only

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Background: (Background) Cochlear implants have evolved to further utilize residual acoustic hearing that combines to electric stimulation, known as electric acoustic stimulation (EAS). However, there are mixed expectations about the benefits of EAS. A positive perspective expects that contributions from acoustic hearing provide better access to acoustic features and cues that can be helpful for separating auditory objects from a mixed auditory scene, which may improve EAS users' speech-in-noise perception. An opposing view concerns potentially poorer spectral resolution of EAS electrodes' stimulation as those electrodes often inserted close to

lateral wall, which may cause poorer speech-in-noise perception. This study aimed to directly compare neural processes of speech-in-noise perception between EAS and E-only CI users to provide an answer to the above alternative expectations.

Methods: We used 64-channel EEG to measure cortical evoked responses to 1) background noise and 2) target word while listeners perform a word-in-noise task. Then we compared the amplitude ratio of evoked responses to the target word and background noise, henceforth referred to as "internal SNR," which is the index that reflects how well target sound is unmasked from the mixture of speech and noise.

Results: Based on the comparison of 29 EAS and 29 E-only CI users, we found that internal SNR was significantly larger in EAS CI users.

Conclusions: This result may indicate that EAS provides enhanced neural process for speech unmasking, as it provides better access to acoustic features for improving auditory scene analysis.

Attention Effects on Neural Encoding of Temporal Envelope and Periodicity in Continuous Speech
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Background: Natural speech contains several temporal cues that evolve at different time scales. Prominent temporal cues include slow temporal envelope (1-30 Hz) and temporal periodicity (70-300Hz). A wide range of studies have shown attentional effects on the neural encoding of temporal envelope of target speech in tasks where there ignored energetic and/or informational auditory maskers. However, earlier studies have not explored parametric attentional effects without the added acoustic confounds of the presence of an auditory masker. Further, attention effects on temporal envelope and periodicity have not been assessed in the same task, which would help in identifying how the auditory system engages differentially with temporal cues at multiple timescales given attentional demands. To this end, we examined the neural encoding of envelope and periodicity using electroencephalography (EEG), and also assessed their correlation with behavioral speech comprehension performance.

Methods: Sixteen normal-hearing young adults participated in the study. Sixty-four channel EEG was recorded at a high digitization rate (25000Hz) to sample neural responses to both slow temporal envelope and periodicities. Participants listened to 45 mins (15 mins per each condition) of the audiobook narrative "Alice in wonderland", while they performed a behavioral task. The behavioral task involved three varying attentional foci; auditory-attention to narrative, visual 0-back task, and visual 3-back task. In each condition, the speech envelope was extracted using an auditory filterbank and the periodicity was extracted using empirical mode decomposition. Separate encoding models were fitted to estimate neural tracking of the two temporal features. The correlation of neural tracking with speech comprehension performance was assessed to investigate how listeners differentially weigh the two cues across attentional demands.

Results: Speech comprehension did not differ among auditory attention and 0-back condition and was significantly lower in the 3-back condition. However, neural tracking of the envelope was significantly lower in the 0-back condition relative to the 3-back condition or the auditory-attention condition. No specific attention effects were seen for the neural tracking of periodicities. In the 0-back condition, neural tracking of envelope showed a significant negative correlation with speech comprehension, while an opposite pattern was seen with periodicity. No significant correlations were seen in the other attentional conditions. The 0-back condition presumably engages attention to both the target speech stimulus and the visual tokens such that listeners perform top-down mediated differential cue-weighting to perceive speech sounds. This indicates that while temporal cues are vital for speech comprehension, attentional networks may prioritize periodicity information over the envelope to maximize performance.

Conclusions: Attention differentially engages the temporal cues at different timescales to aid in speech comprehension. Such naturalistic tasks can be used study neural tracking of temporally disparate acoustic cues simultaneous in a single task, which makes research translation to practice easier.

Audio-Visual Synchrony Perception: Influence of Exposure to the Ambient Language and to Early Auditory Deprivation and Use of Cochlear Implants
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Background: Audiovisual (AV) speech is a special case of AV integration and serves as an apparatus for the acquisition of speech in infancy. Moreover, the temporal relations between the auditory and the visual signals are an important feature affecting whether they will be integrated or segregated. The sensitivity to the temporal misalignments (termed stimulus onset asynchrony, or SOA) can be represented by a temporal window (TW) that provides a measure for the temporal range in which two signals are likely to be integrated. To date, SOA has been studies with non-verbal and verbal stimuli in the English language and in postlingually deafened adults with cochlear implants. The purpose of this study was twofold: (1) to assess the influence of exposure to the ambient language on the SOA and TW by using verbal signals of a language that differs from English in the articulatory-acoustics of the voice-voiceless category (in Hebrew voiced plosives e.g. /b/, the onset of the vocal fold vibration precedes the release of the burst by 80-100 ms whereas in English they occur at the same time); and (2) to assess the influence of early auditory deprivation in prelingual CI users who received their CI at an early age (long-term users).

Methods: A total of 35 participants: 13 native normal-hearing Hebrew speakers (NH-Heb), 10 native American-English speakers (NH-Eng) and 12 Heb prelingually deafened CI. SOA was determined using two stimuli: verbal /b/ produced by a Hebrew speaker and a non-verbal 'flash-beep'. For each type of stimulus, participants were asked to judge if the auditory and visual signals appeared in synchrony or not. Response rates were fitted with a Gaussian distribution, with the mean representing the point of subjective simultaneity (PSS) and the standard deviation representing the TW width.

Results: No significant differences were found between the groups with the non-verbal stimul ($p>0.05$). However, with the speech stimuli, average TW (in ms) was significantly wider for the NH-Heb (370.06) compared to NH-Eng (273.30) and to the Heb-CI (255.25) ($p=0.027$ and 0.013, respectively), with no differences between NH-Eng and Heb-CI. Also, no differences in PSS were found between groups.

Conclusions: These data suggest: (1) exposure to the articulatory-acoustics of the language influences audio-visual integration as determined by the differences between the English and Hebrew speakers to the verbal but not to the non-verbal stimuli; and (2) early auditory deprivation to ambient speech and the use of a CI device influenced the development of the TW to the voiced speech category. These outcomes provide novel insight to the effect of hearing loss on audio-visual integration of speech signals and may explain the complaints of our CI users who have had difficulties in communicating via zoom, chats and face time, especially since COVID-19 pandemic.

Speech Perception in Noise With Electro-Vibrational Stimulation

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Background: Because of recent evolutions, the number of cochlear implant (CI) users with residual hearing after implantation is rapidly growing. Despite the success of CIs in restoring hearing in profoundly deaf people, many still have very limited ability to enjoy music or understand speech in a noisy environment.

Interestingly, stimulating the low-frequency residual hearing in addition to the electrical stimulation by the CI has been shown to improve speech and music perception. Unfortunately, with electro-acoustic stimulation (EAS) as the only rehabilitation strategy available, much of this residual hearing remains unexploited.

Here we present the preliminary results of a study exploring the potential of a new combined stimulation strategy: Electro-Vibrational Stimulation (EVS). This combination consists of the electrical stimulation by the CI and vibrational stimulation provided by a bone conduction (BC) actuator.

Methods: So far, four participants with a unilateral CI and symmetrical residual hearing participated in this explorative study. A commercially available BC actuator was mounted on an elastic headband at the side of the CI to deliver transcutaneous BC stimulation. The actuator was fitted to provide maximal stimulation up to 1 kHz, with minimal stimulation at higher frequencies.

The experiments consisted of speech-in-noise (SPIN) measurements in which the speech reception threshold (SRT) was determined through an adaptive procedure. We presented a Dutch speech material (LIST sentences) in both steady-state (SWN) and modulated noise (ICRA 250). In a within-subject repeated measurement design, each subject was tested with the CI and EVS condition in random order. Only for the two participants that used EAS and a contralateral hearing aid in daily life, this condition was additionally tested.

Results: The participants had a mean low-frequency pure-tone average (125-250-500-1000Hz) of 67 dB HL (SD: 16dB) in the ear of implantation with a mean air-bone gap of 28 dB HL (SD: 17 dB). The SRT in SWN with CI alone was 0.4 dB SNR (SD: 1.9 dB).

We observed an average improvement of the SRT with EVS compared to CI alone of 1.8 dB SNR (SD: 1.0 dB) and 1.6 dB SNR (SD: 1.6 dB) in SWN and ICRA noise, respectively.

The EAS and contralateral hearing aid condition tested in two participants resulted in similar SRTs for EVS and EAS with mean differences of 0.2 dB SNR (SD: 2.69 dB) and 0.7 dB SNR (SD: 0.5 dB) in SWN and ICRA noise, respectively.

Conclusions: These preliminary data show for the first time that EVS could be a valuable alternative for EAS for people with residual hearing after CI implantation. Even larger effects are expected with more efficient BC stimulation techniques or if participants can have a longer period to adapt to the new stimulation technique. Statistical power will further increase as the number of participants expands.

Talker Identification Based on Covariance in Voicing Cues
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Background: We can usually recognize an individual from the pitch and loudness of their voice and we often use these cues to segregate their voice from others speaking at the same time. In natural speech, however, these cues often covary so that the information they provide for identification may at different times be redundant. In emotive speech, for example, voice fundamental frequency F0 (pitch) and level will tend to increase or decrease together depending on whether the talker wishes to express they are happy or sad. This study undertook to test the predictions of four models for how listeners make use of voicing cues and their covariation in talker identification. The four models were (1) listeners use only one of the two cues, (2) listeners use both cues assuming statistical independence, (3) listeners focus largely on the covariation of cues (Gestalt theory) and (4) listeners combine information from the two cues optimally (theory of ideal observers from signal detection theory)

Methods: Fifteen normal-hearing students from the University of South Florida (ages 19-30 yrs.) participated in a single-interval, two-alternative, forced-choice talker identification task for which the talkers differed in mean voice F0 and level. The stimuli were naturally-spoken, recorded sentences drawn at random for each talker on each trial from 200 neutral exemplars of the Emotional Speech Database (https://arxiv.org/abs/2105.14762). Four conditions were examined in which the correlation r between voice F0 and level for both talkers were positive (++),negative (--), orthogonal (-+) or independent (00).

Results: For the specific conditions of the study Model (1) predicted d' performance near 2 for all conditions. Model (2) predicted improved performance over (1) by a factor of 21/2 in all conditions except ++. Model (3) predicted improved performance over (2) in condition -+. Model (4) predicted d' performance near 2 for condition ++, a factor of 21/2 better for condition 00 and near optimal performance for conditions -- and -+. The results clearly supported Model (4)

Conclusions: The results of this study are consistent with a model in which listeners make near optimal use of voicing cues for talker identification by adapting their listening strategy to the different ways in which these cues can covary for each talker. The results are inconsistent with models in which the listener only ever uses one cue, assumes the cues are statistically independent or distinguishes talkers based predominantly on the covariation in voicing cues. The results are considered in the context of other studies of talker identification where the results are understood in terms of the theory of ideal observers (Lutfi et al. 2021, Trends in Hearing, in press).

Speech-Driven Facial Animations Improve Speech-in-Noise Comprehension of Humans
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Background: Comprehension of speech in noise can be substantially improved by looking at the speaker's face, and this audiovisual benefit is even more pronounced in people with hearing impairments. This effect is thought to be linked to the temporal and categorical cues carried by the visual component of speech. In particular, speech rhythms, evident in the amplitude modulations of a speech signal, play an important role in speech processing. These amplitude modulations correlate with the opening and closing of the mouth, and are involved in audiovisual speech perception. Information about a speech signal can be obtained from further aspects of lip movement as

well. However, the precise contributions of the different aspects of lip motion to speech comprehension, as well as the neural mechanisms behind the audio-visual integration, still remain unclear.

Methods: We considered both the natural video of a speaker as well as a variety of synthesized visual signals. The synthesized videos were designed to capture features of lip movements of increasing complexity, from the amplitude modulations of speech to realistic facial animations generated by deep neural networks. We then assessed the speech comprehension of participants for the different types of videos. We also recorded their brain activity through EEG while they listened to the audiovisual speech stimuli.

Results: We found that simple visual features such as the size of the mouth opening, related to the speech envelope, modulated the neural response to the speech envelope. However, they failed to enhance speech comprehension. More complex videos including the realistic synthesized facial animations did improve the comprehension of speech in noise significantly, albeit not as much as the natural videos.

Conclusions: Taken together, our results suggest that categorical cues in the texture of realistic facial animations drive the audiovisual gain in speech-in-noise comprehension. Although the amplitude modulation of speech matters for speech processing, and although simplified visual signals that track these amplitude modulations influence the neural response, these signals do not aid in understanding speech in background noise.

Influence of Hearing Aid Experience on Behavioral and Electrophysiological Measures of Speech Detection, Discrimination and Comprehension

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Background: The goal of this study was to investigate the effects of auditory deprivation (untreated hearing loss) and auditory stimulation (treated hearing loss) on different levels of speech processing using behavioral and electrophysiological measures.

Methods: A between-groups design with three groups of older participants was used: (1) participants with hearing thresholds <25 dB HL from 250-8000 Hz, (2) participants with mild-to-moderately-severe sensorineural hearing loss but no hearing aid experience, and (3) participants with mild-to-moderately-severe sensorineural hearing loss and at least 2 years of hearing aid experience. In terms of behavioral measurements, speech detection thresholds (SDT), speech recognition thresholds (SRT), and speech comprehension scores (SCS) were measured. In terms of electrophysiological measurements (EEG), speech-evoked cortical potentials (N100, P300, N400 and Late Positive Complex, LPC) were measured. The N100 and P300 responses were evoked using an active oddball paradigm, while the N400 and LPC responses were evoked using audio-visual (bimodal) and audio-only (unimodal) test paradigms. All measurements were performed in the free field in the presence of stationary speech-shaped noise. For the hearing-impaired participants, hearing aids were used to compensate for their individual hearing losses.

Results: Regarding the behavioral measurements, the SDT are expected to be similar across the three groups, while for the SRT and SCS the group with untreated hearing loss is expected to show poorer results. Regarding the electrophysiological measurements, the N100 responses are expected to be similar across the three groups, while for the P300, N400 and LPC responses the group with untreated hearing loss is expected to show smaller amplitudes. No group differences in terms of mean latencies are expected.

Conclusions: The hypothesized group differences in the behavioral and electrophysiological measures of discrimination and comprehension would be indicative of changes in cortical speech processing due to (lack of) hearing aid treatment.

Middle Ear Muscle Reflexes Are Potential Biomarkers of Peripheral Neural Dysfunction in Individuals With Chronic Tinnitus

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Background: US-based epidemiological studies have suggested that between 8% and 25% of the total population experience tinnitus. While the pathophysiology of tinnitus remains incompletely understood, there is growing evidence that peripheral auditory dysfunction may lead to cascading maladaptive changes in central auditory function and the tinnitus percept. The aim of this study was to identify a comprehensive test battery of sensitive

and objective biomarkers for tinnitus and to explore the manifestation of peripheral neural dysfunction in people with tinnitus. Specifically, there is preclinical evidence demonstrating that analysis of middle-ear muscle reflexes (MEMRs) and auditory brainstem responses (ABRs) may offer sensitive biomarkers of peripheral neural dysfunction due to cochlear synaptopathy. Several clinical studies have found that ABR and/or MEMR amplitudes are reduced in people who are diagnosed with tinnitus, suggesting that tinnitus may be associated with synaptopathy in some patients. Building on previous studies, we asked whether ABR and MEMR growth functions were depressed in people living with mild to severe chronic tinnitus when hearing sensitivity was controlled in an experimental design.

Methods: Forty-two individuals with (n=21, average age=36 years) and without (n =21, average age=46 years) tinnitus participated in the study. Severity of tinnitus was assessed using the Tinnitus Functional Index. We used a comprehensive audiological test battery to explore objective biomarkers of auditory deficits underlying tinnitus and its functional consequences. In addition to standard audiometric tests (pure tone air- and bone-conduction thresholds, and tympanometry), we assayed function of sensory hair cells using otoacoustic emissions and afferent auditory nerve function using ABRs. We also assayed efferent auditory function as a proxy for affected auditory nerve function (MEMR magnitude). To study the potential impact of audibility on these measures, pure tone hearing thresholds and extended high frequency hearing thresholds were used as covariates in the group comparisons.

Results: At the group level, individuals with tinnitus showed lower middle ear muscle reflex magnitudes than those without tinnitus. The lower group amplitude difference on the MEMR in people with tinnitus persisted even after controlling for audibility. Early indications of lower wave I amplitudes were also observed in individuals with tinnitus. These results confirm the existence of peripheral dysfunction in individuals with tinnitus that cannot be simply accounted for by peripheral loss of hearing sensitivity. Ongoing work is aimed at employing multivariate modelling approaches to ascertain the relationships between various biomarkers of auditory processing and to provide a comprehensive differentiation of the cochlear and peripheral neural deficits in individuals with tinnitus.

Conclusions: Findings from this study confirmed that middle ear muscle reflexes and auditory brainstem responses are depressed in some individuals with mild to severe chronic tinnitus. Our work has implications towards building a sensitive test battery for identifying peripheral neural dysfunction in individuals with chronic tinnitus.

Comparing Tinnitus Questionnaire Scores, Self-Reported Loudness Ratings, and Psychoacoustic Loudness Measurements

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Background: Tinnitus, the phantom perception of sound, affects approximately 15% of the adult population. Tinnitus is commonly assessed using self-report questionnaires as well as pitch and loudness matching tasks. The questionnaires are designed to assess the degree of distress caused by tinnitus, and the psychoacoustic tasks provide a measurement of the tinnitus loudness. The purpose of this study is to investigate the relationship between tinnitus questionnaire responses and psychoacoustic measurements of tinnitus loudness.

Methods: 88 adults (aged 22-70) were recruited as part of a clinical trial investigating a novel tinnitus treatment. At their screening appointment, subjects completed the Tinnitus Functional Index questionnaire (TFI), and at their baseline appointment they completed Tinntester loudness matching software (Roberts et al, J. Assoc. Res. Otolaryngol., 2008). The TFI is a 25-question survey comprised of multiple subsections that provides an overall measure of tinnitus distress as well as individual subsection scores. Question #2 (Q2) in the TFI asks participants to rate how strong or loud their tinnitus was over the past week. Tinntester software was used to obtain loudness matching and minimum masking level (MML) measurements. In addition, the software included a visual analog scale (VAS) that required participants to rate tinnitus loudness on a scale from 0-100. For loudness measures, auditory stimuli comprised of puretones and narrowband noise ranging from 500 Hz – 12 kHz were presented through headphones and participants were instructed to adjust the level of the sound until it matched the loudness of their tinnitus. Each frequency was measured twice and measurements were averaged to obtain an overall loudness value. For the MML, participants determined the level at which a 2kHz high-pass noise masked their tinnitus. Two runs were completed and averaged to obtain an MML value. Correlations were performed between

all questionnaire scores, subjective ratings, and loudness measurements to evaluate the relationship between the measures.

Results: Both objective loudness measures (Tinntester Loudness and MML) were significantly correlated with one another, and the subjective measures (TFI, TFI Q2, and Tinntester VAS) were all significantly correlated with each other. While there was a significant positive correlation between the Auditory subsection of the TFI and the MML, none of the other subjective measures correlated with either loudness measure.

Conclusions: The findings of this study showed that participants' subjective survey responses and VAS tinnitus scores were related to tinnitus distress. The psychoacoustic measurements were consistent with each other but were not correlated to the subjective responses. This finding suggests that overall tinnitus loudness measures are not systematically related to perceived tinnitus distress.

Cortical Thickness and Volume Associated With Tinnitus Severity
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Background: Although hearing loss is associated with a reduction of grey matter in the auditory cortices (Lin et al. 2014), the grey matter changes associated with tinnitus have not been replicated across studies (Adjamian et al. 2014; Elgoyhen et al. 2015). The choice of comparison groups and/or the failure to consider clinical comorbidities may explain the low replicability. To overcome these challenges, we reanalysed the anatomical brain images of participants from two previous studies (Boyen et al. 2012; Koops et al. 2020), stratifying participants into three groups according to the level of tinnitus severity (slight, mild, or moderate–catastrophic).

Methods: Sixty-six participants with tinnitus and hearing loss were included. Participants were stratified into three groups based on the severity of their tinnitus assessed using the Tinnitus Handicap Inventory (THI, Newman et al. 1996) : (1) THI score 0–16 (slight, n=20, 59.7±12.1 years, 17M/3F), (2) THI score 18–36 (mild, n=22, 59.5±8.5 years, 14M/8F), and (3) THI score 38–100 (moderate–catastrophic, n=24, 57.7±6.9 years, 18M/6F). T1-weighted images with a resolution of 1x1x1mm were acquired using a Philips Intera 3T MRI scanner. Whole-brain group comparisons, including age, handedness, sex, and hearing loss (pure tone average) as covariates, were performed to examine changes in cortical morphometric features (FreeSurfer v7.1.1). Cortical thickness and cortical volumes were investigated as independent metrics of grey matter integrity. Control analyses evaluated if these changes could be explained by depression, anxiety or hyperacusis, which were correlated with tinnitus-related severity. All results were corrected for multiple comparisons (cluster size pFWE<0.05, with p=0.001 cluster-forming threshold).

Results: Compared to participants with both slight and mild tinnitus severity, participants with moderate–catastrophic tinnitus severity had unexpectedly higher cortical volumes in the primary visual cortex (V1/V2). In contrast, participants with mild tinnitus severity showed lower cortical thickness and volumes of the medial prefrontal cortex (MPFC) compared to those with slight tinnitus severity. The same contrast also revealed decreased cortical thickness in the right inferior partial lobule (IPL). Whole-brain correlation analyses suggest that these effects were not the result of anxiety, depression, or hyperacusis. We furthermore found no significant association between THI scores and cortical thickness or volumes across the whole group with our strict statistical threshold.

Conclusions: The association between tinnitus severity and changes in visual cortex grey matter were unexpected. Decreased grey matter in the medial prefrontal cortex (MPFC) of tinnitus participants has been reported in two previous studies (Muhlau et al. 2006; Husain et al. 2011). Our results suggest that mild but not moderate–catastrophic tinnitus severity is associated with decreased MPFC grey matter thickness and volumes. Our results together with previous conflicting evidence suggest that grey matter integrity might not be the best proxy for tinnitus or tinnitus-related distress (Vanneste et al. 2015).

Exploring the Middle Ear in Control Subjects and Patients Reporting Tinnitus and a Cluster of Other Otic Symptoms
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Background: After moderate to severe acoustic trauma, it has been described that some patients can develop a cluster of symptoms in addition to tinnitus, i.e. hyperacusis, pain in and around the ear, earfullness, acoustic

distortion and/or middle ear myoclonus. Some of these symptoms (pain in and around the ear, earfullness and myoclonus) suggest that the middle ear is involved in this clinical condition. Some authors have hypothesized that a tensor tympani dysfunction may play a critical role in this syndrome. Our present study is aimed at exploring the middle ear in control subjects and patients reporting the cluster of symptoms described above using two methods: the acoustic admittance and pressure.

Methods: This study was carried out in 12 control subjects (including 5 subjects who can contract the middle ear muscles voluntarily) and 9 patients reporting diverse symptoms among tinnitus, hyperacusis, pain in and around the ear, earfullness, acoustic distortion and/or middle ear myoclonus. The acoustic admittance was measured in a soundproof room or a quiet office using the Madsen Zodiac (Natus Medical, formerly Otometrics, Denmark). The pressure was measured using an in-house device developed in our lab.

Results: At 0daPa in the external auditory meatus, the voluntary and stimulus-induced MEM contractions (vMEMC and sMEMC, respectively) were both associated to a decrease in admittance. In terms of the changes in pressure, the sMEMCs were without effects or associated to an increase in pressure (in 30% of recordings), while the vMEMCs were always accompanied by a decrease in pressure. Our results suggest that sMEMCs are dominated by stapedius muscle contraction, while vMEMCs are dominated by tensor tympani muscle contraction. In a second study, we explored the MEM using the two methods in 9 patients reporting tinnitus and other otic symptoms (see above). We observed changes in admittance and/or pressure while patients were doing somatic maneuvers (n=5), when activating trigger points (n=2), during sound stimulation (n=1) or at rest (n=1). These changes seemed dominated by tensor tympani contraction (large changes and decrease of pressure).

Conclusions: Using acoustic admittance and pressure measurements, we were able to differentiate between the tensor tympani muscle contraction from the stapedius muscle contraction. Our results suggest that sMEMCs are dominated by stapedius muscle contraction, while vMEMCs are dominated by tensor tympani muscle contraction. Moreover, we were able to demonstrate changes in admittance and pressure while patients were doing somatic maneuvers or were pushing on trigger points. These latter observations indicate that MEMs play a role in patients' symptoms and that the MEMs are abnormally connected to the somatosensory system.

CRISPR/Cas9-Mediated Knock-In of WDPCP Mutation rs61734468 in Human Neural Progenitor Cells to Investigate Morphological Disparities in Differentiated Neurons

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Background: Tinnitus, the phantom perception of sound that seemingly originates from within the ear, affects more than 10% of the population. Of the tinnitus-afflicted population, around 5% report particularly severe and debilitating tinnitus, which often leads to significant tolls on cognitive function and quality of life. The current mechanisms underlying tinnitus remain unknown; however, recent studies have identified hyperactivity along the auditory pathway as a consequence of malfunctioning neural plasticity responses as one possible mechanism underlying tinnitus. Recently, we completed a genome-wide association study which revealed a significantly implicated single nucleotide polymorphism (SNP) (rs61734468, p=3.959x10-10) in the planar cell polarity effector gene WDPCP. This gene codes for the WDPCP protein, which has been found to be responsible in mediating septin cytoskeletal complexing, especially during collective cell migration and ciliogenesis. Additionally, our identified SNP was found to be previously implicated in a neuronal ciliopathy, Bardet-Biedl syndrome-15, and may serve as a possible mutation predisposing individuals to developing tinnitus after significant noise or environmental trauma. Subsequently, to investigate the results of our GWAS in vitro, as well as to identify whether malfunctioning cytoskeletal organization may underly the development of tinnitus, we aimed to analyze the morphological characteristics of rs61734468-mutant neurons derived from human neural progenitor cells (NPCs).

Methods: Using CRISPR/Cas9, we generated a WDPCP-mutant knock-in human neural progenitor cell line, which was screened for off-target gene edits using Sanger Sequencing.

Results: Rates of knock-in NPC apoptosis activity were compared to control NPCs to measure cell viability. Knock-in NPCs were further differentiated into either neurons or astrocytes, and we subsequently conducted morphometric assays to analyze dendritic spine numbers and average number and length of neuronal processes.

Conclusions: The results of this study may serve to validate the findings of our original GWAS. Furthermore, they may demonstrate that malfunctioning cytoskeleton rearrangements could underly the development of tinnitus, possibly through misdirected neuronal migration during development or through maladjusted plasticity responses

in both childhood and adulthood. Our study also serves as a potential strategy for testing other SNPs associated with tinnitus.

Cochlear Electrical Stimulation to Suppress Tinnitus in Rats

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Background: Cochlear electrical stimulation (CES) via cochlear implants is known to generate promising results in managing patients' tinnitus. However, the underlying mechanisms of CES-induced tinnitus suppression remains unclear.

Methods: In this study, we set out to implant the rat cochlea via the round window or the modiolus. An operant behavioral paradigm of Conditioned Licking Suppression was used to test for tinnitus following noise exposure and CES. Electrophysiological recordings were conducted in the left auditory nerve (AN) and right auditory cortex (AC) simultaneously.

Results: In the acute experiments, we found that noise exposure (8-16 kHz, 110 dB SPL, 1 hour) resulted in a decreased spontaneous firing rate in the AN and an increased activity rate in the AC. In the chronic experiments, we found that after noise exposure to induce tinnitus, rats with behavioral evidence of tinnitus manifested with a decreased spontaneous firing rate in the AN and increased activity rate in the AC. We also found that CES yielded suppression of behavioral evidence of noise-induced tinnitus. The behavioral result was accompanied by the reversal of activity changes in the AN and AC.

Conclusions: The results have demonstrated that noise-induced tinnitus may result from decreased spontaneous activity in the AN and increased spontaneous activity in the AC and that CES-induced tinnitus suppression may result from CES-induced reversal of the noise-induced decrease in the AN activity and the increase in AC activity rates.

A Qualitative Characterization of Perspectives among Dizzy Patients on Social Media

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Background: Vertigo attributed to vestibular dysfunction has a lifetime prevalence of 7.8%, with approximately 80% of affected individuals reporting interruption of daily activities and work absenteeism due to symptoms. To help patients experiencing such symptoms, the Vestibular Disorders Association (VeDA) support group was created in 1985. One of the organization's initiatives was to create a Facebook group in 2015 titled "Vestibular Disorders Support Group," which has grown to more than 17,000 users. This group is primarily composed of individuals suffering from balance, dizziness, vertigo, or any diagnosed vestibular disorder. Previous studies have explored how social media has been utilized in the medical realm, however, there have been no investigations in quality and content of social media use by patients experiencing vestibular symptoms. Our study aims to qualitatively evaluate the content of communication in the largest Facebook community dedicated to vestibular disorders.

Methods: Based on content analysis strategies, we generated descriptive codes from a sample of 100 posts and developed a coding scheme. Three study personnel will then manually code 50 posts in this sample to establish sufficient interrater agreement. These coders will proceed to categorize all posts from December 1st, 2020 to December 1st, 2021. Discrepancies will be resolved by the senior author.

Results: From an initial set of 100 Group posts, we found 27% of posts to be "Shared experiences – symptoms/diagnosis," containing questions or comments regarding symptoms or diagnostic testing experienced by others or by self. An example of this is a question asking if other patients experience lightheadedness in certain scenarios. Fourteen percent of posts were classified as "Information or education – question," containing questions regarding specific information, news, or other educational material regarding diagnosis or treatment. Eleven percent of posts were designated as "Shared experiences – treatment" and "Illness narrative." These categories refer to questions or comments regarding medications/treatments used by others or by self, and posts describing experiences with vestibular disorders, respectively. Posts describing negative emotions or requesting emotional support constituted 9% of posts. There were no posts that attempted to disseminate information regarding non-evidence-based diagnoses, diagnostic procedures, or treatments.

Conclusions: Thus far, we have found that users post primarily about symptoms or diagnostic procedures they or others experience. The second most common category contains specific questions regarding diagnosis and

treatment. These findings indicate lack of patient education, perhaps due to lack of knowledge transfer during clinic visits. Characterizing information-seeking and information-sharing behavior of vestibular disorder patients has multiple benefits. For example, providers can hone their clinical practice by focusing on commonly-posted concerns and policymakers can work towards better health infrastructure to address unmet needs. Ultimately, this data has the potential to improve quality of life in the vestibular patient population.

Glutamate Decarboxylase Immunoreactivity in the Human Inner Ear: A Pilot Study

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Background: The two glutamate decarboxylase (Gad) genes, Gad1 and Gad2, are differentially expressed and produce GAD67 and GAD5, respectively, in neurons and astrocytes in the central nervous system (CNS), in amacrine, horizontal cells and Müller glia of the retina, and in pancreatic islet cells of Langerhans. GAD enzymes provide a crucial role of synthesizing gamma-aminobutyric acid (GABA), which is a major inhibitory neurotransmitter in the CNS and contributes to the efferent pathway of the vestibular and auditory systems. The localization of GADs their regulation of GABA synthesis is not well known. Recent data from transgenic mice demonstrate Gad1 and Gad2 expression patterns with unique GAD67 and GAD65 immunolabeling in the sensory epithelium of the crista and utricle. We hypothesize that cells in the human vestibular and cochlear sensory epithelium also express Gad1 and Gad2 contributing toward GAD67 and GAD65 GABA synthesis.
Methods: Eight human temporal bones were examined in accordance with the University of California, Los Angeles Institutional Review Board. Preparation of temporal bones for immunohistochemistry was previously described by Lopez et al (2016). Commercially available primary antibodies were used in this study; GAD65 (MAB351, Sigma), GAD67 (PA5-21397, ThermoFisherScientific), GABA (A2025, Sigma), vesicular GABA transporter (VGAT), Tubulin beta 3 (801209, BioLegend). Secondary antibodies were either fluorescently conjugated (ThermoFisherScientific) or streptavidin-biotin HRP/DAB (Vector Laboratories, Inc.). Confocal microscopy was acquired with either an Olympus FV1000 or Airyscan (Zeiss), and image analysis was performed using FluoRender.
Results: We observed GAD65 puncta colocalizing with beta-tubulin in spiral ganglion cells. In the utricle, GAD65 puncta were present beneath hair cells close to nerve terminals and adjacent to GABA. In the crista ampullaris GAD65 was observed in cells surrounding and beneath sensory hair cells. Additionally, immunolabeling for VGAT demonstrated puncta within vestibular nerve fibers below the crista ampullaris. GAD65 and GAD67 were present in discrete populations of cells in the cochlea and vestibular organs.
Conclusions: Immunolabeling of GAD65 and GAD67 in the human adult inner ear suggests these enzymes are present in the peripheral organs and provide important spatially and temporally regulated GABA synthesis.

Comparative Analyses of Synaptic Ribbon Distributions in Vestibular Epithelia: Insights Into Their Functional Architectures

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Background: Synapses within hair cells of vestibular epithelia exhibit broad heterogeneity in the architectures of presynaptic ribbon complexes. Through serial ultrastructure analyses of mouse utricular hair cells presynaptic complexes were found to exhibit architectures ranging from single ribbons to ribbon aggregates forming clusters. While ribbon architectures cannot be explicitly determined through ribbon immunolabeling and light microscopy imaging, insights can be gleaned through the application of super-resolution confocal imaging and reconstruction to obtain estimates of ribbon size. These approaches provide opportunities for broader sampling across vestibular epithelia than can be achieved by relying upon ultrastructural methods. Having achieved initial results from murine cristae, the present investigation was undertaken to investigate the distribution of ribbon synapses in vestibular epithelia from chinchillas. In addition to their use as a model of vestibular hypofunction (Sultemeier and Hoffman, 2017), they represent animals exhibiting greater behavioral agility than other rodents. Such enhanced agility may place greater demands on the vestibular periphery to provide high-fidelity head movement

coding for sensory gaze stabilization. Consequently, the present investigation harbors the potential to provide insight into the contributions synaptic ribbon size distributions supporting contrasting levels of behavioral agility. **Methods:** Following euthanasia crista epithelia were harvested from adult chinchillas and rapidly exposed to fixative by infusing 4% paraformaldehyde (in 0.1M phosphate buffer) into the oval window and incubated for 3 hours. Following microdissection epithelia were either processed intact or embedded into low melting point agar and vibratome-sectioned. Neuroepithelia were processed for immunohistochemistry by incubating first in a standard blocking solution followed by primary (1:250 each of anti-CtBP2, anti-beta III-tubulin, and anti-oncomodulin) and secondary antibody solutions. Specimens were mounted with anti-fade medium, after which they underwent super-resolution confocal microscopy analyses (Zeiss LSM880 Airyscan). Surface reconstructions of ribbon puncta and calyces were created using intensity and local signal:noise (i.e. k-means) criteria. For this analysis, only ribbons within crista central zones and within 1μm of a calyx or parent axon were included. Distributions of ribbon volumes, surface areas, and sphericities were compared through resampling analyses of the relative entropies (Kullback Leibler divergence), comparing morphometric parameters between the two species.

Results: Striking differences in the distributions of ribbon morphometries were in central zone ribbons between chinchillas and mice. Inner and outer face ribbons in chinchillas exhibited a much narrower range of volumes and surface areas, and the larger ribbons found in mice cristae were not found in chinchilla cristae ($p < 10^6$).

Conclusions: The results of the present analyses demonstrate that the functional attributes of presynaptic architectures associated with the largest immunolabeled ribbon puncta are not associated with the high-fidelity head movement coding seemingly in demand for highly agile animals. This result provides further rationale for detailed investigations of the diversity in functional architectures found in mammalian vestibular epithelia.

Vestibular Evoked Myogenic Potentials in Equestrians

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Background: Engaging in balance demanding physical activities (e.g., gymnastics) or changes in the human-environment dynamics (e.g., microgravity) leads to vestibular adaptation. Increasing evidence suggests sports-induced adaptation in sacculo-collic reflex. After years of experience performing high demanding balance tasks, yoga practitioners and dancers exhibited shortened latency and increased amplitude in cVEMP. Professional divers also exhibited shortened cVEMP latency. Such vestibular adaptation suggests increased vestibular sensitivity for enhancing performance. Experienced equestrians exhibit outstanding postural stability during upright stance in a dynamic environment yet experience extensive body movement in the sagittal plane while riding. How the equestrian experience shapes vestibular responses remain unclear. This study attempted to objectively evaluate whether there might be sports-induced adaptation of sacculo-collic reflex and utriculo-ocular reflex associated with equestrian practice.

Methods: Sixteen active equestrians (AE; 14F; 23.3±3.8 years) with 15.6±5.7 years of equestrian experience and fifteen non-equestrians (NE; 8F; 28.9±5.4 years) with regular exercise routine participated. Subjects were excluded for history of concussion, neurological or musculoskeletal disorders, hearing or vestibular deficits, and corrected visual acuity < 20/40. cVEMP and oVEMP were performed using Interacoustics EP25 (Denmark) with subjects in supine and 30° trunk elevation. Air-conducted sound stimuli consisted of 500Hz, 100dB nHL tone-burst (2ms rise/fall, 2ms plateau), a repetition rate of 5.1Hz, and 100 sweeps/trial. For cVEMP, electromyographic activity was obtained from sternocleidomastoid; referenced to sternum; grounded to forehead. Participants turned their heads away from the testing side and lifted their head off the pillow to reinforce sternocleidomastoid contraction. P13 and N23 were identified for latency and normalized amplitude. For oVEMP, inferior obliques activity was obtained; referenced to forehead between eyebrows; grounded to upper forehead; with a 20° upgaze fixating at a target on the ceiling. N10 and P16 were identified for latency and amplitude.

Results: The response rate or oVEMP and cVEMP corroborates with prior findings at 90-100%. For oVEMP, significantly reduced N10 amplitude, P16 amplitude, and peak-to-peak amplitude were found in AE (ps<.01); suggesting sports-induced adaptation in utriculo-ocular reflex. No group difference emerged in N10 latency or P16 latency (ps>.05). For cVEMP, no group difference emerged in any of the parameters (ps>.05).

Conclusions: The main finding around the adaptation in utriculo-ocular reflex and its pathway associated with equestrian engagement is novel. Prior work showed increased VEMP amplitude due to increased balance threats while standing on elevated surface. Reduced oVEMP amplitude in AE may suggest that equestrians become less prone to balance threats as they routinely perform balance tasks on horseback. Alternatively, reduced oVEMP

amplitude in AE could be due to repetitive impact although none reported any concussion history. Whether sports-induced adaptation has a long-term impact for good or bad on balance control into middle-age or older adulthood awaits further investigation.

Assessment for the Utricular Function Using Centered and Eccentric Rotation--Comparison With Ovemp--

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Background: Vestibular evoked myogenic potential (VEMP) is now established as a reliable examination for the otolith organs. However, since the organs are essentially linear accelerometers, it is not a physiological response. Despite centered rotation (CR) causes angular acceleration, eccentric rotation (ER) causes centrifugal acceleration additionally that stimuli otolith organ especially utricle. There are some reports to assess the utricular function by means of comparison between vestibulo-ocular reflex (VOR) in CR and that in ER. The aim of this study was to confirm the consistency of the two assessments for utricular function.
Methods: The utricular disorder group used 6 subjects with unilateral absence of ocular VEMP and normal results of video Head Impulse Test. They aged 45 to 58. The control group consisted of 5 healthy adults ranged in age from 29 to 48. The VOR gains for CR and ER were measured in both groups. Their heads were placed on the axis of the rotation during CR and shifted 30 cm forward from the axis during ER. VOR gain was distinguished by rotation toward the affected side and rotation toward the unaffected side for the utricular disorder group. Sinusoidal rotation with a maximum velocity of 50 degrees/sec was employed at 0.1, 0.2, and 0.5 Hz in each trial.
Results: For the control group, while the gain did not change with frequency in CR, the gain of 0.5Hz was higher than that of 0.1Hz and 0.2Hz in ER. For the utricular disorder group, there was also no frequency-dependent gain difference in CR. On the other hand, higher gain in ER at 0.5Hz were observed in the rotation toward the affected side but not in the rotation toward the unaffected side.
Conclusions: As the angular frequency increases, the centrifugal acceleration increases and the stimulation to the otoliths increases. Thus, the higher gain at 0.5 Hz in ER was derived from utricular reaction. This higher gain was interestingly observed in the rotation toward the affected side but not in the rotation toward unaffected side. The fact showed the laterality of the VOR induced by the utricle. This study indicates that the results of oVEMP and results of the eccentric rotation may be of equal value. The limitation of this study is a small number of cases series. Study with a larger number is required.

Effects of Different Anesthetic Regimens on Vestibular Afferent Responses to Efferent-Stimulation in Inbred Mouse Strains

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Background: The responses of mammalian vestibular afferents to electrical stimulation of vestibular efferent neurons rely on multiple cholinergic mechanisms. To date, three efferent-mediated afferent responses have been identified according to their kinetics and pharmacology: (1) a fast excitation mediated by alpha4/alpha6/beta2-containing nicotinic ACh receptors ($\alpha4\alpha6\beta2$*nAChRs), (2) a slow excitation mediated by muscarinic ACh receptors (mAChRs), and (3) an inhibition presumably driven by the activation of $\alpha9/\alpha10$AChRs and SK2 channels. Recently, we have demonstrated that efferent-mediated afferent responses in mice are generally similar to those recorded in squirrel monkey, chinchilla, and cat. However, efferent-mediated fast excitation in mice anesthetized with urethane-xylazine is noticeably smaller and less-frequently observed when compared with response data from these other species. Since our anesthetic regimen in mouse differs from anesthesia protocols used in these previous preparations, we hypothesized that efferent-mediated fast excitation may be attenuated by a component of our anesthetic cocktail. Here, we compared efferent-mediated afferent responses in four common strains of mice under different anesthetic regimens to probe if urethane or xylazine exhibited any pharmacological effects on efferent-mediated fast excitation.
Methods: We performed extracellular recordings from vestibular afferents during electrical efferent stimulation in C57BL/6, Balb/C, FVB/N, and CD-1 mice anesthetized with Urethane/Xylazine (1.6 g/kg/20 mg/kg), Avertin (i.e., TBE, 250 mg/kg), or Ketamine/Xylazine (90 mg/kg/10 mg/kg). Intraperitoneal (IP) or intrabullar (IB) administration of selective cholinergic compounds were used to pharmacologically characterize efferent-mediated fast excitation, slow excitation, and inhibition. Urethane or xylazine was administered via the IB route to probe their direct pharmacological effect on efferent-mediated fast excitation.

Results: All mouse strains anesthetized with Avertin or Ketamine/Xylazine showed that efferent-mediated fast excitation is more common and several fold larger in amplitude than in mice anesthetized with Urethane/Xylazine. Furthermore, direct application of urethane to the middle ear of avertin-anesthetized mice antagonized efferent-mediated fast excitation without effects on efferent-mediated slow excitation. IB xylazine administration, however, did not impact either efferent-mediated fast or slow excitation. In mice anesthetized with Avertin or Ketamine/Xylazine: 1) efferent-mediated afferent responses predominantly consisted of fast and slow excitation, whose response parameters including amplitude, duration, and activation time constants were comparable; and 2) mean response amplitude for the efferent-mediated fast excitation is comparable to other mammalian species. Pharmacology, however, appeared unaffected across the three anesthetic conditions where efferent-mediated fast excitation was selectively blocked by the nAChR antagonists DHβE and 5-iodo-A-85380 while efferent-mediated slow excitation was sensitive to mAChR agents muscarine, oxotremorine, glycopyrrolate and scopolamine. Finally, efferent-mediated inhibition was completely blocked by the α9/α10nAChR antagonist strychnine.

Conclusions: Collectively, the kinetics and pharmacology of efferent-mediated afferent responses are comparable across multiple mouse strains and various anesthetic protocols; however, considerations about avoiding the use of urethane anesthesia in mice might be warranted when characterizing efferent-mediated fast excitation.

Different of Brain Metabolite and Structural Volume of Central Vestubular System According to the Dominant Hemisphere in Healthy Subjects

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Background: It is well known that compensation like structural change of the central vestibular system plays an important role in the recovery of patients with unilateral vestibular loss. However, most of the studies were conducted through comparison of lesions and normal without considering dominant vestibular cortex. This study was designed to confirm baseline characteristic feature of hemispheric dominance of the vestibular cortical system of healthy controls who were expected to compare with unilateral vestibular failure patients. To this end, gray matter (GMV), white matter (WMV), and brain metabolites of left and right central vestibular system in right-handed healthy person who have never experienced dizziness were compared

Methods: We included 23 healthy volunteers who visited Kyung-Hee university hospital at Gang-Dong from Mar.2016 to Mar.20180. We performed magnetic resonance imaging for calculating GMV and WMV of central vestibular network of both side and 1H MR spectroscopy for collect brain metabolite in the parietal operculum2

Results: In the result of structural comparison, GMV of Rt side parietal operculum 2, caudate, Insula, percuneous area were significantly higher than those of Lt side (p<0.001). WMV of Rt sided caudate, cuneous, percunous area were significantly higher than those of Lt side (p<0.001) but WMV of Rt sided thalamus , Insula were significantly lower than those of Lt side.

Following metabolites of MR spectrography concentration of left side was significantly higher than those of right side. (Total Choline ratio, Glx ratio) In contrast, following metabolites showed apposite result. (N-acetylaspartate(NAA), N-acetylaspartate to creatinin ratio(NAA/Cr)) Among metabolites, left NAA, Rt glutamate, Rt NAA/Cr showed significant correlation that those metabolite values were decreased as one grows older. Furthermore, Lt NAA, Lt glutamate, showed proportional correlation to GMV.

Conclusions: There were significant difference of structural volume and concentration of brain metabolite in central vestibular system according to dominant hemisphere in healthy subject, So the study for vestibular compensation, we should consider this difference of baseline characteristic feature

Repair of Surviving Hair Cells in the Damaged Mouse Utricle

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Background: Sensory hair cells of the utricle are mechanoreceptors required to detect linear acceleration. While cochlear hair cell loss in mammals is irreversible, the utricle is capable of regenerating lost hair cells. Although regenerated hair cells are few and immature, recovery of function as measured by vestibular stimuli evoked potential (VsEP), a brainstem response as a result of a linear acceleration exerted on the head, has been reported. Whether native surviving hair cells contribute to this recovery is unclear. Here, we fate-mapped hair cells prior to hair cell ablation and characterized these surviving hair cells during the recovery of VsEP thresholds.

Methods: We injected tamoxifen and diphtheria toxin in P1 Atoh1-CreERT2; Rosa26tdTomato; Pou4f3-DTR transgenic mice and used Atoh1-CreERT2; Rosa26tdTomato mice as control. Animals underwent VsEP testing at P15 and P30 prior to tissue harvest. Antibodies against Osteopontin (type I), AnnexinA4 (type II), and Myo7a (both) were used to determine hair cell subtypes, and Tuj1 for identifying neurites. Fluorescence-conjugated phalloidin was used to label hair bundles. Animals with and without VsEP recovery were separately analyzed.
Results: After damage, no VsEP responses were detected in any P15 animals, with 57% of damaged animals recovering partial function at P30. Approximately 44% of hair cells were fate-mapped at P3, and about 23% of which survived at P15 after damage. 14% of survived at P30 in the VsEP recovery group while only 4% survived in the non-recovery group. In the VsEP recovery group, surviving Atoh1-tdTomato+ hair cells consisted of both type I and II hair cell subtypes (~50% each). The numbers of hair cells and each hair cell subtype were significantly lower in the utricles without VsEP recovery. Most fate-mapped type I hair cells lost calyces after damage with many regaining calyces in the P30 recovery group (37%), while all surviving type II hair cells-maintained innervation. Both subtypes maintained co-localized pre- and post-synaptic proteins. Finally, after damage, most surviving hair cells had short stereociliary bundles at P15 (91%), while many (41%) displayed long bundles in the P30 recovery group.
Conclusions: Surviving hair cells in the damaged utricle maintain subtype specialization, regain innervation, and display more mature bundles with time as the organ recovers function. This contrasts with regenerating hair cells previously reported to be primarily type II hair cells with short and immature bundles, and the few type I hair cells regenerated were devoid of calyces. These results suggest that mammalian vestibular hair cells are capable of repair and may contribute to the recovery of organ function.

A Novel Epitope-Tagged Clarin-1 Knock-In Mouse Displays Congenital Deafness

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Background: Usher syndrome type III (USH3) is a recessive inherited disorder caused by mutations in the Clarin-1 (CLRN1) gene, leading to progressive hearing loss and retinal degeneration. In this study, we report an unexpected phenotype in a C-terminal hemagglutinin (HA) epitope-tagged Clrn1 knock-in mouse that was generated to allow detection of CLRN1 protein in sensory organs.
Methods: Hearing function in age-matched C-HA tagged Clrn1 knock-in and corresponding wild-type controls was assessed by acoustic brainstem response (ABR) testing. Immunohistochemistry and scanning electron microscopy (SEM) were used to evaluate the auditory hair cells survival and morphology.
Results: Unexpectedly, we have found that homozygous C-HA Clrn1 knock-in mice display profound congenital hearing loss. Remarkably, the auditory phenotype of the homozygous C-HA Clrn1 knock-in mice is even more severe than that of Clrn1 knockout (KO) or N48K knock-in mice, which display an early onset hearing loss. Additionally, cochlea histology in the HA Clrn1 knock-in mice shows abnormal stereocilia hair bundles and progressive inner and outer hair cell loss. Taken together, these results indicate that the placement of the HA-tag at the C-terminal end of CLRN1 interferes with its biological functions in the cochlea. We detect a characteristic diffuse HA-tagged CLRN1 protein band by Western blot analysis in retinal lysates from C-HA Clrn1 knock-in mice throughout development and adulthood. However, CLRN1 protein in the cochlea tissue was below the detection limit of immunoblotting or immunohistochemistry assays, suggesting it is expressed at very low levels.
Conclusions: Our study shows that the presence of the C-HA tag in the wild-type CLRN1 sequence increases the severity of the auditory phenotype relative to mouse models of USH3, highlighting the importance of the C-terminal sequence for CLRN1 function in vivo.

Auditory Function in Dementia Mouse Mutants

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Background: Hearing loss and dementia are two of the most common age-related disorders. The World Health Organization estimates that 466 million people suffer from hearing loss, and that a third of people >65 are hearing impaired. Dementia is estimated to affect 50 million people, with the most common type being Alzheimer's

disease (AD) and vascular dementia (VaD) being the second most common. Hearing impairment has been reported to be a risk factor for dementia(1), but it is not clear if there is a causal relationship. Our goal with this project was to determine if any cochlear functional deficits preceded or followed the onset of dementia.
Methods: We used the 5xFAD mouse and littermate controls to study AD. The mutant carries a transgene expressing mutations known to predispose to AD in humans: four familial AD- (FAD-) associated mutations of the APP gene (K670N, M671L, I716V, V717I) and two FAD-associated mutations of the PSEN1 gene (M146L, L286V). This mutant has been shown to accumulate intra-neuronal Aβ42 from 1.5 months, have abundant amyloid plaque deposits and gliosis from 2 months-old(2), and neuronal loss from 6-9 months-old(3). The 5xFAD mouse demonstrated impaired auditory startle response at 4 months-old(4) and cognitive decline beginning around 3 months-old(5,6). We used a double mutant Apoe<tm1Unc>/Nos3<tm1Unc> mouse to study VaD, as these mutations deplete APOE(7) and eNOS(8) proteins. APOE-deficient mice had cognitive decline from 8 months-old(9) and eNOS-deficient mice showed decline at 14-15 months-old(10). We used age-matched C57BL/6N as controls for our VaD mutants. Auditory brainstem response thresholds and wave I input/output functions across 3-42kHz from 4 weeks-old to 12 months-old were analysed. Additional tests were used to assess frequency tuning, forward masking, click-repetition rate, tone-in-noise, and intertrial coherence.
Results: Our results for the 5xFAD colony showed hemizygous and homozygous mutants had no difference in hearing thresholds and wave I response amplitudes and latencies when compared to each other and littermate controls at 4, 8, and 14 weeks-old and at 6 months-old. The Apoe<tm1Unc>/Nos3<tm1Unc> homozygotes showed no hearing impairment compared to controls from 1-9 months, but did show deficits in suprathreshold function and temporal processing at 12 months-old. Longitudinal testing for the 5xFAD colony is still ongoing for older ages.
Conclusions: In summary, we see little or no evidence of impaired hearing before reported signs of dementia. This work was supported by the NIH/NIDCD.

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Identification of Galectin-3 as a Cochlear Molecule for Sex-Dependent Auditory Dysfunction in Aging Mice
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Background: Sex differences in the development of sensorineural hearing loss have been recognized in certain inner ear disorders, but the molecular basis for those differences is not clear. We previously reported cochlear expression of galectin-3 (Gal-3), a member of the β-galactoside-binding protein family that is involved in multiple biological functions, including immune response, apoptosis, and cell adhesion. In the cochlea, we have shown that Gal-3 is expressed in supporting cells and immune cells and participates in cochlear responses to acoustic trauma. The current study examined the role of Gal-3 in the preservation of auditory function and sensory cell integrity in aging mice.
Methods: Gal-3 knockout (Lgals3-/-) (B6.Cg-Lgals3tm1Poi/J, homozygous), heterozygous (Lgals3-/+) and age-matched wild-type (Lgals3+/+) mice were examined at 1 month, 3 months and 6 months of age. Heterozygous mice were generated by crossbreeding B6.Cg-Lgals3tm1Poi/J mice with C57BL/6J mice. Auditory brainstem responses (ABR) were measured to assess hearing sensitivity, distortion-product otoacoustic emissions (DPOAE) were quantified to evaluate outer hair cell function, and acoustic startle reflexes were collected to examine the amplitude of the brainstem auditory-motor circuit. Sensory cells were quantified to determine the level of cochlear pathogenesis.
Results: At 1 month of age, Lgals3-/- mice and Lgals3+/+ type mice displayed similar ABR thresholds with no significant sex differences. From 3 to 6 months, while both Lgals3-/- mice and Lgals3+/+ mice displayed an

elevation in ABR thresholds, female Lgals3-/- mice displayed significantly greater ABR threshold shifts as compared to both male and female Lgals3+/+ mice as well as to male Lgals3-/-. DPOAE input/output (I/O) function revealed a similar pattern of functional reduction, with the female Lgals3-/- mice showing a significantly greater reduction in DPOAE amplitude with increasing age. Further acoustic reflex testing revealed that the slope of startle reflex amplitude I/O functions in Lgals3-/- females was less than in Lgals3+/+ females but there was no significant difference in the slope in males. Comparison of ABR thresholds among homozygous, heterozygous, and wild-type mice revealed that homozygous female mice displayed the most significant auditory dysfunction. Heterozygous mice also showed increased threshold shifts with age, but the level of the shift was smaller than that observed in homozygous mice. Consistent with the functional results, females displayed greater hair cell loss compared to males in both the Lgals3+/+ and Lgals3-/- groups. Noticeably, female Lgals3-/- mice had the greatest number of missing hair cells in aging ears.

Conclusions: Female Lgals3-/- mice exhibit accelerated age-related hearing loss and cochlear degeneration compared to their male counterparts. These results suggest that Gal-3 expression in females preserves auditory function and sensory cell integrity. Thus, loss of Gal-3 function could serve as a molecular mechanism for female-associated auditory dysfunction. Our finding provides new insights into the molecular mechanisms underlying sex-dependent hearing loss.

Central Gain With Degraded Neural Synchrony: A Translational Model of Aging Auditory Deficits
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Background: The auditory nerve (AN) degrades with age, leading to decreased afferent input, poorer neural synchrony, and auditory processing deficits. Acute injury to the inner ear can also lead to AN loss, resulting in decreased response amplitudes and a loss of neural synchrony. The auditory midbrain compensates for this loss of peripheral AN activity via amplification (central gain). However, even though amplitudes are increased, neural synchrony remains disrupted. It is unknown whether the same process occurs with age. We hypothesized that older mice and humans exhibit central gain without an improvement in neural synchrony (measured by phase locking value) in the midbrain.

Methods: AN and midbrain function were assessed via electrophysiologic responses (CAP and ABR). Measurements of amplitude and neural synchrony in the AN and midbrain were compared across younger and older age groups of mice (CBA/CaJ; 2.5 [SD = 0.6] months: n=24 ears; 26 [3.5] months: n=16 ears) and humans (24 [3] years: n=39; 66 [6.6] years: n=57). The neural synchrony across trials was assessed by calculating inter-trial phase-locking values (PLV).

Results: In both mice and humans, results were consistent with central gain, with larger age-related amplitude reduction observed at the level of the AN than at the midbrain. In contrast, measures of neural synchrony were significantly lower in both the auditory nerves and midbrains of older mice and humans, compared to younger groups.

Conclusions: These translational findings demonstrate that age-related peripheral neural degeneration contributes to central gain, largely preserving midbrain potentials, but central gain fails to recover losses in neural synchrony. Persistent deficits in neural synchrony may contribute to auditory processing deficits observed in older mice and humans. Furthermore, we can use this translational model to further investigate the specific sites of pathology and underlying mechanisms that contribute to functional deficits in the aging auditory system.

Neural Representation of Auditory Selective Attention in EEG Signals
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Background: Electroencephalography (EEG) has long been used to study auditory selective attention by measuring how different types of attention affect brain activity. Selective attention modulates both evoked responses (such as the N1 and P1 waves, whose amplitudes change in the presence of attention), as well as induced responses (such as oscillatory power in the alpha frequency band, 8-14 Hz). However, it is not well established exactly what features of the attentional state are represented at different points in time. In this study, we extracted representational similarities from EEG signals and examined their dynamics when auditory attention is deployed.

Methods: We designed an auditory experiment that required spatial, non-spatial or no attention from listeners (n=30). The experiment had many (21) conditions so that we could build rich representational dissimilarity matrices (RDMs). At each timepoint during the experiment, for each pair of conditions, we trained a classifier algorithm to discriminate between trials belonging to one or the other condition using either (1) the EEG time course at that timepoint or (2) alpha band power at that timepoint. Leave-one-trial-out cross-validation protected against overfitting. The classifier's accuracy served as the estimate of dissimilarity between those conditions at that timepoint. This gave us two time series of RDMs, one derived from EEG time course and the other derived from alpha power. Finally, we characterized patterns in these RDMs using multidimensional scaling and comparison to conceptual models.

Results: RDMs derived from the EEG time course exhibit transient information encoding; attention can be decoded from the EEG time course only for a brief period after a cue or target stimulus was presented. However, RDMs derived from the alpha power show persistent information encoding; we could decode the type of auditory attention throughout the cue and the stimulus periods. In addition, information about the attended location and the gender of the attended talker is encoded in different EEG features and in different time intervals.

Conclusions: Stimulus-evoked neural states show only transient differences between conditions, but the induced activity encodes information for much longer. Results are in line with attention modulating sensory evoked responses in a transient way, while ongoing oscillations are related to effecting a particular attention state. As a whole, our results demonstrate that representational similarities in EEG signals are a viable approach to study the dynamics of auditory attention.

Effects of Spectro-Temporal Degradation on the Cortical Tracking of the Temporal Envelope of Speech
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Background: Cortical tracking of speech envelope is being extensively utilized to assess the speech processing in the auditory system as opposed to the conventional syllable evoked event-related responses. The use of longer stimuli as in cortical tracking experiments offers an ecological valid assay for speech processing that can be used to evaluate outcomes from hearing rehabilitative devices. However, before translation of cortical tracking measures into the clinic, it is vital to thoroughly understand the factors influencing cortical tracking metrics. The current study was aimed to evaluate if cortical tracking of the speech envelope is influenced by the intelligible content in speech. Thus, we sought to assess the cortical tracking of the temporal envelope while we parametrically varied speech intelligibility by degrading the spectral content in speech using chimerization.

Methods: Twenty-nine normal hearing young adults participated in the study. Cortical tracking was assayed using 64 channel surface electroencephalography (EEG). Sentence stimuli were used to assess cortical tracking. The intelligibility of the sentences was parametrically modified by altering the temporal fine-structure and spectral content using speech-speech and speech-noise chimerization. These approaches aided in degrading speech perception while keeping the broadband envelope of the stimuli constant. Cortical tracking was quantified using multivariate linear ridge regression which mapped the stimulus envelope information onto the EEG. Cross-validated regression model fits were used as metrics of cortical tracking. The regression model, i.e., the temporal response functions was also assessed to trace the differences in temporal properties of the brain aid in maintain the neural envelope representations.

Results: The results showed that cortical tracking of the temporal envelope is seen even when speech is not intelligible. However, manipulation of speech intelligibility modulated this entrainment. Speech-noise chimeras with intelligibility comparable to natural speech showed higher cortical tracking the natural speech. Speech-speech chimeras on the other hand, showed overall lower cortical tracking of the envelope. Within each type of chimera, cortical tracking increased with speech intelligibility. We used another unintelligible control chimera where the temporal envelope of speech was reversed, while preserving the temporal fine-structure. Though control stimulus was totally unintelligible, it resulted in cortical tracking metrics that were comparable to natural unprocessed speech. The temporal response functions however showed a graded change in morphology and temporal properties that were characteristic of the type of stimulus degradation.

Conclusions: Cortical tracking of speech envelope is affected by speech intelligibility manipulation, however, the strength of cortical tracking metric was not always linked to intelligibility of the stimulus. Cortical tracking of speech envelope shows a complex interaction with the stimulus intelligibility and the type of spectrotemporal

degradation. The inferences about speech processing should be approached in a more nuanced fashion considering the stimulus spectrotemporal features and underlying neurophysiological computations.

Enhancement of Speech-In-Noise Comprehension Through Transcranial Alternating Current Stimulation

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Background: Auditory cortical activity tracks speech rhythms, in particular at the rate of words (1 - 4 Hz, delta band) and at the rate of syllables (4 - 8 Hz, theta band). The modulation of this cortical tracking through alternating current stimulation with the speech envelope has been found to influence the comprehension of speech in noise. However, the stimulation can be performed with different parameters, such as a temporal delay, a particular frequency band and a potential phase delay. The influence of these stimulation parameters on speech comprehension remains insufficiently understood.

Methods: We presented human volunteers with single sentences that were embedded in noise to assess their speech comprehension. Simultaneously we stimulated the subject's auditory cortices through transcranial alternating current. The current waveforms were obtained from the speech envelope and were shifted by different temporal delays as well as phases. We also explored currents that were obtained from the speech envelope filtered in the delta and in the theta frequency bands.

Results: We first investigated two characteristic delays that emerge in the cortical tracking of speech rhythms, a short delay of 100 ms and a longer delay of 250 ms. We found that current stimulation at both delays influenced speech comprehension. Next, we applied current waveforms that followed either the delta- or the theta-band portion of the speech envelope. The theta-band current stimulation had a significant effect on speech comprehension, while the delta-band stimulation did not. We also investigated the influence of different latencies of the theta-band stimulation on the comprehension of speech in noise. We found that no latency yielded the highest speech comprehension, which was higher than under a sham stimulus. Last but not least, we also stimulated with the envelope of a distractor speaker. Surprisingly, this type of stimulation affected the comprehension of the foreground speaker. The phase dependency of the modulation was opposite to that when stimulating with the envelope of the target speech signal, hinting at a competition for neural resources between the cortical tracking of a target and a distractor speaker.

Conclusions: Our results show that the modulation of speech comprehension through transcranial alternating current stimulation is driven by the theta- but not by the delta band. Speech-in-noise comprehension can be enhanced when the current waveform is temporally aligned to the speech signal. Moreover, the rhythms of a distractor speaker are tracked by cortical activity as well, and influencing this tracking through transcranial current stimulation can affect the comprehension of a target speech signal.

Holographic Single-Cell In Vivo Stimulation of Circuits in Primary Auditory Cortex

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Background: In the developing nervous system, millions of neurons coordinate and refine their connections to give rise to incredibly complex behavior, such as speech and language. These behaviors require intricate networks within the auditory cortex. While the cellular components of these networks have been extensively characterized in brain slices and activity dynamics have been captured in awake, behaving animals using two-photon imaging and electrode arrays, our ability to manipulate these networks in vivo has been limited. Previous studies have relied on large disruptions in network activity, primarily through cooling, focal electrical stimulation, injection of GABAA agonists, or widespread activation of neurons expressing light-activated ion channels (opsins). However, to understand functional networks, the ability to selectively manipulate the activity of specific individual neurons is critical. Several recent advances make this level of manipulation possible. First, the development of red-shifted opsins permit "all-optical" experiments, where individual neuronal activity can be both observed with green genetically-encoded calcium indicators (like GCaMP) and manipulated with red-shifted stimulation. Additionally, coupling high-powered amplified lasers to spatial light modulators (SLM) allows focal stimulation of multiple points within 3D volumes, allowing unprecedented access to manipulate these circuits.

Methods: Here, we examine the efficacy of modern, red-shifted opsins (ChRimson, ChRmine, and ChroME) and their practicality to manipulate activity in the auditory cortex in awake behaving mice. Opsins are delivered via viral vectors or using the Ai167 (Cre-dependent ChRimsonR) mouse model to label subpopulations of neurons in

the auditory cortex. Cranial window implantation is performed to expose the auditory cortex, allowing visualization of neuronal activity and the ability to focally stimulate individual neurons.

Results: Using these approaches, we observed widespread neuronal expression of opsins within the auditory cortex and were able to focally stimulate groups of neurons, however, preliminary results with ChRimson viral vectors and transgenic animals revealed low ability to photostimulate these neurons, consistent with recently published reports. Development of near real-time approaches within the lab to both analyze neuronal networks and stimulate neurons of interest to investigate these cortical circuits will also be discussed.

Conclusions: Using these experiments as a guide, we seek to understand how cortical networks function in normal adult animals, how these networks change as a result of developmental perturbations such as hearing loss, and how these networks guide behavior. Understanding how these circuits function and what goes awry when these networks are altered may provide insight into developmental causes of dysfunctional wiring that leads to hypersensitivity to sounds and inability to discriminate speech in noisy environments.

Auditory Cortical Projection Neurons Exhibit Distinct Organizational Motifs

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Background: The auditory pathway is a beautifully complex series of brain stations that are dedicated to the processing and interpretation of our sense of sound. Incoming sensory signals traverse an ascending hierarchy from the cochlea to the primary auditory cortex (ACtx), before being propagated brain-wide through networks of excitatory projection neurons to inform emotion, attention, decision-making, and action. These neurons fall into three broad classes: intratelencephalic (IT), extratelencephalic (ET), and corticothalamic (CT). Of these classes, ET cells are unique as they form the only direct connection between the ACtx and myriad sub-cortical targets. Their distinct morphology, with prominent apical dendrites and diverse axonal targets, has led to their portrayal as canonical "broadcast" neurons that pool inputs and transmit signals throughout the brain. ET cells typically exhibit "one-to-many" connectivity motifs, placing them in a privileged position to broadcast sensory signals to multiple downstream targets simultaneously. However, the extent of their axonal collateralization, the spatial organization of their projections in downstream brain regions, and whether or not these distinct organizational motifs receive differential synaptic input remains unknown.

Methods: To address these questions, we characterized the input/output circuitry of ACtx ET cells and compared their anatomical organization to that of IT and CT populations. Using an intersectional strategy utilizing both Cre- and Flp-recombinase, we drove selective expression of adeno-associated viruses in distinct sub-populations of ET cells, allowing us to both quantify downstream projection densities with high spatial resolution and identify local and long-range synaptic input through monosynaptic rabies tracing.

Results: Our preliminary results indicate that the majority of ET neurons collateralize to the non-lemniscal regions of the inferior colliculus and thalamus, confirming previous reports (Chen et al, 2019). Monosynaptic rabies tracing demonstrated widespread synaptic inputs to both ET and CT cells from many cortical and subcortical areas, including the thalamus, contralateral ACtx, as well as ipsilateral visual, somatosensory, and retrosplenial cortices. Our ongoing experiments are focused on extending these findings to distinct ET organizational motifs.

Conclusions: This work will provide a foundation for understanding how brain-wide interactions between distinct areas cooperate to orchestrate sensory perception, guide behavior, and become disrupted in perceptual and neurological disorders.

Diverse Inhibitory Circuits in Layer 1 of the Mouse Primary Auditory Cortex

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Background: Layer 1 (L1) interneurons in rodent primary auditory cortex convey behaviorally relevant information by integrating sensory-driven inputs with neuromodulatory signals. These interneurons are therefore in a privileged position to regulate auditory perception and learning in a context-dependent manner. We and others have shown that these interneurons are heterogeneous and can be subdivided into two major subtypes, defined by the expression of neuron-derived neurotrophic factor (NDNF) or vasoactive intestinal peptide (VIP). However, the

unique circuitry of these two L1 interneuron subtypes is not fully understood. It remains unknown whether NDNF and VIP interneurons receive differential inputs from the thalamus and local excitatory and inhibitory neurons. Additionally, it is unknown whether they send distinct outputs to cortical targets.

Methods: We performed fluorescence-guided whole-cell electrophysiology in mouse thalamocortical slices to understand the inputs and outputs of NDNF and VIP interneurons in L1. Specifically, we recorded from L1 interneurons while electrically activating thalamocortical projections or while optogenetically activating subsets of L1 interneurons that express channelrhodopsin (ChR2), and from labeled cortico-striatal projecting neurons while optogenetically activating L1 NDNF or VIP interneurons.

Results: We found that NDNF and VIP interneurons receive distinct monosynaptic thalamic excitatory inputs. Our recordings also revealed GABAA-mediated synaptic connections within networks of NDNF and VIP interneurons. Finally, we characterized the inhibitory projections of these interneurons, and found that NDNF and VIP cells target distinct groups of excitatory cortico-striatal projecting neurons in L2/3 and L5 of the auditory cortex.

Conclusions: Together, our results suggest that L1 interneurons are differentially targeted by thalamic and local cortical inputs and inhibit different groups of excitatory projection neurons. We therefore propose that NDNF and VIP interneurons act within specialized circuits to perform different functions during auditory processing. By understanding the microcircuits in L1, we may shed light on the function of this distinct cortical layer in sound perception and cortical plasticity mechanisms.

Neural Decoding From Auditory Cortex Reveals Integration of Sound Texture Statistics in Behaving Mice

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Background: Identifying sounds of interest embedded in background noise is a common problem faced by the auditory system. Despite being a heavily researched topic in auditory neuroscience, so far, this problem has mostly been approached by using relatively simple classes of sound. Statistically defined auditory textures make up a large portion of what constitutes noise in naturalistic settings and present an opportunity to study this problem using more ethologically relevant sounds.

Methods: In this study, we utilized a behavioral paradigm where mice were trained to detect conspecific vocalizations embedded in sound textures at a random time in order to understand how this type of noise is processed in the mouse auditory cortex (ACX). Concurrently, we recorded populations of neurons in field AI of the left ACX while monitoring the animal's pupil diameter and licking responses.

Results: Mice learned to detect the vocalizations as evidenced by temporally aligned lick responses relative to target onset. The performance of the mice increased as a function of time since noise onset, measured by a temporally resolved d' measure, which suggests that they could use stimulus exposure to improve their detection ability. We observed an analogous increase in the neurally represented information specific to the target sound in relation to stimulus onset, which was quantified by reconstructing the target sound from the neural population response. Animals performed better and exhibited increased pupil dilation for auditory textures that contained higher correlations between frequency channels. Correspondingly, these stimuli could be reconstructed more precisely, although this result is preliminary.

Conclusions: We provide evidence for integration of statistical information during the processing of complex acoustic stimuli for the purpose of detecting a target sound. Furthermore, we show that mice were able to exploit across-frequency correlations beyond marginal statistics for improving their performance.

Neural Correlates of Stream Formation During Active Listening in the Ferret Auditory Cortex

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Background: Listening in the real world involves making sense of mixtures of multiple overlapping sounds. The brain decomposes such scenes into individual objects, and a sequence of related auditory objects forms a stream. How auditory streams are formed within the brain is not well understood. We are investigating the role of the auditory cortex (AC) plays in the formation and maintenance of auditory streams. The temporal coherence theory (Shamma et al., 2011) has provided one explanation for stream formation, postulating that the brain creates a multidimensional representation of the sounds along different feature axes, and then groups them based on their

temporal coherence, to form streams. Supporting this idea, neural correlates of the differences in perception elicited by synchronous and alternating tone have been found in the primary auditory cortex of behaving ferrets (Lu et al., 2017). However, the temporal coherence theory has yet to be tested with more naturalistic sounds composed of multiple streams. In humans performing a multi-talker listening task, the primary auditory cortex represents both attended and non-attended streams, whereas non primary auditory areas showed an enhanced representation of the attended speech (O'Sullivan et al., 2019). The literature on auditory stream segregation is lacking animal models to understand the neural mechanisms of target enhancement / distractor suppression.
Methods: To test the temporal coherence principle, we trained ferrets to detect a target word in a stream of repeating distractor words, spoken by the same talker, played in a background of spatially separated noise (White, pink and speech shaped noise (SSN)). Preliminary data were collected in the auditory cortex of behaving ferrets using Omnetics WARP32 chronic implant.
Results: Three ferrets trained are able to identify the target word in silence (F1702: 55% hit rate on target trials/26% false alarm on catch trials, F1903: 63%/20%, F2102:44%/28%; chance performance = 33% hit rate), and two of them are able to identify the target word in noise at 0dB SNR (F1702: white noise: 55%/21%, pink noise: 59%/22%, SSN: 52%/40%; F1903: white noise: 64%/23%, 56%/29%, SSN: 54%/31%). Preliminary data are being collected on negative SNR (-5 and -10dB).
Conclusions: Ferrets can be trained to detect a target word in a stream of distractor words in both silence and in noise. Population level analysis are currently being implemented to identify correlation structures in the neural data. We hypothesize that when the animal can successfully segregate noise and speech streams, the neural population will be a uniform structure near trial onset, but that there will be two distinct correlation structures later in the trial. Stimulus reconstruction will be performed on the different clusters of neurons to investigate whether they encode for different auditory streams.

Histological Analysis of Auditory-Nerve Damage in the Budgerigar Induced by Kainic Acid

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Background: The loss of auditory-nerve (AN) fibers is a prevalent problem in noise-damaged and aging ears. Kainic acid (KA), an analog of glutamate, has been used to study the impact of selective AN loss without hair-cell (HC) damage. The budgerigar is a small avian species that has been used in previous behavioral studies of AN loss because of its human-like low-frequency hearing and ability to perform complex auditory discrimination tasks with operant-conditioning procedures. Previous studies show that budgerigars exposed to KA have reduced auditory brainstem response (ABR) wave 1 (the compound AN response) and envelope following responses, but surprisingly, normal behavioral tone detection thresholds in quiet and in noise. There is still a lack of histological evidence for the impact of KA infusion in this species, that can link the status of AN fibers and HCs with physiological and behavioral assessments.
Methods: The present study used confocal microscopy to examine the impact of KA on the budgerigar cochlea. Some of the birds were used previously in behavioral studies. Cochleae were processed as frozen sections using standard immunohistochemical techniques. Intracochlear infusions of KA (1-2 mM, 2.5 µL) were performed either bilaterally or unilaterally. The densities of HCs, AN fibers, and AN ganglion neurons were quantified as a function of their position along the tonotopic axis of the cochlea. ABRs and compound action potentials (CAPs) were measured in control ears, as well as ears before and after KA infusion, to examine the correlation between AN anatomical damage and the amplitude of compound AN potentials.
Results: Histological analysis showed that the densities of HCs, AN fibers, and AN ganglion neurons were relatively consistent in normal ears, including ears from control birds and unilateral-infused birds. In the KA-infused ears, the density of AN fibers and AN ganglion neurons was reduced significantly. The amount of cell density reduction reflected the amplitude reduction of the ABR and CAP. On the other hand, hair cells were unaffected by KA-infusion, with no difference found in HC density between KA-infused ears and normal ears.
Conclusions: These results demonstrate that, in budgerigar as in other species including mammals, KA infusion selectively damages AN ganglion neurons and fibers without affecting HCs. Associations were found between histological measures of AN loss and the amplitude of compound AN potentials, which illustrates the effectiveness of using ABR wave I and CAPs to assess AN loss in this model speci¬es. Taken together with previous behavioral studies, these results show that histologically validated AN loss has little impact on behavioral tone detection in quiet and in noise.

Cochlear Optical Encoding With Ultrafast Targeting Optimized Chronos

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Background: Spatially confined optogenetic stimulation of the spiral ganglion neurons (SGNs) represents a prospective alternative to electrical stimulation currently used in cochlear implants (CI). The reduced spread of excitation promises to increase the number of independently excitable channels in the future optical cochlear implant. We and others have aimed to overcome the shortcoming of traditionally used channelrhodopsins (ChR): slow closing kinetics upon light off which challenges the high temporal fidelity of SGN stimulation desired for bionic sound encoding. Chronos, the fastest naturally occurring ChR (Klapoetke et al., 2014) that shows sub-millisecond closing kinetics (Keppeler et al., 2018), is a promising candidate and has been successfully applied to SGNs (Keppeler et al 2018., 2018, Duarte et al., 2018).
Methods: Here, we used the trafficking- optimized version Chronos-ES/TS (Keppeler et al., 2018) and expressed it in mouse SGN using early postnatal viral gene transfer (AAV-PHP.B, human synapsin promoter, titer = 3.3-8.4 x 1012 GC/ml). We then recorded from single SGNs in response to patterned fiber-based optical stimulation.
Results: By combining single neuron and population analysis, we highlighted optimal stimulation parameters (pulse duration, stimulation rate and recovery time).
Conclusions: Our results will be used to analyze neural network function in the auditory brainstem and to design coding strategies of the future optical CI.

The Role of Auditory Nerve Phase-Locking in Audition: Evidence From Deep Neural Networks

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Background: The auditory nerve encodes sound with precise spike-timing that is phase-locked to the temporal fine structure of sound. The role of phase-locking in hearing remains controversial because physiological mechanisms for extracting this information (especially monaurally) remain unknown. Nonetheless, temporal fine structure has been proposed to support hearing in noise, with recognition difficulties of hearing-impaired listeners in noise potentially reflecting an inability to use fine temporal structure. Here, we investigate the perceptual role of auditory nerve phase-locking with deep artificial neural networks. We used artificial neural networks in the spirit of ideal observer analysis, optimizing them for natural tasks and examining whether phase-locking in a network's cochlear input was necessary to obtain human-like behavior.
Methods: We trained networks to recognize words, voices, and environmental sounds as well as to localize sounds from simulated auditory nerve representations of natural stimuli. Training stimuli for the recognition tasks consisted of speech excerpts superimposed on recorded auditory scenes. Training stimuli for the localization task consisted of stereo recordings of natural sounds rendered at different spatial localizations in a virtual environment. We manipulated the upper limit of auditory nerve phase-locking in our networks' ears via the lowpass filter cutoff in simulated inner hair cells and measured the effect on task performance in different acoustic conditions.
Results: Networks whose input featured high-frequency phase-locking replicated key aspects of human auditory behavior: task performance was robust to sound level and remained good even in noisy conditions. Degrading phase-locking impaired performance, but more on some tasks than others. Reducing the upper frequency limit of phase-locking to 50 Hz (eliminating virtually all temporal fine structure in the peripheral representation) had little effect on network word and environmental sound recognition, but substantially impaired voice recognition. Network localization performance (in both azimuth and elevation) was even more reliant on spike-timing, benefiting from phase-locking upwards of 1000 Hz.
Conclusions: The results suggest that auditory nerve phase-locking to temporal fine structure is critical for accurate sound localization and voice recognition -- but less so environmental sound and speech recognition -- in natural environments. The results also suggest auditory nerve phase-locking is critical for level-robust hearing.

Macrophages Facilitate Repair of Damaged Ribbon Synapses After Noise-Induced Cochlear Synaptopathy

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Background: Exposure to noise causes rapid loss of ribbon synapses between presynaptic inner hair cells (IHC) and postsynaptic peripheral axons of spiral ganglion neurons (SGNs). Such synaptic loss leads to gradual axon degeneration and ultimately death of SGNs and contributes to auditory perceptual dysfunctions leading to difficulty in speech recognition and listening in noisy environments and may be key to the generation of tinnitus or hyperacusis. Moreover, absence of functional SGNs limits the performance of hearing aids and cochlear implants or any future hair cell regeneration strategies. We have recently demonstrated that macrophages migrate immediately into the damaged IHC-synaptic region and directly contact the damaged synaptic connections following noise trauma (Kaur et al., 2019). However, the functional consequences of these contacts remain unclear. Here, we seek to determine the role of macrophages in degeneration or preservation of loss of synapses and/or repair of damaged synapses after noise trauma.

Methods: Cochlear macrophages were eliminated using colony stimulating factor 1 receptor (CSF1R) oral inhibitor, PLX5622. First cohort of mice unexposed or synaptopathic noise exposed treated with control or PLX5622 chow were euthanized at 1-day after exposure to determine if absence of macrophages prevent or exacerbate synaptic damage. A second cohort of mice were be euthanized at 15-days after exposure to determine if absence of macrophages impair the spontaneous repair of damaged IHC-ribbon synapses. A third cohort of PLX5622-treated mice lacking cochlear macrophages were noise exposed to damage synapses followed by immediate substitution of PLX5622 chow with control chow to allow macrophage repopulation. Mice were euthanized at 30-days post exposure to examine whether macrophage repopulation in the cochlea lacking macrophages is sufficient to repair the noise-damaged synapses. Auditory function, macrophages, juxtaposed ribbon synapses, and orphan ribbons and AMPA receptor density per IHC were analyzed.

Results: PLX5622 treatment led to a robust elimination (~98%) in cochlear macrophages without any adverse effects on peripheral leukocytes, normal cochlear function, structure, or inflammation. Lack of macrophage did not affect the degree of loss of ribbon synapses (~40-50%) at 1-day after noise trauma when compared to damaged cochlea with intact macrophages. By 15-days after noise trauma, damaged synapses underwent spontaneous partial repair in the presence of macrophages. However, in the absence of macrophages such repair of synapses was significantly impaired. Repopulation of macrophages into the cochlea lacking macrophages partially repaired the noise-damaged synapses and increased the density of the postsynaptic AMPA receptors on axon nerve terminals per IHC which positively correlated with partial recovery of auditory brainstem response thresholds and wave 1 amplitudes at suprathreshold sound levels.

Conclusions: The data imply that macrophages do not prevent or worsen synaptic loss but are necessary and sufficient to facilitate the repair of damaged ribbon synapses after synaptopathic noise trauma.

The Importance of Quantification Method and Scale Selection for Interpreting the Interphase Gap Effect on the Electrically Evoked Compound Action Potential

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Background: The number of surviving neural elements in the cochlear nerve (CN) and their responsiveness to electrical stimulation (i.e., functional status of the CN) has been shown to be important for cochlear implant (CI) outcomes (Pfingst et al., 2017, 2015; Schvartz-Leyzac, et al., 2020b; Schvartz-Leyzac and Pfingst, 2018; Skidmore et al., 2020; Zhou and Pfingst, 2014). The sensitivity of the electrically evoked compound action potential (eCAP) to changes in the interphase gap (IPG) (i.e., the IPG effect) is associated with the survival of spiral ganglion cells (SGNs) in guinea pigs (Prado-Guitierrez et al., 2006; Ramekers et al., 2014, 2015; Schvartz-Leyzac et al., 2019) and with the functional status of the CN in human CI users (He et al., 2018; He et al., 2020a; Hughes, et al., 2018; Kim et al., 2010; Schvartz-Leyzac and Pfingst, 2016, 2018; Skidmore and He, 2021). However, there were substantial differences in how the IPG effect was quantified across these studies, which makes it challenging to compare results across studies. This study demonstrated the effects of using different quantification methods and parameter scales on the IPG effect measured using seven eCAP parameters.

Methods: The IPG effect measured in two groups of CI users varying in CN health status (i.e., children with cochlear nerve deficiency and children with normal-sized CNs) using seven eCAP parameters was quantified using an absolute and a proportional difference method. The eCAP parameters evaluated in this study included 1) the eCAP threshold quantified using linear scaling units, 2) the eCAP threshold quantified using logarithmic scaling units, 3) the maximum slope of the eCAP input/ouput (I/O) function estimated using the window method in a logarithmic input scale, 4) the overall slope of the eCAP I/O function estimated using linear regression in a

linear scale, 5) the maximum eCAP amplitude, 6) the N1 latency of the eCAP recorded at the maximum stimulation level, and 7) the stimulation level offset.

Results: The direction and magnitude of group differences in the IPG effect size were greatly affected by the quantification method and varied for different eCAP measures. The IPG effect on the eCAP threshold was also affected by the scale in which it was calculated.

Conclusions: The IPG effect provides an indicator for the functional status of the CN in human CI users. Specifying how the IPG effect is quantified is critical for accurate result interpretation.

Removing the Variance Explained by Hearing Sensitivity in Common Measures of Auditory Nerve Pathology and Hidden Hearing Loss

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Background: Audiometric hearing loss is often considered a confounding factor in the search for synaptopathy and other auditory nerve pathologies. Most studies attempt to circumvent the effects of hearing sensitivity on other auditory measures by recruiting only participants with thresholds within normal limits. This, however, assumes that thresholds within normal limits do not have an effect on the auditory measures studied. Furthermore, sampling from only normal hearing populations to study synaptopathy and auditory nerve pathologies reduces statistical power by lowering the incidence of disease in the study sample, because it is more likely that auditory nerve pathologies are comorbid with pathologies causing audiometric hearing loss. The present study reports the variance explained by audiometric thresholds in a number of common auditory measures in human studies of auditory nerve pathology and hidden hearing loss and presents a method to remove this variance in study samples.

Methods: Derived metrics of the audiogram were compared in their ability to explain variance in several behavioral and physiological auditory measures, including thresholds-in-noise at two frequencies, frequency-modulation detection threshold, word recognition in four listening conditions, distortion-product otoacoustic emissions (DPOAE), tone burst-evoked auditory brainstem response (ABR) waves I and V amplitudes at two frequencies, and the frequency following response (FFR) of the speech-evoked ABR. The residual values after regressing audiometric variables and auditory measures may serve as functional proxies of the threshold-independent portion of auditory measures that can be used in place of the original variables in studies searching for auditory nerve pathology or hidden hearing loss. Derived metrics of the audiogram included the first component of a principal component analysis of all audiometric thresholds, the speech intelligibility index, four-frequency pure-tone average, and the threshold at corresponding stimulus frequencies for auditory measures.

Results: The best-fitting model for almost all auditory measures was a 2nd degree polynomial using the first component of a principal component analysis of audiometric thresholds .25-8 kHz. Audiometric thresholds accounted for 5-53% of the variance, with the least variance explained in the FFR, and the most explained in DPOAE strength.

Conclusions: The residuals of the auditory measures after regressing the first component of a principal component analysis of audiometric thresholds can serve as threshold-independent portion of the auditory measure, allowing researchers to sample people with hearing loss in studies of auditory nerve pathologies and hidden hearing loss.

Intraoperative Monitoring of Cochlear Function During Cochlear Implantation Using Simultaneous Intra- And Extracochlear Electrocochleography

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Background: The objective to preserve residual hearing during cochlear implantation (CI) has recently led to the use of electrocochleography (ECochG) as an intra-operative monitoring tool. As the recording electrode moves during insertion with respect to the different signal generators in the cochlea, response changes can result solely from a changing contribution of the underlying generators in the absence of cochlear trauma. We hypothesized

that intracochlear ECochG recordings show signal changes not reflected in simultaneous extracochlear ECochG recordings.

Methods: Eleven subjects with residual hearing were enrolled in this study. The CI electrode array was inserted in a stepwise manner. At each step intracochlear ECochG responses (through the most apical electrode of the CI using back-telemetry) and extraECochG responses (needle electrode placed close to the round window) were simultaneously recorded. The acoustic stimulus was a 500 Hz tone burst at 110 to 120 dB SPL with alternating starting phases.

Results: Abrupt or slowly progressing phase changes in intracochlear recordings were observed in the difference curves of all subjects, without corresponding phase changes in extracochlear recordings. Abrupt phase shifts of approximately 180 degrees occurred in three cases. Our results show that an amplitude decrease with associated near 180-degree phase shift and harmonic distortions in the intracochlear difference curve during the first half of insertion was not accompanied by a decrease in the extracochlear difference curve's amplitude (n = 1). Late amplitude decreases in intracochlear difference curves (near full insertion, n = 2) did correspond to extracochlear amplitude decreases.

Conclusions: Phase shifts and amplitude decreases in intracochlear ECochG recordings can be observed without associated changes in extracochlear recordings, likely caused by movement of the recording electrode with respect to the different signal generators. Our findings suggest that comparison of intracochlear ECochG recordings with simultaneous extracochlear recordings could enhance the interpretation of ECochG changes and potentially allow for differentiation between traumatic and atraumatic changes in intracochlear recordings.

Acoustic Processing Abilities of New Cochlear Implant Recipients

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Background: Speech understanding after cochlear implant (CI) activation is not immediate; it requires frequent use of the CI for listening practice in daily life. The neural substrates for sound processing need to be reestablished in reaction to novel mode of auditory stimulation that is different from acoustic hearing before implantation. Frequency discrimination, which is essential for speech perception and music appreciation, is particularly challenging for new cochlear implant recipients due to the poor spectral resolution of the CI. This study will examine the frequency change detection abilities of new cochlear implant patients within two months of activation.

Methods: Twelve CI ears (8 adult CI recipients) participated in electrophysiological and behavioral testing. All participants were new CI users who had been activated within two months (mean: 44 days) before the testing date. The frequency Acoustic Change Complex (fACC), recorded using EEG technique, was evoked by a frequency change embedded in a pure-tone. In addition to objective measurements, behavioral assessments were used to examine the frequency change detection threshold and speech understanding (Consonant-Nucleus-Consonant word lists and AzBio sentences in quiet and noise).

Results: Data analysis is ongoing. The fACCs will be evaluated in terms of morphology, peak amplitudes, and peak latencies. If possible, the correlations between the fACC and behavioral assessments will be examined.

Conclusions: The current study demonstrates the large variability of frequency change detection, speech perception, and cortical processing of frequency changes in new CI recipients. The results will reveal if the fACC can be utilized as an objective tool to document brain plasticity following cochlear implantation.

Effects of Carrier Pulse Rate and Estimates of Peripheral Neural Health on Cortical Encoding of Silent Gaps in Cochlear Implant Users

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Background: Electrically-evoked compound action potentials (ECAPs) relate to the density of surviving spiral ganglion neurons (SGNs) in cochlear implanted animals. Related work in humans show that these same ECAP measures are often related to speech recognition performance in cochlear implant (CI) users. We hypothesize that greater SGN density is important for auditory temporal encoding. Furthermore, we hypothesize that increasing carrier pulse rate may improve temporal coding, but only in channels with SGN densities sufficient to accurately encode faster rates. We tested these hypotheses using ECAPs and cortical evoked potentials evoked by silent gaps in the pulse train. We predict that cortical encoding of temporal gaps is more precise when measured using pulse

trains with higher rather than lower carrier pulse rates, and that this improvement in precision is greater when using electrodes estimated to stimulate a higher rather than lower density of SGNs.

Methods: Participants included adult post-lingually deafened (>18 years old) CI recipients who used Cochlear ™ implant systems and had at least 3 months' CI experience. ECAP amplitude-growth functions (AGFs) were measured using two interphase gaps (IPGs) of 7 and 30 µs. The difference in AGF slope between the two IPGs for each electrode was calculated ("IPG effect"). For each participant, we identified the electrodes estimated to stimulate the highest and lowest SGN survival rates, indicated by the highest and lowest IPG effects, respectively. Cortically auditory evoked potentials were recorded using direct stimulation and a 64-channel Neuroscan Quickcap connected to a SynAmps RT. The acoustic change complex (ACC) was recorded to fixed duration of silent gaps, using two carrier pulse rates (500 and 3500 pps) on two electrodes within each participant.

Results: Preliminary results suggest that, for loudness balanced stimuli, cortical encoding of temporal gaps is more precise when using a higher carrier pulse rate, but this effect is modulated by the condition of the auditory nerve. Specifically, results thus far suggest that stimulating with a higher pulse rate to an electrode estimated to excite a higher density of SGNs in the cochlea results in more precise encoding of temporal cues in the auditory cortex. However, a similar improvement in the encoding of temporal cues is not noted when higher pulse rates are delivered to electrodes estimated to excite a lower density of neurons.

Conclusions: Preliminary studies demonstrate the extent to which auditory nerve health drives cortical representation of temporal cues within individuals. Importantly, these results may provide novel therapeutic targets for improving speech recognition in CI users based on estimated SGN survival and subsequent cortical representations.

UltraHearing: Comparison of Stimulation Modalities for an Ultrasound-Based Hearing Technology
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Background: Ultrasound stimulation (US) is an exciting new technique to non-invasively modulate neural activity with high spatial precision. Recently, studies have demonstrated that US, when coupled to the subject, activates the auditory system (Guo et al., Neuron 2018). The current hypothesis posited by these studies suggests that the method of activation is via a fluid pathway, in which the ultrasound waves vibrate the cerebrospinal fluid and travel into the cochlea via the cochlear and vestibular aqueducts. Due to the differing mechanism and pathway of activation, US of the auditory system will have different characteristics from traditional air- and bone-conduction stimuli. Our lab has previously reported on the comparisons between air-evoked and US-evoked neural activity, and how US can effectively evoke activity when modulated with relevant information. However, these experiments have not explored the complexity of information which can be encoded, nor compared the responses to bone conduction.

Methods: In this study, we investigated the neural activity in response to complex signals transmitted via bone-, air-, and fluid-conduction (ultrasound). Neural activity was recorded using a two-shank 32-channel NeuroNexus electrode array placed in the central nucleus of the inferior colliculus (ICC) of anesthetized guinea pigs. Air- and bone- stimulation consisted of guinea pig vocalizations presented via a speaker or a B-81 bone conduction device coupled to the skull via a screw-nut system (Curthoys et al., Exp Brain Res, 2006). For fluid-stimulation, we extracted the envelope of the vocalizations using the Hilbert transform, and then modulated a 220 kHz sinusoid. The signal was presented via a SonicConcepts ultrasound transducer coupled to the brain with agarose. To compare the neural activity, we created post-stimulus time histograms (PSTHs) and utilized the Earth Mover's Distance metric as well as the Normalized Root Mean Square Error to compare the different stimuli.

Results: Our results demonstrate that the ICC is able to detect the vocalizations' envelope, even when encoded in ultrasound. In particular, high frequency channels have a more similar response than low frequency channels when comparing ultrasound and air-conducted stimuli. Bone conduction stimuli have a more varied response, and further analyses will explore and quantify the differences between the response of bone to ultrasound- and air-stimulation modalities.

Conclusions: In conclusion, ultrasound can viably deliver complex information encoded in its envelope, and has some meaningful differences from traditional bone conduction stimulation. Further research will investigate the variability of these responses, and how varying the stimulus intensity can lead to higher, or lower, degrees of similarity between air-, bone-, and fluid- evoked activity. Better understanding of how ultrasound stimuli act on

the auditory system can help guide the development of next-generation hearing devices, possibly even a combination of technologies leveraging the different stimulation modalities

Dexamethasone Eluting Arrays Suppress MHCII Mediated Antigen Presentation in Cochlea Following Cochlear Implantation

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Background: Cochlear implants (CIs) provide auditory rehabilitation to indicated patients with sensorineural hearing loss and can considerably improve their quality of life. Surgical implantation of a CI invariably induces an inflammatory and foreign body response (FBR) that can contribute to loss of residual acoustic hearing in hearing preservation post-CI and may impact CI performance and hearing outcomes. Studies from temporal bone records of implanted humans and animal models have shown that both macrophages and lymphocytes are involved in the FBR following implantation. With single-cell RNA-sequencing in a murine model, we have found evidence for involvement of lymphocytes and MHCII mediated antigen presentation in the post-CI inflammation in the cochleae. MHCII mediated antigen presentation by macrophages to lymphocytes has been shown to play a key role in implantation in other organs. The role of MHCII mediated antigen presentation in post-CI inflammation is unknown. Moreover, we and other authors have shown in animal studies that corticosteroid-eluting CIs can mitigate intracochlear macrophage infiltration and FBR. Using a murine model, this study aims to explore whether cochlear implantation activates MHCII mediated antigen presentation within the cochlea and how dexamethasone-eluting CI electrode arrays affect that presentation.
Methods: 12-week-old CX3CR1-GFP Thy1-YFP C57BL6 mice were implanted with either regular or dexamethasone-eluting CIs in the left ear with the contralateral ears acting as controls. The implants were stimulated from postoperative day 7 for up to 28 days and sacrificed at 10, 28, or 56 days postoperative. The cochleae were fixed with 4% PFA and cryosectioned at 30 micrometer thickness. Macrophages and neurons were intrinsically labeled, and mid-modiolar sections were labeled with DAPI for nuclei and immunostained with antibodies against MHC class II. Images were taken using confocal microscopy, and quantitative image analyses were performed. The cochlear regions of the scala tympani, Rosenthal's canal, lateral wall, and modiolus were manually traced, and the quantification of three parameters of MHCII+ macrophages were automated using IMARIS: density of CX3CR1+ MHCII+ macrophages, ratio of total and MHCII+ macrophages, and intensity of MHCII expression on macrophages.
Results: Cochleae implanted with regular, non-dexamethasone-eluting CI electrodes developed a robust immune response with significantly increased density of CX3CR1+ MHCII+ macrophages in the scala tympani of the basal cochlear turn and lateral wall across the whole cochlea up to 56-days postoperative. Specimens implanted with dexamethasone-eluting cochlear implant electrodes displayed significantly reduced the density of CX3CR1+ MHCII+ macrophages at all time points in scala tympani, lateral wall, modiolus, and spiral ganglion. The ratio of MHCII+ and total macrophages and the expression of MHCII on macrophages are reduced by dexamethasone-eluting implants.
Conclusions: This study suggests that activation of MHCII mediated antigen presentation is involved in the inflammatory and FBR following implantation and can be mitigated by immunosuppressive dexamethasone-eluting cochlear implants.

Gene Expression Changes and Mechanisms of Fibrosis Following Events Associated With CI Surgery and Stimulation in Rat Model

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Background: Cochlear fibrosis is a common repair mechanism occurring after cochlear implantation. Overgrowth of fibrosis can alter the functioning of cochlear implant, but also promote to the loss of residual hearing. Nowadays, the respective parts of insertion trauma, the presence of intracochlear implant, and electrical stimulation involved during cochlear fibrosis are unknown.

In a previous study we observe that fibrosis development after cochlear implantation is induced by the presence of a foreign body rather than by the insertion trauma only. We have highlighted some signaling pathway potentially implicated in fibrosis development such as inflammatory pathway, Smad independent signaling pathway, metalloproteases and their inhibitors, and gene involved in extracellular secretion matrix.

Methods: In the present study, we analyzed the gene expression induced by an electrical stimulation using a cochlear implant and compared it with the implanted yet non stimulated condition. It allowed to evaluate the effects of the foreign body reaction and the electrical stimulation respectively. Then, by cochlea clearing and 3D imaging, we quantified fibrotic tissue into cochlea in both conditions.

We used female rat Wistar, implanted with an array specifically designed for rodents with 5 electrical contacts (Cochlear Ltd). Animals were stimulated with ACE coding strategy for 4 hours a day during 1 month after surgery.

We compare the expression of 84 transcripts known to be involved in fibrotic processes using qPCR from 1 days to 28 days after cochlear implantation, and with deep learning algorithms, we selected signaling pathways and gene clusters supposed to be involved in fibrotic processes.

Next, we used cochlear clearing methods for 3D imaging of whole cochleae with Ultramicroscope. We are able to quantify the volume of fibrotic tissues into cochlea and compared the fibrotic development between conditions.

Results: The first results seem to indicate an overactivation of signaling pathways with electrical stimulation, already known to be activated by the foreign body reaction such as inflammation and extra cellular matrix secretion without no gene activation only induced by electrical stimulation.

Conclusions: Altogether, these results seem to indicate that te electrical stimulation plays a role in fibrosis process.

Ipsilateral Residual Acoustic Hearing is Important for Spatial Masking Release in Simulations of Cochlear Implants With Bilateral Residual Hearing

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Background: Binaural hearing plays an important role in segregating spatially separated competing speech. However, bilateral and bimodal cochlear implant (CI) patients often do not benefit from spatial cues to segregate competing speech. The present study evaluated whether residual acoustic hearing (AH) in the implanted ear contributes to the binaural benefit, defined as the binaural advantage over the better ear alone.

Methods: Speech recognition thresholds (SRTs) for a target sentence were measured in the presence of two competing sentences in normal-hearing adults listening to CI simulations with residual AH in the contralateral ear (bimodal) or in both ears (biEAS). Non-individualized head-related transfer functions (HRTFs) were used to create a virtual auditory space for headphone presentation. The male target sentence originated directly in front of the listener, and the two male masker sentences were either co-located with the target or presented to the left and right of the target. After HRTF processing, the stimuli presented to the left ear were bandpass filtered to simulate the contralateral residual AH and the stimuli presented to the right ear were processed by sinewave vocoding and bandpass filtering to simulate electric hearing (EH) and AH. Four conditions were tested: 1) "bimodal match," with no frequency overlap between AH and EH, but with speech information loss in EH, 2) "bimodal mismatch," with an interaural frequency mismatch between AH and EH, but with maximum speech information in EH, 3) "biEAS low," with no interaural frequency mismatch between binaural AH and EH, but with limited extent of residual AH in the ipsilateral ear, and 4) "biEAS high", with no interaural frequency mismatch between binaural AH and EH, but with the same extent of residual AH across ears.

Results: For co-located target and maskers, SRTs were significantly lower with the bimodal match than with the bimodal mismatch condition. There was no significant improvement when residual AH was added to the ipsilateral ear (biEAS low or high). For spatially separated target and maskers, SRTs were significantly lower with the bimodal match than with the bimodal mismatch condition, and significantly improved when residual AH was added to the ipsilateral ear (biEAS low or high). Negative spatial release from masking (SRM) was observed for the bimodal mismatch, bimodal match, and biEAS low conditions, with a positive SRM observed only for the biEAS high condition.

Conclusions: These simulation data suggest that hearing preservation in the implanted ear may significantly benefit the utilization of spatial cues when segregating competing speech, especially when the amount of residual AH is comparable across two ears. Also, the benefits of residual AH in the ipsilateral ear may be best ascertained when target and masker speech are spatially separated.

High-Resolution Volumetric Analysis of the Auditory Pathway Supports Genetic Variation Affects Auditory Structure Size and Aging

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Background: Hearing loss is the most common sensory deficit worldwide. Recently, volumetric analysis in humans has revealed associations with hearing impairments and brain volume changes. Highly conserved genetics between humans and mice make the murine model a high-yield candidate for characterization of the underpinnings in hearing loss; however, investigations into neural structure analysis remain sparse due to difficulties of doing so in the mouse. Imaging technologies can achieve a spatial resolution adequate to perform such studies, potentially lending invaluable headway into mechanistic basis in the relationships observed between hearing and brain volumes.

Methods: Twelve different BXD recombinant inbred mice strains were aged into two cohorts, young (3 months) and old (15 months). Following sacrifice, the mice brain and skull were fixed in formalin and mounted. Magnetic resonance (MR) images were acquired utilizing a protocol providing an isotropic spatial resolution of 45 µm. A three-dimensional bilateral label set was subsequently imposed, creating spatial mapping including 175 labelled regions per hemisphere. This was screened down to twelve regions comprising of the auditory cortex and the central auditory nuclei structures and their divisions, including the cochlear nucleus, superior olivary complex, lateral lemniscus, inferior colliculus, and medial geniculate nuclei. A voxel-based assessment of individual structure volume in both mice cohorts was then performed. Published auditory brainstem response (ABR) threshold data was utilized to perform correlational analysis with structure volume in old mice.

Results: 184 total mice were imaged across all twelve strains. Differences arise in the absolute volume of all auditory structures between every BXD strains in both young and old mice. With the exception of BXD strains 60 and 101, auditory cortex (AUD) size decreased with age. 111/132 (84.1%) of structure-strain pairings in central auditory nuclei demonstrated an increase in size after aging; the 21 exceptions were primarily in BXD strains 48a, 51, and 62. Inferior colliculus central nucleus (Icc) and AUD volumes correlated with ABR thresholds (Icc vs 16kHz: r=-0.59, p=0.04; Icc vs 32kHz: r=-0.52, p=0.08; AUD vs 32kHz: r=-0.52, p=0.08).

Conclusions: We present a novel approach in the volumetric analysis of the auditory pathway in the murine model. The heterogeneity of our results between BXD strains suggest genetic variation in both the volume of auditory structures and the effect of aging upon mentioned structures. ABR correlates portends the Icc and AUD as likely targets for future hearing phenotypic research. High resonance MR imaging provides a promising efficacious avenue in mouse model hearing loss investigations.

Single-Sided Deafness Asymmetrically Disrupts Binaural Integration in the Developing and the Adult Auditory Midbrain

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Background: Asymmetric hearing during early development can disrupt the hemispheric balance of central auditory circuits that support directional hearing. The degree of neural vulnerability following asymmetric hearing loss suffered during adulthood is less understood. Here, we evaluate whether disruptions of normal binaural experience during various developmental stages or during adulthood similarly impact binaural integration in the auditory midbrain.

Methods: Using unilateral intrascalar application of aminoglycosides, we induced single-sided deafness (SSD) at several experimentally determined stages during gerbil auditory midbrain development (P15, P19, P23) and during

adulthood (P65). After 15 days of deafness, animals received bilateral round window electrodes, and single neuron responses to monaural and binaural biphasic electric pulses were recorded in the inferior colliculi of both hemispheres. Normal hearing adult gerbils (NH) served as controls.

Results: In NH animals, response strength is typically stronger in the hemisphere contralateral to the stimulated ear (contralateral dominance). In SSD animals, response strength to stimulation of the hearing ear was not affected in either hemisphere. However, SSD weakened response strength to stimulation of the deafened ear in the contralateral hemisphere, but not in the ipsilateral hemisphere. As a result, SSD animals showed a loss of contralateral dominance in the hemisphere contralateral to the deaf ear. This effect occurred in all age groups, but was strongest in animals with early-onset deafness.

Changes in contralateral dominance in SSD animals were paralleled by disruptions of binaural integration of interaural time differences (ITD). Animals with early-onset SSD had lower fractions of ITD sensitive neurons when compared to later-onset SSD and NH animals. Among ITD-sensitive neurons, SSD degraded neural ITD discrimination thresholds and ITD discriminability (d'). Reductions in neural ITD discrimination performance occurred in both hemispheres, but were more pronounced in the hemisphere contralateral to the deafened ear. In accordance, population rate-ITD coding in SSD animals was distorted and highly asymmetric between hemispheres. Degradations in neural ITD discrimination were observed across all SSD age groups, but were most prominent in animals with early-onset deafness.

Conclusions: The observed results reveal that hemisphere-specific imbalances in binaural integration following SSD are not exclusively governed by critical periods during development, but also occur, although to a lesser degree, in animals with adult-onset SSD. According to 'two-channel' models of directional hearing, imbalances in binaural integration between the two hemispheres are predictive of poor ITD discrimination acuity. Taken together, the reported findings may explain perceptual deficits in directional hearing observed in both pediatric and adult SSD subjects using unilateral cochlear implants.

Combining an Auditory Nerve Model With Information Theory to Simulate Modulation Detection by Cochlear-Implant Users

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Background: Computational models can help in understanding the limitations of cochlear-implant (CI) users when performing various hearing tasks and aid in designing new algorithms to improve listening outcomes. Models can also help identify factors (e.g., neural health, current spread, insertion depth, etc.) that may affect the transmission of information from the acoustic stimulus to the auditory nerve of the CI user. Here, we combine a computational model of the auditory nerve response to electrical stimulation with an information-theory algorithm to investigate factors that may affect the perception of amplitude and rate modulation for CI users.

Methods: In a generic implementation, the model simulates auditory-nerve spike trains (for up to 10,000 fibers) evoked by an acoustic signal passed through a CI. The acoustic signal is first converted into sequences of electrical pulses (electrodogram) according to a selected processing strategy. A current-spread model is then used to calculate the electrical current waveform reaching each auditory nerve fiber. Lastly, each fiber generates a spike train in response to the received current waveform using the point-process model of Goldwyn et al. (2012, J. Neurophysiol.). An information-theory algorithm is then used to assess the amount of information transmitted from the acoustic stimulus to the auditory nerve. The algorithm aims at optimally reconstructing the acoustic stimulus from the simulated spike trains, and the quality of the reconstruction reflects how much information is present in the nerve. In this particular study, the model is used to reproduce experimental amplitude and rate modulation detection thresholds for CI users tested using direct stimulation via a single electrode.

Results: It will be shown that (1) a model with generic parameters is sufficient to predict the average experimental data; (2) current spread can improve the neural coding of amplitude and rate modulation; (3) fiber loss impairs the encoding of amplitude modulation, particularly at high modulation rates.

Conclusions: Altogether, results suggest that both increased current spread and lower neural loss improve the sensitivity of CI users to amplitude and rate modulations. [We thank Attila Fráter and Monita Chaterjee for useful discussions. Work supported by Oticon Medical and The William Demant Foundation.]

Neural Correlates of Auditory Spatial Processing in Unilateral deafness: Relation to Sound Localization in Noise and Cortical Plasticity

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Background: People with unilateral deafness (UD) typically complain of impaired ability to locate a sound location, especially in noise. However, recent studies have suggested that paying attention to sound could mitigate the degradation of spatial processing in noise. To investigate cortical processing to spatially varied sounds under active listening, the current study examined cortical evoked responses to quantify the brain activities while at the same time measured the accuracy of sound localization to relate behavioral performances in UD individuals.
Methods: 20 adult UD patients (10 right-sided deafness, all right-handed) and 32 normal-hearing (NH) controls participated. Among the NH participants, one ears of 21 people were earplugged and masked with noise (11 left earplugged) to simulate acute unilateral hearing loss (AUHL). Cortical potentials were recorded from 64 scalp electrodes while actively detecting sound locations at different azimuth angles including +60, +15, 0, -15, and -60. N1, P2, N2, and late positive complex (LPC) were analyzed as a function of angles. Relationships between cortical responses and behavioral sound localization and audiological factors were examined for UD groups.
Results: In behavioral data, UD group showed poorer localization ability than NH and AUHL groups. The AUHL groups revealed prolonged N1 latency and greater LPC amplitudes compared to NH and UD groups. P2 amplitudes in UD groups were positively correlated with sound localization accuracy. The lateralization index calculated by N2 source activity indicated the left-hemispheric asymmetry for NH. In AUHL groups, stronger activity ipsilateral to the intact ear was revealed, regardless of the side of hearing loss. Unlike AUHL, UD groups showed distinct patterns of the laterality such that people with the left-sided UD had greater activity on the hemisphere contralateral to the hearing side, whereas no hemispheric asymmetry was found for the right-sided UD subjects. The cortical source activities in UD groups were negatively correlated with the onset of deafness in the area encompassing the right frontal cortex, suggesting that earlier onset of deafness induces more extensive cortical reorganization.
Conclusions: Our finding suggests that AUHL yields longer processing time and higher cognitive efforts to process spatial cues. Moreover, the AUHL can exert immediate cortical reorganization evidenced by the change of hemispheric laterality. In people with UD, patterns of cortical plasticity are different depending on the side of deafness. In addition, the earlier onset of deafness relates to stronger cortical activity in the frontal regions in which are mainly recruited for attention.

Spatial Hearing Performance for Single-Sided Deaf (SSD) Listeners With Contralateral Routing of Signal (CROS) Systems: Does Amplification in the Non-Serviceable Ear Impact Localization?

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Background: Single-sided deafness (SSD) refers to the clinical scenario in which hearing loss in one ear is non-serviceable with traditional amplification due to profound degree or poor word recognition abilities. With an incidence rate of 12–27 per 1,000,000 persons annually (Snapp and Ausili 2020), SSD is a common clinical problem. The management of expectations for this population is critical because of difficulty listening in background noise and decreased ability to localize sounds (Giolas and Wark, 1967). Historically, rehabilitation efforts have focused on non-surgical and surgical options for contralateral routing of signal (CROS) to the normal- or better-hearing ear with mixed performance outcomes and failure to gain widespread enthusiasm. One intriguing possibility to augment the CROS hearing aid system is the addition of amplification to the deafened ear in a stimulation paradigm known as ampCROS. This approach has gained anecdotal interest for its potential not only to suppress bothersome tinnitus, but also to provide spatial awareness and hypothetically offer the opportunity to improve speech performance in noisy environments. There are currently no controlled studies investigating the efficacy of ampCROS for individuals with SSD to improve spatial hearing. Here, we sought to investigate sound localization ability in patients with SSD during use of ampCROS stimulation. Performance was compared, within-subjects, to that in unaided and traditional CROS conditions.

Hypothesis: Addition of amplification to the non-serviceable ear in a CROS system paradigm will provide patients with SSD improved spatial awareness reflected in increased accuracy in sound source localization when compared with performance in unaided or standard CROS conditions.

Methods: Patients with SSD undergoing fitting of CROS systems were recruited for participation. Standard audiometry and device fitting data were collected from the electronic medical health record in accordance with IRB guidelines. Psychoacoustical data was collected in a double-walled, hemi-anechoic chamber equipped with a 24-channel, 360o surround loudspeaker system. Azimuth localization responses were collected as head positions measured via an electromagnetic position tracking system (Polhemus Fastrak) in the dark. Stimuli consisted of broadband, high-frequency narrowband (4 kHz) and low-frequency narrowband (0.5 kHz) noise bursts with a duration of 0.5 seconds at 70 dB SPL presentation level.

Results: Localization errors were quantified in degrees relative to target location. Performance for SSD subjects was predictably poorer compared with a database of responses from normal-hearing listeners. Errors for individual subjects were computed across the three stimulus types between the listening paradigms (unaided, standard CROS, ampCROS), quantifying the prospective spatial hearing benefits of ampCROS technology.

Conclusions: Results presented here describe the impact of two CROS paradigms on localization performance and may influence clinical decision making for both clinician and patients with SSD. Additionally, results from this study will inform counseling regarding expectations for real-world device performance on spatial hearing.

Integration of In Vivo Like Inputs in the Lateral Superior Olive: A Dynamic-Clamp Study

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Background: Excitatory and inhibitory synapses are major components of interneuronal information processing. A robust interplay of both types is utterly important in the lateral superior olive (LSO), a prominent brainstem hub. LSO neurons integrate excitatory and inhibitory inputs from the ipsilateral and contralateral ear, respectively. They are remarkably sensitive to interaural level differences (ILD) and time differences (ITD) in the tens of microseconds range (recent paper: Franken et al, 2021). The sensitivity to both cues makes LSO neurons particularly suitable for sound source localization. Together with their input neurons (cochlear nucleus (CN) and medial nucleus of the trapezoid body (MNTB)), LSO neurons form an ideal circuit to explore the mechanisms of behaviorally relevant integration of excitation and inhibition. How the relative strength and the relative timing of excitatory and inhibitory inputs are integrated in the LSO is not fully understood.

Methods: Here we use computational modeling to mimic the relative timing of in vivo like excitatory and inhibitory inputs (clicks, bursts). Modeling was combined with in vitro dynamic-clamp experiments in the LSO of immature and young-adult mice. One focus was laid on the effect of (de)synchronized inputs on spike generation. Each spike pattern was convolved to time-varying conductances using a unitary synaptic conductance template. Total excitatory and inhibitory conductances thus simulate the input activity from converging CN and MNTB neurons, respectively. This enabled us to analyze the sensitivity of LSO neurons to different virtual sound paradigms.

Results: In immature neurons, click-like inhibitory inputs most effectively blocked spike generation when they arrived ~200 µs before excitatory inputs. The integration window during which spikes were blocked ranged from 1-3 ms, depending on the inhibitory input strength. Experiments on young-adult mice are ongoing. Also ongoing are experiments involving stimulation of LSO neurons with burst-like patterns that are more desynchronized than clicks. Preliminary results confirmed a high sensitivity of LSO neurons to the rate of depolarization, i.e., unitary excitatory inputs must converge within a short temporal window of ~1 ms.

Conclusions: Together, our results confirm previous findings that LSO neurons are sensitive to ITDs (Franken et al, 2021). Furthermore, we find that the strength of inhibition prolongs its effect in reliably blocking AP generation. The precise interplay between excitatory and inhibitory strength therefore tunes the sensitivity of LSO neurons to ITDs. However, we cannot exclude the effects of local enhanced transmission due to spatial distribution of excitatory and inhibitory inputs to fine-tune ITD integration. We further show that excitatory inputs must occur in a small temporal window to reliably generate spikes in LSO neurons. These results confirm the hypothesis of Ashida and colleagues (2016) of the LSO as a coincidence detector for excitatory inputs.

Binaural Brainstem and Spatial Hearing Deficits in a Guinea Pig Model of Noise-Induced Cochlear Synaptopathy

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Background: Hearing loss is normally characterized by permanently raised auditory thresholds due to cochlear dysfunction. However, recent animal studies have revealed that brief, moderate-level noise exposure causes a permanent loss of ribbon synapses between inner hair cells and auditory nerve fibers, but only temporary threshold shifts that recover within a couple of weeks. Such noise-induced cochlear synaptopathy has therefore been called 'hidden hearing loss' because while there is a significant degeneration of ribbon synapses, the resulting hearing dysfunction is effectively hidden from typical clinical assays.

Methods: Here we used Guinea pigs to study the mechanisms leading to hearing deficits resulting from synaptopathy caused by a 2-hour 106 dB SPL 4-8 kHz octave band noise. We measured distortion product otoacoustic emissions (DPOAEs) and auditory brainstem responses (ABRs) to assay peripheral hearing. Next, we tested spatial hearing ability through the prepulse inhibition (PPI) of the acoustic startle reflex. Finally, we performed immunohistochemistry to confirm synaptopathy. ABR and DPOAE recordings were made prior to noise exposure, 24 hours post-exposure, and 1, 2, 4, and 8 weeks following exposure. Brainstem circuits subserving spatial hearing were assessed physiologically via the binaural interaction component (BIC) of the ABR and behaviorally through PPI (pre-noise and 8 weeks post-noise). PPI was used to measure hearing-in-noise ability, or spatial release from masking, where the animal must detect a target (broadband chirp) at varying SPLs from one spatial location in the presence of background noise presented from various other spatial locations. This task approximates the 'cocktail party' effect, where the listener focuses on the speech of a specific talker while trying to ignore background noise.

Results: Results show that the noise exposure induces no permanent hearing threshold shift, as measured by the DPOAEs and ABR thresholds, but results in a persistently depleted BIC amplitude relative to pre-noise exposure. Despite recovery of normal audibility, spatial hearing deficits persisted concomitant with the depleted BIC of the ABR. Cochlear synaptopathy was objectively confirmed by visualizing the loss of ribbon synapses in the cochlea. Labeling for the presynaptic ribbon (Ctbp2) and post synaptic ribbon (GluA2) allowed us to visualize any permanent changes to ribbon synapses induced by the noise exposure.

Conclusions: The results demonstrate that cochlear synaptopathy causes deficits in brainstem circuits known to be critical for binaural and spatial hearing and that these alterations lead to deficits in hearing abilities that require integration of inputs from both ears.

Investigating the Efficacy of a Bilateral Mixed-Rate Cochlear Implant Strategy for Speech Understanding in Noise

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Background: Listeners with bilateral cochlear implants (BiCIs) demonstrate benefits compared to unilateral listeners in speech understanding and sound localization in quiet. Further, in noisy situations they perform poorer than normal hearing (NH) listeners. One explanation is that BiCI listeners have no access to spatial hearing cues, such as interaural time differences (ITDs), when using clinical processors. This is largely because clinical processors lack coordination across the ears and use high rates of stimulation, which can result in unreliable ITDs. High rates are needed for achieving good speech understanding, but low rates are needed for ITD sensitivity. A potential solution is to use a "mixed-rate" strategy with high- and low-rate stimulation at different electrodes. Previous work in our lab using research processors has found that speech understanding with unilateral mixed-rate strategies can be comparable to that with all-high rates. Here, we evaluate the efficacy of a new bilateral mixed-rate strategy for preserving speech understanding in quiet and in noise. Our new implementation of the mixed-rate strategy is capable of real-time processing and can yield ITD sensitivity. We hypothesized that a greater improvement in speech recognition in noise will be achieved compared to using high rates across all electrodes if a) a mixed-rate strategy yields speech understanding comparable to an all-high CIS strategy, and b) BiCI listeners can utilize an ITD in a mixed-rate strategy to spatially separate a target word from a masker.

Methods: Closed-set speech understanding was measured in BiCI listeners using the CCi-MOBILE, a bilaterally-synchronized real-time research processor. Listeners were tested with all-high (1000 pulses per second [pps]) and mixed-rate (with 1000 pps on some electrodes and 125 pps on the other electrodes) strategies. Stimuli were CNC words combined with speech-shaped noise. Stimuli were presented in quiet and noise. An ITD of either 0 μs or +800 μs was applied to the target. A binaural benefit was calculated as the difference in performance between the

0 µs and +800 µs ITD conditions. Speech understanding in quiet was also measured using the Advanced Combination Encoder strategy as a reference.

Results: Preliminary data suggest that speech recognition in noise is comparable across processing strategies, though the binaural benefits obtained in listeners using the mixed-rate strategy are unclear. The current data will be discussed in the context of how a bilateral mixed-rate strategy impacts speech understanding in quiet and noise and the potential contribution of ITDs.

Conclusions: These data suggest that a bilateral mixed-rate strategy can provide speech understanding similar to conventional processing strategies. Furthermore, our mixed-rate strategy may have the potential to convey ITDs for spatial separation of speech from noise.

Beta-4 Spectrin at the Heminode is Essential for Temporal Fidelity of Firing, Conduction, and Central Auditory Processing in the Developing Brain

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Background: Beta-4 (β4)-spectrin, a protein encoded by the gene Sptbn4, is part of the nodal and axon initial segment (AIS) complexes that include voltage-gated sodium (Nav) channels and ankyrin G. Spontaneous or targeted mutations in Sptbn4 result in neurological dysfunctions, such as myopathy and auditory neuropathy in human and mouse models. However, the cellular mechanisms of how the loss of β4-spectrin causes deficits in auditory transmission and processing in the brain is not well understood.

Methods: Using Sptbn4geo mice, which have a ROSAβgeo insertion in the Sptbn4, we investigated how the loss of β4-spectrin impacts structural and functional properties of auditory neurons in the auditory brainstem. An in vitro analysis using immunohistochemistry on Sptbn4geo mice examined Nav channel clustering at specific axon segments such as AIS, nodes, and the heminode in the medial nucleus of the trapezoid body (MNTB). Whole-cell patch-clamp recordings of the calyx of Held terminals were performed at P14-P16 of Sptbn4geo mice. In addition, we performed in vivo auditory function tests using distortion product otoacoustic emissions (DPOAEs), auditory brainstem responses (ABRs), and acoustic startle responses (ASRs) in Sptbn4geo mice at P21-P24.

Results: The loss of β4-spectrin did not impact structural properties of the nodes nor the AIS, but the clustering of Nav channels at the heminode were disrupted. Presynaptic terminal recordings in Sptbn4geo mice showed an elevated threshold of AP, larger rheobase, lower maximum dv/dt, and increased failures during AP train at high-frequency. The results indicate that the presence of β4-spectrin in the heminode is critical for maintaining the reliability and temporal fidelity of presynaptic spikes at the nerve terminal during early postnatal development. In the ABRs of Sptbn4geo mice, central conduction was significantly slower, whereas peripheral conduction was not affected. The DPOAEs showed that the cochlea function was normal in Sptbn4geo mice. Intriguingly, Sptbn4geo mice did not show detectable startle responses when presented with loud noise stimuli (at 120 dB), indicating central auditory processing deficits.

Conclusions: During postnatal development, the lack of β4-spectrin disrupts Nav channel clustering at the heminode along the nerve terminal and impacts the temporal fidelity and reliability of presynaptic spikes, leading to central auditory processing deficits.

Modulation of the Acoustic Startle Response by Background Sound

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Background: The acoustic startle response is a reflex elicited by intense auditory stimuli with rapid onset times. The acoustic startle circuit can be modulated by various factors to produce either attenuation or potentiation (e.g. prepulse inhibition or fear, respectively). Background noise can increase startle amplitudes, by a heretofore unexplained mechanism. Incremental levels of background sound increase startle amplitudes in a 'reverse-u' pattern, where the increasing background intensity increases startle amplitude after which a reversal is seen (Ison and Silverstein, 1978). It has been postulated that the startle potentiation is due to arousal, whereas the attenuation of this effect at high background intensities is due to masking of the startle by background noise.

Methods: N/A

Results: Using 3D motion tracking of the guinea pig pinna reflex, we show a reliable increase, followed by decrease in startle amplitudes with increasing background noise. Further, using a combination of single-unit recordings in the cochlear nucleus, and models of the auditory nerve and inferior colliculus (Zilany et al., 2014)

we demonstrate that the nonmonotonic effect of background noise on startle responses is not observed in the auditory brainstem and midbrain. Finally, preliminary recordings indicate the presence of this non monotonic effect in the human auditory cortex.

Conclusions: While the precise mechanism by which background sound increases startle amplitudes is still unknown, these data suggest that descending modulation from cortical areas to startle generating areas such as the caudal pontine reticular nucleus or medial facial nucleus might play an important role.

The Unique Developmental Differentiation of Neurons in Ultra-Low Frequency Nucleus Magnocellularis

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Background: The distinct tonotopic organization of auditory brainstem neurons in the chicken nucleus magnocellularis (NM) permits the precise temporal and spatial encoding of different sound frequencies. Recent work on the most caudal-lateral, ultra-low frequency region of NM (denoted NMc) describes neurons that demonstrate structural and functional differences from their higher-frequency NM counterparts. While we have characterized the functional phenotype of NMc neurons in late-developing chicken embryos (E20-21), little is known about the properties of NMc neurons at earlier developmental stages.

Methods: Using whole-cell patch clamp electrophysiology, we recorded from the most caudal-lateral region of NM in E13-14 chicken embryos, a developmental period just prior to the onset of hearing.

Results: We report here a distinct phenotype of NMc neurons that differ from both higher-frequency NM at a similar developmental period as well as more mature NMc. Early developing NMc neurons demonstrate significant differences in passive properties, such as input resistance and time constant, compared to NM neurons at the same age. NM and NMc at this developmental stage also exhibit differences in active properties, such as action potential (AP) current threshold, sodium current magnitude, and AP firing properties to square-pulse and sinusoidal somatic current injections. In addition to active and passive differences, a subset of NMc neurons demonstrate intrinsic spontaneous activity in the absence of synaptic contribution. While this is similar to mature NMc neurons, the spontaneity in younger neurons is broader in frequency distribution. Additionally, the total proportion of spontaneously active neurons in early-developing NMc is lower than in mature NMc, suggesting intrinsic developmental changes that work to increase spontaneity in maturation.

Conclusions: Taken together, our results identify the NMc phenotype in embryonic stages before the onset of hearing; in doing so, we demonstrate distinctions between early- and late-developing NMc and raise questions about how and why such differences arise.

Importance of Synaptic Vesicle Recycling in Ongoing Neurotransmission at Synapses Involved in Sound Localization

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Background: The lateral superior olive (LSO) is essential for computing interaural time and level differences during sound localization. LSO neurons weigh excitatory glutamatergic inputs from the cochlear nucleus against inhibitory glycinergic inputs from the medial nucleus of trapezoid body (MNTB). The glycinergic inputs are tuned for resilience, reliability and precision, even during sustained high-frequency stimulation (> 100 Hz). It has been shown that these features are achieved by a high quantal content and a rapid refilling of empty release sites. The mechanisms governing the vesicle replenishment at MNTB-LSO synapses are poorly understood.

The neurotransmitter refilling of recycled vesicles is driven by a proton-electrochemical gradient generated by a vacuolar H+-ATPase (V-ATPase). To investigate the dependency of vesicle replenishment at MNTB-LSO synapses on RRP recycling, we studied the synaptic performance upon V-ATPase blockade during long-lasting high-frequency stimulation.

Methods: Whole-cell recordings were performed on LSO principal neurons in acute coronal slices from P11±1 C57BL/6 mice at 37±1°C, and inhibitory postsynaptic currents (eIPSCs) were recorded. eIPSCs were evoked by electrical stimulation of MNTB fibers at 1-200 Hz in 60-s trains. Each train was followed by a 60-s recovery period at 1 Hz. In another series of experiments, MNTB-LSO synapses were challenged at 100 Hz for 4 min, followed by a 3-min recovery period at 1 Hz. V-ATPase activity was blocked pharmacologically by perfusing the V-ATPase inhibitors bafilomycin A1 (2 µM) or folimycin (concanamycin A; 1 µM). eIPSCs were recorded in control condition, after 10 min (short wash-in), and after 30-60 min (long wash-in) perfusion of the inhibitors.

Results: Unexpectedly, eIPSCs amplitudes were unaffected upon short wash-in of the inhibitors, during both challenge and recovery periods. The RRP, the vesicle release probability, and the sIPSCs amplitudes also remained unchanged compared to control. In contrast, synaptic plasticity and recovery from synaptic depression were significantly reduced upon long wash-in. However, neurotransmission did not completely collapse. Compared to controls, eIPSCs amplitudes reduced □2-fold more at 50 Hz. Despite a significant reduction of both RRP (□5-fold) and an increase in the release probability (□1.5-fold), MNTB-LSO synapses were still able to recover from synaptic depression, although only partially (□60% of the baseline level at ≥ 50 Hz). The resilience of MNTB-LSO synapses upon V-ATPase blockade was also demonstrated during 4-min long high-frequency stimulation at 100 Hz.

Conclusions: Our results suggest that the synaptic replenishment at MNTB-LSO synapses is not exclusively dependent on RRP recycling. It is tempting to search for other molecular mechanisms that form the basis for the remarkably indefatigability of these synapses.

Kv3.3 Subunits Control Presynaptic Waveform and Improve Timing at a Central Excitatory Synapse

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Background: High-voltage activated potassium channels of the Kv3 gene family are key in rapidly repolarizing action potentials (AP), supporting fast spikes and enabling high firing rates (Rudy and McBain, 2001; Kaczmarek and Zhang, 2017). Of the four Kv3 gene family members, Kv3.1 and Kv3.3 are highly expressed throughout the auditory brainstem including the medial nucleus of the trapezoid body (MNTB). Expressing multiple Kv3 subunits in the same neuron could be functional redundancy or imply subunit-specific roles; for example the shaping of presynaptic vs. postsynaptic APs. It has been shown that MNTB neurons possesses functional Kv3 channels that are composed of either Kv3.1 and/or Kv3.3 (Choudhury et al., 2020), and the deletion of either subunit caused a small increase in postsynaptic AP duration, consistent with functional redundancy of either subunit in the postsynaptic MNTB cell body. Here we test for Kv3 subunit-specific roles at the presynaptic calyx of Held terminal innervating MNTB neurons using in vivo single unit recordings in Kv3.3 knockout mice.

Methods: Adult Kv3.3 knockout mice of either sex and age-matched wild type mice were anesthetized by MMF, placed on a temperature-controlled heating pad in a soundproof chamber and stabilized in a custom stereotaxic device. An incision was made at the top of the skull, followed by a craniotomy just anterior to the lambda suture intersection. The skull was tilted to provide access to the auditory brainstem. A ground electrode was placed in the muscle at the base of the neck. Glass capillaries were pulled to a resistance of 5-20 MOhm when filled with 3M KCl solution. Signals were amplified, filtered and recorded with a Fireface UFX audio interface (HoerrTech). Pure tones (100ms duration, 5ms ramps) of varying intensities (0-90dB SPL) were presented at CF through hollow ear bars connected to the speakers.

Results: Extracellular recordings from MNTB neurons exhibited a typical complex waveform, comprised of a presynaptic and a postsynaptic component. The time between the peak and trough of extracellular APs are compelling markers for AP half-width and showed that the presynaptic APs were significantly longer in Kv3.3 knockouts (0.24 ±0.09ms; n=13) compared to WT recordings (0.17 ±0.02; n=20; P=0.036). Synaptic delays as measured by peak-to-peak times in the complex waveform were also significantly prolonged in Kv3.3 knockouts (0.58 ±0.17ms; n=13) compared to WT controls (0.45± 0.05ms; n=20; P=0.013).

Conclusions: These results suggest that Kv3.3 is the presynaptic 'delayed rectifier', enabling fast presynaptic APs and precisely timed synaptic delays. The changes in presynaptic AP duration and synaptic delay in the Kv3.3 knockout are likely to affect temporal processing in the MNTB output, such as first spike latency and jitter. However, the longer presynaptic APs will also affect transmitter release and hence spontaneous and sound driven firing rates.

The Organization of Auditory Nerve Fiber Synapses on Octopus Cells of the PVCN

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Background: Octopus cells (OCs) of the posteroventral cochlear nucleus (PVCN) receive inputs from approximately 60 tonotopically-organized auditory nerve fibers (ANFs) in mice. Each synaptic input is relatively weak, requiring summation of many inputs for OCs to fire action potentials. Together, well-timed ANF inputs and the remarkably fast OC membrane properties create a circuit ideal for coincidence detection. Previous work has

shown that ANFs have branches within the octopus cell area (OCA) and each ANF makes many synapses on an OC. However, the precise number of ANF synapses on OCs in mice is unknown. Additionally, new insights into molecularly distinct populations of spiral ganglion neurons (SGNs) have revealed our lack of knowledge about the organization of subtype-specific OC-ANF synapses and the interaction with the tonotopic organization of ANF inputs.

Methods: We used Cre recombinase-expressing mouse lines to specifically target anatomically and molecularly distinct subpopulations of SGNs and expressed Cre-dependent fluorescent reporters such as tdTomato (Ai14 strain) or synaptophysin-tdTomato fusion protein (Ai34D strain). Ai14 was used for validation of Cre-line specificity and Ai34 was used for subtype-specific labeling of synaptic puncta. We sparsely labeled OCs using a Thy1-YFP-H strain. We reconstructed putative synaptic puncta from subpopulations of ANFs onto sparsely labeled OCs in adult (P28-35) mice. Putative synaptic puncta were labeled with a Cre-dependent synaptophysin-tdTomato fusion protein (Ai34D mouse line). We then made 100 micron sections of the OCA in the parasagittal plane and collected confocal images of optically cleared (CUBIC) tissue. 3D reconstructions of ANF synapses on the dendrites and somas of OCs were made in Imaris.

Results: We validated the specificity and coverage of Cre-dependent fluorescent reporters in SGN subpopulations by reconstructing the innervation of SGNs along the pillar-modiolar axis of inner hair cells. We based our SGN subtype classification on previous work which demonstrated that type Ic SGNs are biased toward the modiolar face of the inner hair cell, like canonical low-SR SGNs, while type Ia SGNs are biased toward the pillar face of the inner hair cell, like high-SR SGNs. Our results indicate we can target type Ib and Ic SGNs with excellent specificity and coverage. We also found that we can sparsely target type Ic SGNs with high specificity. In addition, our preliminary data show subtype-specific differences in the dendritic innervation of ANF-OC synapses. Ongoing experiments and analysis are focused on differences in ANF-OC synaptic organization on the soma, the trunk-like proximal dendrites, and the distal ends of dendritic trees.

Conclusions: Our results suggest that synaptic puncta from distinct ANF subtypes are not evenly distributed along the somas and dendrites of OCs. Ongoing experiments will investigate this innervation pattern and examine how differences among ANFs influence the timing and pattern of OC synaptic integration and therefore OC computations.

Subcortical Responses to Continuous Music in Human Listeners
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Background: Music and speech are sounds that are unique to human beings. It has been shown that the Auditory Brainstem Response (ABR) can be derived from the EEG signal when people listen to continuous speech using deconvolution methods. The responses are robust and share a similar morphology with the ABR derived from repeated clicks using the conventional method. However, little is known about the subcortical processing of music, which has acoustically rich signals, even when compared to speech. Theoretically, deconvolution can be generalized to any continuous sound, and thus we used it to investigate the subcortical encoding of music.
Two years ago, we reported that derived subcortical responses for speech and music are different when using deconvolution with rectified stimulus as the regressor: ABR wave V was shown in speech at about 7.5 ms, but was absent at that latency for music. We offered two possible explanations: 1) subcortical auditory processing truly differs for music and speech; 2) acoustical differences between speech and music affected the deconvolution, leading to different derived responses.

Methods: To test the second possibility, here we introduce a new regressor that takes into account the peripheral effects of the acoustical differences – the Auditory Nerve Model responses (ANM). The ANM regressor is generated from Zilany et al (2014), which models the detailed transformation from acoustic signals to the AN representation of the stimulus. We created the regressor by summing the model neuron responses to the stimulus across characteristic frequencies.
To generalize the response for music and speech, 6 types of music that span different genres (hiphop, classical, pop, metal, jazz and acoustic music) and 6 types of speech from different scenarios (English audiobook, Chinese audiobook, lecture, news, interview and talk) were used in this study. Click trains generated from a Poisson process were also presented for comparison. Each type of stimulus was presented over forty 12-second epochs without repetition. Combined EEG with an ABR set-up and 32-channel system for cortical response was simultaneously recorded.

Results: From the results of 24 normal hearing subjects, we found that using the new ANM regressor in deconvolution, the ABR showed clearly for all types of speech and all genres of music with the same wave V latency and amplitude. Beyonds the subcortical response, the cortical responses for speech and music from deconvolution were also very consistent with the same major peaks and similar scalp topography.

Conclusions: Our results indicate that speech and music brainstem responses are very similar when accounting for acoustical differences' nonlinear effects on peripheral encoding. Further, although previous studies have highlighted differences between the auditory cortex's encoding of music and speech, our results seem to suggest that unaccounted for peripheral effects could be playing a role in those findings.

Four Sources of Cholinergic Input to the Nuclei of the Lateral Lemniscus

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Background: The dorsal and ventral nuclei of the lateral lemniscus (DNLL and VNLL) are large brainstem nuclei that provide the majority of ascending inhibitory inputs to the inferior colliculus. These nuclei express high levels of cholinergic receptors, suggesting that acetylcholine could modulate the ascending inhibitory signals to the IC. Four groups of cholinergic cells have been implicated in innervation of subcortical auditory nuclei, including the superior olivary complex (SOC), the pedunculopontine tegmental nucleus (PPT), the laterodorsal tegmental nucleus (LDT) and the lateral paragigantocellular nucleus (LPGi). However, it is not known which nuclei provide the cholinergic input to the DNLL and VNLL.

Methods: We injected adeno-associated virus containing a Cre-dependent fluorescent protein gene into brainstem cholinergic nuclei in a normal-hearing ChAT-Cre mouse. After 4 weeks to allow for gene expression, brains were fixed by perfusion with formaldehyde and sectioned for fluorescence microscopy.

Results: Following viral injections, we observed fluorescent axons in VNLL and DNLL. Many of the axons had long trunks parallel to the lemniscal fibers. In both VNLL and DNLL, the axons frequently exhibited en passant boutons as well as terminal boutons at the end of short side branches and, occasionally, more complex multi-branched arbors. Projections from the PPT and the LDT terminated bilaterally in both DNLL and VNLL. Projections from the LPGi also terminated bilaterally, with moderately dense terminations in the VNLL and sparser terminations in DNLL. Finally, injections in the SOC (which labeled cholinergic cells mainly in the lateral superior olivary nucleus and the ventral nucleus of the trapezoid body) revealed bilateral projections to the VNLL and no projections to the DNLL.

Conclusions: The VNLL receives cholinergic input from the PPT and LDT of the pontomesencephalic tegmentum, the LPGi, and the SOC. The DNLL receives input from PPT, LDT and LPGi, but not from the SOC. Each of these inputs could modulate the ascending inhibitory signals sent to the inferior colliculus. The various sources of acetylcholine likely serve different functions. The PPT and LDT have been associated with arousal, reward, sleep-wake cycle, sensory gating and cortically-driven plasticity. The SOC is likely to provide information more narrowly tuned to stimulus frequency, and may be particularly important for adjusting neuronal gain or maintaining temporal precision. Response properties of LPGi cells are unknown. The LPGi, like the PPT, LDT and SOC, receives ascending auditory input from brainstem centers and descending input from higher (e.g., auditory cortex) centers. Thus, all four cholinergic groups could contribute to bottom-up and top-down modulation of these inhibitory nuclei.

A Computational Model of Integration of Afferent Input Onto Medial Olivocochlear Neurons

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Background: Biologically informed computational models of individual neurons are important tools in the study of cell physiology. Computational models give the experimentalist dynamic and nearly unlimited control of potential variables of interest, including the presence and timing of synaptic inputs from other parts of a neuronal circuit. In auditory circuits, where sound is encoded through precisely timed neuronal activation, the computational control of input timing enables direct interrogation of synaptic effects on neuron function impossible in traditional brain slice preparations. Medial olivocochlear (MOC) neurons provide efferent feedback to the cochlea and modulate outer hair cell (OHC) electromotility via cholinergic signaling. MOC neurons receive excitatory inputs from neurons of the cochlear nucleus and recently described inhibitory inputs from the medial

nucleus of the trapezoid body (MNTB). The function of inhibitory vs excitatory input timing in neuronal activity remains to be explored.

Methods: We have constructed an anatomically detailed computational model of a single MOC neuron using python and the NEURON modeling framework. Ion channels used in the model neuron were chosen based on general principles of excitable membranes and modified to replicate measured action potential (AP) height, halfwidth, and threshold validated against electrophysiological measurements of MOC neurons from brain slices of ChAT-IRES-Cre x tdTomato mice. Inhibitory and excitatory postsynaptic potentials in the model neuron (IPSPs and EPSPs, respectively) were validated against EPSPs and IPSPs measured from MOC neurons.

Results: We applied models of inhibitory and excitatory synaptic conductances to the model neuron. We found the experimentally measured amplitude, kinetics, and timing of inhibitory inputs relative to excitatory inputs was sufficient to suppress or delay action potential generation in the model neuron. This result held for both single stimuli and trains of stimuli at 100 Hz.

Conclusions: Our computational model supports the ability of inhibitory synaptic inputs from MNTB neurons to suppress action potentials generated by excitatory inputs to MOC neurons.

Development of Patient-Specific Drug-Releasing Implants in Otorhinolaryngology Using Additive Manufacturing Techniques

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Background: There are anatomical structures in the head that are hard to access for a local and sustained drug delivery. Due to the individual sizes and shapes of the structures, an optimal pharmacotherapy adjusted for the individual patients needs is hard to achieve. We see a growing unmet clinical need for individually shaped drug-releasing implants for applications in the external ear canal, the middle ear, the round window niche, and the sinuses. These individualized implants will achieve a personalized drug therapy with a site-specific release of active compounds, extending the drug application time to span up to several weeks to get a much more dwell time and therefore keep the local drug delivery time extended.

Methods: We develop individualized drug-releasing implants for different anatomical regions in the ENT area utilizing 3D printing technology. Diagnostic imaging techniques and 3D reconstructions are employed to capture the anatomy of the region of interest. Different materials such as Poly(ethylene glycol) diacrylate (PEGDA) and UV-silicone loaded with e.g. dexamethasone and ciprofloxacine are investigated. To ensure the safety of the used materials and drugs we run in vitro tests, determined the drug release kinetics and tested for microbiological contamination.

Results: MTT tests and TNFα reductions tests were performed to evaluate the biocompatibility and bioactivity. Microbiological inhibition tests were performed as well and showed different zone of inhibition results with different concentrations of the drugs. The drug-loaded implants were also investigated for release rates and tested for microbiological contamination and showed no microbiological growth after curing in the UV crosslinker. In addition, the anatomical accuracy as well as the handling of different prototypes of implants were evaluated in cadaver experiments and showed perfect fit in the desired anatomical regions.

Conclusions: Using different additive manufacturing processes such as Digital Light Processing (DLP) and extrusion printing using the EnvisionTEC VIDA DLP-device and EnvisionTec 3D-Bioplotter (EnvisionTEC, GmbH, Gladbeck, Germany) drug-loaded individualized implants can be manufactured and implanted into the patient. The goal is to integrate the developmental steps in one workflow in the operating room during the surgery to offer individualized drug-eluting implants to the patients.

Audiovestibular Symptoms at the Intensive Care Unit: A Scope Review

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Background: Individuals in the intensive care unit (ICU) are exposed to several environmental, biological and mechanical factors that may affect hearing and balance. Some of these include noise, ototoxic medications, sepsis, electrolyte abnormalities, dehydratation and malnutrition. Others described include exposition to led monitors, ventilation equipment, changes on the ventilation and vital capacity, limited body movements, and medical complications associated to systemic patient's disorders. However, limited data and studies are focused on how hearing and balance may be affected at ICU. Here in, we performed a scope review to determine the frequency of audiovestibular symptoms at the ICU and the related events and factors associated to their presentation.
Methods: This narrative review was conducted between August and September 2021. We used the MeSH headings "vertigo", "hearing loss","tinnitus", "intensive care unit",   and "critical care", to search PubMed, Embase, and Google Scholar databases for case reports, case series, retrospective chart reviews, prospective studies, cohort and case control studies. This search aimed to 1) Describe the frequency of audiovestibular symptoms at the intensive care unit and, 2) Describe the audiometric and vestibular testing findings in patients who complained of audiovestibular symptoms at the intensive care unit.
Results: Seven studies including 2 case reports, 3 prospective studies and 2 retrospective chart reviews with 571 patients were included. The age average of studies included was 60.8 ±8.1 with range between 23-84.8 years. Five studies (one case report, one retrospective study and three prospective studies) revealed hearing loss as one of the most common audiovestibular symptom in patients at the intensive care unit. Primary causes of admission to the intensive care unit in patients with hearing loss included multiple trauma, sepsis, single system failure and, cardiovascular and pulmonary disorders. Abnormal Distortion product otoacoustic emissions were detected in 77.8% of patients at ICU as well as tympanometric curves type A and C. Vertigo and tinnitus were also found with lower prevalences at ICU, mainly associated to cardiovascular disorders and central nervous system injury. Males presented more vestibular symptoms than females. Primary etiologies associated to vertigo and tinnitus at ICU were focal intracerebral hemorrhage, septic shock, arteriosclerosis and cardiogenic cerebral infarction. No available vestibular testing were described in the studies found.
Prolonged use of ventilator was described in patients with hearing loss and tinnitus.
Conclusions: Hearing loss, tinnitus and vertigo are some of the audiovestibular symptoms reported on patients at ICU. Most of them were found in patients with cardiovascular and pulmonary disorders. Abnormal testing in hearing loss suggest auditory changes during ICU stay. Further studies are needed to understand the clinical progression of these symptoms and the clinical characteristics of each.

Sleep Disturbance and Dysregulation of Circadian Clock Machinery in Sudden Sensorineural Hearing Loss: A Preliminary Study

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Background: Circadian rhythm is driven by genetically programmed mechanisms that regulate the circadian clock genes in the sleep-wake cycle with a 24h periodicity. The communication between central (i.e., suprachiasmatic nucleus) and peripheral circadian oscillators regulates biological functions essential in our body. Specifically, the cochlea contains a robust biological clock associated with auditory function, exhibiting diurnal sensitivity to noise trauma or ototoxicity. However, in humans, little is known about how dysregulation of circadian rhythms links to hearing loss. We herein examine the relationship between disrupted circadian rhythm and altered expression of circadian clock genes in patients with sudden sensorineural hearing loss (SSNHL) and explore whether the circadian clock genes serve as a prognostic biomarker.
Methods: We herein examine the relationship between disrupted circadian rhythm and altered expression of circadian clock genes in patients with sudden sensorineural hearing loss (SSNHL) and explore whether the circadian clock genes serve as a prognostic biomarker.
Results: Compared to healthy controls without hearing loss, most of the circadian clock genes were markedly downregulated, coupled with the low sleep quality and disturbing patterns, in the patients with SSNHL. Intriguingly, a weak tendency of correlation between hearing improvement following steroid treatment and altered levels of circadian clock genes was observed.
Conclusions: This study provides additional basis for the recently proposed relevance of disrupted circadian rhythm to SSNHL and suggests a prognostic biomarker towards better audiological outcomes, possibly opening avenues for chronotherapy.

Bilateral Cholesterol Granulomas of the Maxillary Sinus
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Background: Cholesterol granulomas are a common benign pathology classically found in the mastoid antrum and air cells of the temporal bone and less commonly found in the skull and paranasal sinuses. As of 2020, only 54 cases of cholesterol granulomas in the maxillary sinus have been reported in literature. This is the second case to our knowledge of bilateral maxillary sinus cholesterol granulomas. This report and literature review should serve as an updated resource for otolaryngologists regarding cholesterol granulomas of the paranasal sinuses and an imaging reference for bilateral lesions.
Methods: In an effort to provide an update to this comprehensive review, we examined the literature regarding cholesterol granulomas of the paranasal sinuses from January 2011 through 2021. Twelve abstracts were identified with a total of the 18 cases. The mean age was 45.4 years with a male to female ratio of 0.7:1.
Results: Combining the results from our review with that of Durgam et al, approximately 59% (91/153) of the reported cases of cholesterol granulomas originated in the frontal sinus, 33% (51/153) in the maxillary sinus, 4% (6/153) in the ethmoid sinus, and 3% (5/153) in the sphenoid sinus.
Given the variability in location and features of cholesterol granulomas in the paranasal sinuses they are amendable to various surgical approaches. Classically, there is the external approach with the Culdwall-luc operation and an internal approach with endoscopic sinus surgery. Other procedures such as intranasal antrostomy, lateral wall osteotomy, incisional biopsy of maxillary vestibule were also reported. The open approach has essentially become obsolete due to the advancements of endoscopic sinus surgery that allow most lesions to be removed endoscopically. The Durgam et al. review reported on definitive management for 65 cases which consisted of an open procedures in 80% (52/65) of cases and an endoscopic approach in 20% (13/65) of cases. Of the 18 cases reported in the literature since 2011, 61.1% (11/18) where treated with an open approach and 38.9% (7/18) where treated via an endoscopic approach. The Drugam et al. review reported a recurrence rate of 8.2% and in our review of cases since 2011, there were reportedly zero cases of recurrence. Although our patient developed recurrence on the left, she continues to be asymptomatic and therefore elected for close observation before considering revision ESS.
Conclusions: In conclusion, we present the second known case of a bilateral cholesterol granuloma of the maxillary sinus. This case reinforces that upon presentation, cholesterol granulomas can resemble multiple pathologies and histology is needed for diagnosis. Additionally, we illustrate that although rare, cholesterol granulomas should be kept in mind as a possible etiology for bilateral masses of the paranasal sinuses.

Modeling Neurofibromatosis by Schwann Cell Differentiation From a NF2-Mutant Bearing Induced Pluripotent Stem Cell Line
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Background: Neurofibromatosis is a genetic disorder that leads to the formation of Vestibular schwannomas (VSs). Although these tumors are normally benign, depending on the tumor location and microenvironment, patients with VS often suffer from progressive hearing loss (HL). Genotype-phenotype analysis of NF2 patients has identified point mutations that cause a range of severity in clinical outcomes. In previous work, a natural-occurring mutation (L64P) in patients, located in exon 2, impaired the ability of merlin protein to interact in a complex essential for its signal transduction function. Human induced pluripotent stem cells (iPSC) will be used to generate functional Schwann cells (SC) to model the molecular consequences of this variant on SC physiology. Here, we describe the differentiation of control and L64P mutant-bearing iPSCs to self-renewing SC precursors (SCP) and, in turn, mature myelinating SCs.
Methods: iPSCs were differentiated into SCPs and mature SCs using lineage-specific chemically defined medium conditions. The identification and functionality of SCPs and SCs were confirmed by staining for lineage-specific markers for immunocytochemistry (ICC), as well as molecular analyses. Western blot analysis was used to determine merlin protein expression levels throughout the differentiation protocol.
Results: iPSCs were differentiated into SCPs as demonstrated by the positive staining for Sox10, Nestin, and GAP43. Subsequently, derivation of mature SCs was determined by staining for myelination-specific markers Myelin basic protein (MBP) and Myelin protein zero (MPZ). Finally, we demonstrated that merlin protein, the

product of the NF2 gene, was expressed in both the control and L64P variant-bearing iPSC-derived SCs following 15 days of differentiation.

Conclusions: Tissue engineering of SC has led to an in vitro disease model that allows investigation of the VS tumor biology. In this study, we successfully demonstrated the SC differentiation protocol to further study the phenotypic manifestations of the L64P mutation. In previous studies, several immunological proteins that were highly associated with HL were identified in the perilymph proteomic studies of patients with VS. This work is significant as it will complement previous in vivo studies in the discovery of biomarkers associated with these tumors in individual VS patients by using patient-derived iPSCs. In the future, this will lead to development of personalized identification of biomarkers followed by therapeutic treatments on an individual basis for VS-associated HL.

Control of Cochlear Innervation by Pou3f4 and Otic Mesenchyme

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Background: Spiral ganglion neurons (SGNs) interact with otic mesenchyme cells prior to forming synapses with hair cells during cochlear development. The Pitt-Oct-Unc (POU)-domain transcription factor Pou3f4 is expressed specifically by otic mesenchyme cells in the inner ear. POU3F4 mutations are associated with X-linked nonsyndromic hearing loss (DFNX2). Previously, we found that POU3F4 and otic mesenchyme cells control aspects of SGN guidance and survival (Coate et al., 2012; Brooks et al., 2020). Here, we report on POU3F4 in the control of Efna1 (EPHRIN-A1) and Efna2 (EPHRIN-A2) by otic mesenchyme cells at E15, and the role of these factors in SGN fasciculation. We also report on POU3F4 in the regulation of SGN survival cues, and activity in the early postnatal cochlea.

Methods: These studies include transgenic mouse models, SGN explant cultures, confocal imaging, RNAscope™, chromatin immunoprecipitation (ChIP), in situ hybridization, and Ca2+ imaging.

Results: Results from RNAscope™ and ChIP suggest POU3F4 normally inhibits Efna1 and Efna2 expression in the otic mesenchyme at E15. Compared to controls, Efna1-/-Efna2-/- cochleae show reduced axon outgrowth and subtle fasciculation defects. In-vitro assays show both EPHRIN-A1 and -A2 recombinant proteins help attract growing SGN processes and inhibit fasciculation of the growing neurites. In ongoing work, in situ hybridization is being used to examine expression patterns of factors misregulated in Pou3f4 null cochleae, as elucidated by single cell RNA sequencing. In addition, Snap25-GCaMP6s and Ca2+ imaging is being used to examine patterns of spontaneous activity in Pou3f4 null cochleae.

Conclusions: Taken together our findings suggest that POU3F4 is an important regulator of SGN axon guidance and survival, and may be necessary for pre-hearing activity. Understanding how SGNs form proper connections to hair cells through these studies and others will be crucial in developing new therapeutic strategies to help patients with sensorineural hearing loss.

Retinoic Acid Signaling Guides the Efficiency of Inner Ear Organoid-Genesis and Governs Sensory-Nonsensory Fate Specification

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Background: The early developmental of inner ear organoids—from germ layer to otocyst formation—are highly controlled with timed chemical cues that recapitulate major signals in vivo. In contrast, later stages of differentiation—from otic vesicle (OV) to organoid formation—are self-guided with little outside influence, even though these stages are guided by several key morphogens in vivo. In prior work, we found that late-stage cultures were sensitive to retinoic acid (RA) signaling, an essential morphogen in normal inner ear development. In this study, we sought to gain greater control over RA signaling during OV formation in organoid cultures.

Methods: Mouse embryonic stem cells carrying a lacZ transgene under the control of three RA response elements were adapted to the organoid culture paradigm and used to report on RA response. Exogenous control over RA signaling was achieved by inhibiting endogenous RA synthesis combined with exogenous application of all-trans RA (atRA). RA level was modulated from culture day (D) 8 to D12, during OV formation but prior to organoid

development. LacZ transgene expression was monitored by X-gal staining. RA-dependent changes in sensory and nonsensory fate were examined by quantitative PCR using the biomarkers Lfng and Lmx1a, respectively. Organoid efficiency was quantified as the percentage of spheroids producing one or more cyst-like organoids by culture D20.

Results: LacZ expression was asymmetrically distributed around the OVs suggesting that RA signaling was polarized, with a greater response in the portion facing the outer epithelium of the spheroids that contain these vesicles. The intensity of the lacZ staining varied widely between individual OVs; the highest levels were equivalent to those from cultures treated with 500 nM atRA on D8 to D12, a treatment that completely inhibited organoid-genesis. When endogenous RA synthesis was inhibited and atRA was added at increasing doses from 0.5 to 500 nM, the expression of Lfng decreased and Lmx1a increased, consistent with a shift in sensory to nonsensory fate specification—anterior to posterior fate—in the OVs. Notably, moderate levels of atRA (~50 nM) increased the efficiency of organoid production compared to control, non-modified cultures.

Conclusions: Late-stage organoid development is regulated by RA, a key morphogen guiding organogenesis in vivo. Observations of extremely high RA signaling in some cultures—levels similar to conditions that inhibit organoid-genesis—suggested that variations in endogenous RA levels could negatively impact organoid culture efficiency. By gaining greater control over RA signaling, we improved the efficiency of organoid production and could shift the expression of sensory and nonsensory biomarkers. Further study is needed to determine whether modulation of RA level can increase organoid hair cell production and whether this platform can help catalog the RA-responsive genes driving organogenesis and cell fate specification.

A New Transcriptional Regulator of Medial–Lateral Sensory Cell Patterning in the Developing Organ of Corti Identified by Multi-Omics Analysis

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Background: We previously used ATAC-seq analysis of the embryonic cochlea to identify transcription factor binding motifs in open chromatin regions of prosensory cells. This analysis (1) confirmed the presence of motifs for transcription factors already known to be important in cochlear development and (2) revealed the presence of motif enrichment for numerous transcription factor families not previously known to be important in hair cell and support cell development.

Methods: Here, we used parallel scRNA-seq analysis to determine the expression patterns of transcription factors with the motifs that were specifically enriched during prosensory cell development. We identified one highly-expressed member of a transcription factor family not well studied in the inner ear previously, Ebf1, and then tested its role in cochlear development. To conditionally delete Ebf1 in prosensory cells, we crossed an existing floxed mouse strain for Ebf1 to the Sox2-CreER strain and injected pregnant dams with tamoxifen at E11, prior to the formation of the cochlea.

Results: By E16, we observe extra Atoh1+ inner hair cells in the tamoxifen-treated mice. At E18, we observed extra Myo7a+ inner hair cells both in flox/flox homozygous and in flox/+ heterozygous mice after tamoxifen treatment, indicating haploinsufficiency of Ebf1. We found extra Myo7a+ hair cells at E18 in tamoxifen-treated flox/flox mice, but not in the untreated mice, indicating they are independent of Sox2 haploinsufficiency in the Cre line. In mutants, increased inner hair cell density is evident in the apical half of the cochlea by E16, and EdU+ inner hair cells and medial support cells were observed in the apex after daily injections at E15, E16 and E17. Other aspects of cochlea formation that we measured including cochlear length and outer hair cell numbers were unaffected.

Conclusions: We surmise that medial restriction of the phenotype relates to the expression pattern of Ebf1, which is greater in medial prosensory cells than in lateral prosensory cells. It is unclear whether the phenotype is stronger in the apical region because of a difference in basal–apical gradient of hair cell differentiation or is a phenotypic delay caused by the timing of Cre induction. Although the importance to hearing function remains unclear, our findings confirm a novel regulator of medial–lateral sensory cell patterning. Loss of function was associated with delay of cell cycle exit in the apical/medial region of the cochlea on the medial side of the organ of Corti. Analysis of appropriate ATAC-seq and RNA-seq datasets is a valuable strategy to predict previously unknown regulators of developmental patterning in the cochlea.

Modulation of Neural Excitability by Acetylcholine in the Developing Gerbil MNTB
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Background: The medial nucleus of trapezoid body (MNTB) has been well studied as the primary source of contralaterally derived inhibition to the brainstem auditory circuitry. MNTB-originated inhibition plays a critical role in the computation of sound location as the temporal aspects of sounds are precisely conveyed through calyx of Held/MNTB synapse. We have previously shown that in adult gerbils, cholinergic signaling modulates sound-evoked responses of the MNTB (Zhang et al. 2021). One of the key findings is that cholinergic signaling contributes to maintaining sustained MNTB responses for optimized encoding efficacy. However, the cellular mechanisms through which ACh influences MNTB neurons remains obscure. To investigate these questions, we used in vitro whole cell patch clamping to record from MNTB neurons in developing gerbils. In addition, we performed immunolabeling to document developmental expression patterns of cholinergic receptors on pre- and postsynaptic elements in MNTB.

Methods: Gerbils aged P9-36 were used for immunolabeling and whole cell patch clamping. The excitability of MNTB neurons in response to cholinergic activity was assessed using current clamp with 50pA steps. Voltage clamp with 10mV steps was used to study the cholinergic effect on outward membrane conductances. Measurements were taken with bath application of cholinergic agonist acetylcholine (ACh), nicotinic receptor antagonist mecamylamine, and muscarinic receptor (mAChR) antagonist atropine. Antibodies directed against M1 and M3 muscarinic receptors were used to profile expression during this developmentally consequential age range.

Results: Our anatomical studies demonstrate a developmental pattern in the expression of key cholinergic markers. M3 receptors were prominent in presynaptic terminals prior to hearing onset while M1 receptors were primarily postsynaptic. Whole cell physiological results show that ACh has a potent positive effect on the excitability of MNTB neurons via mAChRs, with the effect being the most prominent just before hearing onset. During this period and following hearing onset, we observed a mAChR-mediated decrease in voltage dependent outward conductances at depolarized voltages. These findings are complemented by high levels of postsynaptic M1 receptor expression that may underlie the cholinergic increase in membrane excitability that we observed.

Conclusions: Our results indicate that cholinergic function is prominent in the developing MNTB and is likely mediated in part by M1 and M3 muscarinic receptors. These data demonstrate candidate cellular mechanisms of cholinergic signaling that may contribute to the known modulation observed in adults. Further, the dynamic mAChR expression and physiology of cholinergic signaling early in development suggests a potential developmental role in shaping MNTB circuitry.

Cellular and Molecular Basis of Neurite-Glia Interaction in the Developing Cochlea

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Background: In the sensory epithelium, sound frequency is encoded in the spatial organization of hair cells and spiral ganglion neurons (SGNs). During development, SGN peripheral processes of similar characteristic frequencies coalesce into radial bundles. Aberrant radial bundle formation has been implicated in hearing loss yet the cellular and molecular mechanisms underlying radial bundle formation remains unknown. In live images of murine embryonic cochlea explants, the initial outgrowth of SGN peripheral processes were facilitated by close interaction with Schwann cell precursors (SCPs). Previous work also shows that glia depletion from the cochlea impairs radial bundle formation, but the SGN cell bodies were also misplaced. Strikingly, radial bundle formation is also disrupted upon conditional loss of the transcription factor, Gata3, from SGNs. We will investigate the hypothesis that precise radial bundle formation is mediated by interaction between SCPs and SGN peripheral processes, and that these interactions are disrupted in Gata3 conditional mutant mice.

Methods: Sparse labeling of SGN peripheral processes was used to characterize the patterns of interactions with SCPs during outgrowth. We examined how these interactions were disrupted on Gata3 deletion or on loss of glia. To label SGN peripheral processes, Ngncre was used to drive expression of a red fluorescent protein. To delete Gata3 from SGNs, we crossed Ngncre into a mouse homozygous for Gata3 floxxed allele. We collected cochleae at embryonic day 14.5 (E14.5), when SGN peripheral processes first reach hair cells. For glia ablation, we used an inducible Plp1cre to drive diphtheria toxin expression in glial cells which express Proteolipid 1 protein. We performed wholemount cochlea immunostaining with TUJ1/Neurofilament to label SGN peripheral processes. Images of stained cochleae were acquired on a confocal microscope and analyzed in ImageJ software. To identify genes enriched in SCPs interacting with SGN peripheral processes, we analyzed a single cell RNA sequencing dataset of E14.5 cochlea glia.

Results: Our analysis of neuron-glia interaction in cochlear development highlighted a glia bridge which SGN peripheral processes grow on towards hair cells. Preliminary data shows that perturbations of Gata3 expression in SGNs affect neurite glia interactions. Analysis of the single cell data showed enrichment of Gata2 and Npy in the glial bridge, which we have confirmed by immunostaining. No Gata2 transcript was detected in neurons. On diphtheria toxin ablation of glia, SGN peripheral processes were highly disorganized. More analyses are needed to determine how Gata3 mediates neuron-glia interaction.

Conclusions: Our data confirm the importance of SCPs for SGN peripheral process outgrowth. Further, Gata2 and Gata3 may play complementary roles in neuron-glia interaction in the cochlea. Future studies will be aimed at closer analysis of the roles of Gata2 and Gata3 by examining their downstream target genes and identifying adhesion molecules that may mediate these interactions.

Pou4f3 is Required for the Development of Type I Hair Cells in the Mouse Utricle

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Background: POU class 4 transcription factor 3 (Pou4f3) is predominantly expressed in the sensory hair cells of the inner ear (Xiang et al., 1997). Germline deletion of Pou4f3 (Pou4f3−/−) leads to profound hearing loss and balance deficits, due to rapid and progressive loss of hair cells during development. Xiang et al., showed complete loss of hair cells in the hearing organ (cochlea) of Pou4f3−/- mice. In addition, deletion of downstream targets of Pou4f3, such as Gfi1 and Lhx3, also negatively affects the development of cochlear hair cells. Although the cochlear manifestations of Pou4f3 mutations have been characterized, the role of Pou4f3 in vestibular hair cell development is unclear.

Methods: Studies used Pou4f3huDTR mice (Tong et al., 2015) in which a portion of the Pou4f3 coding region is replaced with the human diphtheria toxin receptor (huDTR) gene. Homozygous huDTR mice (Pou4f3DTR/DTR) lack functional Pou4f3 protein (referred as Pou4f3-/-). We also used homozygous Gfi1 GFP/GFP mice, in which a portion of the Gfi1 gene is replaced by the green fluorescent protein (GFP) gene (referred as Gfi1-/-). Temporal bones were collected at P0 and P30, and cochleae and utricles were processed as whole mounts. Specimens were immunolabeled with combinations of Myosin VIIa, SOX2, TUJ1/Neurofilament, Annexin A4 (ANXA4), MAPT antibodies, and nuclei were stained with DAPI. Confocal microscopy was used to image specimens and subsequent analysis used ImageJ and Velocity software. We also examined stereocilia bundles using phalloidin labeling and by Scanning Electron Microscopy (SEM).

Results: We found that germline deletion of Pou4f3 and its downstream target Gfi1 led to near complete loss of all hair cells in the adult cochlea. Although we also observed some reduction in the numbers of vestibular hair cells, a significant number of hair cells were still present in utricles of adult Pou4f3−/− mice. No hair cell loss was observed in utricles of Gfi1−/− mice. Remaining hair cells in utricles of Pou4f3−/− mice expressed immunomarkers consistent with a type II phenotype, and the reduction in hair cell number suggested the loss of type I hair cells. We also noted abnormally small and truncated stereocilia bundles on the apical surfaces of the type II hair cells in Pou4f3−/− utricles.

Conclusions: These findings suggest that Pou4f3 and its downstream target Gfi1 are required for the development and survival of cochlear hair cells. However, Gfi1 is not essential for the development of vestibular hair cells and Pou4f3 is essential for generation and survival of type I – but not type II - hair cells.

The Single-Cell Transcriptional Landscape of Neuronal Fates in the Developing Mouse Cochlea

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Background: The spiral ganglion neurons (SGNs) of the cochlea are essential for auditory perception by transmitting complex auditory information from hair cells (HCs) to the brain, yet the molecular mechanisms generating their diversity are unknown.

Methods: Here we used single cell RNA sequencing to reconstruct the developmental trajectories of SGN cell fates in mouse embryos.

Results: We identified genes and gene regulatory networks that participate to changes in developmental competence and cell states, and in specification of each major cell types. Our analysis also identified gene modules associated with the sequential binary decisions that delineate neuron fate choices along the diversification

tree. Transcriptome analysis of both developing SGN and HC types further revealed cell-state specific cell-cell signaling potentially playing a role in the differentiation and connectivity profile of SGNs as well as human deafness-associated genes, both previously known and novel.

Conclusions: Overall, our results identify molecular principles that shape SGN differentiation and will facilitate further studies of SGNs development, physiology and dysfunction.

The Effects of Age on Outcomes of Cochlear Implant Use in Children and Adolescents Wit Short Durations of Single-Sided Deafness

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Background: The aim of the present investigation was to identify effects of age at cochlear implantation in a cohort of children/adolescents with limited durations of single-sided deafness (SSD). Many children with SSD use their cochlear implants (CI) consistently and develop auditory function in the implanted ear (Polonenko et al, 2017; Zeitler et al, 2019; Ganek et al, 2020). Early implantation may provide better hearing outcomes (Arndt et al, 2015; Deep et al, 2021) but it is not clear whether this is particular to children with pre-lingual onset of SSD. To study this, 57 children with limited durations of both pre-lingual (PL-SSD) and later onset (LO-SSD) SSD were implanted. We hypothesize that, when duration of SSD is limited, outcomes of CI improve as age at implant decreases.

Methods: The cohort had limited durations of deafness [1.94-years (1.56 SD)]; 40 had PL-SSD [Age/Implant = 2.47-years (1.58 SD)] and 17 had LO-SSD [Age/Implant = 11.67-years (3.91 SD)]. Outcome measures were consistency of CI use (datalogging in 37 PL-SSD; 16 LO-SSD), spatial-release-of-masking (SRM) [9 PL-SSD; 7 LO-SSD], speech-perception-accuracy (RAU) [17 PL-SSD; 8 LO-SSD] in age-appropriate tests, self-reported hearing (Speech, Spatial and Qualities of Hearing Scale (SSQ) [35 PL-SSD; 14 LO-SSD], and localization of stationary and moving sound [3 PL-SSD; 9 LO-SSD] (band-pass filtered white noise presented within 120° arc in frontal-azimuthal/horizontal plane). Linear mixed effect models assessed group effects on each outcome.

Results: Datalogs revealed slight decreases in daily CI use with ongoing CI experience [Estimate (SE) = -0.039 (0.014) daily hours/month, $p = 0.003$]. Of note, daily CI use across groups decreased during the pandemic compared to pre-pandemic levels [Estimate (SE) = -1.47 (0.70) daily hours, $p = 0.04$]. Speech perception (RAU) on the side of the implanted ear was better in children with PL-SSD than LO-SSD [Estimate (SE) = 23.9 (8.9) RAU, $p = 0.008$] and SRM was more symmetric in the PL-SSD group [Estimate (SE) = 5.61 (1.55), $p = 0.003$]. Location of stationary sound showed high root-mean-squared-error (RMSE) [CI on: 26.6°-30.4°; CI off: 25.8°-40.0°] which improved slightly with CI especially on the implant side [Estimate (SE) = -10.4° (5.55), $p = 0.06$]. SSQ revealed hearing challenges [SSQ total = 6.4 (out of 10)] which increased significantly as RMSE increased.

Conclusions: Children/adolescents with late onset/short duration SSD can have challenges maintaining consistent daily CI use which are exacerbated during the pandemic period with associated school closures. This group experienced more asymmetrical hearing than the younger children with pre-lingual/short duration SSD which is consistent with declining cortical representation from the deaf ear in the older group (Lee et al. 2020). The subtle improvements in spatial hearing with CI use could be valuable in these children given increased self-reported hearing challenges with poor sound localization.

The gEAR Portal (umgear.org) – New Features and Updates!

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Background: The gene Expression Analysis Resource (gEAR, umgEAR.org) Portal has become a familiar resource within the hearing community as a tool to display, analyze and share multi-omics data. Here we describe updates to the platform, including new multi-gene displays, Dataset Explorer, Gene Cart Manager, full data downloads, spatial transcriptomics, support for epigenetic data via EpiViz integration and many smaller improvements and optimizations to better serve this community.

Methods: The portal runs on Google Compute Engine resources, implemented as a web application via Python3, Javascript, HTML5, CSS3, the Scanpy module, and visualizations using D3.js, Plotly and Dash Bio. Data are stored in a combination of MariaDB and H5AD binary files.

Results: After the gEAR Portal was published in Nature Methods (Orvis et al., 2021), our team has continued to add new features based in great part on user feedback. Previously focused on single-gene displays, we have added a toggle on the primary search page which updates any configured displays to show multi-gene displays such as heatmaps, which show expression for multiple genes simultaneously. To support this, we have also created a Gene Cart Manager, allowing users to create collections of genes called carts. These carts can be unweighted (simple gene symbol lists) or weighted (gene symbol lists with expression values across an array of conditions.)

A completely redesigned Dataset Explorer allows users to find public datasets, organize them into thematic profiles, as well as manage their own private datasets. Furthermore, each dataset window on the main page now has additional external links and download options, allowing users to download the originally submitted data and analysis for most public datasets.

As single-cell transcriptomics continues to evolve, we now provide support for graphical display of spatial transcriptomic data - a data modality well suited also for inner ear research given the specific organization of the inner ear cell types.

Conclusions: The gEAR portal, which now has over 162 curated multi-omic datasets from the ear field organized in thematic displays, is an actively growing repository that continues to update based on user feedback. With over 1,300 registered users and thousands of monthly entries, it is now the primary resource for multi-omic data visualization, sharing and analysis in the ear field.

Utility of Exome Sequencing for Genetic Diagnosis of Hearing Loss in a Large, Clinically Heterogeneous Cohort of Pediatric Patients

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Background: Hearing loss (HL) is a common sensory deficit, affecting 3/1000 newborns. Pinpointing the etiology of pediatric HL in a timely manner is critical in order to provide prognostic information, ensure access to appropriate habilitation as early as possible, and allow for time-sensitive counseling. Pediatric HL may be attributed to infectious, anatomic, or genetic causes, with over 50% of instances of sensorineural HL being genetic in etiology. Due to its genetic heterogeneity, the standard of care for identifying the cause of pediatric HL is a targeted panel that sequences a list of known deafness-causing genes. However, with recent developments in technology, exome sequencing (ES), which sequences all protein-coding regions of the genome, has become more accessible and presents an alternative strategy for obtaining a molecular diagnosis for HL. Several studies have investigated the efficacy of ES for genetic diagnosis of HL, with overall diagnostic rates from 31%-47.3%. The objective of the current study was to build upon previous literature by investigating the efficacy of ES for genetic diagnosis in a large clinically heterogeneous cohort of pediatric patients affected by HL.

Methods: Patients seen in the Boston Children's Hospital Department of Otolaryngology and Communication Enhancement with confirmed HL, without a known genetic or environmental etiology, as well as their biological relatives were eligible for enrollment. Written informed consent was obtained from all participants, and ES was performed using DNA derived from buccal swabs. Variant filtering and analysis focused on 366 known and candidate deafness-causing genes as well as ACMG59 secondary findings for interested participants. Clinically confirmed results were reported back to patients who elected to receive them, and appropriate follow-up care was coordinated for patients with syndromic and secondary findings.

Results: Exome analysis has been completed for 161 probands to date. This was a highly heterogeneous cohort consisting of patients with known anatomic abnormalities; various lateralities, degree, configuration, and age at onset of HL; and various familial inheritance patterns. Positive findings were identified for 51 (31.7%) patients in 28 different genes, with inconclusive findings identified in an additional 13.7% of patients. The diagnostic (positive) rate was highest for patients with a positive family history, symmetric HL, and prelingual onset of HL. 19.6% of children with positive findings were diagnosed with syndromic forms of hearing loss. Secondary findings were identified in seven patients in six different genes.

Conclusions: ES offers several advantages over HL gene panel testing, including an effective diagnostic rate, identification of secondary findings, and an efficient research pipeline for discovery of novel deafness-causing

genes. Furthermore, the results of this study support increased access to genetic testing for patients affected by HL in order to provide valuable prognostic information and facilitate timely access to appropriate habilitation and molecular therapies.

Single-Cell RNA-Seq Reveals Transcriptional Changes in Pou4f3 Mutant Vestibular Hair Cells

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Background: The transcription factor POU Class 4 Homeobox 3 (Pou4f3) plays a critical role in the development of the sensory cells of the inner ear. In the cochlea, Pou4f3 appears to be essential for hair cell survival, as the cochlear hair cells of Pou4f3-/- and Pou4f3DTR/DTR mice degenerate around the time of birth, resulting in profound deafness. Interestingly, while these mice also display clear signs of vestibular dysfunction, including circling behavior and ataxia, their vestibular organs retain MYO7A-positive hair cells into adulthood. While Pou4f3 is therefore not required for the survival of vestibular hair cells, recent work showed that utricular hair cells lacking functional Pou4f3 are Type II-like and appear immature, with their stereocilia and innervation rudimentary or absent.
Methods: To investigate the transcriptional effects of a lack of functional Pou4f3 on utricular hair cells, we performed single-cell RNA sequencing on utricular hair cells from heterozygous and homozygous Pou4f3 mutants.
Results: Consistent with reported antibody staining, mouse utricles without functional Pou4f3 lacked Spp1-positive Type I hair cells. Instead, these utricles possessed hair cells that expressed some Type II markers, but also retained expression patterns consistent with immaturity.
Conclusions: Our data suggest that functional Pou4f3 is necessary for Type II hair cell maturation and, when absent, utricular hair cells remain in a developmentally arrested state. Ongoing analyses should reveal additional insights into the role of Pou4f3 in the regulation of hair cell development.

Deep Learning-Based Transcriptional Characterization of Endocochlear Potential Development in the Mammalian Stria Vascularis Using Single Nucleus RNA-Sequencing

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Background: The stria vascularis (SV) is a heterogenous tissue located in the lateral wall of the cochlear duct. It plays an important role in normal hearing, by maintaining ion homeostasis and generating the endocochlear potential (EP). EP development initiates at around P8 and reaches adult levels at P15 in the mouse. We aim to identify critical regulators of EP generation by identifying genes relevant to the development of the EP.
Methods: In this study, we characterize transcriptional profile changes in SV cells during EP development using snRNA-Seq on mouse SV samples collected at three different time points during EP development (P8, P15 and P30). Samples were sequenced using the 10X Genomics Chromium platform, and reads were mapped to GRCm38 (mm10) mouse reference genome. Cell annotation are performed using a supervised deep learning (DL)-based method, which uniformly labeled the cells in all three datasets. Differentially expressed genes in major SV cells are identified by DESingle, and trajectory analysis are performed using Palantir tools. Gene regulatory network analyses are performed using published bioinformatics tools.
Results: We demonstrate that a supervised deep learning-based based cell annotation methodology can label individual cells and overcomes some of the disadvantages of modularity-based clustering approaches. We identify differentially expressed SV cell type-specific genes during EP development and correlate these results with developmental trajectory analysis. We identify active regulons during the period of EP development and correlate these results with trajectory analysis.
Conclusions: Identification of differentially expressed genes and putative gene regulatory networks will inform efforts to identify critical regulators of EP generation and provide a basis for understanding SV-related hearing dysfunction. These analyses will establish a basis for perturbation-based experiments to validate critical regulators of EP development and generation.

Sporadic Meniere Disease Molecular Phenotypes May Be Defined by Dna Methylation Signature in Mononuclear Cells

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Background: DNA methylation is a stable epigenetic mechanism necessary to gene expression regulation and cell phenotype definition. However, little work has been done to evaluate the role of epigenetics in hearing. As epigenetic modifications could be important to phenotypic differences, in this study, we carried out whole genome bisulfite sequencing (WGBS) in Meniere Disease (MD) patients and healthy controls to identify an MD methylation signature and potential disease mechanisms.

Methods: WGBS was performed on fourteen MD patients and six healthy controls. Differentially methylated cytosines (DMC) were mapped with methylKit R package; differentially methylated regions (DMR) were identified with Methpipe software, and undermethylated regions (UMR) were identified with methylSeekR R package. To identify biological pathways, processes and function, functional analyses were carried out using Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) databases, and Genomic Regions Enrichment of Annotations Tool (GREAT).

Results: We observed two UMRs in PHB gene exclusive to MD patients. PHB encodes Prohibitin, a protein with a role in B cell receptor signaling, T cell maturation, antigen-stimulated signaling in mast cells, and mitochondrial integrity. We observed a high number DMC when comparing MD patients to controls (n= 9545), various mapped to hearing loss genes, such as PCDH15, ADGRV1 and CDH23, which encode proteins forming ankle links in the stereocilia bundle. IL32 gene was found to have a DMR (DM = −0.35) and a DMC (DM = −0.41) in the promoter region when comparing MD patients with high levels of cytokines (MDH) to controls. IL-1β is increased in MDH patients, which could induce IL-32. Furthermore, increased serum levels of IL-32 have been linked to various auto-immune and allergic diseases. GREAT was used to predict the function of cis-regulatory sites, and revealed that the identified DMCs have predicted phenotypes associated with cochlear and organ of Corti degeneration, and abnormal synaptic current.

Conclusions: DNA methylation allows to distinguish MD patients from controls. Our results support previous findings of a chronic inflammatory process underlying MD. Transcriptomic data should be generated to facilitate the functional interpretation of methylation status in these genes.

Comparative Exploration of Mammalian Deafness Homologues in the Drosophila Auditory Organ Reveals Conservation Between Insect and Vertebrate Hearing

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Background: The Drosophila auditory organ, Johnston's organ (JO), is located within the second segment of each antenna and is comprised of around 200 stretch receptor units called scolopidia. These scolopidia contain mechanosensitive neurons that respond to gravity and sound from vibrations of the outermost antennal segment. Johnston's organ is anatomically very different from the mammalian organ of Corti. However, recent evidence indicates significant cellular and molecular similarities may exist between vertebrate and invertebrate hearing, suggesting that Drosophila may be a convenient platform to determine the function of the many mammalian deafness genes whose biochemical function is poorly characterized.

Methods: We used bioinformatic comparisons to screen all reported human and mouse deafness genes and found that 156 genes had orthologues in Drosophila. We then used fluorescent imaging of T2A-GAL4 gene trap lines (to

identify gene expression) and fluorescent protein trap lines (to identify protein localization) for 54 of the Drosophila orthologues and found 38 genes to be expressed in different cell types in Johnston's organ. We compared this expression with published RNA sequencing data of the fruit fly antenna to validate the results of our expression/localization screen. Of the genes that were identified from our localization/expression screen, we initially selected 3 genes with null alleles that were viable until adulthood as homozygotes to functionally test. We later identified 13 homozygous viable gene trap lines which also act as loss of function alleles. To characterize function of the hearing organ in these flies, we plan to perform electrophysiological field recordings of Johnston's organ neurons, test courtship behavior of mutant females with wild type Canton-S flies and perform climbing assays to assess defects in balance and coordination.

Results: Behavioral screening data from 3 orthologous mutant fly lines showed a statistically significant delay in time to copulation. Orthologs of Sun1 and Nesprin 4, which form the LINC complex in mammals and is necessary for outer hair cell health and hearing function, and the ortholog of Pcdh15, which is a member of the tip complex in mammals and causes deafness and Usher syndrome, were all affected. We are performing electrophysiological recordings of these mutants to confirm that these observations are due to defects in Johnston's organ.

Conclusions: Our findings show that roughly 80% of the genes tested had orthologs in the fruit fly with conserved expression/localization in Johnston's organ. Our experimental pipeline shows that the fruit fly provides a model to quickly and efficiently study mechanisms of deafness in orthologs of some mammalian genes.

Novel Candidate Genes CEP250 Cause Cilia Dysfunction and Hearing Loss

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Background: Hearing loss is the most common sensory disorder in humans. Hearing loss caused by autosomal dominant diseases accounts for 22-25% of hereditary hearing loss, and about 30 causative genes have been identified. However, the exact association and mechanism for this are not yet known. In addition, there are many related genes that have not been discovered yet, and research is underway to discover them. In this study, we uncovered CEP250 as a newly discovered deafness gene through whole exome sequencing in a family where affected individuals showed hearing loss. (YUHL cohort)
Therefore, we studied how it affects centrosome and primary cilia when CEP250 clone transfection into NIH3T3 cells through immunocytochemistry.

Methods: WES analyses on the DNA samples of the proband showed compound heterozygous variants, c3511C>T, Q1171X in the CEP250.
For immunofluorescence, cells were fixed with ice-cold 4%PFA for 10 min, blocked with 5% (v/v) bovine serum albumin (BSA) and 0.05% Triton X-100 in PBS for at least 1 hr. Cells were subsequently incubated with primary antibodies with 5% BSA and 0.05% Triton X-100 in PBS for overnight followed by three washes with 0.1% Triton X-100 in PBS for a total of 30 min and another 1 h incubation with secondary antibodies.

Results: High frequency sensorineural hearing loss was confirmed in YUHL251-21 through hearing measurement. The autosomal recessive CEP250 c.3511 C>T,p.Gln1171Ter gene mutation was discovered through whole exome sequencing. Stained using γ-tubulin and C-myc antibodies in NIH3T3 cells to confirm the expression of CEP250. Confirmation of localized expression of CEP250 protein in WT. It was confirmed that the CEP250 protein was not localized and expressed in the Q1171X mutation. Confirmation of CEP250 expression site in cochlea of WT mouse by immunohistochemistry analysis. Expression of CEP250(green) was confirmed in inner hair cells, outer hair cells, and spiral ganglion.

Conclusions: In order to investigate the effect of CEP250 as a deaf gene on cilia and centrosomes, ICC experiments were conducted using NIH3T3 cells derived from mouse fibroblasts. It was confirmed that CEP250 Wild type was localized to the centrosome in the transfected cells. On the other hand, it was confirmed that CEP250 Q1171X was expressed without being localized to the centrosome in the transfected cells. Also, the expression of CEP250 mutation does not completely block the generation of primary cilia, but it is presumed that it affects the length and the distance between centrosomes.
When the location of CEP250 was confirmed through IHC in cochlea of WT mouse, it was confirmed that it was expressed in hair cells and spiral ganglion.

Insights Into the Pathophysiology of DFNA44 Hearing Loss Associated With CCDC50 Variants

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Background: CCDC50 maps to the DFNA44 locus and encodes Ymer protein, an effector of epidermal growth factor (EGF)-mediated cell signalling. Mutations in CCDC50 cause progressive non-syndromic hearing loss. It has been reported that the c.866_873dup (p.(Phe292Hisfs*37)) mutation, which segregates with the hearing impairment in a Spanish family, affects Ymer subcellular localization in NIH 3T3 transfected cells. While wildtype Ymer is a soluble cytoplasmic protein, the mutant protein accumulates in the perinuclear area. This study aims to identify new mutations in CCDC50 causing hearing impairment and investigate the underlying mechanisms.

Methods: We have used a custom NGS targeted panel (OTO-NGS-Panel V1) to screen 111 Spanish independent familial cases. This panel uses the HaloPlex technology (Agilent) to capture 71 genes involved in hearing loss. To gain insight into the role of CCDC50 in the inner ear, we generated a Ccdc50<tm1b> mouse mutant and performed Auditory Brainstem Response (ABR) recordings at 14 weeks and 6 months old. To study the pathological mechanism of CCDC50, we created a set of 6 artificial mutants by site-directed mutagenesis sharing different parts of the protein tail. Then, the Ymer cellular distribution in each mutant was assessed by transfecting NIH 3T3 cells with the different plasmids, followed by immunohistochemistry.

Results: We identified a novel mutation in CCDC50 (c.828_858del, p.(Asp276Glufs*40)) segregating with the hearing impairment in a Spanish family. This frameshift mutation produces an aberrant protein, 39 amino acids longer than the wildtype. Pairwise alignment revealed that the protein tail generated by this mutant was identical to the one generated by p.(Phe292Hisfs*37) mutant. In addition, we observed that p.(Asp276Glufs*40) mutant led to a cellular distribution pattern similar to the p.(Phe292Hisfs*37) mutant, suggesting that the effect of the mutation might be mediated through the aberrant protein tail.

Ccdc50<tm1b> homozygous and heterozygous mutant mice showed normal ABR thresholds up to 6 months old, suggesting the hearing loss is not due to a loss of function effect. Finally, in vitro cellular studies revealed that only the mutants containing the five amino acid sequence CLENG as part of their aberrant protein tail showed perinuclear aggregates of Ymer.

Conclusions: Therefore, we conclude that the CLENG sequence is necessary to form the aggregates, although further studies are needed to determine the biological impact of the protein aggregates. Together with the absence of phenotype in the Ccdc50<tm1b> mutant mice, we determined that mutations in CCDC50 exert their effect through a dominant-negative or a gain of function mechanism.

Coch Mutations Cause Autosomal Dominant Hearing Loss via Different Pathogenic Mechanisms According to the Affected Domain

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Background: Mutations in COCH (coagulation factor C homology) causes not only autosomal dominant (DFNA9) but also autosomal recessive (DFNB110) nonsyndromic hearing. COCH encodes cochlin which contains an N-terminal LCCL domain and two copies of Von Willebrand factor A (vWFA) domains. Cochlin is cleaved by aggrecanse-1 upon bacterial infection, and the cleaved LCCL domain is secreted to perilymph space and induces bacterial aggregation in the scala tympani. However, the physiological role of C-terminal vWFA domains is not clear; therefore, the pathogenicity of mutations which occur in vWFA domains is also not well understood.

Methods: 155 families with NSHL predicted as DFNA9 were analyzed by exome sequencing. Phenotype-genotype based on COCH domains were also analyzed. Functional studies for COCH variants were performed.

Results: Here we reported seven families with DFNA9 and four of them had COCH mutations which had not been previously reported (p.A67T, p.F230L, p.G403C, and p.G447D). Functional study revealed that p.A76T mutation located in the LCCL domain formed a dimer and p.G403C, p.G447D, and p.F527C mutations located in the vWFA2 domain significantly reduced cleavage of cochlin. In addition, p.F230L mutation located in the vWFA1 domain did not affect posttranslational processing of cochlin, thereby producing normal cleaved LCCL domain, but incubation of cell with media containing p.F230L cochlin resulted in cytotoxicity.

Conclusions: These results suggest that COCH muations have different pathogenic mechanisms according to the domain affected by variants.

Hair-Cell-Specific Conditional Knockout of Tmtc4 Demonstrates Progressive Hearing Loss and Hair-Cell Death.

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Background: We recently identified Transmembrane and tetratricopeptide repeat 4 (Tmtc4) as a novel deafness gene in mice. Mice lacking Tmtc4 have normal onset of hearing at postnatal-day 13 (P13), with rapid progression to complete deafness by P26. Tmtc4 deficiency is also associated with dysregulation of Ca2+ flux between the cytoplasm and endoplasmic reticulum (ER), ER stress, and the unfolded protein response (UPR). We previously showed that Tmtc4 expression is present in multiple cochlear cell types. In this study, we sought to develop Tmtc4 conditional knockout (cKO) mice to understand the cell-type-specificity of Tmtc4 -associated progressive hearing loss.
Methods: We used CRISPR/Cas9 to introduce loxP sequences flanking exon 3 of Tmtc4. Tmtc4-loxP mice were bred with ROSA26CreER, Myo15Cre and Prox1CreER for inducible/ubiquitous, hair-cell-specific, and inducible/supporting-cell-specific Tmtc4 knockdown, respectively. RNAScope was used to measure expression of Tmtc4, Myo7a, and Chop (C/EBP-homologous protein; a marker of pro-apoptotic UPR activation) in P3-5 neonatal mouse cochleae. Serial auditory brainstem responses (ABR) to broadband clicks and pure-tones at 8, 16, and 32 kHz were recorded from the onset of hearing at P13 through P45. At P45, mice were euthanized and whole-mount immunohistochemistry performed to quantify inner- and outer-hair cells in the apical, middle, and basal turns.
Results: We generated cKO mice harboring Tmtc4 with exon 3 flanked by loxP. Sequencing confirmed germline transmission with no off-target mutations. Mice breed true without morbidity. For initial validation of the conditional construct, we bred Tmtc4 cKO with mice harboring tamoxifen (TMX)-inducible ubiquitous Cre (ROSA26CreER). E13 pregnant dams were treated with TMX and pups were sacrificed at P0. Brain and other tissue were then screened for recombination, which confirmed loss of exon 3. We then established Myo15Cre/Tmtc4loxP and Prox1CreER/Tmtc4loxP (induced with TMX at E16) lines and validated Tmtc4 knockdown using RNAScope, with Myo7a as a hair-cell marker and Chop as a concurrent marker of pro-apoptotic UPR activation. Myo15Cre/Tmtc4loxP homozygous mice exhibited normal hearing at auditory onset (P13) and rapid progression to complete deafness by P26, similar to constitutive Tmtc4 KO mice, and significant loss of cochlear hair cells at P45. Myo15Cre/Tmtc4loxP heterozygous mice had normal hearing. Supporting-cell-specific Tmtc4 knockdown in Prox1CreER/Tmtc4loxP mice yielded normal ABRs through P45 and no hair-cell loss.
Conclusions: Tmtc4 is a novel progressive hearing-loss gene. Tmtc4 deficiency permits normal initial cochlear development and hearing at P13, but rapid progressive hearing loss due to overactivation of the UPR. Here, we demonstrate successful development of cell-type-specific conditional knockout of Tmtc4. Using these mice, we show that Tmtc4-associated hearing loss is a hair-cell-specific phenotype. This demonstrates the importance of Tmtc4 and the UPR in hair-cell physiology. These mouse models will be useful for future studies of progressive hearing loss due to UPR dysfunction and subsequent rescue in hair cells.

Usher Syndrome Type IV: Clinically and Molecularly Confirmed by Novel ARSG Variants

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Background: Usher syndrome (USH) is an autosomal recessively inherited disease characterized by sensorineural hearing loss (SNHL) and retinitis pigmentosa (RP) with or without vestibular dysfunction. It is highly heterogeneous both clinically and genetically. Recently, variants in the arylsulfatase G (ARSG) gene have been reported to be causal to USH type IV. This distinct type of USH is characterized by both late-onset RP and SNHL without vestibular dysfunction. In this study, we describe the USH type IV phenotype in three unrelated subjects.
Methods: For a complete description of the subjects' phenotypes, we retrospectively collected demographic data and general medical history, ophthalmic data, audiological data, and data on vestibular function.

Whole exome sequencing (WES) was performed in one and whole genome sequencing (WGS) in two subjects to obtain a genetic diagnosis. Biallelic variants in other USH-associated genes were excluded.

To determine the effects of the variants on the protein level, immunoblot and sulfatase activity assays were performed. In addition, minigene splice assays were conducted for variants with a predicted effect on splicing.

Results: All subjects had SNHL with an age of onset ranging from 20 to 40 years and were diagnosed with RP with a midlife age of onset (40 to 60 years). Remarkably, not only peripheral but also central retinal aberrations and visual field loss occurred. Ne vestibular problems were reported.

Genetic analyses of the three cases revealed six heterozygous ARSG variants: one previously reported (likely) pathogenic single-nucleotide deletion (c.1326del (p.(Ser443Ala fs*12)), four novel pathogenic variants of which a nonsense variant (c.588C>A (p.(Tyr196*)), a canonical splice site variant (c.1212+1G>A (p.(Val405Ilefs*41)), a missense variant (c.275T>C (p.(Leu92Pro)) and a deletion of two exons (c.705-3940_ 982+2952del (p.(Ser235Arg fs*29)), and one novel likely pathogenic missense variant (c.1024C>T (p.(Arg342Trp))).

Five identified variants were shown to cause loss of enzyme activity. The sulfatase activity of one variant was not tested, but this variant (c.1212+1G>A (p.(Val405Ilefs*41)) is also assumed to result in loss of sulfatase activity because it leads to premature protein truncation. Genetic diagnostics revealed no other explanatory diagnoses.

Conclusions: In conclusion, our observations confirm the newly described USH type IV caused by biallelic variants in ARSG. This type differs from other USH types by the late onset of SNHL. Also, the onset of RP is later compared to other USH types and not only the peripheral but also the central retina is involved.   Unlike atypical USH, which we believe is an aberrant phenotype of a known USH gene, the ARSG phenotype is a consistent phenotype distinct from the original USH types I, II, and III.

This study contributes to the determination of genotype-phenotype correlations that are important not only for patient counseling, but also for the purpose of developing therapeutic strategies.

Dimensions of a Living Cochlear Hair Bundle

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Background: The hair bundle, the mechanosensory organelle of hair cells, consists of a group of stereocilia – "hair-like" insertions in the hair cell's apex. Hair bundles from different species, sensory organs, positions within an organ, and hair-cell types respond best to different kinds of stimulation due to differences in their stereociliary heights, widths, and organization, including their insertion-point separations. While hair-bundle dimensions dictate bundle mechanics, these measurements have only been determined to a limited degree. In particular, mammalian cochlear data are either incomplete, lack control for age or position within an organ, or have artifacts owing to fixation or dehydration. Here, we report the most complete, accurately controlled, and precise blueprint of a living mammalian cochlear hair bundle to date, a postnatal day (P) 11 mouse apical inner hair cell. We rigorously quantified artifacts owing to fixation and dehydration and determined how they affect calculations of the hair bundle's mechanical properties.

Methods: Hair bundles from P11 C57Bl6/J mouse apical inner hair cells were measured from tissue treated with a membrane-stain for live imaging, a mild PFA-fixative and phalloidin for fluorescent imaging, or a strong PFA/glutaraldehyde fixative and subsequently dehydrated for scanning electron microscopy (SEM). The left and right ears of each mouse were treated differently – either for live-cell or mildly-fixed imaging – and SEM imaging was performed on littermate cochleae, allowing us to determine the scaling factors between preparations. Each protocol permitted us to collect large numbers of stereociliary measurements from bundles located in a precise region between the 90th-160th inner hair cells from the cochlea's apex. Measurements include stereociliary heights, widths, organization, insertion separations, and numbers of stereocilia. Using these measurements, we determined how fixation and dehydration affect calculations of hair bundle mechanical properties.

Results: We found that hair bundles mildly fixed for fluorescence had the same dimensions as living hair bundles, whereas SEM-prepared hair bundles shrank uniformly (66 +/- 4% on average) in their stereociliary heights, widths, and insertion separations. By determining the shrinkage factors, we imputed live dimensions from SEM that were too small to observe optically. Accordingly, we created the first complete blueprint of a living inner-hair-cell hair bundle. SEM-prepared measurements greatly affect calculations of the bundle's mechanical properties – overestimating stereociliary deflection stiffnesses (200-300% of live) and underestimating the fluid coupling between stereocilia (55-75% of live).

Conclusions: By comparing live-cell, mildly-fixed, and SEM-prepared conditions, we have shown that mildly-fixed tissue can be used as an alternative to live-cell imaging for many bundle measurements and have created a

tool to impute live hair-bundle dimensions from SEM-prepared tissue. These conversion techniques will be particularly useful for measurements of rare samples, such as human hair bundles. Finally, we have shown that accurate and precise measurements are critical for understanding hair-bundle mechanotransduction.

Centrin-2 is a New Member of the Elongation Complex Required for Sensory Function in Inner Ear Hair Cells

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Background: Many deafness-associated genes alter the structure of the hair cell sensory organelle, which is comprised of organized rows of membrane protrusions called stereocilia. A group of five proteins, referred to as the elongation complex (EC), localizes to stereocilia tips and is restricted to the tallest row (row 1) in mature hair cells. The EC is required for row 1 elongation and the precise patterning of stereocilia into a hair bundle with rows of graded height. Consequently, hair bundle dysmorphology in EC mutants results in congenital deafness. Here, we identify a new EC candidate, centrin-2 (CETN2), a small calcium-binding protein with homology to unconventional myosin light chains. We propose that CETN2 acts as a binding partner and regulator of the critical hair cell myosin motor and EC member MYO15.
Methods: We used a Cetn2-Egfp transgenic mouse line paired with immunostaining and confocal microscopy to track CETN2 and compare its localization to other EC members in wild-type mice and in the EC mutants Myo15 shaker-2 and Whrn whirler. We used a trafficking assay in COS-7 cell filopodia and cultured cochlear explants to assess the MYO15-CETN2 interaction. We engineered mice with deletions in Cetn2 and its paralog Cetn3 and recorded Auditory Brainstem Response (ABR) to assess their hearing.
Results: We discovered that CETN2, although best known as a centriolar component, is unexpectedly enriched at stereocilia tips. We show that CETN2 precisely colocalizes with MYO15 and EPS8, being present at tips in all stereocilia at embryonic to early postnatal stages, and then gradually restricting to row 1 tips as hair cells mature. This profile is distinct from other EC proteins (WHRN, GPSM2, GNAI) that show a later enrichment at stereocilia tips, limited to row 1. We found that MYO15 motor activity is required for CETN2 localization at stereocilia tips: Cetn2-Egfp signal is absent at tips in Myo15 shaker-2 hair cells and is diminished but not eliminated in Whrn whirler hair cells, which have reduced MYO15 dosage. We established that CETN2 is a direct binding partner to MYO15 and that this interaction is dependent on MYO15's third putative light-chain binding IQ domain, as well as a subset of CETN2's 4 calcium-binding EF hand domains. We found that Cetn2, but not Cetn3, knock-out mice display elevated ABR thresholds.
Conclusions: We propose CETN2 as a novel member of the sensory hair cell EC. We show that CETN2 is a binding partner to and cargo of MYO15, and that it could be a first identified endogenous light chain associated with MYO15 in hair cells. We show that CETN2 plays a role in hearing, in line with our conclusion that it is an important newly discovered member of the EC.

Calcium and Integrin-Binding Protein 2 (CIB2) is Essential for Force Transmission to the Mechanotransducer Channels of the Mammalian Auditory Hair Cells

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Background: Calcium and Integrin-Binding Protein 2 (CIB2) is an essential subunit of the mechano-electrical transducer (MET) in the mammalian auditory hair cells (Giese et al., 2017; Liang et al., 2021). Recent data show that it is required for TMC1/2 localization to the stereocilia bundle (Liang et al., 2021). Since CIB2 binds not only to MET channel subunits TMC1/2 (Giese et al., 2017) but also to whirlin (Riazuddin et al., 2012), it may provide a Ca2+-dependent mechanical link between the plasma membrane and the actin core of stereocilium. Unfortunately, so far, it was not possible to test this idea due to the fact that CIB2 knockout mice or mice with point mutations disrupting CIB2-TMC1/2 interaction have no mechanotransduction at all. Therefore, we generated a mouse strain carrying p.R186W variant in Cib2 that results in deafness but does not disrupt CIB2 binding to TMC1/2, potentially preserving MET function.
Methods: We measured MET currents evoked by deflection of stereocilia bundles in young postnatal auditory hair cells either with a piezo-driven rigid probe or with a fluid-jet that is driven by known pressure stimuli. The movements of fluid-driven stereocilia and the probe were recorded by a high-speed camera at 3,000 and 30,000

fps, correspondingly. These records were used to determine the relative changes of the hair bundle stiffness, the current-displacement relationships, and the speed of the rigid probe that had a rising time of ~30 µs (10-90% of 1 µm). We also examined stereocilia ultrastructure with scanning electron microscopy (SEM) and serial sectioning with focused ion beam (FIB-SEM).

Results: Both inner (IHCs) and outer (OHCs) hair cells of Cib2R186W/R186W mice had measurable MET current, which is consistent with a recent report on another p.R186W mutant (Liang et al., 2021). However, Cib2R186W/R186W hair cells exhibited only "slow" but no "fast" adaptation. The time constant of MET current activation in Cib2R186W/R186W hair cells was at least four times slower than in control littermates, which allowed us to demonstrate its dependence on intracellular voltage. Additionally, p.R186W mutation resulted in a decrease in the stiffness of the hair bundles. FIB-SEM showed disruption of the lower tip link density in Cib2R186W/R186W mutants. Finally, SEM examination revealed over-elongation of the second (transducing) row stereocilia in both IHCs and OHCs that cannot be explained by known MET-dependent remodeling of stereocilia bundles.

Conclusions: We concluded that CIB2 is not only essential for mechanotransduction but also for establishing proper mechanical connections of the MET channel at the tips of stereocilia.

Single Particle Cryo-Em Structure of the Outer Hair Cell Motor Protein Prestin

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Background: The mammalian outer hair cell (OHC) protein prestin (Slc26a5), a member of the solute carrier 26 (Slc26) family of membrane proteins, differs from other members of the family owing to its unique piezoelectric-like property that drives OHC electromotility. OHCs require prestin for cochlear amplification, a process that enhances mammalian hearing. Despite substantial biophysical characterization, the mechanistic basis for the prestin's electro-mechanical behavior is not fully understood.

Methods: We have used cryo-electron microscopy at subnanometer resolution (overall resolution of 4.0 Å) to investigate the three-dimensional structure of prestin from gerbil (Meriones unguiculatus). To test a number of hypotheses of its structure generated by our data we used constructs of Slc26a9 and mutated specific residues of prestin that were transfected into HERK cells to test electromotility (eM) and nonlinear capacitance (NLC) by whole-cell patch clamp recordings.

Results: Our studies show that prestin dimerizes with a 3D architecture strikingly similar to the dimeric conformation observed in the Slc26a9 anion transporter in an inside open/intermediate state, which we infer, based on patch-clamp recordings, to reflect the contracted state of prestin. The structure shows two well-separated transmembrane (TM) subunits and two cytoplasmic sulfate transporter and anti-sigma factor antagonist (STAS) domains forming a swapped dimer. The dimerization interface is defined by interactions between the domain-swapped STAS dimer and the transmembrane domains of the opposing half unit, further strengthened by an antiparallel beta-strand at its N terminus. The structure also shows that each one of its two transmembrane subunits consists of 14 transmembrane segments organized in two inverted 7-segment repeats. Structural features of prestin are quite similar to that of SLC26a9 and other family members. Despite this similarity, we find that SLC26a9 lacks the characteristic displacement currents (or NonLinear Capacitance(NLC)) found with prestin, and we show that mutation of prestin's Cl- binding site removes salicylate competition with anions in the face of normal NLC

Conclusions: Prestins structure in the contracted state is closely aligned with that of Slc26a9. Our data refute the yet accepted extrinsic voltage sensor hypothesis and any associated transport-like requirements for voltage-driven electromotility.

Effects of Dexamethasone on Hair Cell Homeostasis and Function

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Background: In larval zebrafish lateral-line organs, the steroid drug dexamethasone has been shown to enhance both hair cell regeneration and afferent nerve reinnervation. Yet the effects of prolonged dexamethasone on regenerating or uninjured hair cells had not been previously examined. We therefore wanted to address whether prolonged dexamethasone exposure altered hair cell homeostasis and function.

Methods: Free-swimming 5-day-old larvae were briefly exposed to 3 µM copper sulfate (CuSO4), which induces both loss of lateral-line hair cells and considerable retraction of nerve terminals. Both lesioned and lateral-line intact larvae were then exposed to either 10 µM dexamethasone—a dose shown to promote lateral-line regeneration—or carrier (0.1% DMSO) alone for 48 hours. We subsequently examined hair-cell morphology, mechanotranduction, mitochondrial potential, and evoked calcium influx at hair bundles and hair cell synapses.
Results: In dexamethasone-treated larvae, we made the following observations: 1) hair cell mechanotransduction, as measured by FM1-43 uptake, appeared comparable to that of control DMSO-treated fish, however 2) in response to a fluid-jet delivered saturating stimulus, influx of calcium through hair cell mechanotransduction channels, as measured by the calcium indicator gCAMP6sCAXX, was significantly reduced, yet presynaptic calcium influx was unchanged 3) hair cell mitochondrial membrane potential, as measured with TMRE labeling, was hyperpolarized relative to controls, indicating changes in mitochondrial metabolic state and, 4) hair cells exposed to dexamethasone were more vulnerable to neomycin-induced cell death. Cumulatively these observations support that, while dexamethasone positively influences lateral-line organ regeneration in zebrafish, it also disrupts hair cell homeostasis in both regenerating and intact lateral-line organs, which makes hair cells more vulnerable to ototoxic insults and may negatively impact hair cell function.
Conclusions: Dexamethasone is a steroid commonly used for treating sudden hearing loss and is considered a promising treatment for protection from ototoxicity from cisplatin chemotherapy. Identifying strategies to retain high levels of dexamethasone for long periods within the inner ear is currently an active area of research. Considering that our data suggest prolonged exposure to dexamethasone may have an adverse effect on hair cell homeostasis, we believe further studies on the effects of long-term, localized dexamethasone treatment on mammalian cochlear function are indicated.

N-Terminus of GRXCR2 Interacts With CLIC5 and is Essential for Auditory Perception
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Background: Stereocilia of cochlear hair cells are specialized mechanosensing organelles that convert sound-induced vibration to electrical signals. Glutaredoxin domain-containing cysteine-rich protein 2 (GRXCR2) is localized at the base of stereocilia and is necessary for stereocilia morphogenesis and auditory perception. However, the detailed functions of GRXCR2 in hair cells are still largely unknown. Here, we report that GRXCR2 interacts with chloride intracellular channel protein 5 (CLIC5) which is also localized at the base of stereocilia and required for normal hearing in human and mouse.
Methods: Immunolocalization analyses suggest that GRXCR2 is not required for the localization of CLIC5 to the stereociliary base during development, or vice versa. Using clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system, we deleted 60 amino acids near the N-terminus of GRXCR2 essential for its interaction with CLIC5. Interestingly, mice harboring this in-frame deletion in Grxcr2 exhibit moderate hearing loss at lower frequencies and severe hearing loss at higher frequencies although the morphogenesis of stereocilia is minimally affected.
Results: Yeast-two-hybrid and co-immunoprecipitation data show that GRXCR2 interacts with CLIC5 and that at least two regions in CLIC5 are essential for its interaction with GRXCR2. The immunostaining suggests that CLIC5 is not required for the GRXCR2 localization in hair cells during development. Disrupting the interaction between GRXCR2 and CLIC5 has minimal effects on stereocilia morphogenesis. Brain stem response results suggest that the interaction between GRXCR2 and CLIC5 is required for normal hearing especially for hearing at high frequencies.
Conclusions: In this study, we found that GRXCR2 interacts with CLIC5. The localization of these two proteins at the stereociliary base is independent of each other. Disrupting the interaction between GRXCR2 and CLIC5 in vivo has minimal effects on stereocilia morphogenesis but leads to hearing loss in mice. Thus, the interaction between GRXCR2 and CLIC5 is crucial for normal hearing.

Central Gain is Invariably Triggered by Reduced Cochlear Input but may be Insufficient to Account for Tinnitus Perception
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Background: The nervous system is known to adapt in many ways to changes in the statistics of the inputs it receives. An example of such plasticity observed in animal models is that central auditory neurons tend to retain their driven firing rate outputs despite reductions in peripheral input due to hearing loss. This "central gain" has been demonstrated to occur even when the peripheral loss is not accompanied by audiometric threshold shifts, i.e., following noise-induced or age-related loss of afferent synapses and nerve terminals innervating the cochlea (cochlear synaptopathy) despite intact sensory hair cells. Down-regulation of inhibitory neurotransmission is thought to contribute to such plasticity. Pathological versions of such central gain are thought to underlie disorders such as tinnitus and hyperacusis.

Methods: To investigate central gain, we designed an electroencephalogram (EEG)-based paradigm that concurrently elicits separable responses from different levels of the auditory pathway. We applied this measure to a large cohort of individuals spanning a wide age range (18–60 years). To test whether central gain contributes to tinnitus perception, we applied the same measures to a separate group of subjects reporting persistent tinnitus. To directly test whether central gain is triggered by reduced peripheral input, we performed the same measures in a cohort of subjects with temporary (1-week-long) hearing loss in one ear induced via moldable silicone earplugs.

Results: We find that (i) individual differences in response amplitudes that are as large as 20 dB at the auditory nerve level were reduced to less than 2 dB at the cortical level, (ii) individuals exhibiting shallower rates of auditory nerve response growth with stimulus intensity showed progressively steeper rates of growth at higher levels of the pathway, and (iii) a central gain metric defined as the size of the cortical response relative to the size of the auditory nerve response monotonically increased with age consistent with age-related cochlear deafferentation and consequent central gain. Middle-aged individuals with higher central gain also exhibited reduced ability to use temporal coherence cues to derive masking release, consistent with downregulation of inhibitory neurotransmission thought to be important for coherence processing. However, when comparing individuals with self-reported persistent tinnitus to those reporting no tinnitus or occasional tinnitus, we found the degree of central gain was similar, suggesting that central gain per se may be insufficient to account for tinnitus perception. Preliminary results show that subjects with temporary hearing loss also exhibit increased central gain suggesting causal links.

Conclusions: Together, our findings suggest that peripheral deafferentation invariably triggers central gain in the human auditory system, and may influence auditory scene analysis, but by itself is insufficient to account for tinnitus perception.

Behavioural Evidence for a Relationship Between Auditory Object Formation and Speech-In-Noise Processing in a Cochlear Implant Population

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Background: A persistent problem within the study of hearing impairment (HI) is the fact that peripheral measures do not fully account for outcome variability for natural listening tasks like speech in noise (SIN). One hypothesis to account for variability in performance is that the detection of SIN depends critically on central mechanisms for auditory object formation. In this study we assessed cross-frequency grouping mechanisms that are a first stage of auditory object formation. We have previously demonstrated that these tasks predict speech-in-noise detection in normal listeners [PMID 31728002]. In this study we tested the hypothesis that a measure of cross-frequency grouping predicts speech in noise ability in electrical hearing (cochlear implant users with mixed device configurations) independently of measures that capture peripheral analysis.

Methods: Measures to account for peripheral acoustic ability consisted of spectral ripple detection and temporal modulation detection based on adaptive 3AFC paradigms. For the AOD stimulus condition, subjects performed a single-interval detection task in which the background was heard first and comprised a sequence of random complex tones which on half the trials continued to the end (background-only) or on figure-present trials contained a figure based on six fixed-frequency components (background + figure). The SIN measures included the Iowa Test of Consonant Perception (ITCP), a 4AFC CVC word recognition task, which embedded words in multi-talker babble at two signal to noise ratio values (+7.5 and +15 dB) and the AzBio sentence-in-noise task (+5dB).

Results: In a cohort of 47 cochlear implant listeners, no relationship was found between the ITCP word-in-noise task and AOD scores. A significant correlation was demonstrated between AOD performance and AzBio (r = 0.447. p = 0.001). In a linear regression model that accounted for peripheral hearing using the participant's scores on spectral ripple and temporal modulation tasks the AOD performance accounted for 12% of the variance (r = 0.342, p = 0.019).

Conclusions: The correlation between AOD and SIN performance may form a bridge between peripheral auditory measures and speech outcomes. AOD ability could allow specification of deficits in hearing impaired listeners in a way that reflects how people listen in the real-world.

Accessibility to Hearing Healthcare in Rural and Urban Populations of Alabama: Perspectives and a Preliminary Roadmap for Addressing Inequalities

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Background: Hearing loss is a growing public health concern and has been associated with poor cardiovascular health, diabetes, increased social isolation and poor cognitive functioning. Addressing this issue, especially in rural communities, will require increased awareness of hearing loss and its link to emotional and physical well-being. The purpose of this study was to understand the challenges that those with hearing loss living in rural and urban communities experience and to examine the feasibility of using primary care physicians to assist with improving access to hearing healthcare in rural communities.

Methods: One hundred thirty-four participants were recruited from rural and urban counties in West Central and South Alabama. All participants completed a hearing evaluation and a Healthcare and Hearing Healthcare Accessibility Questionnaire.

Results: Over half of the adults in the study with hearing loss did not have access to hearing healthcare because of distance to a hearing healthcare professional. Other reasons for participants not having access to hearing healthcare included financial constraints, lack of awareness of having a hearing loss, lack of time to see a hearing healthcare provider, and not knowing how to access a provider. Results, however, revealed that most adults in the study had access to a primary care professional. The primary care provider, therefore, could be a valuable resource for the dissemination of information related to hearing healthcare.

Conclusions: Collaborative work with primary care providers will help to develop and expand hearing healthcare awareness, research and services provided through the Here Hear Alabama project, a rural outreach initiative in West Central and South Alabama.

Effects of Age-Related Hearing Loss on Visual Stimulus Encoding

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Background: Auditory deprivation is associated with intra-modal plasticity that modifies connections in the visual cortex and cross-modal plasticity where auditory cortical neurons are repurposed to respond to visual stimuli. These changes are thought to underpin improvements for visual abilities in deafness that are analogous or relevant to hearing, such as motion discrimination, face discrimination, and visual localization. However, less is known about forms of compensatory visual plasticity under conditions of partial or incomplete hearing loss, such as age-related hearing loss, where auditory and multisensory brain regions still receive some level of afferent input. While some studies have shown evidence of cross-modal plasticity in adults with partial hearing loss that negatively relates to speech-in-noise (SIN) listening, it is unclear if adults with hearing loss show changes in visual perception as has been shown in deafness. Here, we test the hypothesis that degrees of hearing loss and SIN listening ability associate with peripheral motion detection and face recognition in middle-aged and older adults.

Methods: Participants aged 40 to 80 participated in the study. All participants underwent pure-tone audiometry to 16 kHz, and QuickSIN was used to measure SIN listening. Participants completed a visual motion detection task and a face recognition task. The visual motion detection task presented two sinusoidal gratings presented for 500 ms horizontally 10° from a central fixation point. For each trial, one of the two gratings moved up or down, and participants reported the grating (left or right) that they perceived to move. The 50% detection threshold was obtained using parameter estimation by sequential testing (PEST). The face-matching task consisted of 120 target identities taken from the Chicago Face Database. In each trial, a target face with a Gaussian noise mask was

presented with two comparison faces presented below. Participants decided whether the target was in the comparison array (50% probability) and, if so, indicated which face they perceived it to be.
Results: Data collection is ongoing.
Conclusions: These findings will potentially clarify if visual perceptual abilities are affected by age-related hearing loss, and how they relate to speech in noise outcomes. For instance, research on hearing prosthesis users has shown that forms of compensatory plasticity may be deleterious for SIN listening. Our results can be used to infer if compensatory visual plasticity is a source of variability in SIN listening in listeners with age-related hearing loss.

Parvalbumin Immunolabel for Quantifying Type II Afferent Morphology
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Background: Immunohistochemical study of type II afferent neurons has been hampered by the availability of antibodies that label both the fibers and bouton endings. Previous use of antibodies, such a peripherin, or genetic reporters would only label subsets of type II afferent neurons (Vyas et al., 2019). Here we use a commercially available antibody to Parvalbumin that labels type II afferent processes and terminal boutons in the adult mouse (Maison et al., 2016; Webber et al., 2021). Combined with synaptic ribbon labels, parvalbumin immunohistology further characterizes the afferent innervation of outer hair cells (OHCs) and the impact of acoustic trauma.
Methods: Mice of both sexes were exposed to 110 dB SPL of broadband noise exposure as previously described (Wood et al., 2021). Auditory brainstem responses were recorded for a subset of the mice to show this noise exposure caused an average threshold shift (across all frequencies tested) of 23.99 ± 2.79, 7 days after damage. Excised cochlear segments were labeled with anti-Parvalbumin, anti-CtBP2, anti-Tuj1, and DAPI. Images were taken at 60x magnification and 0.15 micron z-step size in 4 frequency locations – 8, 16, 32, and 45 kHz. The virtual reality software, syGlass was then used to quantify the number of synaptic ribbons and type II afferent endings at the base of each OHC.
Results: Parvalbumin robustly labels type II afferent endings and IHCs (Webber et al., 2021). When co-labeled with Tuj1, both antibodies label the fibers of the neurons, but only Parvalbumin labels the dendritic endings and boutons of the type II afferent neurons. This creates a staining pattern underneath the OHC where MOC efferents are stained with only Tuj1 and type II afferent endings are labeled with Parvalbumin at the bouton. Unlike OHC synaptic ribbons which increased in number after noise exposure (Wood et al., 2021), type II endings did not change in number except in the most basal region of the cochlea. The pairing of CtBP2- labeled ribbons with parvalbumin-positive type II boutons also was not significantly altered by acoustic trauma. Thus, these results do not show a change in the innervation density (as opposed to ribbon numbers per se) of OHCs post trauma.
Conclusions: Antibodies to parvalbumin can be used to study type II afferent ending morphology without the need for a genetic reporter. This allows for the expansion of our understanding of how type II afferents may be affected by noise exposure or other interventions.

Animal Models of Alport Syndrome
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Background: Alport syndrome (AS) is a hereditary disease characterized by progressive renal failure and sensorineural deafness. It is caused by variants in the COL4A5 gene with the absence alpha3,4,5 networks in basement membranes (BM). The collagen IV genes (COL4A1-6) produce six collagen IV chains, which form three distinct networks (alpha 1,1,2; alpha 3,4,5; alpha 5,5,6). The triple helical protomers self-associate to form the insoluble meshwork of the BM with other macromolecules such as laminin. While the alpha 1,1,2 (IV) network is ubiquitous in BMs, the alpha 3,4,5 (IV) protomer has a restricted distribution in the kidney and the cochlea. Mutations of COL4A5 gene in AS prevents the assembly of the alpha 3,4,5 protomer and 70% of people with AS have the X-linked pattern due to variants in the COL4A5 gene and exhibit kidney failure and deafness. The purpose of this research is to determine the mechanisms involved in hearing loss in animal models of AS.
Methods: An X-linked mouse model of AS was developed at the University of Minnesota; on a C57BL6 mouse strain, which also contains the early, age-related hearing loss variant and dies early due to kidney failure. To produce a mouse model of AS that doesn't die due to kidney failure, we have used CRISPR-Cas9 system. A

floxed COL4A5 mice with two loxP sites flanking the COL4A5 exon mouse were mated with Foxg1-Cre mice causing a frameshift mutation that inactivates COL4A5 expression in the cochlea.

Results: Histology of the X-linked AS mice revealed holes in the basement membrane of the basilar membrane. These holes show a defect in the BM that may be related to the loss of the more highly cross-linked alpha 3,4,5 type IV chains and its replacement by the weaker alpha 1,1,2 network. This location is also where, in human temporal bone studies with AS, showed a zone of separation between the organ of Corti and the basilar membrane along the basement membrane.

Conclusions: Hearing threshold studies on the X-linked mouse model showed hearing loss that could only be attributed to the age-related hearing mutation. These mice also died early due to kidney failure; however, histology revealed holes in the basement membrane of the basilar membrane which suggest a mechanism for the zone of separation and hearing loss in AS. Results from the new Alport mouse model showed that of the 19, F0 mice that were produced, 3 pups showed the presence of COL4A5 exon 20 flanking 3' and 5' LoxP sites. Unfortunately, the F1 generation of animals were born at the start of the COVID 19 pandemic and the LoxP sites were lost in subsequent offspring. We have restarted the project and will report histology and hearing tests in this new animal model of Alport syndrome.

Two Photon Fluorescence Microscopy of the Unstained Human Cochlea Reveals Organ of Corti Cytoarchitecture

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Background: Diagnostics and therapies for sensorineural hearing loss (SNHL) remain limited in part because of a historical lack of methods for visualizing the cochlea's interior at the cellular level in living patients. While conventional computed tomography (CT) and magnetic resonance imaging (MRI) can reveal gross anatomical defects of the cochlea and may be sufficient for guiding otologic surgeons in planning their surgical access, they do not afford the resolution necessary to enable visualization of the individual cells and auditory nerve fibers that are known from animal and human autopsy studies to be damaged in the progression of SNHL. Here we demonstrate the ability of two-photon fluorescence microscopy (TPFM) to facilitate visualization of sensory cells and auditory nerve fibers in an unstained, non-decalcified cochlea from a former adult patient.

Methods: For TPFM imaging, the otic capsule over the cochlea's apical, middle, and basal turns was slowly and carefully drilled away to expose the sensory epithelium. The specimen was fixed to a glass petri dish with dental cement, submerged in phosphate-buffered saline solution, and observed under a light microscope to confirm that (a) the structure had remained grossly intact during the drilling process, and (b) bone dust had been adequately flushed away. The dish was then secured to the surface of a 3-axis goniometer, and the entire system was then positioned under a Thorlabs Bergamo II Series Multiphoton microscope (Thorlabs, Newton, NJ, United States) for investigation using TPFM. The light source was a Spectra-Physics Mai Tai HP Ti:Sapphire laser (Spectra-Physics, Santa Clara, CA, United States). Emitted fluorophores were detected using Hamamatsu photomultiplier tubes (Hamamatsu Photonics, Hamamatsu City, Shizuoka, Japan). The objective lenses used were the Nikon CF175 LWD 16X and Apochromat 25XC water immersion lenses (Nikon, Minato, Tokyo, Japan). To verify images obtained using TPFM, cochlear wholemounts were prepared for confocal immunohistochemistry.

Results: Individual sensory cells and bundles of auditory nerve fibers were clearly visualized, in addition to winding vasculature containing small donut-shaped cells consistent with erythrocytes in regions more proximal to the modiolus. The identity of the sensory cells detected with TPFM in the unstained specimen was confirmed using confocal microscopy applied to the specimen immunostained for hair cells.

Conclusions: We demonstrate that TPFM obviates the need for laborious, lengthy, and artifact-prone processing by providing cellular and subcellular resolution of the cochlea's interior in a non-decalcified, unstained specimen. This strongly motivates further investigation into the sources of endogenous fluorescence in the inner ear and how they might be tapped for diagnostic imaging without contrast dyes or radiation in living humans, e.g., via intracochlearly inserted microendoscope. TPFM could accelerate progress in understanding cellular-level pathology in precious human temporal bone specimens and may lead the way toward much-needed personalized therapy recommendations in living patients suffering from SNHL.

Seasonal Synaptic Changes in the Inner Ear and Auditory Efferent System of a Vocal Fish

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Background: Structural plasticity in the nervous system often occurs in conjunction with reproductive and circadian cycles. The plainfin midshipman, Porichthys notatus, is a marine fish which has provided decades of insight into neuroendocrine mechanisms of auditory plasticity. Males migrate to intertidal zones to breed during the late spring and summer, producing advertisement calls. Females are strongly attracted to this call, locating males at night for mating. Both males and females undergo an increase in inner ear auditory sensitivity coincident with the breeding season, particularly within the range of the harmonics of the male call. Causally linked to changes in circulating gonadal steroids, this increase in auditory sensitivity is associated with increased hair cell density, transcriptional changes within hair cells, a reduction of dopaminergic input to the inner ear and an increase in dopaminergic input to the cholinergic hindbrain octavolateralis nucleus (OE, homologous to the olivocochlear nulceus). Here we investigate whether the synaptic architecture of the saccule, the main endorgan of hearing in midshipman, and its associated efferent systems undergo seasonal remodeling.

Methods: Females in non-reproductive (n = 6) condition were collected by otter trawl in the winter from deep waters offshore and in reproductive condition (n = 6) by hand from summer intertidal nesting sites northern CA. Nervous system tissue was processed using pre-embedding tyrosine hydroxylase (TH) immunohistochemistry combined with transmission electron microscopy (TEM) to visualize dopaminergic cells and processes. Serial sections from the saccule and the OE were sampled random-systematically and imaged by TEM. After tracing image sets with TrakEM2, reconstructed 3D volumes were analyzed to extract changes in ultrastructure as a function of reproductive condition.

Results: Consistent with our previous confocal microscopy study, reproductive females have fewer dopaminergic varicosities that were smaller in volume than in non-reproductive females. We find for the first time direct dopaminergic terminals contacting saccular hair cells. These terminals also frequently abut afferent and cholinergic efferent synapses on hair cells. Reproductive females have significantly fewer direct dopamine-hair cell terminals compared to non-reproductive females. Reproductive females had more direct synaptic and non-synapse-forming dopamine terminals within the OE.

Conclusions: As supported by a recent pharmacological study, the summer reduction of dopamine innervation likely results in enhanced inner ear sensitivity, while increased terminals in the OE may reduce cholinergic inhibition in the ear, altogether improving the ability of females to find and evaluate potential male mates. Because increased auditory sensitivity can be induced in winter, nonreproductive females with testosterone or estradiol, we hypothesize that the synaptic plasticity we report here is likely hormone-mediated.

Investigating the Action of GABA on Type II Spiral Ganglion Neurons

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Background: Outer hair cells (OHCs) play a role in the amplification of responses to sound and are innervated by both medial olivocochlear efferent neurons (MOCs) and type II spiral ganglion afferent neurons (SGNs). MOC efferent neurons contacting OHCs inhibit amplification but the function of the type II innervation is less clear. In addition to the known circuitry in the outer spiral bundle (OSB), evidence of more complex circuitry exists. Electron microscopy has provided evidence of direct synaptic contacts between type II SGNs and several other cell types, including MOC efferent neurons. Markers of GABA and GABA synthesis have been localized to MOC efferent neurons and terminals in multiple species, however OHCs do not respond to GABA. Our work will inform whether a functional GABAergic synapse could exist directly between MOC efferent fibers and type II SGNs.

Methods: The current work uses immunohistochemistry to localize indicators of GABAergic signaling in the mouse cochlea and whole-cell patch-clamp electrophysiology in mouse cochlear preps to investigate whether type II SGNs respond to GABA. To investigate through which receptor type responses are mediated pharmacology was combined with whole-cell patch-clamp recordings.

Results: Histological data from our lab confirms that genetically labelled MOC terminals contain the GABA synthesis enzyme, glutamate decarboxylase, suggesting they may engage in GABAergic signaling. Further data from our lab shows that type II SGNs express GABA A β3 receptor subunits apposed to MOC terminals, suggesting these fibers are equipped to respond to GABA, potentially released from MOC efferent neurons. Furthermore, whole-cell patch-clamp recordings from the dendrites of type II SGNs show evoked currents with an average amplitude of ~42 pA when treated with 1 mM exogenously applied GABA. These currents reverse at ~-12

mV which is near the chloride reversal potential of the experimental solutions used, suggesting neurotransmission through ionotropic GABA or glycine receptors. Application of 30 µM of the GABA A receptor blocker gabazine caused a reversible block of the GABA-evoked chloride currents, while blocking GABA B or C receptors had no effect, supporting the hypothesis that type II SGNs have GABA A receptor-mediated chloride currents.
Conclusions: Together these data show that type II SGNs respond to GABA, potentially released from MOC efferent neurons, suggesting a functional GABA-A receptor mediated synapse between type II SGNs and MOC efferent neurons. Identifying and characterizing previously unexplored functional inputs to type II SGNs will enable us to better understand the connectivity of the OSB and elucidate a role for type II SGNs in local OSB function.

Transverse Vibrations of the Reticular Lamina and Basilar Membrane in the Basal Turn of Gerbil Cochleae

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Background: The reticular lamina and basilar membrane vibrations at the cochlear base have been measured through the round window in living gerbil cochleae. It was found that the reticular lamina vibration is more robust and compressive than the basilar membrane vibration, and the low-frequency reticular lamina vibration is physiologically vulnerable. The reticular lamina and basilar membrane vibrate approximately in phase at the best frequency and out of phase at low frequencies. These data suggest a global hydromechanical mechanism for cochlear amplification. However, the phase difference between the reticular lamina and basilar membrane vibration has been thought to result from a measurement error caused by the non-perpendicular angle between the incident light beam and the cochlear partition. This study is to determine whether the phase difference between the reticular lamina and basilar membrane vibration exists when the vibration is measured approximately in the transverse direction through the cochlear lateral wall.
Methods: Young Mongolian gerbils of either sex with normal hearing at the age of 4 to 8 weeks were used in this experiment. The bulla on the left side was opened through a ventrolateral surgical approach, and the stapedial artery was partially removed from the bony surface of the cochlea. A small opening was made in the lateral wall of the scala tympani of the basal turn, and the cochlear partition was positioned approximately in the horizontal plane. When the object beam of a heterodyne low-coherence interferometer was focused on the reticular lamina or the basilar membrane, the vibration magnitude and phase were measured as a function of frequency at different sound pressure levels.
Results: The reticular lamina and basilar membrane vibrations both show sharp response peaks at the best frequency at low sound pressure levels. The magnitude of the reticular lamina vibration is significantly larger than that of the basilar membrane. As the sound level increases, the reticular lamina vibration at the best frequency increases at a rate smaller than that of the basilar membrane vibration. The phase data shows that the reticular lamina and basilar membrane vibrate in opposite directions at low frequencies and in the same direction at the best frequency. The phase difference between the reticular lamina and basilar membrane vibration indicates that the reticular lamina vibrates after the basilar membrane vibration, which is consistent with that measured through the round window.
Conclusions: The present result shows that the phase difference between the reticular lamina and basilar membrane measured through the round window is not caused by a measurement error. Instead, the phase difference is an important manifestation of the cochlear mechanical processing.

Mechanisms of Tonic Outer Hair Cell Length Changes in the Mouse Organ of Corti

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Background: Mammalian cochlear amplification is thought to depend on cycle-by-cycle force generation by outer hair cells (OHCs). This force generation is primarily mediated by prestin, a voltage-sensitive motor protein that allows OHCs to rapidly change length in response to variations in membrane potential. In addition to fast electromotility, OHCs also exhibit slow or tonic length changes in response to a variety of electrical, mechanical, or chemical stimuli in vitro, and in response to sound in vivo. However, the underlying mechanisms and functional significance of these tonic length changes have yet to be carefully examined. Here we tested whether sound-evoked tonic OHC length changes depend on the same mechanisms responsible for cycle-by-cycle motility –

namely, prestin-mediated electromotility and the stereociliary mechanotransduction currents that generate the OHC receptor potential.

Methods: We used optical coherence tomography to measure sound-evoked displacements from the ~9 kHz region of the adult mouse cochlea in vivo. Displacements of the OHC region were obtained in wild-type (WT) mice and mice with abnormal prestin (Prestin 499 knockin mice) or impaired mechanotransduction. The latter included salsa mice, which lose stereociliary tip links by adulthood, and TriobpΔex8/Δex8 mice, which lack a stereociliary rootlet and exhibit widespread bundle degeneration.

Results: Tonic displacements of the OHC region were reliably elicited in WT mice. The displacements were consistent with somatic contractions and saturated at ~20-30 nm. These motions were likely driven by prestin, as tonic displacements in Prestin 499 knockin mice were reduced by >90%, in agreement with the amount of electromotility previously observed in these mice. Tonic displacements were absent in most salsa mice, suggesting that stereociliary mechanotransduction is also required. Interestingly, however, tonic displacements were measurable in over half of TriobpΔex8/Δex8 mice examined. These tonic displacements were observed in the absence of cycle-by-cycle amplification, could be larger than those in WT mice, and exhibited variable degrees of adaptation. Tonic displacements were mainly observed in TriobpΔex8/Δex8 mice before postnatal day 40, possibly indicating a dependence on bundle integrity, which progressively declines in these mice.

Conclusions: Sound-evoked tonic OHC length changes depend on prestin-based electromotility but do not require completely normal mechanotransduction. Nevertheless, the absence of tonic displacements in most salsa mice suggests that conventional, tip-link-mediated gating of mechanotransduction channels is involved. Thus, tonic displacements in TriobpΔex8/Δex8 mice may simply reveal the presence of residual stereociliary mechanotransduction, albeit with altered kinetics, perhaps owing to reduced bundle stiffness. Alternatively, tonic displacements in TriobpΔex8/Δex8 mice may reveal the contribution of other mechanically gated channels. One candidate is PIEZO2, a stretch-activated channel localized to the OHC's apical surface. Regardless, whether tonic OHC motility plays a functional role in the normal or impaired cochlea remains to be determined.

Comparison of Passive Cochlear Tuning Between Humans and Laboratory Animals

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Background: The live healthy mammalian cochlea is capable of exquisitely sharp frequency tuning to sound at low intensities. Increasing sound intensity results in a broadening of cochlear frequency tuning which becomes similar to that observed in the deceased cochlea. Tuning in a deceased cochlea is referred to as "passive" tuning. Recent work has demonstrated that the motion along different radial positions of the basal cochlear partition (CP) shows several key differences between fresh cadaveric humans and laboratory animals. The structural architectures also differ between human and laboratory animals at the cochlear base. These differences may have significant implications for frequency tuning. In this study, we compare passive CP tuning between laboratory animals and humans.

Methods: Human temporal bones were obtained from recently deceased patients (<36 h post-mortem). Optical coherence tomography (OCT) was used to visualized the cochlear partition through the round window and displacement of the basal cochlear structures was measured in response to applied tones of various frequencies. Displacement was normalized to ear canal sound pressure measured with a calibrated probe-tube microphone near the tympanic membrane. This human OCT data were compared to previous laser Doppler vibrometry (LDV) measurements of the human scala tympani CP surface and to OCT vibrometry data from laboratory animals.

Results: Using temporal bones of recently deceased patients, we obtained OCT images of the human CP and were able to identify several structures within the human CP including the basilar membrane (BM), osseous spiral lamina (OSL), bridge (soft tissue between BM and OSL), OoC, tectorial membrane (TM), reticular lamina (RL) and spiral ligament. Similar to the LDV data, the human OCT measurements showed fairly sharp passive tuning in the human cochlea with the largest motion near the junction of the BM with the bridge. Many reports show that tuning in laboratory animals soon after death varies considerably across species and differs from the tuning we found in cadaveric humans.

Conclusions: OCT vibrometry results in very fresh human cadaveric cochleas are similar to vibrometry results measured with laser Doppler. The tuning of the passive human CP is surprisingly sharp. The passive tuning in human cochlea (17 – 72 hours post-mortem) can be sharper than in laboratory animals 10 - 15 minutes after death. This study shows that the mechanics responsible for passive tuning differ across species.

Visualizing Collagen Fibers in the Basilar and Tectorial Membranes Using CNA35-OG, a Fluorescently Labelled Bacterial-Adhesion Protein.

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Background: The tectorial membrane (TM) is a complex acellular structure that extends from the spiral limbus and covers a part of the organ of Corti, running along the entire cochlear spiral. It is required for normal hearing, interacting with the stereocilia of the hair cells that are essential for mechanoelectrical transduction. The TM is comprised of 97% water, collagen fibrils (II, V, IX and XI), and various noncollagenous glycoproteins such as a-tectorin, b-tectorin, ceacam16, otogelin, otogelin-like, and otoancorin. As an acellular matrix, the TM is not easily stained with regular dyes and is therefore difficult to visualize. We produced a fluorescently labeled collagen-specific probe using a technique that takes advantage of the inherent specificity of collagen-binding protein domains present in a bacterial adhesion protein (CNA35), obtained from E. coli, which allows for a detailed view of collagen fibers. In this study we present confocal images of regions of gerbil cochleae stained with CNA35-OG.
Methods: Recombinant poly-histidine-tagged CNA35 (from the Maarten Merkx lab in the Netherlands) was expressed in E. coli and purified by cobalt-affinity chromatography before labeling with OregonGreen488-succinimidyl ester. OregonGreen488-labeled CNA35 (CNA35-OG) was further purified by gel filtration chromatography. Mongolian gerbils of both sexes were used. Freshly harvested bullae were harvested at the end of experiments. The bullae were opened, middle ear ossicles were removed, and the cochleae were irrigated with 1-2 uM CNA35-OG through the oval window. The samples were immersed in approximately 1.5 ml of CNA35-OG and stored at room temperature overnight. The cochleae were then placed in 4% formaldehyde and fixed at 7°C for about 24 hours, and then decalcified in EDTA (120mM) for 3 days. Dissection was done under phosphate buffer saline (PBS), and samples from the basal and middle turn were obtained. The dissected turns were imaged using the 488 nm laser on a Nikon A1R laser scanning confocal microscope.
Results: CNA35-OG dye stains both the basilar membrane and tectorial membrane. In addition, volumetric images allowed us to visualize the shape of these structures and the organization of the collagen fibrils within them.
Conclusions: The confocal images illustrated in this report represent a unique view of the tectorial membrane in the fixed and decalcified gerbil cochlea. The use of CNA35-OG dye allowed a detailed visualization of the TM collagenous fibers and the attachment of the TM to the spiral limbus. Future studies will analyze the effect of injecting collagenase into scala media, and its impact on TM structure, cochlear mechanics and cochlear amplification.

Intracochlear Sound Pressure Measurements Under Intracranial Stimulation and Bone Conduction Stimulation

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Background: The frequency-dependent contributions of the non-osseous bone conduction pathways are poorly understood, especially the fluid pathway. The aim of this work is to measure and investigate sound pressure propagation from the intracranial space to the cochlear fluid.
Methods: Stimulation was provided sequentially to the bone (BC) or directly to the intracranial contents (hydrodynamic conduction, or HC) in four cadaver heads. Each ear was tested individually, for a total of 8 samples. The intracochlear sound pressure levels were monitored via custom-made intracochlear acoustic receivers (ICAR), while intracranial pressure was generated and monitored via commercially available

hydrophones. In parallel, the spatial motion of the cochlear promontory and stapes were measured via a 3D Laser Doppler Vibrometer (3D LDV).

Results: The ratios between the otic capsule velocity, the stapes volume velocity (relative to the cochlea), and the intracochlear pressure were very similar under BC and HC stimuli. This was regardless of the significant differences, between the two excitation methods, in the absolute values of each of the measured parameters. The cochlear fluid appears to be activated by an osseous pathway, rather than a direct non-osseous pathway from the cerebrospinal fluid (CSF), under HC. However, the osseous pathway itself is activated by the acoustic pressure within the CSF.

Conclusions: Data at high frequencies indicates that the skull bone surrounding the brain and CSF could play a role in the interaction between the CSF and the cochlea, under both stimulation conditions, while inertia is a dominant factor at low frequencies. Further work should be focused on the investigation of the solid-fluid interaction between the skull bone walls and the intracranial content.

Finite Element Model of Residual Hearing and Balance After Cochlear Implant Surgery

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Background: The inner ear's sensory systems include the cochlea and the vestibular apparatus which provide the senses of hearing and equilibrioception, respectively. Connected by the reuniting duct, pathologies of one system often impact the other. One such example is the trauma from cochlear implant (CI) surgery which can damage residual hearing and balance function. In the last decade substantial progress has also been achieved in the design of the cochlear implant. However, no model has had the potential to simulate the effect of CI surgery on both residual hearing and balance. Such a model could provide information on the effectiveness of CIs and evidence for further improving their design.

Methods: The computational model includes a three chambered cochlea and a vestibular system with utricle, saccule, cupulas, and maculae. The source of the model's geometry is μ-CT and μ-MRI imaging of a chinchilla bulla, coreferenced to obtain good definition on both osseous and soft tissues. Each image was segmented using 3D Slicer yielding the geometry of the temporal bone, cochlea, and vestibular system. Details like the dimensions of the membranous labyrinth were verified with literature. All components were meshed separately and carefully connected to maintain a consistent element size in Hypermesh. Dimensions of a MED-EL FLEXSOFT electrode array were scaled to create an analogous implant which was placed in accordance with a round window insertion in the scala tympani of the cochlea. The length of this electrode is variable to test the effect of different insertion angles. Harmonic acoustic simulations with stapes displacement, linear acceleration, and angular acceleration as inputs were conducted for both the normal and implanted model.

Results: Cochlear function was investigated through analysis of basilar membrane displacement in harmonic acoustic simulation with displacement of the stapes footplate as input. Vestibular function was assessed through simulations of linear and angular acceleration of the whole inner ear; cupula and macula displacements were collected. Hearing and balance function in the implanted ear model was significantly reduced compared to the those of the normal model.

Conclusions: The simulation results verified the model's correlation with experimental values and revealed marked reduction in residual hearing and vestibular function after CI surgery. Future studies could pinpoint the precise hearing threshold where CI surgery is optimal, focus on other diseases which effect both hearing and balance like Meinere's disease, or incorporate the nervous system to study sensorineural hearing loss.

Degradation of Extracellular Matrix Proteins around Cochlear Inner Hair Cells after Exposure to Synaptopathic Noise or Mild Head Injury

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Background: Synapse degeneration at the junction of the cochlear inner hair cells (IHCs) and auditory nerve fibers has been linked to deficits in processing complex sounds and is predicted to contribute to reduced speech comprehension in noise, tinnitus, and "hidden hearing loss". This "synaptopathy" has been shown to arise in surviving hair cells after exposure to noise, ototoxic drugs, and aging processes. However, neither the full extent of environmental causes, nor the mechanisms by which this synaptic degeneration occurs are fully elucidated.

Methods: Here we propose that mild traumatic brain injuries (mild TBIs) may also lead to reduced numbers of IHC synapses. Furthermore, we propose that extracellular matrix proteins that typically contribute to perineuronal nets play an important role in the maintenance of IHC synapses. To test whether mild TBI or noise cause synapse loss and corresponding changes to the perineuronal net-like structures that surround IHCs, we performed immunofluorescent staining for several extracellular matrix proteins known to be components of perineuronal nets, then imaged the cochlear samples at high resolution in three dimensions using confocal microscopy, and performed volumetric and intensity analyses. For mild TBI experiments, 4-5 month old mice were exposed to a low-energy, closed-head impact or to an anesthesia-only control condition and cochleae were collected at 1 or 7 days post-impact. For noise experiments, 3-5 month old mice were exposed to octave-band noise (8-16kHz) at 100dB for 2 hours (or placed in a silent noise chamber for 2 hours as a control) and euthanized at 1, 7, or 14 days after this exposure. Samples were stained using antibodies against several perineuronal net proteins including brevican, aggrecan, HAPLN4, and Tenascin-R.

Results: Counts of immunopositive cells, fluorescence intensity measures, and volumetric analyses using IMARIS software suggest that there are decreases in the amounts of these proteins around the IHCs after noise and after mild TBI.

Conclusions: Future studies will aim to determine the mechanisms underpinning these reductions and whether or not changes to these perineuronal net-like structures contribute directly to the loss of IHC synapses after noise or mild TBI. The identification of such mechanisms could lead to novel therapeutic approaches to prevent or reverse cochlear synaptopathy.

Effects of Selective Inner Hair Cell Loss on Auditory Evoked Potential Measures in Chinchillas

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Background: Pre-clinical physiological studies measuring the auditory brainstem response (ABR) have shown little-to-no change in thresholds following cochlear damage, such as selective loss of inner hair cells (IHC) or synaptopathic damage to IHCs, if outer hair cells (OHC) remain intact. In contrast, ABR wave-1 amplitudes are typically reduced at suprathreshold presentation levels, suggesting a reduction in cochlear output despite an absence of elevated thresholds. In addition to changes in ABR wave-1 amplitude, the envelope following response (EFR) has been suggested as a potential assay that is sensitive to loss of IHC or synaptic damage. The EFR is a steady-state evoked response that is used to assess the auditory system's ability to phase-lock to the stimulus envelope. Here, we evaluated the relationship among ABR wave-1 amplitudes and EFR magnitude-level functions following carboplatin-induced selective IHC loss in the chinchilla. We hypothesized that IHC loss would reduce both ABR wave-1 and EFR amplitudes with EFR showing a greater degree of sensitivity to loss of IHCs.

Methods: Adult, free feeding, male and female chinchillas (1-4 years of age) were used for this study. Distortion product otoacoustic emissions (DPOAE) were obtained from awake study subjects to confirm normal nonlinearity of the cochlea. Sedated ABR thresholds were assessed as a measure of overall hearing sensitivity. Suprathreshold ABR wave-1 amplitudes were measured bilaterally at 90-, 80-, and 70-dB SPL with 1, 2, 4, 8, 12, and 16 kHz tone pips. EFRs were elicited by amplitude modulated (AM) tones presented in quiet with carrier frequencies of 1, 2, 4, 8, and 12 kHz, modulated at 88 Hz, and modulation depths of 100% (deep AM) and 40% (shallow AM). EFR magnitude-level functions were recorded for the right ear at suprathreshold intensities of 90-, 80-, and 70-dB SPL. All pre- and post-carboplatin DPOAE, ABR thresholds and amplitudes, and EFR assessments were obtained using a commercially available clinical system. Following baseline measures, animals were treated with a single dose of 75 mg/kg of carboplatin (i.p., by body weight), a dose reliably shown to produce 50-80% IHC loss with minimal OHC loss. Post-carboplatin assessments were performed three weeks following treatment to allow for recovery time.

Results: Carboplatin had no significant effect on DPOAEs, suggesting survival and function of OHCs. ABR thresholds did not change significantly following carboplatin. ABR wave-1 amplitudes were substantially reduced, even in the absence of elevated thresholds. EFR magnitude-level functions showed differences following carboplatin, specifically, magnitude and phase locking values for shallow AM tones were significantly reduced.

Conclusions: Findings support previous studies suggesting the use of suprathreshold ABR wave-1 and EFR amplitudes as sensitive assays of selective IHC loss.

Determining the Recovery Rate of Hearing Thresholds Across Audiometric Frequencies With Use of Hyperbaric Oxygen Treatment

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Background: Hyperbaric oxygen treatment (HBOT) was first reported to improve auditory outcomes following sudden sensorineural hearing loss (SSHL) in the early 1970s. The proposed mechanism of action is a reversal in the oxygen deficit within the cochlea. Despite more than 40 years of interest in the delivery of HBOT, the therapeutic mechanism and frequency specific recovery shows considerable variability. Further investigation of frequency specific results of HBOT for SSHL may provide a better understanding of the therapeutic mechanism. This study seeks to investigate amount of recovery (in dB) across tested audiometric frequencies (250 – 8000 Hz) before and after HBOT. The overall goal is to retrospectively analyze patient outcomes to better understand how specific frequency regions are affected after HBOT. This work will aid in the understanding of the recovery rate (in dB) of audiometric thresholds with HBOT and provide future directions for HBOT therapy for SSHL.
Methods: A retrospective study was conducted at Dartmouth-Hitchcock Medical Center in Hanover, NH. Inclusion criteria included adults (age range 18-90) with SSHL and seen for HBOT. SSHL was defined as a greater than 30 dB sensorineural hearing loss occurring in at least three neighboring audiometric frequencies with confirmation by an audiologist. Primary outcome variables were audiometric thresholds from 500-8000 Hz before and after HBOT. Analysis included amount of recovery in dB as a response variable in a linear mixed effect model with fixed effects including age, gender, and time between onset of symptoms and first HBOT session.
Results: HBOT resulted in a significant recovery of audiometric thresholds across the audiogram. Increased recovery of low frequency thresholds (250-2000 Hz) was more profound compared to high frequency thresholds (3000-8000 Hz). Time between onset of symptoms and HBOT was also found to be inversely correlated with audiometric threshold recovery.
Conclusions: HBOT was shown to be an effective treatment of SSHL with recovery of thresholds across the audiogram. Increased recovery of low frequency thresholds was apparent in our study, but the reason for these results remains unknown. Possible interpretations include the change in vasculature of the cochlea from base to apex, but future studies will need to confirm this interpretation. Future studies should also assess the combined effect of oral steroids with HBOT and possible spontaneous recovery of thresholds after SSHL.

Investigating Mechanistic Relationships Between Endolymphatic Hydrops, Glutamate, and Cochlear Synaptopathy

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Background: Cochlear synaptopathy refers to the degradation of synaptic connections between inner hair cells and auditory neurons. It is thought to be one of the earliest manifestations of noise-induced hearing loss and may also be involved in perceptual abnormalities such as poor speech recognition and hyperacusis. Current literature suggests glutamate excitotoxicity to be a key mediator of cochlear synaptopathy. In addition, previous findings from our lab showed that endolymphatic hydrops correlates with cochlear synaptopathy both after noise trauma and osmotic modulation of perilymph. However, the specific mechanisms connecting endolymphatic hydrops to synaptopathy are not established. We sought to investigate the mechanistic relationships between endolymphatic hydrops, glutamate excitotoxicity, and cochlear synaptopathy.
Methods: Three separate mouse models were used: wild-type (CBA/CaJ), mice lacking the capacity for glutamatergic transmission (Vglut3KO), and mice with a tectorial membrane that is detached from the outer hair cell stereociliary bundles (TectaC1509G/C1509G). After surgically exposing the middle ear, hypotonic (0 mOsm/kg) or normotonic (304 mOsm/kg) solution was applied to the surface of the intact round window membrane in vivo. A custom-built optical coherence tomography system was used to obtain cross-sectional images of the apical turn of the cochlea prior to and sixty minutes after application of the solutions. Endolymphatic hydrops was quantified by obtaining a ratio of the scala media (SM) and scala vestibuli (SV) cross-sectional areas. Mean SM to SV ratios were compared using paired t-tests.
Results: Exposure to hypotonic solution led to significant increases in SM to SV ratio in all three mouse models, indicating endolymphatic hydrops. In CBA/CaJ mice (n=7), mean ratios before versus after exposure were 0.84 ± 0.04 vs. 1.32 ± 0.14 (p=0.014). In Vglut3KO mice (n=7), mean ratios before versus after exposure were $0.87 \pm$

0.05 vs. 1.13 ± 0.10 (p=0.003). In Tecta mice exposed to hypotonic solution (n=6), mean hydrops ratios before versus after exposure were 1.27 ± 0.25 vs. 1.59 ± 0.28 (p=0.009). Control mice exposed to normotonic solution did not have significant changes in the SM to SV ratio. Counts of synaptic ribbons and dendritic boutons are in progress.

Conclusions: Application of hypotonic fluid to the round window led to endolymphatic hydrops in all three mouse models. This is consistent with the hypothesis that endolymphatic volume after hypotonic challenge is modulated by osmotic gradients between endolymph and perilymph, and is not mediated via the release of glutamate from hair cells. Future work will include synaptic analysis to understand the roles of endolymphatic hydrops and glutamate excitotoxicity in cochlear synaptopathy.

The 226 Hz Acoustic Reflex in Noise-Exposed Chinchillas
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Background: In mammals, moderately loud sounds trigger a reflexive response known as the acoustic reflex (AR). When elicited, the middle ear stapedius muscle contracts and reduces the admittance of the tympanic membrane, which then attenuates acoustic input to the inner ear. Standard diagnostic hearing tests routinely measure the AR in order to assess multiple levels of the auditory pathway, including the middle ear, inner ear, and central auditory nervous system. The AR has been proposed as a possible clinical assay of pathologies that arise from exposure to noise including cochlear synaptopathy or inner hair cell synapse damage. In humans, this damage is thought to produce suprathreshold deficits in the absence of elevated pure tone thresholds such as difficulty understanding speech in noise, tinnitus, and hyperacusis. Previously, we found that ipsilateral AR amplitudes in a chinchilla model remained unchanged following noise exposure when measured via a 226 Hz probe tone at a fixed elicitor level. To further investigate the effects of noise exposure on the AR, AR thresholds were measured in chinchillas before and after noise exposure in both ipsilateral and contralateral conditions using a 226 Hz probe tone.

Methods: Young adult chinchillas (2-3 years-of-age), housed in enriched environments and allowed to free-feed, were used for this study. Ipsilateral and contralateral AR thresholds were measured in awake animals using a Tympstar middle ear clinical analyzer. AR thresholds were obtained via 226 Hz probe tone and multiple pure tone elicitor stimuli (.5, 1, 2 and 4 kHz). As a measure of auditory sensitivity, auditory brainstem response (ABR) thresholds were evaluated from 1-12 kHz. ABR wave-1 amplitudes were recorded at suprathreshold levels as a potential measure of IHC synapse loss. Following baseline data collection, awake animals were exposed to 89 dB SPL octave band noise (center frequency = 4 kHz) for 24 hours in sound field to produce robust temporary threshold shifts (TTS), and no permanent threshold shifts (PTS), consistent with previous studies evaluating the effects of noise exposure. ABR thresholds, DPOAEs, and ABR wave-1 amplitudes were re-assessed 24-hrs post exposure and three weeks post exposure.

Results: Noise exposure produced temporary ABR threshold shifts, reduced DPOAEs and reduced ABR wave-1 amplitudes 24-hrs post exposure. TTS and DPOAEs returned to pre-exposure levels three weeks post exposure, however ABR wave-1 amplitudes did not recover. Following noise exposure, chinchilla AR thresholds at each elicitor frequency did not change.

Conclusions: AR thresholds did not change following noise exposure, suggesting that the reduction in wave-1 amplitude was not associated with changes in the 226 Hz AR. Future studies will assess the effects of noise exposure on the AR using wideband AR, as recent studies suggest that this measure may be more sensitive to noise induced cochlear damage than the 226 Hz probe.

The Human OPA1delTTAG Mutation Induces Adult Onset and Progressive Auditory Neuropathy in Mice
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Background: Dominant optic atrophy (DOA) is the most frequent form of hereditary optic neuropathy, and is caused by heterozygous variants in the OPA1 gene encoding a mitochondrial dynamin-related large GTPase. In the last decade, the clinical spectrum of DOA has been extended to a wide variety of syndromes, including

deafness, called dominant optic atrophy plus (DOAplus). To date, the mechanisms underlying the deafness in DOA remain unknown.

Methods: To gain insights into the pathophysiological mechanisms, we have used a transgenic mouse model carrying a recurrent Opa1delTTAG mutation recapitulating the DOAplus syndrome. the consequence of this mutation on cochlear anatomy and physiology were assessed using complementary approaches combining morpho-physiology, biochemistry and molecular biology.

Results: Our results reveal that the Opa1 delTTAG het mice displayed an adult onset and progressive hearing loss, as attested by the ABR threshold shift over time. However, the mutant mice harbored larger otoacoustic emissions in comparison to WT. Finally, the endocochlear potential, which is a proxy for the functional state of the stria vascularis, was comparable between mutant and WT mice. Ultrastructural examination revealed a selective loss of IHCs together with a progressive degeneration of neurites and soma of primary auditory neurons in mutant mice. These results are therefore in favor of a neuropathy. Molecular assessment demonstrated increased autophagy and mitophagy with impaired autophagic flux, and depletion of MtDNA.

Conclusions: Altogether, these results support a new role for the OPA1 in contributing to the maintenance of inner hair cells and auditory neural structures and opens new perspectives for the exploration and the treatment of OPA-linked deafness.

Nadph Oxidase 3 Deficiency Protects From Noise-Induced Sensorineural Hearing Loss

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Background: The reactive oxygen species (ROS)-generating NADPH oxidase NOX3 isoform is highly and specifically expressed in the inner ear. NOX3 is needed for normal vestibular development but NOX-derived ROS have also been implicated in the pathophysiology of sensorineural hearing loss. The role of NOX-derived ROS in noise-induced hearing loss, however, remains unclear and was addressed with the present study.

Methods: Two different mouse strains deficient in NOX3 or its critical subunit p22phox were subjected a single noise exposure of 2 hours using an 8-16 kHz band noise at an intensity of 116-120 decibel sound pressure level.

Results: In the hours following noise exposure, there was a significant increase in cochlear mRNA expression of NOX3 in wild type animals. By using RNAscope in situ hybridization, NOX3 expression was primarily found in the Rosenthal canal area, colocalizing with auditory neurons. One day after the noise trauma, we observed a high frequency hearing loss in both knock-out mice, as well as their wild type littermates. At day seven after noise trauma however, NOX3 and p22phox knockout mice showed a significantly improved hearing recovery and a marked preservation of neurosensory cochlear structures compared to their wild type littermates.

Conclusions: Based on these findings, an active role of NOX3 in the pathophysiology of noise-induced hearing loss can be demonstrated, in line with recent evidence obtained in other forms of acquired hearing loss. The present data therefore demonstrates that the absence of functional NOX3 enhances the hearing recovery phase after noise trauma, opening an interesting clinical window for pharmacological or molecular intervention aiming at post prevention of noise-induced hearing loss.

Identification of Novel Compounds Against Cisplatin and Aminoglycosides-Induced Ototoxicity

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Background: Cisplatin and aminoglycosides antibiotics are the most ototoxic medications. There are no FDA-approved therapy to prevent either drug-induced ototoxcity.

Methods: Using computational methods we have identified a series of novel compounds that are potential otoprotective. Their hair cell protective effects against both cisplatin and aminoglycosides were examined using the zebrafish lateral line neuromast assay. In vitro cell assays were used to further assess their protective effect as well as whether they attenuate cisplatin anti-tumor and aminoglycosides anti-baterial effects.

Results: Our results show that some of computationally identified compounds were highly protective against cisplatin and aminoglycosides in larval zebrafish. They did not interfere with the intended therapeutic effects of cisplatin and aminoglycosides.

Conclusions: This new class of compounds may have the potential to afford dual protection against the most ototoxic medication. The most potent candidates will be tested in vivo to determine their protective efficacy.

The Effects of Metformin on Protection From Permanent Threshold Shift-Inducing Noise

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Background: Our team recently published an atlas of the cell type-specific transcriptional changes induced by permanent threshold shift (PTS)-inducing noise in the mouse cochlea (Cell Reports, September 2021). A major finding of the study was the identification of candidate therapeutics to prevent noise induced hearing loss by intersecting the list of dysregulated genes with the DrugCentral database. The top-ranking candidate was the FDA-approved antidiabetic drug, metformin. In a literature review, we found some evidence of metformin's potential as an otoprotective drug. However, to date, there exists no comprehensive evaluation of the effects of metformin treatment on protection from PTS-inducing noise in animals of both biological sexes. Here, we examine the physiological and histological outcomes of exposure to PTS-inducing noise in mice of both sexes, with and without metformin treatment.

Methods: Baseline auditory brainstem response (ABR) thresholds were established in 9-week-old male and female B6CBAF1/J mice. Following baseline testing, mice were administered metformin (2 mg/kg/day) or a vehicle (saline) in their drinking water. Treatment continued for the remaining duration of the study. At 10-weeks of age, mice were exposed to a PTS-inducing noise (102.5 or 105 dB SPL, 8-16 kHz, 2h). Auditory thresholds were determined via ABRs at 24-hours post-exposure and 1-week post-exposure. Following the 1-week ABR, the mice were euthanized and cochlear tissue was collected for histological analysis, including cytocochleograms and inner hair cell synapse counts.

Results: Here we present ABR threshold data, cytocochleograms, and inner hair cell synapse counts. ABR thresholds are compared before and after PTS-inducing noise exposure in both male and female mice treated with either metformin or vehicle. Cytocochleograms and inner hair cell synapse counts are compared between treatment groups and sexes to examine the effects of metformin treatment on outer hair cell loss and cochlear synaptopathy following PTS-inducing noise.

Conclusions: This study is the first, to our knowledge, that physiologically and histologically evaluates the FDA-approved antidiabetic drug, metformin, as a candidate treatment for noise-induced hearing loss in mice of both sexes.

Ototoxic Potential of COVID-19 Therapies

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Background: COVID-19 has become a widespread disease for which multiple drugs are in clinical trials. Some of these drugs have the potential to cause negative side-effects. Reports of hearing loss after COVID-19 infection, particularly those cases needing treatment, suggest that some COVID-19 therapies are ototoxic. However, clinical trials do not usually examine auditory function. Our experiments in multiple animal models reveal the potential for ototoxicity in multiple COVID-19 therapies.

Methods: We used the zebrafish lateral line, an established model for hair cell damage, to test for ototoxicity of seven drugs approved or in clinical trials for treatment of COVID-19. We performed hair cell counts to assess lateral line damage after a 24-hr drug treatment. To test the hypothesis that these drugs enter hair cells via the MET channel, we blocked the MET channel with amiloride during the drug treatment.

Ivermectin is not recommended by the FDA for treating COVID-19, yet many people have chosen to take ivermectin without a doctor's guidance and subsequently have been hospitalized. Therefore, we assessed the ototoxic potential of ivermectin in vivo in rats. One week after baseline auditory brainstem response (ABR) recordings, we injected rats each day for 10 days with a clinically relevant ivermectin dose (0.2 mg/kg). We then recorded post-treatment ABRs after a 3-week recovery period.

Results: Over half of the drugs we tested caused damage to zebrafish hair cells, including ivermectin, lopinavir, and remdesivir, one of the few FDA-approved drugs for treatment of COVID-19. Dexamethasone, a recommended treatment for COVID-19, did not cause hair cell damage, confirming previous research. We also

found that blocking the MET channel protected hair cells from lopinavir damage. In contrast to our zebrafish assays, ivermectin did not cause a threshold shift in rats. We plan to look for more subtle inner ear damage in the ears from ivermectin-treated animals.

Conclusions: We discovered that multiple COVID-19 drugs are ototoxic to the zebrafish lateral line, some of which require MET channel activity to cause damage. While ivermectin was toxic to zebrafish hair cells, it did not appear to cause hearing loss in our rodent model. These contrasting results suggest that ivermectin may require a different delivery route to cause inner ear damage, or that the damage was too subtle for detection with ABR recordings. In future experiments we will also examine remdesivir in our rodent model. In addition, we are developing a computational model to predict drug ototoxicity in silico to rapidly identify drugs for validation in the lab. Considering the large number of COVID-19 therapies in clinical trials, our research can help identify drugs with the fewest side-effects and determine which therapies warrant audiometric monitoring.

Interferon Gamma Expression in the Mouse Cochlea After Congenital CMV Infection
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Background: Congenital CMV (cCMV) infection is a leading cause of hearing loss in infants and children worldwide. cCMV occurs in 1/200 births in the USA, and 10% of infected newborns develop hearing loss. Despite years of study, the mechanism by which cCMV causes hearing loss is unknown. Our murine model of congenital CMV infection using peripheral viral inoculation in newborn mice produces hearing loss in approximately 60% of infected mice. This provides an excellent model for studying the effects of cCMV on the cochlea. Our previous work with this model has shown active virus in cochlear macrophages and endothelial cells between 7-14 days post infection.

Methods: In order to learn more about the immune response in the inner ear to cCMV, we examined the production of interferon gamma (IFNg) in the cochlea using IFNg-YFP reporter mice exposed to mCMV at birth. Previous studies of CMV infection in the brain have shown that IFNg plays a critical role in the immune response to infection and potentially to neurodegeneration. We believe that IFNg may play an important role in CMV induced hearing loss. Initially, we sought to determine the timing and location of IFNg expression in the inner ear after CMV infection. IFNg-YFP reporter mice were inoculated with 200 pfu of mCMV at birth. Immunofluorescence microscopy was performed to identify cells that expressed IFNg post CMV. Mice were examined at 7 and 14 days post infection and their cochleae were studied by sections and whole mount preparations. Additionally, bulk-RNA sequencing was used to evaluate differential gene expression in control and mCMV infected mice at p7 and p14.

Results: CMV infected mice at both P7 and P14 showed an increase in IFNg expression when compared to control mice. This finding coincided with the bulk-RNA sequencing data that showed a significant upregulation in IFNg expression in the mCMV infected mouse cochlea. IFNg expression frequently colocalized with CX3CR1+ cochlear macrophages. Additionally, IFNg expression was found in cells among developing cochlear hair cells and auditory nerve fibers. The identity of these cells is currently under investigation.

Conclusions: Previous work with cCMV has shown loss of spiral ganglion neurons 4-6 weeks post infection suggesting a possible interaction between neuronal loss and IFNg expression.

Auditory Effects of Antiretroviral Exposure During Pregnancy and Breastfeeding
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Background: The World Health Organization (WHO) recommends that pregnant and nursing women with human immunodeficiency virus (HIV) take highly active antiretroviral therapy (HAART) in order to avoid transmitting HIV to their children. To date, studies of HAART exposure in utero have indicated that exposed children are at increased risk for neurological abnormalities, including auditory effects. Previous studies have suggested that non-auditory damage resulting from exposure to HAART is caused by mitochondrial damage and endoplasmic reticulum stress, and that these toxicities vary across cells, organs, and tissues. To date, there have been conflicting findings in clinical populations on the auditory effects of post-natal HAART exposure and no published studies of the effects of exposure during pregnancy and breastfeeding (PaB). The first objective of this project was to characterize the auditory effects of these exposures during PaB. Outside of the direct risks of HAART exposure, the use of aminoglycoside antibiotics is more common in people with HIV for the treatment of opportunistic

infections. As such, this study also evaluated how exposure to HAART during PaB may increase future susceptibility to aminoglycoside-induced hearing losses.

Methods: This study utilized a well-understood mouse model of ototoxicity, the CBA/CaJ mouse, to evaluate the effects of HAART exposure during PaB on auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs). 10 female breeding mice were exposed to one of four combinations of WHO-recommended HAART for pregnant and nursing mothers or a control in order to compare the auditory effects of these drugs on their offspring. 96 offspring who were exposed to HAART during PaB underwent ABR and DPOAE testing at wean age after completing breastfeeding. A subset of the offspring were exposed to 900 mg/kg of the aminoglycoside kanamycin daily for 12 days. Auditory measures were repeated on day 7 of kanamycin exposure, 24 hours after the final exposure, and again 28 days after the final exposure. Following data collection, ABR P1-N1 amplitudes and P1 latencies were evaluated to assess the physiologic index of the afferent synaptic pathway between the IHCs and the spiral ganglion neurons.

Results: The findings can be summarized in three major themes: 1) Exposure to zidovudine (AZT)- and efavirenz (EFV)-containing HAART regimens during PaB caused ABR threshold elevation at wean with no significant impairment of DPOAEs; 2) Exposure to HAART during PaB increased the risk of kanamycin-induced mortality, especially in animals receiving EFV; 3) kanamycin-induced hearing loss is worsened by exposure to HAART during PaB, especially in animals receiving EFV and AZT.

Conclusions: These findings suggest that HAART exposure during PaB has direct effects on the developing auditory system that both causes small but significant hearing loss at wean and elevates subsequent auditory risk associated with kanamycin exposures after birth.

The TrkB-Selective Agonist Monoclonal Antibody M3 Promotes in Vivo Spiral Ganglion Cell Survival in Deafened Guinea Pigs
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Background: The auditory nerve degenerates following severe damage to the organ of Corti including loss of hair cells. For optimal hearing performance with a cochlear implant (CI), a healthy auditory nerve is essential. In numerous animal studies it has been established that TrkB and TrkC agonists, such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), can reduce degeneration of spiral ganglion cells (SGCs) after severe loss of cochlear hair cells. In the present study, we investigated the efficacy of the TrkB-selective monoclonal antibody agonist M3 in a guinea pig model of acquired deafness, following the reported beneficial effects of this molecule in rat models of cochlear synaptopathy ex vivo (Szobota et al., 2019, PLoS One 14, e0224022) and following intratympanic administration in vivo (Tsivkovskaia et al, 2021, ARO 2021 abstract).

Methods: Three groups of guinea pigs were ototoxically deafened. Two weeks after deafening a gelatin sponge, soaked in one of three solutions, was placed on the perforated round window membrane (RWM; Vink et al., 2020, Brain Sci. 10, 787). These solutions were: 1) 7 mg/ml M3 in buffer, 2) 0.7 mg/ml M3 in buffer or 3) buffer alone (negative control). The experimenters were blinded with regard to the treatment. Four weeks after treatment, the animals were implanted with a CI and electrically evoked compound action potential (eCAP) recordings were performed to assess nerve responsiveness. Specifically, we analyzed the eCAP inter-phase gap (IPG) effect indicative of neural health (Ramekers et al., 2015, J. Neurosci. 35: 12331–12345). Following animal termination, the cochleae were harvested and SGC survival was quantified.

Results: The guinea pigs exposed to the 0.7 mg/ml M3 dose showed significantly more SGC survival in the basal turn of the cochlea of the treated ear than in that of the untreated contralateral ear. For the 7 mg/ml dose, a trend towards SGC survival was observed that did not reach statistical significance. No differences were observed between ears in the control group. With regard to the IPG effect, no differences were observed among the three groups.

Conclusions: Administration of M3 via the RWM in this guinea pig model of acquired deafness provided a protective effect on SGCs, as measured histologically, consistent with previous studies showing similar effects of neurotrophins (Vink et al., 2020). M3 treatment did not lead to altered neural activity as compared to the negative control group. This was unexpected, since BDNF treatment did lead to significantly altered neural activity (Vink et al., 2020). We conclude that the monoclonal antibody TrkB-selective agonist M3 is able to preserve SGCs in vivo when applied to the RWM.

Cochlear Synaptopathy and Nitrative Stress in Lead-Induced Auditory Dysfunction

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Background: Environmental exposure to heavy metal lead, a public health hazard in many post-industrial cities, causes hearing loss upon long-term exposures. Blood lead levels of ≥2 µg/dl, which is well below the current action level (5 µg/dl) recommended by the Centers for Disease Control and Prevention, and bone lead levels of 15 µg/g (in tibia) were associated with increased odds of hearing loss. Lead-induced cochlear and vestibular dysfunction is also well documented in animal models. Although short-term exposure to lead alone at concentrations relevant to environmental settings does not cause significant shifts in hearing thresholds in adults, moderate-low level lead exposures induce neuronal damage and synaptic dysfunction. Lead-induced neuronal damage is facilitated by glutamate excitotoxicity, excessive Ca2+ influx, and oxidative stress, which are mechanisms attributed to cochlear synaptopathy and neurodegeneration of the auditory nerve. We reported that exposure to lead induces oxidative stress in mouse cochlea. However, potential damage to cochlear ribbon synapses after lead exposure is yet to be fully understood.
Methods: In this study, we evaluated the cochlear synaptopathy and nitrative stress in lead-induced auditory dysfunction in C57BL/6 mice. Young-adult mice (four-week old) were exposed to 2 mM lead acetate in drinking water for 28 days and its effects on hair cells, ribbon synapses, and spiral ganglion cells were assessed. Control animals were given normal drinking water.
Results: Inductively coupled plasma mass spectrometry analysis indicated that this exposure increased the blood lead levels to 29 ± 7 µg/dl (n=6). Assessment of hair cell loss by immunohistochemistry (n=6) and outer hair cell activity by distortion product otoacoustic emissions (n=4-5) indicated that the structure and function of the hair cells were not affected by lead exposure at this level. However, it significantly decreased the expression of CtBP2 and GluA2, pre- and post-synaptic protein markers in the inner hair cell synapses, particularly in the basal turn of the organ of Corti, suggesting lead-induced disruption of ribbon synapses (n=5-8, * $p<0.05$, ** $p<0.01$). In-addition, lead exposure significantly increased the nitrotyrosine levels in spiral ganglion cells suggesting lead-induced nitrative stress in the cochlea (n=4-5, * $p<0.05$).
Conclusions: Collectively, these findings suggest that even exposure levels that do not affect the hair cells induce cochlear nitrative stress and can cause hidden hearing loss.

The Antagonistic Roles of JAK1 and JAK2 Signaling in Response to Amingolycoside-Mediated Ototoxic Stress

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Background: The role of the immune system in relation to the propagation or protection of the inner ear from ototoxic insults has come into focus in the last several years. Central to immune system development, polarization, and proliferation is the important JAK – STAT signaling pathways. This class of signaling molecules has been extensively studied and their respective signaling patterns are well known. There are four different JAK proteins that signal as receptor tyrosine kinases. These proteins are JAK1, JAK2, JAK3, and TYK2. These proteins can interact as homo or heterodimers to activate their downstream signaling and transcription factors, the STATs. There are seven recognized members within the family of STATs. This signaling family has generated interest in past as it pertains to ototoxicity. However, to date, such focus has been on the downstream STAT transcription factors and not the JAK kinases. Here we have utilized specific JAK inhibitors to assess the roles of these upstream receptor tyrosine kinases with respect to lipopolysaccharide (LPS) and kanamycin (KM) mediated cochleotoxicity.
Methods: To investigate the role of JAK signaling in ototoxicity we utilized a clinically relevant aminoglycoside model in C57BL/6 AHL+ mice. In this model, animals were treated with LPS at 1 mg/kg I.P. 3 times over a 14-day period in combination with subcutaneous injection of KM at 500 mg/kg two times a day, 6 hours apart for 14 days. Experimental animals were co-treated with KM and Fedratinib - 50 mg/kg O.G. (JAK2 specific) or Momelotinib - 100 mg/kg O.G. (JAK1 and JAK2 activity). Functional hearing assessments were collected via auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAE). ABRs were tested at 4,8,16,22,32,45, and 64 kHz from 100 dB SPL to 20 dB SPL in 5 dB increments. DPOAEs were tested at 5.6, 8, 11.3, 16, 22, and 32 kHz from 75 dB SPL to 10 db SPL in 5 dB increments.
Results: Momelotinib co-treatment potentiates toxicity associated with KM + LPS treatment in mice. This toxicity was permanent as measured via ABR and DPOAE out to 10 weeks post treatment at the frequencies of 16kHz,

22kHz, and 32 kHz. In contrast, treatment with Fedratinib rescued hearing in animals exposed to this treatment regimen across the middle and basal turn frequencies including 22kHz, 32kHz, and 45kHz. Interestingly, 22 kHz and 32 kHz thresholds were no different than age matched control animals. Similarly, Fedratinib co-treatment resulted in protection of DPOAE thresholds at 16, 22, and 32 kHz.

Conclusions: This study has uncovered, for the first time the opposing effects various components of JAK signaling plays within the inner ear upon ototoxic stress. Specifically, we have identified opposing roles of JAK1 and JAK2 in the maintenance of the inner ear from KM-mediated ototoxic stress.

SENS-401 Inner Ear Exposure is Not Altered by Severe Acoustic Trauma in a Rat Model of Sudden Sensorineural Hearing Loss (SSNHL)

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Background: Studies have demonstrated an impact of inner ear lesioning on local drug exposure upon systemic administration in various hearing loss animal models, which could be model-specific and affect successful dose-translation from preclinical studies to the clinical sudden sensorineural hearing loss (SSNHL) patient population. SENS-401 (azasetron besylate) is currently in phase 2 clinical trial for the treatment of SSNHL as otoprotective treatment. Preclinical otoprotective efficacy with significant improvement of hearing by SENS-401 treatment vs placebo control has been previously demonstrated in a rat animal model of SSNHL (Petremann et al. Otol Neurotol, 2019). Here, the goal is to determine the impact of acute acoustic trauma performed 24 hours prior to SENS-401 oral administration on systemic (blood plasma) and local (inner ear and perilymph) SENS-401 exposure profile.

Methods: Following baseline audiometry (ABRs and DPOAEs), rats were randomly assigned to two groups of noise exposure: 0 dB SPL (sham trauma group) or 120 dB SPL (acoustic trauma group) octave band noise (8-16 kHz) for 2 hours. 24 hours after sham or acoustic trauma, audiometry recordings were performed on both groups in order to assess hearing loss degree. Four hours after anesthesia induction, fully awake rats received a single oral administration of SENS-401 (13.2 mg/kg), and each group was divided in 4 subgroups for each of the 4 sampling time points: 0.5h to 4 hours post SENS-401 treatment. SENS-401 was quantified in temporal bones and blood plasma by high performance liquid chromatography/tandem mass (LC-MS/MS) spectrometry.

Results: As control, T+24h mean ABR threshold shift across frequencies was significantly higher in the acoustic trauma group in comparison to sham control group (mean difference 53 dB, p<0.001), indicative of effective hearing loss induction. Furthermore, no statistically significant differences were observed between rats experiencing acoustic trauma or sham trauma for SENS-401 quantification on inner ear tissue, perilymph and blood plasma (p=0.921; p=0.315 and p=0.313, respectively).

Conclusions: The impact of lesions on variations in local exposure of therapeutic products is an important consideration which merits being assessed in preclinical models and included in development strategies. Here, building on previous preliminary studies, we demonstrate that acoustic trauma-induced hearing loss did not affect local and systemic exposures of orally administered SENS-401 in an animal hearing loss model where blood-labyrinth barrier (BLB) permeability is supposed to be enhanced. Acoustic trauma did not affect inner ear local bioavailability as shown with inner ear tissue, perilymph, and plasma SENS-401 concentrations. The lack of impact of noise trauma on drug local exposure profiles may result from SENS-401 demonstrated good local bioavailability (Petremann et al. Otol Neurotol, 2017).

Investigating a Potential Mechanism of Noise-Induced Synaptopathy

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Background: Noise is the most common cause of preventable hearing loss, affecting at least 31 million Americans. A recently discovered subcategory of noise-induced hearing loss is known as hidden hearing loss, in which the synapses connecting hair cells to afferent ganglion neurons are damaged (termed synaptopathy). However, the exact molecular mechanisms underlying synaptopathy remain unknown and there is currently no FDA approved treatment. Here we investigate a potential mechanism of noise-induced synaptopathy. Other research groups have demonstrated that excess AMPA exposure can induce pre- and post-synaptic damage at the

hair cell synapse, and that calcium influx precedes this damage. Other studies have also shown that cells with weaker calcium buffering mechanisms are more prone to synaptic damage.

Methods: We hypothesize that excess calcium influx through calcium-permeable AMPA receptors is responsible for mediating synaptopathy by disrupting intracellular buffering mechanisms and ultimately damaging the pre- and post-synaptic cells. Overactivation of calcium-permeable AMPA receptors, which lack the calcium-filtering GluA2 subunit, is expected to be caused by copious glutamate release following intense noise exposure.

Results: We tested this hypothesis using noise to damage hair cell synapses in the zebrafish lateral line – an established vertebrate model for studying noise-induced hearing loss. We co-treated fish with various glutamate receptor agonists, antagonists, and calcium chelators, after which synaptic damage was assessed via antibody labeling of pre- and post-synaptic markers. Future experiments will utilize live pre- and post-synaptic calcium imaging to determine the correlation with synaptic damage.

Conclusions: This research sheds light on the suspected mechanism of glutamate excitotoxicity and AMPA-receptor mediated synaptopathy following acoustic trauma, thus uncovering potential pharmacological targets. Given the absence of an FDA approved treatment and the inefficacy of hearing aids in mitigating hidden hearing loss, our research has the potential to fill a health care gap for a currently untreatable condition.

Investigating the Therapeutic Potential of Piperlongumine Derivatives for Noise-Induced Hearing Loss

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Background: According to the World Health Organization, 466 million people globally have disabling hearing loss. The degradation of the inner ear from acquired hearing loss produces a total cost of $980 billion annually. We must develop effective therapies to avoid this financial toll and to mitigate the reduction in quality of life for millions. Currently, there are no FDA-approved drugs that protect the inner ear against noise-induced hearing loss (NIHL). However, preliminary evidence suggests that piperlongumine – an extract from the Indian long pepper (Piper longum) – has therapeutic potential for the treatment of NIHL. Data from our lab shows that piperlongumine protects zebrafish hair cells against antibiotic ototoxicity. Piperlongumine's otoprotective capacity may be linked to NF-κB pathway inhibition. After the ear is exposed to excessive noise, NF-κB is activated and induces apoptosis via the generation of reactive oxygen species (ROS). It's been shown that piperlongumine regulates this pathway in cancer systems. Piperlongumine may be preventing noise-injured hair cells from undergoing apoptosis by, at least in part, inhibiting the NF-κB pathway. We propose that by modifying the chemical structure of piperlongumine, we will 1) improve its efficacy for protection against NIHL; and 2) increase its potency at interfering with the NF-κB pathway.

Methods: We tested 34 piperlongumine derivatives in a zebrafish model for excitotoxicity. For the first aim, we used Tg(brn3c:GFP) zebrafish that express a GFP-bound membrane protein in the neuromast hair cells. Fish were pre-incubated with kainic acid (KA) to induce excitotoxicity, and then with the piperlongumine derivatives. Fish were fixed and stained, and the hair cells were quantified under an epifluorescence microscope. For the second aim, we used Tg(NFKB:EGFP) zebrafish, an NF-κB reporter line that induces expression of GFP when NF-κB is activated. Fish were pre-incubated with kainic acid, and then with the otoprotective derivatives. They were fixed and immunostained for GFP. The total fluorescence intensity was quantified using ImageJ.

Results: We found five piperlongumine derivatives that protected against excitotoxicity better than the original piperlongumine molecule. Out of those five, PG53 showed the best protection (96%) at the lowest concentration (1nM). We also observed a significant reduction in NF-κB activation (compared to KA) when fish were incubated with PG25.

Conclusions: By modifying the chemical structure of piperlongumine, we were able to improve protection against KA excitotoxicity, as well as reduce NF-κB pathway activation. Future experiments will be aimed at addressing the effects of PG53 in a more relevant model for hearing loss.

Multiplex Immunohistochemistry Reveals Cochlear Macrophages Diversity in Cisplatin-Induced Hearing Loss

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Background: The inner ear was previously thought to be immune-free due to the blood-labyrinth barrier, which restricts the entry of most blood-borne compounds. However, recent studies have discovered macrophages within

the cochlea, vestibular system, and the audio-vestibular nerve, suggesting that macrophages are essential for inner ear homeostasis and may be targets for the prevention and treatment of hearing and balance disorders. Tissue macrophages are classically categorized into pro-inflammatory macrophages (M1) and anti-inflammatory macrophages (M2). However, precise information about the types and role of macrophages in the inner ear remains unclear. Therefore, the objective of this study was to clarify the diversity of inner ear macrophages and analyze macrophages both in the normal state and following exposure to an external stimulant, such as cisplatin (CDDP), an ototoxic chemotherapeutic drug.

Methods: Mice were injected with 5mg/kg/day of CDDP intraperitoneally for six consecutive days. Mice cochleae were collected at day 0 prior to CDDP exposure and on days 8 and 15 following CDDP exposure and fixed in formalin and paraffin sections prior to immunostaining with a multiplex immune histochemistry (mIHC) technique, which can stain different markers within the same paraffin section. We used a six-marker panel to identify different macrophages subtypes. In addition, cell cytometry was used to analyze and quantify the macrophages in both the normal state and following CDDP exposure.

Results: CDDP exposed mice developed a hearing threshold shift at day 8 post-CDDP, and this shift started to recover at day 15 post-CDDP. This threshold shift was associated with a decrease in the number of macrophages of monocyte origin (F4/80$^+$). Additionally, there was an increase in the expression ratio of markers for both pro- and anti-inflammatory macrophages in the auditory nerve and spiral ganglia area on day 8 and started to resolve on day 15, suggesting a new subcategory of mixed macrophages in the inner ear. Furthermore, the Iba1$^+$ macrophages ratio was increased at day 8 post-CDDP, suggesting microglial activation in the auditory nerve. These findings propose that CDDP exposure causes a state of temporary neuronal inflammation in the auditory nerve and spiral ganglia that triggers macrophages polarization towards a new subcategory of macrophages with M1, M2, and microglia in the same single macrophage.

Conclusions: This is the first study that provides an in-depth analysis of macrophage diversity within the inner ear under normal conditions and following CDDP exposure. Inner ear macrophages are a new subtype of macrophages, and they are not exclusively M1 or M2 macrophages. Furthermore, an increased Iba1 expression ratio following CDDP exposure suggests that the auditory nerve and spiral ganglia undergo neuronal inflammation. Thus, inner ear macrophages play a significant role in understanding the mechanism of hearing loss onset following CDDP exposure.

Atorvastatin Reduces Cisplatin-Induced Hearing Loss in Mice

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Background: Concurrent use of statin medication during cisplatin-based cancer treatment may offer a low-risk approach to protect the hearing of patients undergoing cisplatin-based chemotherapy. Recently, our lab has shown in a mouse model of cisplatin ototoxicity (Fernandez et al., 2019) that lovastatin can be used to inhibit cochlear injury and reduce hearing loss (Fernandez et al., 2020). In our observational clinical study of patients with head and neck cancer treated with cisplatin, after controlling for age, sex, cisplatin dose, cochlear radiation exposure and pre-existing hearing loss, we determined that atorvastatin use was significantly associated with reduced incidence and severity of cisplatin-induced hearing loss (Fernandez et al., 2021). Here, we examine the extent to which atorvastatin reduces cochlear injury in cisplatin-treated mice with the aim of exploring potential underlying mechanisms of otoprotection.

Methods: Adult CBA/CaJ mice (29M, 22F) were tested using distortion product otoacoustic emissions (DPOAE) and auditory brainstem response (ABR) at baseline. Subsets of mice were treated once daily with saline or atorvastatin (10 mg/kg or 20 mg/kg) while undergoing 3 cycles of cisplatin treatment consisting of 3 mg/kg/day for 4 days followed by 10 recovery days totaling 6 weeks. Upon completion of the 3rd (final) cycle, hearing was reevaluated using DPOAE and ABR to assess any change in hearing. All mice were then held for up to 4 months without additional treatment and hearing tests were repeated. Cochleas were collected for either immunohistochemistry and hair cell quantification or to analyze platinum levels via inductively coupled plasma mass spectrometry (ICP-MS) at separate locations along the length of the cochlea or in the isolated stria vascularis.

Results: Our cisplatin paradigm induced a 35-55 dB SPL threshold shift and significant reduction in outer hair cell function, confirmed by ABR and DPOAE respectively, at all frequencies assayed from 8 to 40 kHz. Consistent with our previous report using lovastatin, atorvastatin offered a dose- and sex-dependent level of otoprotection. Male mice co-treated with 20 mg/kg atorvastatin demonstrated reduced threshold shifts, up to 24

dB, and greater DPOAE amplitudes relative to saline controls treated with cisplatin alone. In contrast, female mice co-treated with atorvastatin at 10 or 20 mg/kg/day did not differ from those treated with cisplatin alone. Further auditory testing is scheduled to assess the stability of otoprotection 4 months after cessation of atorvastatin treatment.

Conclusions: The results of this study will help us develop studies to determine the underlying mechanisms of statin-induced otoprotection. A multi-site phase 3 clinical trial is currently underway to determine the extent to which atorvastatin reduces cisplatin-induced hearing loss in adults with head and neck cancer.

Hair-Cell Neurotransmission Modulates Neomycin Susceptibility

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Background: The aminoglycoside antibiotic neomycin is effective at eliminating not only life-threatening infections but also auditory hair cells and as a result can cause irreversible hearing loss both in human patients and animal models. Recent work has shown that hair cells may be particularly vulnerable to antibiotics because they must cope with a high metabolic load which may sensitize them to insults. Near constant stimulation of hair cells by omnipresent sound calls for steady mitochondrial ATP production, which ultimately can stress mitochondria by leading to the buildup of cytotoxic reactive oxygen species (ROS) mitochondrial byproducts and thus may render hair cells more susceptible to neomycin. Our work aims to understand what aspect of hair-cell activity drives this ROS production. The conversion of mechanical sound stimuli into a chemical signal in hair cells is metabolically demanding. Sound triggers the influx of Ca2+ through presynaptic voltage-gated Cav1.3 channels. This Ca2+ influx has been shown to stimulate ATP production and could therefore impact ROS byproduct production. The protein otoferlin is a Ca2+ sensor that couples Ca2+ influx to vesicle exocytosis. Recent work in neurons has shown that exocytosis alone is a major consumer of ATP and thus could also significantly impact ROS production.

Methods: For our study we used cav1.3-/- and otof-/- mutants to investigate the contribution of Ca2+ influx and exocytosis to neomycin susceptibility. We used larval zebrafish, which offer several advantages over rodent models. Larvae are transparent and possess externally located hair cells. These features allow for easy in vivo and in toto imaging of individual cells following bath pharmacological or fluorescent dye treatment.

Results: We found that both cav1.3-/- and otof-/- mutants exhibit significantly augmented hair cell survival relative to controls when challenged with neomycin. Transient block of Cav1.3 channels with the antagonist isradipine during neomycin exposure has no effect while 24- or 48-hour pre-incubation in isradipine prior to neomycin treatment results in significant hair-cell protection. Similarly, inhibiting exocytosis with Dynole 34-2 over 24 or 48 hours also results in neomycin resistance comparable to that seen in otoferlin mutants. Furthermore, we utilized the cytosolic ROS indicator cellROX Orange as well as larvae expressing the genetically encoded mitochondrially-localized ROS indicator mitoTimer to show a reduction in oxidative stress in both mutants. The mitochondrial potential indicator TMRE also revealed reduced mitochondrial activity likely resulting from reduced ATP demand in both mutants.

Conclusions: These results suggest that processes associated with calcium influx or even exocytosis alone can significantly contribute to ROS byproduct buildup and metabolic stress that modulate hair-cell neomycin susceptibility. These findings bring us closer to creating targeted therapies to prevent hearing loss, particularly in the context of slow long-term damage associated with aging.

Differential GFP Expression in Mouse Cochlear Cells in Response to AAVDJ-CMV Delivery to Semicircular Canal

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Background: Gene delivery into the cochlea has applications in clinical treatment and research. Adeno-associated viruses (AAVs) are commonly used for inner ear gene delivery due to their low levels of pathogenicity and immune response, and their long-term expression in various cells (Naso et al, 2017). AAV types have been shown to infect cochlear cells (Tan et al, 2019; Ivanchenko et al, 2021). We are interested in exploring the differences in cell responses to a viral challenge based on cell type and location with the goal of enhancing specific gene delivery for research and translational purposes.

Methods: 1 ul AAV DJ (Grimm et al, 2008) with CMV promotor expressing GFP was injected into the semicircular canal of C57/BL6 mice at P1 (Talaei et al, 2019; Salt et al, 2012). Mice were sacrificed and cochleae collected and stained with DAPI and Myo7a between 1 week and 1-year post-injection. FIJI was used to designate regions of interest in cells and collect the mean gray value of GFP expression in consistent areas for inner pillar cells (IPCs), Deiters' cells (DCs), inner hair cells (IHCs), and outer hair cells (OHCs). Cells were analyzed for raw intensity scores and percent infected above threshold.

Results: We compared expression over time at a given apical turn. We found that IHC expression increased to almost 100% over ten weeks with intensity increasing 100-fold. In contrast, there was little change in either the number or intensity of OHC expression. IPCs significantly increased in intensity and percentage of cells infected from 2 to 10 weeks ($P<0.05$). From 3 to 10 weeks, DCs had no change in mean intensity but did significantly increase the percentage of cells infected ($P<.05$). For all cell types, GFP expression was greatly reduced at 1 year. We next investigated tonotopic differences in expression. At both 5- and 10-week time points, all cell types showed a reduction in cell infection and cell intensities at basal cochlear regions as compared to apical turns.

Conclusions: We found differences in onset of expression, percent of cells infected, and changes in intensity over time between cell types in response to a common injection despite using a ubiquitous promoter and broad spectrum AAV. Hair cells showed expression at 1 week with IHC infection rates and expression levels increasing over the ten weeks and OHCs showing constant infection and levels. IPCs had delayed infection and both IPCs and DCs showed increases in expression over time. Cells responded in a similar pattern tonotopically, with infection and expression levels reduced uniformly in basal regions. We conclude that cells have different mechanisms for coping with infection both in the short and long term and these mechanisms need to be identified and addressed to increase the efficacy of gene therapy approaches.

Novel Dual Lumen Microneedle for Simultaneous Intracochlear Injection and Aspiration

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Background: We have previously shown that microneedles 3D printed via Two-Photon Lithography (2PP) can create precise perforations in the round window membrane that heal within 72 hours, and cause no functional or physiological consequences. These microneedles were also used to aspirate 1µL of perilymph, enough for proteomic analysis. In the current work, we demonstrate that a novel dual lumen microneedle can be used for simultaneous aspiration and injection at constant volume into the inner ear to facilitate delivery of large volume of therapeutic agent.

Methods: 100-µm-diameter microneedles with two interior channels running parallel with diameters of 30µm were produced using 2PP. The designed microneedle was mounted onto the tips of two 30 Gauge syringe needles that, in turn, were connected to two pressure sources. The tympanic bullae of Hartley guinea pigs were excised and perforations were made on these samples using an in-house built microindenter system.

Results: Microneedles were successfully produced with the novel design, and simultaneous constant volume aspiration and injection were achieved. When used with DI water, volume flow rates of up to 0.20 microliters per second for injection, and 0.12 microliters per second for aspiration could be achieved without damaging the microneedles, corresponding to Reynolds numbers of 4.31 and 7.19, consistent with laminar flow.

Conclusions: Dual lumen microneedle can facilitate simultaneous constant volume aspiration and injection from a single 100µm diameter, circular cross-section microneedle. These results are promising for precise direct intracochlear delivery of large volume of therapeutics without causing damage.

Development of a Round Window Niche Implant for Guinea Pigs as Favored Animal Model in Cochlear Pharmacotherapy Research.

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Background: There is an unmet need for precise inner ear drug delivery. Drug deposition in the middle ear cavity and diffusion via the round window membrane (RWM) into the cochlea is widely accepted as a tolerable compromise between application that is as non-invasive as possible and the greatest possible entry of the active ingredient into the cochlea without risking side effects from high-dose systemic administration. However, intratympanic applications are uncontrolled with regard to the amount of active ingredient solution applied, the

duration the substance stays in situ and the rate of penetration into the cochlea. We aim to develop individualized drug eluting round window niche implants (RNIs). In order to test these in the guinea pigs as established animal model cochlear pharmacology studies for bioactivity and biocompatibility, RNIs must firstly be developed for this species.

Methods: Micro-CT scans (Xtreme CTII, Scanco Medical) of 4 Dunkin-Hartley guinea pigs were taken. The data sets were transformed into DICOM (digital imaging and communications in medicine) files and the round window area was segmented manually using 3D SlicerTM version 4.11 (http://www.slicer.org). Since guinea pigs do not have a round window niche such as it is present in humans, we decided for a one-size-fits-all RNI model to reduce the variabilities in the animal model. The height and diameter of the individual digital models were measured and an average model was designed from the mean values of the 8 data sets. A handle which additionally illustrates the orientation of implantation and keeps the RNI in situ was added. The segmented model was exported as STL (standard tessellation language) file and 3D printed (3D-Bioplotter® Manufacturers Series EnvisionTEC, GmbH, Gladbeck, Germany) using EnvisionTEC UV silicone. The accuracy and precision of the printed implants were checked microscopically and by weighting the samples. Finally, the implantability of the printed samples was evaluated by inserting the RNI into n = 6 fresh guinea pigs cadavers.

Results: It is possible to print the used silicone in the small dimension as needed for the RNI. The used extrusion printing technology results in a slight blurring of the print planes and the repeated printing shows that the printing is precise. The accuracy is given to the extent that the RNI could be implanted in the free space between the RWM and the middle ear ossicles as well as the facial ridge without damaging one of the structures.

Conclusions: Using additive manufacturing, we developed a RNI, which can be used for precise RWM drug delivery in guinea pigs. Drug release studies are already started and future in vivo application of this drug delivery system will explore its biological efficacy.

'Mini-PCDH15' Gene Therapy Rescues Hearing in a Mouse Model of Usher Syndrome Type 1F

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Background: Usher syndrome is a devastating hereditary deafness and blindness caused by mutation of any of nine genes. Mutations in one gene, PCDH15, cause Usher syndrome type 1F, manifesting as profound deafness and lack of balance at birth, and blindness developing over several decades. Currently, treatment for Usher 1F is limited to cochlear implants, and there is no treatment for the blindness or balance disorder. Similarly, mice lacking PCDH15 are deaf and have a severe balance deficit. Gene addition to cochlea or retina is an attractive therapeutic strategy; however, the PCDH15 coding sequence, at 5.8 kb, is too large for a single AAV. The extracellular domain of PCDH15 contains 11 link-like "EC" repeats which convey tension to the transduction channel. We hypothesized—based on atomic structure of a full-length hsPCDH15 model—that some EC repeats may not be essential for function, so that a "mini-PCDH15" lacking some of them could be encoded in a single AAV capsid.

Methods: We engineered eight different mini-PCDH15s by deleting up to five EC repeats. We assessed targeting to the plasma membrane in an HEK293 cell line with immunofluorescence microscopy and immunogold scanning electron microscopy (SEM) and assessed binding to CDH23 using a cell aggregation assay. To test the ability of mini-PCDH15s to rescue hearing, three versions were packaged in AAVs. Pcdh15 conditional knockout mice were injected with AAVs at P1 through the round window membrane. ABR and DPOAE tests, and comprehensive histological analyses, were performed four weeks after injection.

Results: Immunofluorescence microscopy and immunogold SEM of HEK293 cells transfected with mini-PCDH15 genes showed that all PCDH15s went to the cell surface with their extracellular domains positioned outside, and that they mediated cell aggregation by binding to CDH23-expressing cells. These were similar to the positive-control full-length PCDH15 in HEK293 cells.

Pcdh15 conditional knockout uninjected mice were deaf at age P30, and had degenerated hair bundles. Mice treated with one mini-PCDH15 version demonstrated hearing rescue to wild-type thresholds at low and middle frequencies. With confocal imaging and immunogold SEM, strong mini-PCDH15 signal was detected at the tips of stereocilia, with gold beads labeling tip links in P30 cochleas. SEM showed robust rescue of hair bundle morphology and tip links at the tips of stereocilia. FM1-43 labeling at P30 demonstrated robust rescue of mechanotransduction in inner and outer hair cells, comparable that in normal hearing littermates.

Conclusions: Together, these data suggest that a PCDH15 mini-gene strategy is a promising approach for therapeutic gene delivery to the human inner ear. Because structural demands on PCDH15 are likely greater in the cochlea than in the retina, these results are also encouraging for the treatment of the progressive blindness in Usher 1F.

Developing Treatment Modalities for the Preservation of Residual Hearing Post-Cochlear Implantation

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Background: Cochlear implantation (CI) is widely used to provide auditory rehabilitation to individuals having severe to profound deafness. However, insertion of cochlear implant induces electrode insertion trauma (EIT) leading to activation of apoptosis and inflammatory pathways in the cochlea damaging sensory cells and consequently loss of residual hearing. Pharmaceutical interventions targeting these apoptotic and inflammatory pathways holds a great potential in the preservation of residual hearing. The objective of this study was to determine the efficacy of a novel drug in providing otoprotection using ex vivo and preclinical in vivo models of cochlear implant trauma.

Methods: For in vitro studies, the organ of Corti was dissected from postnatal day 3 (P-3) rats and then subjected to EIT followed by incubation in the presence and absence of different concentrations of drug. EIT was induced using a custom designed electrode that was introduced into the inner ear through the small cochleostomy located next to the round window area, allowing for an insertion of between $110°$ and $150°$. The organ of Corti not subjected to EIT and unexposed to drug served as the control group. The organ of Corti explants were subjected to FITC phalloidin staining to visualize hair cells using confocal microscopy. The number of surviving hair cells were counted. For in vivo studies, a preclinical animal model of EIT was established. Animals were treated with drug or left untreated followed by implantation. Hearing thresholds were determined by auditory brainstem recordings (ABRs) at different days post-implantation.

Results: The number of surviving hair cells were significantly higher in organ of Corti explants subjected to EIT and treated with drug compared to EIT group alone. Hearing thresholds were significantly lower in implanted animals treated with drug compared to implanted and untreated animals. The molecular mechanisms underlying otoprotection involved attenuation of activation of apoptotic and inflammatory pathways.

Conclusions: Our results suggest that identified drug provides significant otoprotection for EIT both in ex vivo and in vivo models. As drug treatment during cochlear implantation surgery is a feasible approach, the identified compound merits evaluation in clinical trials in future studies. The findings of this study will lay the foundation to develop novel treatment modalities for the preservation of residual hearing post cochlear implantation in pursuit of improving quality of life of implanted individuals and their families.

In Vitro Transfection of Primary Fibroblasts From the Inner Ear of Postnatal Rats With Recombinant Plasmids

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Background: Cochlear implants are widely used to restore hearing through electrical stimulation of the auditory nerve. Typically, sensorineural hearing loss is accompanied by a loss of spiral ganglion neurons (SGN). Delivery of neurotrophic factors (NF) to the inner ear can help the neurons to survive. To provide a long-term support to the SGN, also a long-term delivery of NF might be necessary. As fibroblasts are naturally found in the cochlea but also part of the tissue reaction after insertion of the electrode array, the aim of the current study was to establish a transfection method of cochlear fibroblasts to produce and deliver brain-derived neurotrophic factor (BDNF).

Methods: A recombinant plasmid containing td-tomato red and BDNF sequences (5.6 kB) was used in an in vitro transfection model using Lipofectamine™3000. Fibroblasts were isolated from the spiral ganglion and cultured under standard conditions. The influence of different seeding densities, incubation times and plasmid concentrations was evaluated. Transfection efficiency was characterized by counting fluorescent cells versus

DAPI stained cells. Additionally, BDNF concentration released by genetically modified cells was determined by ELISA.

Results: Results revealed transfection efficiencies between 15 and 20 %. The most effective transfection rates were achieved at a cell seed between 2000 and 4000 cells as well as at 300-500 ng of the recombinant plasmid. In accordance with the number of transfected cells, an increasing BDNF concentration up to 22 ng/ml was found in the supernatants.

Conclusions: Having this system for transfection of primary cochlear fibroblasts successfully established, it can provide the basis for a long-term delivery of drugs in the cochlea.

Combined AAV Gene Therapy Improved Hearing in a Mouse Model of DFNB42

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Background: Hearing loss is one of the most common communication disorders affecting the world's population today. Over the past few years, studies have shown that inner ear gene therapy is capable of improving the auditory function in several mouse models of hereditary hearing loss. In most of these studies, the genetic variants only affected a small cell population in the inner ear (e.g., hair cells). In this study, we applied inner ear gene therapy to a mouse model of DFNB42 (Ildr1w-/-), which carries a gene trap in intron 2. ILDR1 is an integral part of tricellular tight junctions and is strongly expressed in both the organ of Corti and lateral wall. To affect both organ of Corti and the lateral wall simultaneously, we applied two synthetic adeno-associated viruses (AAVs) with full length Ildr1 cDNA, one targeting the organ of Corti (AAV2.7m8), and the other targeting the lateral wall (AAV8BP2).

Methods: Neonatal (P0-P5) Ildr1w-/- mutant mice were used in this study. Synthetic AAV2.7m8 and AAV8BP2 each carrying the full-length cDNA of mouse Ildr1 were injected through the posterior semicircular canal simultaneously. Auditory brainstem response (ABR) testing was used to assess auditory function. Atomic force microscopy (AFM) was applied to the lateral wall epithelium and the organ of Corti to assess tissue mechanical integrity. Immunohistochemistry was performed to examine localization of ILDR1 and tricellulin in the lateral wall and cochlear tight junctions.

Results: Ildr1w-/- mutant mice injected with both AAV2.7m8-Ildr1 and AAV8BP2-Ildr1 showed restoration of ILDR1 expression and improved tight junction architecture. Improvements in tricellulin localization in the tricellular tight junctions was also observed. AFM of the lateral wall epithelium revealed recovery in tissue integrity following the combined AAV injection. In addition, investigation of the organ of Corti inner pillar cells revealed improvement in cell apical stiffness in the treated group. Finally, Ildr1w-/- mutant mice injected with both AAV2.7m8-Ildr1 and AAV8BP2-Ildr1 showed improvement in hearing at P14 versus non-injected mutant animals. At 8 weeks, although non-injected mice were profoundly deaf, mutant mice treated with combined AAV2.7m8-Ildr1 and AAV8BP2-Ildr1 maintained recordable ABR thresholds.

Conclusions: Our study showed that simultaneous delivery of AAV2.7m8-Ildr1 and AAV8BP2-Ildr1 restored the expression of ILDR1 in the tricellular tight junctions in Ildr1w-/- mutant mice. Treatment of AAV2.7m8-Ildr1 and AAV8BP2-Ildr1 resulted in increased tissue biomechanical integrity of the lateral wall and organ of Corti in the treated mutant mice compared with non-treated mutants. In addition, treated mutant mice maintained recordable ABR thresholds at 8 weeks post injections.

Brain Oscillations and Spiking Responses in the Auditory Space Map of Awake Barn Owls

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Background: Brain oscillations evoked by sensory stimuli are fluctuations in field potentials reflecting the combined activity of neural populations. Specifically, gamma oscillations (25-140 Hz) have been linked to

inhibition which shapes population activity in the barn owl's midbrain nuclei involved in sound localization. Earlier in vivo recordings in the owl's optic tectum (OT) have shown that gamma oscillations are spatially tuned to both visual and auditory information. The OT of barn owls, a well established model for sound localization, provides a unique opportunity to evaluate the role of brain oscillations in coding space and salience. Gamma oscillations are sensitive to anesthesia and have been implicated in awake processes, like attention and stimulus selection. However, previous studies characterizing gamma oscillations in the barn owl have relied on light tranquilization with nitrous oxide or anesthesia with ketamine - affecting gamma oscillations to different extents. Therefore, recordings in awake owls are imperative to understanding the role of gamma oscillations in the owl's sound localization and stimulus selection network.

Methods: In a novel approach, we chronically implanted drives loaded with tetrodes in OT and recorded spikes and local field potentials. We investigated spontaneous and sound evoked neural activity in the midbrain of awake barn owls and compared this to recordings from anesthetized state in the same animal.

Results: Preliminary results show that phase locking of spikes to both gamma and delta oscillations are stronger for stimuli at the neurons' preferred location. In addition, anesthesia increases spontaneous delta power and decreases gamma power but does not impact phase locking of spikes in both spontaneous or stimulus evoked firing. These results are consistent with previous reports that gamma power represents spatial tuning and further demonstrate that phase locking of spikes to both gamma and delta oscillations match the spatial tuning of nearby neurons. Additionally, we find that spatial tuning of oscillations are comparable across awake and anesthetized states, suggesting that the functional properties of oscillations in organizing spike patterning remain consistent across states and thus support the use of anesthesia for extensive experiments requiring more precise placement of electrodes in OT, like population recordings.

Conclusions: In this work, we demonstrate the technical feasibility of chronically implanted electrodes to record from the avian midbrain. This will bridge our understanding of auditory processes between awake and anesthetized states and further our knowledge about the role of brain oscillations in midbrain computations. Comparisons between recordings from awake and anesthetized animals demonstrate generalizability between the two states in the midbrain. In the future, we plan to record from forebrain regions, possibly simultaneously with OT, to shine light on the interactions across the brain, which have been historically difficult to study in anesthetized animals.

Modulation Content of Interfering Natural Background Sounds Affects Neural Population Synchrony and Response Power During Vocalization Processing

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Background: Selectively listening to a target sound within a noisy environment is a challenge to the auditory system. Studies using simple sounds such as a tone in noise have attempted to understand how sound segregation is accomplished during normal listening. However, in everyday settings natural background sounds confront listeners with far more complex listening conditions.

Methods: During sound masking, the low-order sound structure, such as overlapping power spectra, can inhibit the strength of neural responses. However, the contribution of higher order statistics in the modulation content of natural backgrounds to masking at the neural level is less understood. Here we manipulated the spectrum and modulation content of five natural backgrounds (babble, machine noise, water, fire, birds) and white noise to determine their contribution to energetic and modulation masking. To dissociate masking attributed to the spectrum versus modulation content, we generated phase randomized (PR) backgrounds which have the power spectrum of the original sounds but with whitened modulation content. Furthermore, to compare masking effects attributed to the modulation content of different background sounds, we used spectrum equalized (SE) backgrounds that have a power spectrum identical to pink noise, but retain the original sound modulation content. Electrophysiological recordings were carried out in the inferior colliculus of awake Dutch Belt rabbits. Neural population responses to speech and animal vocalizations were measured in the presence of natural and perturbed backgrounds.

Results: We demonstrate that vocalizations in the presence of PR backgrounds reduce the neural population signal strength and synchrony of the encoded foreground vocalizations, when compared against the unaltered backgrounds. Despite this, and paradoxically, the neural output signal-to-noise ratio of the encoded foreground is higher for the PR sounds. This suggest that PR backgrounds produce sparser neural activity but allows for a higher fidelity neural representation of the foreground vocalization. This was particularly true for response fluctuations

for frequencies < 20 Hz. In contrast, SE backgrounds have little effect on neural responses when compared to unaltered backgrounds. Masking differences observed across the original background sounds remained for the SE condition.

Conclusions: Collectively, these results suggest that modulation statistics in natural background sounds can have varied interfering effects and that the unique modulation content of each of the interfering background sounds can strongly influence the encoding of masked vocalizations.

Representation of Mouse Vocalizations across Subdivisions of the Inferior Colliculus in Males and Females
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Background: We report neuronal responses within the three main inferior colliculus (IC) subdivisions to mouse ultrasonic vocalizations (USVs) and several categories of non-USV, mostly broadband calls. We address: 1) whether and how frequency tuning properties of neurons affect their vocalization responses, 2) whether factors other than frequency tuning influence vocal selectivity, 3) how frequency tuning and vocalization response properties are distributed, and 4) how these responses might differ between males and females.
Methods: We used multichannel probes to extracellularly record single and multiunit responses in head-fixed, urethane-anesthetized adult CBA/CaJ mice (~4-8 months). Frequency tuning and sound level responses were assessed using tones (4-90 kHz) and broad band noise (4-80 kHz) at 0-80 dB SPL. Recording sites were then tested for responses to both pre-recorded and synthesized syllables containing a broad range of spectral components.
Results: For both males and females, the frequency tuning of IC neuronal populations displayed a broad peak centered in the 16-20 kHz range, with a secondary peak above 40 kHz most evident in the central nucleus. At moderately high sound levels (70 dB SPL peak), most IC neurons across the tonotopic range were responsive to non-USV, mostly broadband calls with low fundamental frequency. The response of high characteristic frequency (CF) neurons to these lower frequency calls was likely the result of call energy in the low frequency tails of high-CF tuning curves. The response to both tonal and stepped USVs was, as expected, most common among neurons tuned above 32 kHz, but was also observed in some neurons with CFs well below the frequencies within these calls. These responses in low CF (< 16 kHz) neurons may result from low frequency distortion products introduced by cochlear processing, as proposed by Portfors and colleagues. There were several key features of these low-CF responses to USVs: 1) they occurred much more commonly in response to stepped USVs than tonal USVs such as flat or chevron calls, 2) they occurred in a larger proportion of neurons from the external (ECIC) and dorsal (DCIC) cortex than in the central nucleus, and 3) there were substantially larger percentages of these responses among females than males, especially in the DCIC and ECIC. For example, responses to stepped USVs in the DCIC of males was ~25% versus ~80% in females.
Conclusions: To our knowledge, this is the first report exploring responses in the mouse lemniscal and non-lemniscal IC to a broad range of vocalizations in males and females. These results can provide a basis for understanding the nature of projections from the IC through the auditory thalamus to structures such as the amygdala and cortex, and how this information may be processed differently between the sexes.

Matrix Efferents of the Lateral Cortex of the Inferior Colliculus During an Early Critical Period
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Background: The lateral cortex of the inferior colliculus (LCIC) is a multimodal shell nucleus of the midbrain that consists of a series of discrete modular domains surrounded by an encompassing matrix. In mice, this compartmental framework emerges over an early critical period (postnatal day, P0-P12) and can be easily visualized with a host of neurochemical stains (e.g. GAD, an established modular marker). Multisensory afferents of somatosensory and auditory origin initially overlap at birth in the LCIC, prior to segregating into modality-specific compartments (modules and matrix, respectively). Comparatively less is known about the LCIC efferent system, its organization, and in particular, its development. Recent evidence in adult mice suggests LCIC compartments project to distinct midbrain and thalamic targets (Lesicko et al., 2020; doi: 10.1523/jneurosci.0646-20.2020). Utilizing retrograde tracing approaches in GAD67-GFP living brain preparations, the present study examines the arrangement of nascent LCIC efferent systems during its defined critical period.

Methods: Dual tracer placements were made in the superior colliculus (SC; biocytin, DyLight 549 streptavidin) and contralateral central nucleus of the IC (CNIC; dextran AlexaFluor 647 direct conjugate) in a developmental series (P0, P4, P8, P12) of GAD67-GFP knock-in mice. Living midbrain slices were bubbled (95% O2, 5% CO2) overnight in artificial cerebrospinal fluid at room temperature to ensure adequate tracer transport. GAD67-GFP labeling enabled the characterization of retrogradely-filled LCIC cells as either GAD+ or GAD-, as well as their relative position with respect to emerging LCIC compartments (i.e. modular or matrix).

Results: The results indicate that distinct LCIC cell populations target the contralateral IC and the SC bilaterally. Each of these efferent cell classes were almost exclusively found in regions of the matrix by the critical period peak (P8-P12). The vast majority of back-filled cells were large with extensive dendritic arbors, many of which appeared to hug or wrap modular borders. Evidence of some GAD+ cells for each identified projection class suggests at least a partial GABAergic component for each pathway. LCIC matrix cells double-labeled for both tracers were not observed, indicating that collateral branching to both the IC and SC is unlikely.

Conclusions: Similar to previously described afferent arrangements, major LCIC outputs appear to be largely segregated into discrete compartments early in development. Future studies are needed to determine if the mechanisms that instruct order in the developing afferent system also influence that of its emerging efferent connections.

Separate Origins of Commissural and Intrinsic Circuits in the Inferior Colliculus

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Background: The inferior colliculus (IC) has extensive intrinsic connections as well as commissural connections between left and right ICs. Together, the intrinsic and commissural projections are considered the most numerous inputs to IC cells, reflecting the extensive neural processing considered to take place in the IC. Tract-tracing studies have shown symmetry in the intrinsic and commissural connections, suggesting closely related functions for these two projection systems. Moreover, Golgi studies and intracellular labeling have demonstrated that many IC cells have local axonal branches, leading to a common view that intrinsic axonal connections are ubiquitous among IC cells. However, direct evidence for IC cells with axons that branch to provide both intrinsic and commissural projections is limited. Here, we use double retrograde tracing methods to identify IC cells with intrinsic and commissural axon branches.

Methods: We injected retrograde tracers bilaterally in the IC of four mixed background B6/J;CBA/CaJ mice. All mice carried at least one copy of the wild-type cdh23 gene and were assumed to lack early-onset hearing loss. Fast Blue (FB) was injected in one IC to label the commissural pathway and red or green fluorescent Retrobeads were injected into the opposite IC. After 3-5 days survival, mice were perfused with fixative. Brains were postfixed overnight then sliced at 40 μm on a microtome and mounted onto glass slides. Every third section through the IC with the Retrobeads injection was examined for FB-, Retrobead-, or double-labeled cells. The area encompassing the Retrobeads injection site and the area immediately adjacent to it were excluded from analysis. A total of 4,967 cells were counted across the four cases.

Results: The FB injections typically included all major IC subdivisions and labeled cells throughout the contralateral IC. Retrobead injections were smaller, but typically included the central nucleus plus parts of dorsal cortex and/or lateral cortex. The Retrobead injections labeled many cells across IC subdivisions. In each case, commissural (FB-labeled) and intrinsic (Retrobead labeled) cells were intermingled. Nonetheless, very few double-labeled cells were observed. The double-labeled cells made up, on average, 0.85% of the cells that projected within the ipsilateral IC and 0.93% of the commissural population. These numbers reflect the IC as a whole, but the observations were similar for individual IC subdivisions; i.e., in each IC area, few cells projected both intrinsically and through the commissure.

Conclusions: Intrinsic connections within the IC and commissural connections between the two ICs arise from different populations of cells. Separate origins of these pathways could allow for different information to be transmitted to each target, and for differential modulation of intrinsic versus commissural pathways.

Synergy Between Neural Sensitivities to Amplitude Modulation (AM) and Dynamic Interaural Correlation Yields Robust Temporal Coding of AM in Reverberation

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Background: Reverberation is ubiquitous in rooms and in nature but the neural mechanisms for robust sound recognition and localization in reverberant settings are poorly understood. Because AM plays a key role in speech perception, AM noise is a useful stimulus for investigating neural coding in reverberation. Slama and Delgutte (J Neurosci. 35:4452) found that about one third of neurons in the inferior colliculus (IC) showed a "reverberant advantage" in the temporal coding of AM in that the modulation in the neural response was significantly larger for reverberant AM stimuli than for "anechoic" (diotic) stimuli having the same modulation depth at the inputs to the ears. In reverberation, the fluctuating amplitude of AM sounds results in variations in the ratio of direct and reflected sounds reaching the ears, causing the correlation between the waveforms at the two ears, the interaural correlation (IACC), to fluctuate periodically. Because IC neurons are sensitive to this cue, we hypothesized that the reverberant advantage is caused by a synergetic interaction between neuronal sensitivities to AM and dynamic IACC.

Methods: We recorded from single units in the IC of two unanesthetized rabbits in response to AM noise (4-64 Hz modulation frequency) presented in reverberation simulated for a typical classroom. We also measured responses to artificial stimuli that had the same AM envelope waveform and the same dynamic IACC as the reverberant stimuli but in which the relative phase of the two forms of modulation was systematically varied.

Results: Consistent with the results of Slama and Delgutte, about 20% of IC neurons showed an advantage in the temporal coding of AM for reverberant stimuli relative to stimuli having the same modulation waveform and the same average IACC at the ear inputs. A smaller fraction (13%) showed the opposite pattern (a "reverberant disadvantage"). For artificial stimuli having periodic modulations in both amplitude and IACC, the strength of temporal coding of AM (vector strength) was dependent on the relative phase between the two types of modulations in most neurons. Consistent with our hypothesis, the signed reverberant advantage was correlated with the amount of phase-dependent modulation in AM coding across the neuronal population, such that the reverberant advantage was greater for neurons in which the phase between AM and dynamic IACC that optimized temporal coding matched the phase in the reverberant stimulus.

Conclusions: Interaction between neuronal sensitivities to AM and dynamic IACC provides a mechanism for robust coding of AM important for speech reception in reverberation that does not require sensitivity to monaural temporal fine structure. The observed variability in the neural effect, including neurons showing a reverberant disadvantage, might be explained by differences among IC neurons in both ITD sensitivity (peak vs. trough) and AM tuning (bandpass vs. band-reject).

Independent Contributions of Age and Audiometric Profile to the Middle Ear Muscle Reflex

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Background: Recent cross-species experiments suggest that the middle ear muscle reflex (MEMR) may be a sensitive indicator of cochlear synaptopathy. However, because risk-factors for synaptopathy often co-vary with risk-factors for audiometric hearing loss, it is difficult to disentangle their independent contributions to the MEMR from low-powered lab-based studiesl. In contrast, large datasets allow for the effect of any one factor (e.g., age) to be analyzed by tightly restricting the range of variation of other factors (e.g., audiometric thresholds). This allows us to ascertain the contributions of each factor without the confounding influence of the others, and without invoking strong assumptions (e.g., linear dependence) that may be needed for conventional regression analyses. Here, using data from the National Health and Nutrition Examination (NHANES) repository, we analyzed how age and audiometric thresholds affect clinical MEMR amplitudes.

Methods: Audiological examination data was downloaded from the NHANES database for the years 2011-2012 (n=3817). Data was then organized to include age, MEMR amplitude, and audiometric thresholds (PTA of 4 and 8 kHz thresholds) for each participant. Linear models were used to examine the effects of age on MEMR amplitude while sub-selecting individuals with audiometric thresholds in different narrow ranges, and vice versa.

Results: Preliminary analyses revealed that MEMR amplitudes declined as a function of age for those who have normal to mild hearing losses; however, when the hearing loss is of a moderate to severe degree, age no longer impacts MEMR amplitudes. Similarly, when sub-selecting individuals to have a narrow age-range, MEMR amplitudes decrease with degree of audiometric hearing loss.

Conclusions: These preliminary results are consistent with the interpretation that age-related cochlear synaptopathy reduces MEMR amplitudes independently of audiometric hearing loss. However, when the degree of

audiometric elevation is higher, the MEMR is already reduced in young individuals either due to concomitant synaptopathy or owing to the reduced audiometric sensitivity by itself. Consequently, further age-effects that may remain are no longer apparent with this measure. Our results illustrate the advantage of big-data approaches for characterizing the prevalence of cochlear synaptopathy in humans and quantifying the sensitivity of an assay in the presence of extraneous factors.

Conductive Hearing Loss in the Hyp Mouse Model of X-Linked Hypophosphatemia is Accompanied by Hypomineralization of the Auditory Ossicles

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Background: X-linked hypophosphatemia (XLH) is a hereditary musculoskeletal disorder caused by loss-of-function mutations in the PHEX gene. In XLH, increased circulating fibroblast growth factor 23 (FGF23) levels cause renal phosphate wasting and low concentrations of 1,25-dihydroxyvitamin D, leading to an early clinical manifestation of rickets. Importantly, hearing loss is commonly observed in XLH patients.
Methods: To decipher the underlying pathophysiology of hearing loss in XLH, we utilized the Hyp mouse model of XLH and measured auditory brain stem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs) to functionally assess hearing and carried out an in-depth histomorphometric and scanning electron microscopic analysis of the auditory ossicles, and present clinical data from XLH patients.
Results: As evidenced by the increased DPOAE/ABR threshold shifts in the mid-frequency range, the measurements indicated a predominantly conductive hearing loss in Hyp mice compared to wild-type (WT) mice. An increased SP/AP ratio in ABR waveforms consistent with endolymphatic hydrops (ELH) in Hyp mice was histologically confirmed as grade 1 ELH. Quantitative backscattered electron imaging (qBEI) indicated a severe hypomineralization of the ossicles in Hyp mice, evidenced by lower calcium content (CaMean) and higher void volume (i.e., porosity) compared to WT mice. Histologically, voids correlated with unmineralized bone (i.e., osteoid), and the osteoid volume per bone volume (OV/BV) was markedly higher in Hyp mice than WT mice. The density of osteocyte lacunae was lower in Hyp than in WT mice, whereas osteocyte lacunae were enlarged. Likewise, XLH patients exhibited profound conductive hearing loss and we measured lower CaMean compared to age-matched controls. Moreover, histomorphometric analysis of transiliac biopsies indicated an elevated OV/BV compared to controls, pointing to severe osteomalacia.
Conclusions: Taken together, our findings highlight the importance of ossicular mineralization for hearing conduction and point towards the potential benefit of improving mineralization to prevent hearing loss in XLH.

Ex Vivo Investigation of Different Prototypes of a Eustachian Tube Stent

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Background: Chronic otitis media is often connected to Eustachian tube dysfunction (ETD). As successful treatment cannot be guaranteed with the currently available options, we want to develop a stent for the Eustachian tube (ET). In the course of this development, different prototypes were generated and tested in ex vivo experiments.
Methods: Five different prototypes of an ET stent and one commercially available coronary stent were implanted in the ET of human cadavers. The position of the stents was verified by cone beam CT. The implanted tubes were harvested, embedded in resin and ground at 200 μm steps. Resulting images of the single steps were used to generate 3D models of each implanted ET. The 3D models were then evaluated regarding position of the stent in the ET, its diameters, amount of squeezing, orientation of the axes and other parameters.

Results: Virtual reconstruction of the implanted ET was successful in all cases and revealed one malinsertion. The cross-section increased for all metal stents in direction from the isthmus towards the pharyngeal orifice of the ET. Depending on the individual design of the metal stents (open or closed design), the shape varied also between different positions along a single stent. In contrast, cross-section area and shape remained constant along the polymeric prototype. For all metal stents, some changes in the orientation of the main axes was detected.
Conclusions: With the current investigation, insight into the behavior of different prototypes of ET stents was gained, which can help in defining the specifications for the ET stent.

A Sensitive and EMI-Resistant Fully Implantable Microphone

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Background: The development and widespread adoption of fully implantable hearing devices is hindered by the lack of suitable implantable microphones. Despite decades of research, current implantable microphone candidates suffer from some combination of poor sensitivity, reliability, and susceptibility to electromagnetic interference. Our microphone design promises sensitivity comparable to commercially available hearing aid microphones, adequate EMI rejection, linear mechanical impedance, and a robust and straightforward implantation procedure.
Methods: Our design consists of a piezoelectric cantilever built from polyvinylidene difluoride (PVDF) and a low noise differential charge amplifier. The cantilever's rigid base is mounted to the bony wall of the middle ear space and the tip rests under the umbo. The cantilever comprises a flexible polymer with a piece of PVDF affixed with conductive epoxy on either side. By constructing the device with PVDF layers of opposite polarity and grounding the outside of the device, we obtain a shielded differential-mode signal in response to bending. Most electromagnetic interference is common-mode, giving differential mode signals an advantage.
In order to use the differential signal from the sensor, we built a low-noise differential charge amplifier. The charge amplifier topology features gain independent of parasitic resistances and capacitances while maintaining a noise floor comparable to high fidelity instrumentation amplifiers. Our design uses two matched charge amplifier input stages fed into a difference amplifier.
The microphone's sensitivity was measured by deflecting the tip of the cantilever with a piezoelectric stack. Noise floor was measured by recording the microphone's output in an electrically and acoustically quiet room. We have not yet implanted the microphone in a human temporal bone, but we have a series of experiments planned to evaluate the implanted device performance, testing both acoustic sensitivity and crosstalk from an implanted cochlear implant.
Results: Our sensor design has stiffness comparable to that of the human ossicular chain over a wide range of frequencies, and this stiffness is reasonably independent of how the sensor is installed. Our sensor and amplifier combination had an A-weighted RMS noise floor from 100 Hz to 20 kHz equivalent to approximately 40 pm of deflection of the cantilever. At 1 kHz the umbo moves approximately 30 nm/Pa (referenced to ear canal pressure), giving an equivalent input noise of roughly 32 dB SPL. A good hearing aid microphone has an A-weighted EIN of roughly 25 dB SPL.
Conclusions: Our microphone topology shows promise for improving cochlear implants and other implantable hearing devices. Although there is still significant work to do honing the device fabrication and developing the implantation procedure, the design is fundamentally sound and could be a strong contender for the cochlear implants of the future.

Neuronal Insm1 Regulates the Arrangement of Presynaptic Efferent Boutons Innervating OHCs

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Background: Olivocochlear (OC) neurons provide brainstem-to-cochlea feedback through projection of their axons to the cochlea. Lateral OC neurons (LOCs) project to the area underneath the inner hair cell (IHC) where they make axodendritic contact with type I afferents. Medial OC neurons (MOCs) project into the outer compartment, where they make direct, axosomatic contact with the outer hair cells. How these neurons project to and terminate on their appropriate target is poorly understood.

Methods: INSM1 is a zinc-finger transcription factor that is expressed in all nascent neurons and has fly and nematode counterparts with described roles in axon guidance and dendritic branching. Here, we have used neuronal specific Cre lines to ablate Insm1 in mouse efferent neurons to assess whether there are any defects in LOC or MOC axon branching and/or termination.

Results: We find that in Insm1 conditional (neuronal) knockout mice, IHC and OHC contact with type I afferent neurons is unperturbed. Afferent fibers innervate IHCs, and form ribbon synapses as in control littermates. In contrast, we observe a marked increase in the distribution and size of MOC terminals that contact the OHCs. For some OHCs, innervating MOC terminals appear to coalesce into a single large (and flat) terminal. In other cases, MOC terminals appear to cluster tightly together (with very little space between them), when compared to the spacing of MOC terminals that innervate OHCs in control animals. Finally, preliminary measurements suggests that Insm1 conditional knockouts display age-related reductions in auditory brainstem responses and distortion product otoacoustic emissions. Since efferent neurons are known to protect against age-related hearing loss, this result is indicative of a potential reduction in efferent function in the Insm1 conditional knockouts.

Conclusions: Our results highlight a novel role for Insm1 in MOC presynaptic terminal branching, which we suggest when perturbed may contribute to a functional decline in the MOC efferent system.

Gap Detection Response Time as Measure of Cognitive Processing Speed

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Background: Cognitive processing speed is an essential executive function that is affected by the normal aging process and various neurocognitive disorders. Central auditory tests may offer the ability to assess more than just perception of complex auditory stimuli, but also cognitive processing speed. Previous studies show elevated gap detection thresholds in individuals with HIV-related neurocognitive deficits, which cannot be attributed to peripheral hearing loss. We have been performing a battery of central auditory and cognitive measures on a cohort of HIV+ and HIV- individuals in Dar es Salaam, Tanzania to understand how the central auditory system could be used to identify, track, and potentially predict neurocognitive dysfunction. Specifically we have been using an adaptive gap detection test to measure temporal resolution and processing speed in those with HIV. The goal of the project was to determine the relationship between gap detection response time and validated measures of cognitive processing speed in those diagnosed with HIV.

Methods: Participants included HIV-positive (N = 79) and HIV-negative (N = 54) adults ages 18-45 years who had clinically normal hearing. Gap detection was measured using an adaptive algorithm that changed the gap length based on subjects' responses. The procedure started with a long gap length (20 ms) and converged on the subject's gap threshold (lowest detectable gap length). Gap responses were recorded by a button press device. Cognitive processing speed was measured using the Tests of Variables of Attention (TOVA) and the Groton Maze Learning (GML - moves per second) from The Cogstate test battery. Primary analyses focused the relationship between gap response time at threshold and processing speed measures of the TOVA and GML. Gap response time was also modeled across all gaps. Data were analyzed using a linear- and non-linear regression models.

Results: Gap detection response time at threshold was significantly related to cognitive processing speed on the TOVA and GML (all p<.03). Subjects with longer response times on the gap test also displayed significantly slower measures on the TOVA and GML. The interaction of age and processing speed on gap response time was also significant (p<.001) consistent with increased processing speed and gap response time with increased age, even in our narrow age range (18-45 years). Across gap length, response times increased incrementally, and were highest at threshold for most subjects.

Conclusions: Gap detection response time was significantly related to validated measures of cognitive processing speed. Results suggest an adaptive gap detection test could provide a dual metric of temporal resolution and cognitive processing speed. This measure, with further validation, could be potentially used in audiology clinics as a simple measure of processing speed or potentially a sensitive screening measure to monitor cognitive decline in patients.

Simulation Based Study of Contribution of Ventral Pouch and Alar Edge in Production of Mouse Vocalizations

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Background: Vocalizations in mice, both in the audible range and in ultra-frequency (UF) range, are of communicative significance. Ultrasonic vocalizations (USVs) are emitted by pups when they are isolated from the mother and elicit retrieval behavior. Similarly, multiple studies have shown that USVs in male mice are important in social contexts, especially in courtship and mating behavior and multiple aspects of interactions with other mice. Due to their communicative significance and genetic tools available in mice, mouse vocalizations have become an attractive tool to study models of neurodevelopmental disorders with communication deficits like Autism Spectrum Disorders (ASD). Understanding and identification of the mechanisms and role of laryngeal structures in generating USVs is crucial to understand neural control of USV production, which is likely dysfunctional in communication disorders. Using heliox experiments, it has been convincingly demonstrated that mouse USV production is an outcome of a whistle-based phenomena. However there is no consensus on the class of whistle to which mouse USVs belong.

Methods: Recent studies using contrast-enhanced micro-CT scans have been successful in reconstructing the mouse anatomical structures involved in USV production. Taking advantage of the same, we simulated a 2D model in COMSOL Multiphysics. Recent studies have focused primarily on replicating the fundamental frequency (f0) of mouse USVs although mouse USVs have multiple characteristics like pitch jumps, harmonics and frequency modulations that have been characterized parametrically.

Results: The same f0 can be achieved in different ways which may not be realistic. We successfully reproduced some of the crucial aspects of mouse USVs, observed in the spectrograms of simulated vocalizations. We further investigated the impact of the intralaryngeal air sac-like cavity, the ventral pouch and its cartilaginous edge, the alar edge, a recently uncovered anatomical structure, on the simulated USVs. For the same combinations of parameters, removing the ventral pouch resulted in an alteration of the call characteristics dramatically removing the variety of calls observed otherwise.

Conclusions: Our results thus provide evidence supporting hole-edge mechanism and the possible role of the ventral pouch and the alar edge in mouse USV production. Future studies would consider 3D simulations and neural pathways involved to produce the different vocalization patterns.

Loss of PEX1 Affects Inner Hair Cell's Ribbon Synapse Maturation and Auditory Function

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Background: Peroxisome Biogenesis Disorders (PBD) or Zellweger syndrome spectrum disorders (ZSD) are a group of rare genetic multisystem disorders characterized by partial or total defect in peroxisome synthesis, assembly, and/or function. PBD-ZSD is associated with neurosensory hearing loss, retinopathy, multiple organ dysfunction and psychomotor impairment. Mutations in 14 peroxin (PEX) genes have been found to cause PBD-ZSD. Mutations in PEX1 are the most common, representing 70 percent of the cases (Reuber et al. 1997). Based on genotype-phenotype correlations, PBD-ZSD has been classified into class I (less severe, survival of 2 years to above 45 years) or class II (more severe, survival of less than 12 months). Limited research has focused on the impact of peroxisomal disorders on auditory function, hampering the development of treatments for PBD-ZSD patients. As hair cells are particularly sensitive to metabolic changes, we hypothesize that mutations in PEX1 cause hearing loss by affecting hair cell functions and survival along the cochlea.

Methods: Global deletion of the Pex1 is neonatal lethal in mice impairing any postnatal studies. To overcome this limitation, we created a conditional knockout (cKO) mouse by breeding a novel floxed Pex1 mouse with VGlut3-CRE mouse to allow for selective deletion of Pex1 in inner hair cells (IHCs). We measured auditory brainstem responses (ABR) to click and pure tone stimuli from 5.6 to 32KHz in mice from 4 to 16 weeks old. Whole mount cochleae were stained with Myo7a, CtBP2 and GluR2 antibodies to assess IHC's synapse. Samples were imaged using the Zeiss LSM800 confocal microscope and analyses were done using Imaris Cell Imaging software (Oxford Instruments).

Results: Pex1 excision in IHCs leads to progressive hearing loss that is more pronounced in the low frequency range and was associated with significant decrease in ABR wave I amplitudes (P<0.0001). To determine if this change was caused by alterations in IHC-SGN synapses, cochleas were stained with CtBP2 (pre-synaptic) and GluR2 (post-synaptic) markers. We observed a decrease in ribbon synapse number and volume especially in the low frequency region of Pex1 cKO mice.

Conclusions: These results suggest a critical function of Pex1 in development and maturation of IHC-SGN synapses as well as hearing function in the low to mid-frequency range.

CDK2 Inhibitor AZD5438 Protects From Cisplatin-Induced Acute Kidney Injury and Hearing Loss

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Background: Nephrotoxicity and hearing loss caused by cisplatin hinders its use in higher doses that could enhance anti-cancer effects. Novel approaches to mitigate both cisplatin mediated ototoxicity and nephrotoxicity will open new avenues to cancer treatments by enhancing therapeutic efficiency of cisplatin. Recent reports show that, AZD5438, an oral potent CDK2 kinase inhibitor, protects from cisplatin-induced ototoxicity in mice.
Methods: Activation of cyclin dependent kinase 2 (Cdk2) on treatment with cisplatin is identified to mediate cell death in kidneys. Inhibition of CdK2 is a potential target to mitigate the cisplatin mediated nephrotoxicity. High-throughput screens of 187 specific kinase inhibitors done in inner ear cell line identifies AZD5438, a potent CDK2 inhibitor as top hit. Here we investigate the nephroprotective effect AZD5438 in cisplatin treated in vitro and in vivo models
Results: In the present study, we show that AZD5438 protects against cisplatin-induced cell death in human kidney tubular cells (HK2) with an IC50 of 26 nM which was similar to the IC50 of AZD5438 in inner ear HEI-OC1 cells of 28 nM. Next, we examined AZD5438 for nephroprotective effects in FVB adult mice in a regimen that previously conferred protection against hearing loss. AZD5438, at an oral dose of 35 mg/kg body weight, was administered twice daily, for three consecutive days to cisplatin (25 mg/kg body weight) intraperitoneal injected mice. On day 3 after cisplatin treatment, acute kidney injury markers (BUN and lipocalin), kidney histology, apoptosis, and protein expression of cellular proliferation marker PCNA were examined. The cisplatin alone treated mice had significantly elevated serum BUN and lipocalin levels with increase in tissue damage, apoptosis and PCNA expression. Renal function was partially restored upon treatment with AZD5438, as evaluated by BUN and lipocalin levels, PCNA expression, apoptosis, and histology. Mice treated with AZD5438 and cisplatin in this regimen shows significant increase in survival rate compared to cisplatin alone injected mice. Examination of germline CDK2 knockout (KO) mice also shows that the KO CDK2 mice are resistant to cisplatin-induced acute kidney injury compared to CDK2 WT littermates. Genetic deletion of CDK2 in mice phenocopied the level of protection we achieved with AZD5438 administration.
Conclusions: This study demonstrates that therapeutic effect of AZD5438 is not limited to protection from cisplatin-induced hearing loss but is also beneficial for mitigating cisplatin-induced kidney cell damage and improving survival in mice post-cisplatin treatment.

A Hippocampal Role in the Active Adjustment of a Non-Spatial Acoustic Scene

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Background: In addition to supporting declarative memory and navigation, the hippocampus can represent sensory and conceptual spaces (Behrens et al., Neuron 100:490-509, 2018). Rodent hippocampal place cells that increase their firing rate at particular spatial locations can also show tuning to sound frequency when there is an active task (Aronov et al., Nature 543:719-722, 2017). To investigate whether human hippocampus maps auditory features we generated a sound feature space - based on random elements in frequency-time space - in which subjects could adjust the density from sparse ("beepy") to dense ("noisy"). Unlike tone frequency, density is not consistently associated with any physical spatial dimension.
Methods: In a functional magnetic resonance imaging (fMRI) experiment, 29 subjects held in mind the fixed density of a series of ten consecutive 200-ms chords over a subsequent 2-s silent interval. They then adjusted the density of a 8-s sequence of chords to match the target. In other conditions we removed the memory component (adjustments were made freely with no target density), the adjustment component (button presses were instead made for odd/even judgments on spoken digits), or both. The design distinguished activity supporting auditory

memory from that relating to sound adjustment, also controlling for sensory and motor factors. A simplified version of the experiment was conducted in 18 patients intracranially implanted with electrodes to determine the focus of their epileptic seizures, a subset of whom had hippocampal electrode coverage.

Results: In the fMRI experiment, auditory working memory was associated with activity in anterior insula, inferior frontal gyrus and paracingulate cortex. Density adjustment elicited bilateral hippocampal activity - this occurred regardless of whether the subject was navigating toward a fixed target density or making adjustments freely. Medial prefrontal sites were preferentially active for navigation toward a fixed target density. In the intracranial recording experiment, local field potentials from hippocampus contained sustained 1-8 Hz power during the maintenance of targets and sound adjustment.

Conclusions: The data establish involvement of the hippocampus in active navigation along an auditory feature that is not consistently associated with a physical spatial dimension. The control conditions established that the recorded hippocampal activity was not driven solely by acoustics or button presses. Ongoing unit recordings from the intracranial patients, together with multivariate analysis of the fMRI data, will establish whether human hippocampus forms a map of the sound density feature, as rodent hippocampus does for tone frequency. The work adds to a growing body of evidence for a role of this structure in auditory cognition (e.g. Kumar et al., J Neurosci 36:4492-4505, 2016).

Transformation of Acoustic Information to Sensory Decisions in Parietal Cortex

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Background: Integrating sensory information over time guides accurate perceptual choices. The underlying neural process is facilitated by the transformation of stimulus representations into decision variables. Here, we asked how the neural representation of acoustic information is transformed in the auditory-recipient parietal cortex (PC), a region that is causally associated with sound-driven perceptual decisions (Yao et al., 2020).

Methods: Neural activity was recorded wirelessly from PC while gerbils performed an alternative forced choice auditory temporal integration task, and subsequently during passive listening to the identical acoustic stimuli. During task performance, gerbils were required to discriminate between two amplitude modulated (AM) noise rates, 4 versus 10 Hz, as a function of signal duration (100-2000 ms).

Results: As reported previously, task performance improved with increasing signal duration, and reached an optimum at ≥600 ms (Yao et al., 2020). To determine whether PC population activity reflected acoustic information which could contribute to perceptual task performance, we applied a linear classifier read out procedure that assessed AM rate discrimination within simultaneously recorded PC neurons. This "within-session" population analysis showed that PC neurons represent acoustic information during passive listening, and are therefore likely to integrate this information during task performance. To further assess PC population dynamics, a principal component analysis was fitted to trial averaged neural responses. During passive listening, low-dimensional encoding of acoustic information was apparent as neural trajectories (i.e., neural manifolds) differentiated across stimulus conditions. During task performance, population dynamics within PC reflected the temporal progress of stimulus representation into behavioral choices (left versus right). At stimulus onset, neural trajectories started at a similar position, but began to diverge toward the relevant decision subspace after ~300 ms of acoustic stimulation, and clearly diverged by 600 ms. Finally, neural trajectories of incorrect trials tended to course along the opposing decision subspace, reflecting lapse rate or failed evidence accumulation.

Conclusions: Taken together, our findings demonstrate that PC leverages the information encoded in the auditory cortex to guide sound-driven perceptual decisions over a behaviorally-relevant time course. We propose that PC integrates and transforms bottom-up sensory information into decision variables during behaviorally-gated task performance that could be initiated by top-down signaling.

The Effect of Contralateral Filtered Music on DPOAEs in Musicians and Non-Musicians

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Background: Specialized auditory systems in echolocating bats (Suga 2012) have an analogue in musicians. Musicians have an efferent system which modulates the inputs to the cortex through action on cochlea. Its action has been studied through the change in amplitude of OAEs (Brashears et. al. 2003) when a noise is presented to the contralateral ear. Besides ensuring a clean signal to the cortex, the efferent pathway has been linked to

attention(de Boer and Thornton2008). Attention reduces the suppressive effects of the efferent fibers. There are not many studies examining the effect of attention to musical contralateral efferent stimulus on OAEs in musicians and non-musicians and therefore, the current study was taken up to examine the effect of contralateral filtered music on DPOAEs of Carnatic musicians and non-musicians.

Methods: Carnatic Vocal Musicians (n=28) , and Non-Musicians (n=29) in the age range of 18-35 years, with hearing thresholds within 25dBHL, reflex thresholds at 1kHz above 90dBHL were recruited for the study. All musicians had musical training from the age of five years and practiced music for at least six hours a week. Non-Musicians had no formal training in music. Stimulus for contralateral presentation was a filtered musical excerpt with a bandwidth of 570 Hz (1730-2300Hz) from a song "Mile Sur" in 'Desh Raag' frequently broadcasted on National Television network. DPOAEs of 2f1-f2, (L1/L2 intensity ratio of 65/50 and frequency ratio of primaries f2:f1 1.22) were recorded on ILO V6 instrument at 8 points/octave. DPOAEs were measured with and without contralateral stimulus and repeated for replicability. The average DPOAE amplitudes of two baselines and two with contralateral stimulus of 50dBSPL were computed separately and magnitude of difference was calculated as the reflective and distortion components of DPOAEs are affected differently by contralateral stimulation causing phase shifts (Deeter et. al. 2003). The difference magnitude was compared in the two groups with one-way RM ANOVA (f2 frequencies were within subject and group as between subject factors).

Results: Overall mean DPOAEs increased slightly, in the presence of contralateral stimulation in musicians suggesting the effect of attention. This has also been reported by de Boer and Thornton,(2008), and Bhagat and Xu (2008). Repeatability of recordings as assessed by correlation was high (0 .88 to 0 .97).   RM ANOVA was used to compare the magnitude of change in DPOAE amplitude between Musicians and Non-Musicians. Analysis was limited to DPOAEs at f2 frequencies 1685Hz to 2832Hz since the bandwidth of the contralateral stimulus was in this frequency range. A significant difference was found(F=8.913, p=.004) between Musicians and Non-Musicians for the filtered music.

Conclusions: The current study also shows the effect of attention on DPOAEs in Carnatic musicians. The study highlights the role of attention and central modulation of the DPOAEs in musicians.

Distortion-Product Otoacoustic Emission Suppression Tuning Curves Derived From 2D Cochlear Model
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Background: A tone presented simultaneously with stimuli used to measure distortion-product otoacoustic emissions (DPOAEs) may suppress their amplitude. DPOAE suppression tuning curves (DPOAE STCs) represent the suppressor level as a function of its frequency and were suggested to reflect tuning in the cochlea.

Methods: DPOAE STCs are simulated in a 2D cochlear model. Stimuli parameters were chosen according to [Abdala et al.(1996), HearRes98:38] whose experimental data were taken for comparison. Stimulus level was L1=65 and L2=50 dB SPL, f2/f1=1.22, and f2=1.5 or 3 kHz. Suppressing criterion was 6 dB. The suppressor level was changed with a step of 2 dB.

Results: Simulated DPOAE STCs qualitatively resemble experimentally measured DPOAE STCs. The best agreement was reached for the low-frequency side of the STCs. For f2=1.5 kHz, the high-frequency side of the simulated STC contained ripples, i.e., local peaks and dips. The ripples complicate estimation of Q 10 dB above the STC tip, which is in the range between 2.2 and 3, same as in experiment. For f2=3 kHz, the high-frequency side of simulated STC did not contain ripples. However, the simulated STCs indicate larger suppressor levels at frequencies between 2.5 and 3.5 kHz (around the tip) than the experimental data. As a consequence, Q10 is about 2.5 for the simulated STCs at 3 kHz, which is the lowest range in the experimental data. Simulated BM TCs were broader than simulated DPOAE STCs at 1.5 kHz (Q10=2), but narrower at 3 kHz (Q10=3.4). Especially the low-frequency side of the BM TCs was shallower at 1.5 kHz than the low-frequency side of DPOAE STCs and approximately the same at 3 kHz. In contrast, the experimental psychophysical TCs were either equivalent or narrower than DPOAE STCs.

In the model, the analysis showed that DPOAE generation region is only suppressed due to low-frequency (below f2) suppressor. In contrast, high-frequency suppressors shifted the DPOAE generation region apically. In addition, for f2 = 1.5 kHz the generation region seems to be in some cases strongly adjusted, which changes the distribution of the source. Decrease of gain in the model adjusted DPOAE STCs near the tip and the high-frequency side. The effect on DPOAE STCs agrees with data measured in hearing impaired humans [Gruhlke et al.(2012) JASA132:3292].

Conclusions: DPOAE STCs were in the model adjusted by changing the feedback undamping force, which has an effect on BM tuning. The question is how the suppressor effect on the distribution of the DPOAE source distracts the relation between the BM tuning and DPOAE STCs.

Vagus Nerve Stimulation-Mediated Auditory Perceptual Improvement

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Background: Input from the periphery to the central nervous system (CNS) can influence sensory perception. Previous work has demonstrated the vagus nerve plays a key role in transmitting peripheral information to the CNS. This is partially done by activating neuromodulatory areas, including basal forebrain (BF). Here, we examined if vagus nerve stimulation (VNS), and subsequent activation of cholinergic BF neurons, could be used to improve sensory discrimination.

Methods: To study this, we sought to improve auditory perceptual decisions in well-trained mice. Mice were trained to classify tones as a single, center frequency (11-16 kHz) or non-center. In well-trained animals, perceptual decisions were variable across animals, but stable within an animal. We used a custom cuff electrode to stimulate the vagus nerve in blocks of trials in animals to try to improve stable behavior. Additionally, we dissected the contribution of cholinergic signaling to VNS-mediated perceptual improvement, using two photon axon imaging in auditory cortex, anatomical circuit tracing and optogenetics.

Results: After six days of VNS, we observed significant improvements in performance (N=11 animals), in comparison to sham-implanted animals (N=7 animals). We found increased activation of cholinergic BF axons in auditory cortex during VNS. Since VNS activated auditory-cortical projecting cholinergic neurons, we optogenetically activated these neurons to mimic VNS. Activation of cholinergic neurons alone was sufficient to improve behavior (N=5 animals). To test if VNS-mediated improvements were dependent on activity of cholinergic neurons, we optogenetically inhibited cholinergic BF neurons during VNS. This abolished the VNS-mediated improvement previously seen (N=6 animals).

Conclusions: Taken together, these results indicate that VNS improves perceptual discrimination, at least in part, by activating auditory-cortical projecting cholinergic neurons in the BF.

Neural Correlates of Temporally Coherent Figure-Ground Stimuli

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Background: In the cocktail party problem, frequency components of different sound objects are interleaved over a wide range of the frequency spectrum and are not readily separable using spectral coes. Recent studies have suggested that temporal coherence, the precise onset timing of frequency components, could play an important role in streaming processes. The stochastic figure-ground stimulus has recently been used to demonstrate brain responses and perceptual sensitivity to repeated, temporally coherent chords in human listeners. This stimulus is a modified version of random tone clouds, which have long been used to generate neuronal tuning maps similar to STRFs in complexity.

Methods: We use single-unit recordings from the auditory cortex of ferrets to examine neural correlates of a perceptual "pop-out" in response to the stochastic figure-ground stimulus during passive listening. Initial tuning is established in responses to a non-coherent, random tone cloud. After a brief period of "background noise," a subset of inharmonic frequency channels become temporally locked in synchrony.

Results: Cell responses indicate a change in neuronal encoding strategies which develops over the period of a few seconds of the figure presentation. The modulation effect is not persistent and is extinguished when the figure tones return to an in-coherent presentation scheme.

Conclusions: These results indicate that temporal coherence in a sound stimulus modulates neuronal responses to segregate a coherent stream from random background. Furthermore, the inharmonic nature of the temporally coherent figure demonstrates that this mechanism does not rely on spectral structure to form an auditory stream.

Topographical Distribution of Spectrotemporal Receptive Field Properties in Bat Primary Auditory Cortex

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Background: Spectrotemporal modulations are a prominent feature of natural sounds including animal vocalizations and human speech. The neural networks of the mammalian primary auditory cortex (A1) that encode and categorize communication sounds remain incompletely understood in part because of the heterogeneous distribution of interacting neuronal subtypes and microcircuits serving different functions throughout A1. To get around this problem, we investigated the topographical distribution of tuning properties throughout A1 in an auditory substrate homogenously specialized to process one specific acoustic feature – the downward frequency modulated (FM) sweep utilized in bat echolocation. While the majority of A1 neurons in bat auditory cortex are preferentially tuned to downward FM sweeps, the entire cortex is required to capture and categorize patterns of spectral details embedded within each biosonar echo.
Methods: Here, we captured the neural responses of the A1 to complex, biologically-relevant acoustic stimuli using linear, dual shank 32-channel translaminar microelectrode arrays. We analyzed frequency response areas (FRAs), spectrotemporal receptive fields (STRFs), and neural responses to various combinations of downward FM sweeps in adult male and female Mexican free-tailed bats (Tadarida brasiliensis).
Results: We extracted a total of 170 STRFs across the A1 from spike-sorted single units (60) and multiunit activity (110) with most of our sampled STRFs at cortical depths corresponding to input layer 4. STRF best frequencies ranged between 10-55 kHz. We quantified the spectral and temporal modulation transfer functions (sMTF and tMTF, respectively) to determine neural tuning preferences in the spectral and temporal dimensions. All STRFs were classified as either bandpass or lowpass in the spectral and temporal domains, and features such as best frequency, bandwidth, and integration time were evaluated separately for the excitatory and inhibitory components. For simple STRFs, sMTFs and tMTFs were evaluated as a function of best frequency, and spatial location and revealed tonotopic trends similar to those reported for non-specialized animals.
Conclusions: The goal of this project is to compare the responses across cortical layers and throughout the tonotopic axis of A1 to reveal spatial patterns that may in turn shed light on the functional organization of A1 neural networks processing downward FM sweeps.

Function of Cortical Ndnf Interneurons in Sound Frequency Discrimination
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Background: Frequency tuning is a defining property of neurons in the primary auditory cortex, with highly tuned neurons responding to a more narrow range of sound frequencies. Both inhibitory and disinhibitory cortical circuits are known to shape the tuning selectivity of excitatory neurons; however, the full extent of this circuitry has yet to be described. The present study focuses on the potential contribution of a recently identified class of interneurons that express neuron-derived neurotrophic factor (NDNF). In the auditory cortex, NDNF interneurons reside primarily within layer 1 and are known to inhibit the distal dendrites of excitatory neurons while simultaneously disinhibiting their somata via projections to parvalbumin-expressing inhibitory interneurons. This circuitry suggests that subsets of NDNF interneurons are well-poised to shape excitatory neuron tuning via either inhibition or disinhibition.
Methods: We performed in vivo two-photon calcium imaging in transgenic mouse lines expressing GCaMP in NDNF interneurons (NDNF-cre x Ai148-GCaMP6f mice) or layer 2/3 excitatory neurons (Thy-1-GCaMP6s mice). Simultaneous imaging of NDNF interneurons and layer 2/3 neurons was additionally undertaken using multiplane imaging of NDNF-cre x Ai148 mice injected with a pan-neuronal AAV-GCaMP8. Pure tones of varying frequencies (4-32kHz) and intensities (0-80dB) were presented to awake, head-fixed mice to determine frequency-intensity receptive fields. We trained a subset of mice on a Go/No-Go frequency discrimination task, in which they distinguished trains of repeating pure tones from trains of two alternating tones. In some experiments, we expressed an inhibitory chemogenetic receptor (AAV-DREADD-hM4Di) in NDNF interneurons and injected mice with either saline or agonist (CNO or C21) to establish the contribution of NDNF interneuron activity on frequency tuning and discrimination performance.
Results: We first characterized the tuning properties of NDNF interneurons in response to pure tones. We found that a subset of NDNF interneurons exhibit robust frequency and intensity tuning comparable to excitatory neurons in layer 2/3. We next used our behavioral paradigm to establish reliable frequency discrimination

thresholds in mice. We recorded the responses of both NDNF interneurons and layer 2/3 excitatory neurons to the training stimuli under both passive and behaving contexts. We identified individual neurons in both classes capable of discriminating between repeating or alternating tones. Finally, ongoing experiments using chemogenetic silencing of NDNF interneurons will test the necessity of their activity for task performance and tuning specificity.

Conclusions: As tuning selectivity is thought to contribute to frequency discrimination acuity, understanding the cortical circuits that shape tuning will be important for identifying the neural mechanisms underlying perceptual deficits. This work will help establish the function of NDNF interneurons in frequency tuning and further elucidate the contribution of specific inhibitory cortical circuits to sound processing.

Influence of Age and Hearing Loss on Gaussian Noise Disruption

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Background: For brief masker-signal delays (<25 ms), forward masking is generally greater for Gaussian noise (GN) than for low-fluctuation noise (LFN) maskers for listeners with and without sensorineural hearing loss (SNHL). However, the additional masking attributed to inherent fluctuations in the temporal envelope of Gaussian noise (GN disruption) persists over a longer time course (>75 ms) for listeners with SNHL. Because older age and SNHL may affect recovery from masker-envelope fluctuations differently, the current study explored which of these two factors contributed more substantially to the persistence of GN disruption.

Methods: Masked thresholds were obtained for a 10-ms, 4000-Hz pure-tone signal when presented after the offset of 400-ms GN or LFN maskers (⅓ ERBN centered at 4000 Hz) fixed in level at 80 dB SPL for three different masker-signal delays (25, 75, and 150 ms). GN disruption (GN threshold - LFN threshold) was measured for three adult participant groups (n = 54): younger participants with normal hearing (YNH, n = 18), older participants with normal hearing (ONH, n = 14), and older participants with sensorineural hearing loss (OSNHL, n = 20). Statistical analysis consisted of a linear mixed-effects model examining significant differences for threshold by masker-signal delay, masker type, and participant group. The role of underlying mechanisms was tested using a computational model for midbrain neurons, with inputs from a peripheral model with efferent feedback. Mechanisms examined included outer and inner hair cell integrity, modulation filtering, and efferent regulation of outer-hair-cell function (i.e. efferent feedback).

Results: For all three participant groups, significant GN disruption occurred for the 25- and 75-ms masker-signal delays, with less GN disruption at 75 than 25 ms. When considering effects across the three listener groups, significantly greater GN disruption was observed for the ONH than for YNH and OSNHL participants for the 25-ms masker-signal delay. Otherwise, GN disruption did not differ among the participant groups for the two longer masker-signal delays.

Conclusions: The primary results suggest that older listeners may be more susceptible to the deleterious effects of masker envelope fluctuations, even in the absence of hearing loss, when compared to masked thresholds for younger listeners. Model results suggested there may be a larger influence of efferent feedback on forward-masked thresholds, and possibly GN disruption, than previously expected.

Improved Acoustic Frequency Discrimination Acuity in Usher Syndrome Mice Treated With Antisense Oligonucleotides

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Background: Usher syndrome (Usher) is the most common genetic cause of combined deaf-blindness. Type 1C Usher (USH1C) is caused by mutations in the USH1C gene, which encodes the scaffolding protein Harmonin. In cochlear hair cells, Harmonin is necessary for hair bundle development, mechanoelectrical transduction, and synaptic transmission. Although Harmonin is expressed in the brain, its role in the central nervous system is not known. The aim of this study was to determine the effect that an antisense oligonucleotide (ASO) therapy that

rescues hair cell function and restores hearing thresholds has on acoustic frequency discrimination in an USH1C mouse model.

Methods: USH1C mice were treated systemically via intraperitoneal (IP) injection or locally to the inner ear via posterior semicircular canal (SC) injection at postnatal day 2 (P2) with ASO therapy or saline. Frequency discrimination acuity was assessed through prepulse inhibition (PPI) of acoustic startle responses (ASRs) in 1-month-old USH1C-ASO, USH1C-control (saline or untreated), and untreated wild-type (WT)-control littermate mice. 100 dB-SPL startle stimuli were presented in the presence of a constant 70 dB-SPL background pure tone of 8, 16, or 32 kHz. Immediately preceding the startle stimulus, the frequency of the background tone (F1) was decreased by 0-37.5% of F1. Additionally, hearing sensitivity at 8, 16, and 32 kHz was assessed by auditory brainstem response (ABR) analysis.

Results: ABR thresholds were significantly elevated (>80dB SPL) at low, mid, and high frequencies in 1-month-old USH1C-control mice compared with WT-controls (~20dB SPL). Additionally, in USH1C-control mice, prepulse at any frequency failed to reduce post-stimulus activity. In contrast, ASRs were significantly inhibited for frequencies up to 24 kHz in USH1C-ASO mice treated either systemically or locally. ABR thresholds for USH1C-ASO mice were significantly reduced compared to USH1C controls. Robust rescue of ABR thresholds was observed at 8 and 16 kHz, and mild rescue at 32 kHz.

Conclusions: USH1C-control mice did not detect the pure tones tested at low, mid, and high frequencies. Both systemic and local ASO treatments significantly improved detection and discrimination of frequencies up to 24 kHz in USH1C mice. These findings support the efficacy of ASO treatment in restoring peripheral hearing and central auditory processing functions, toward the long-term objective of developing therapies for patients with USH1C.

Measuring Response Latency to Estimate Working Memory Capacity in a Speech Intelligibility Test
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Background: Cognitive domains such as working memory can influence speech perception. Latency-based scoring methods such as n-back tasks have been used to estimate individual differences in working memory capacity. This study used a combined structure of n-back and listen-and-repeat tasks to simultaneously estimate speech perception ability and working memory capacity, by measuring both speech intelligibility and response latency.

Methods: The auditory working memory capacity of each participant (age > 65) was assessed using digit span tasks. The participants performed conventional listen-and-repeat (0-back) and 1-back repeat tasks. Short words (four morae) with low familiarity were used as experimental stimuli. The stimuli were randomly presented with various signal-to-noise ratios. We measured speech intelligibility (ratio of correct responses), perceived listening effort, and response latency (time required to finish one trial).

Results: Our results indicated that the participants had lower intelligibility scores and longer response latencies on the 1-back repeat task than the listen-and-repeat task, which might represent the increased cognitive load on working memory caused by the concurrent maintenance of previous information and updating of new input information. This was supported by a positive correlation between response latency and perceived listening effort in the 1-back repeat task.

Conclusions: This study demonstrates the significance of measuring response latency as well as speech intelligibility for investigating the relationship between speech perception and auditory working memory capacity. Together with the conventional accuracy-based scoring methods, this approach could be used for meaningful prediction in screening for cognitive and hearing impairment.

Characterization of Cochlear Nestin-Expressing Cells After Hair Cell Ablation Using a Triple Transgenic Mouse Model
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Background: Nestin has been well-studied as a stem cell marker in multiple tissues with potential for neuroregeneration, though investigation into its role in the cochlea has only recently begun. There is some evidence that nestin-expressing cells may be progenitor cells in early neonatal mouse cochleae. The objective of

this study is to develop a transgenic mouse model to examine the fate of nestin-expressing cells after hair cell ablation.

Methods: Three separate transgenic mouse lines were bred together over multiple generations to develop a triple transgenic mouse model with two key features. First, this mouse contains a tamoxifen-inducible nestin reporter gene (TdTomato) suitable for lineage tracing. Additionally, it has a Pou4f3 gene linked to human diphtheria toxin receptor (Pou4f3-DTR) capable of inducible hair cell ablation after diphtheria toxin (DTx) administration. Neonatal triple transgenic pups received intraperitoneal tamoxifen injections (180ug/gBW) on P2-3 to activate the nestin reporter gene. On P5-6, they received intramuscular DTx injections (4ng/gBW) to selectively ablate cochlear hair cells. On P12-13, they were euthanized and their cochleae whole-mounted on glass slides. Immunofluorescence with confocal microscopy was performed, viewing MyosinVIIa (hair cells), Sox2 (supporting cells), and TdTomato (nestin-expressing cells).

Results: A triple transgenic mouse model suitable for characterizing the role of nestin-expressing cells in response to hair cell ablation was successfully developed and confirmed genotypically and phenotypically.

Of 50 pups born, 6 expressed all three genes of interest, resulting in a yield of 12%, aligned with the anticipated yield of 1/8 pups, or 12.5%.

In response to tamoxifen, Nestin-expressing cells also express TdTomato (average of 5.5 ± 2.2 nestin-expressing cells per 100µm of Organ of Corti).

In response to DTx, hair cells are ablated when compared to double transgenic mice lacking the Pou4f3-DTR gene (13.3 ± 1.1 vs 4.3 ± 2.5 IHCs per 100µm, respectively, n = 8 middle turns).

Nestin-expressing cells were located inferior and modiolar to the IHCs, consistent with prior studies. Three cellular morphotypes were identified: long, short, and atypical.

Preliminary results obtained thus far (n=8) do not identify any significant changes in nestin-expressing cells between ablated and non-ablated cochleae. Data collection is ongoing to more fully characterize the response of nestin-expressing cells to hair cell ablation.

Conclusions: A novel transgenic mouse line has been developed to study the role of nestin-expressing cells in response to hair cell ablation. Morphologic subtypes of nestin-expressing cells were identified. Further work is ongoing to characterize the role of nestin as a potential cochlear stem cell and the significance of these morphologic subtypes.

Development of an Enhanced Screening Assay to Distinguish Between Hair Cell Regeneration and Otoprotection

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Background: The replacement of mechanosensory hair cells (HCs) lost due to noise, age, or ototoxic exposures has long been a focus of auditory research and drug development; however, the few reported clinical trials have shown minimal efficacy. This slow progress in developing therapeutics with regenerative properties could be due to lack of consistency across drug screening platforms, many of which only quantify HC numbers and thus cannot distinguish actual HC regeneration from acute protection or repair. Since many pathways are known to have modest otoprotective effects, mere HC quantification results in potentially false hits for regeneration. Here we have utilized a cochlear explant model of HC loss combined with fate-mapping of transdifferentiated supporting cells (SCs), the progenitors of regenerated HCs, to establish an improved screening method for compounds that stimulate actual HC regeneration.

Methods: Here, using Prox1CreERT2::Rosa26loxP-stop-loxP-tdTomato reporter mice, we permanently labeled SCs (the pillar and Deiters' cells) that surround outer HCs with a fluorescent marker prior to HC damage. Specifically, mice pups were injected with tamoxifen at postnatal day (P)0 to induce tdTomato expression in SCs, and cochlear explants were established from them at P2. After equilibrating overnight, explants were exposed to 400 µM neomycin for 24h to induce HC death, followed by 3 media washes then treatment with compounds of interest for 96h. Explants were then fixed, immunostained with anti-myosin VIIa and analyzed via confocal microscopy to quantify the regenerated (tdTomato+) HCs.

Results: As positive controls, we used Notch pathway inhibitors with well characterized roles in HC regeneration and differentiation, and confirmed the presence of tdTomato+ transdifferentiated HCs following neomycin damage and treatment with these inhibitors. Interestingly, some compounds or drug combinations (e.g. CHIR99021 combined with valproate) previously reported to show regenerative effects in explants or in vivo,

failed to stimulate HC transdifferentiation/regeneration in our fate-mapping assay. In both our assay and published results, HC numbers were reduced after damage and increased after treatment, but the lack of tdTomato+ HCs suggests this specific combination may be acutely protecting HCs rather than inducing HC regeneration. In contrast, our preliminary work did identify several other compounds with regenerative potential as evidenced by the presence of tdTomato+ fate-mapped SCs that had converted into HCs following treatment.

Conclusions: While the lack of efficacy observed for some compounds could be attributed to subtle differences in screening models, it is possible that these discrepancies could, in part, be due to false positive hits for HC protection rather than regeneration when fate-mapping is not included in initial drug screening. By clearly establishing a compound's mechanism of action prior to translational in vivo studies, assays that include fate-mapping may speed up the identification and validation of compounds capable of HC regeneration vs otoprotection.

Reprogramming of Non-Sensory Cells Into Hair Cell-Like Cells in the Mature Mouse Cochlea by Atoh1, Gfi1, and Pou4f3 Expression

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Background: Hearing loss currently affects 1 in 5 people and is expected to increase to 1 in 4 by 2050. Of particular worry for many is the rise in acquired hearing loss, which occurs when sensory hair cells of the inner ear are damaged beyond repair due to aging, noise damage, and/or illness. Previous studies have shown that overexpression of key transcription factors such as Atoh1, Gfi1, and Pou4f3 in the neonatal mouse inner ear can prompt the ectopic reprogramming of non-sensory cells to become hair cell-like. However the effect of these transcription factor combinations on non-sensory cells of the mature inner ear is not well understood.

Methods: Our lab has targeted the Rosa26 locus to generate mice to conditionally express Atoh1 (Rosa-A) alone, Gfi1 and Atoh1 (Rosa-GA), or Gfi1, Atoh1, with Pouf43 (Rosa-GAP). Our lab also modified the FBXO2 allele to express CreER in non-sensory tissue and combined the Fbxo2CreER mouse with Rosa-A, -GA, or -GAP mice to induce transcription factor expression at three weeks of age. Cochleae were collected at 5 and 6 weeks of age, vibratome sectioned followed by immunolabeling with a Myosin VIIa antibody and anti-Sox2 for hair cell and supporting cell labeling, and DAPI for a general nuclear stain.

Results: In cochleae with Rosa-GA expression, all 6-week-old tissue had reprogrammed hair cell-like cells, whereas some, but not all, of the 5-week-old samples, contained reprogrammed hair cell-like cells. The number of reprogrammed cells increased between 5- and 6-weeks of age. Additionally, increased reprogramming was observed with Rosa-GAP expression compared with GA, while no hair cells were observed in the control Fbxo2CreER:Rosa26tdTomato control mice. Reprogrammed cells were only observed in cells surrounding the spiral limbus and along the inner and outer sulci. Additional work is being done to expand the characterization of reprogrammed hair cells as well as to understand the transcriptomic profile of the reprogrammed cells we have observed.

Conclusions: Identifying the underlying genetic pathways that allow for reprogramming of non-sensory cells to hair cells in mature mouse models could uncover potential therapeutic strategies for acquired hearing loss. Our study highlights a possible strategy of how Atoh1, Gfi1, and Pou4f3 could be utilized in hair cell reprogramming in mature mice models.

Transcriptome and Epigenome Analyses Reveal Three Three Sequentially Activated Gene Activity Modules during Zebrafish Sensory Hair Cell Regeneration

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Abstract: Loss of sensory hair cells in the mammalian inner ear leads to permanent hearing and vestibular defects, whereas loss of hair cells in zebrafish results in their regeneration.
The abundance, accessibility and remarkable speed (3-5h) at which zebrafish hair cells regenerate after injury has allowed us to characterize the gene expression and epigenetic dynamics of a regenerating vertebrate sensory organ at unparalleled levels of resolution (Baek et al., BioRxiv and unpublished). We collected six time points in 30min-several hour intervals which demonstrated that expression changes occur rapidly and enabled us to characterize the molecular changes that occur in different cell types in exquisite detail. We subjected the data sets to rigorous

differential gene expression, gene enrichment term, pseudotime, and in situ hybridization analyses. We discovered that gene expression changes occur within 30min after hair cell death, and that hair cell regeneration consists of three discrete and sequentially activated gene activity modules. Namely, a systemic injury/inflammatory response, a transient activation of regeneration-specific genes, followed by a recapitulation of developmental events. The final reactivation of developmental gene programs, includes hair cell specification, cell cycle activation, ribosome biogenesis, and a metabolic switch to oxidative phosphorylation. In addition, we performed ATACSeq and ChIPSeq with histone marks to determine the epigenetic landscape of regeneration genes and characterize transcription factor binding motifs. Altogether, our integrative analyses allowed us to build a gene regulatory network underlying hair cell regeneration. Our work, therefore, stands to serve as a foundation for devising strategies to induce hair cell regeneration in mammals. The detailed transcriptome characterization and characterization of the above-described modules will also serve as a reference to interrogate if their sequential activation is conserved across species and in response to different injury paradigms.

Feature Characteristics and Signaling Mechanisms Involved in SGN Neurite Guidance

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Background: The cochlea exploits an intricate tonotopic organization of afferent innervation to effectively process highly complex auditory stimuli. To create this precisely organized pattern, the neurites from spiral ganglion neurons (SGNs) navigate a complex milieu of cells, extracellular matrix, and biochemical gradients to reach their peripheral and central targets in the organ of Corti and cochlear nuclei, respectively. This process of a neurite growing through the environment is called pathfinding. In pathfinding, the tip of the neurite, the growth cone, senses, turns, and grows toward a target in response to biochemical and biophysical cues. To study this in vitro, I am using photopolymerization and other engineering techniques to study the biophysical and biochemical factors that direct SGN neurite growth and by which pathways SGNs use to pathfind in response to these cues.
Methods: Spiral ganglion neurons were dissected from neonatal mice, cultured on various micropatterned features, and their growth, alignment, and turning in response to these cues were studied. These features include: 1) topographical features of ridges and grooves made by photopolymerization of methacrylate monomers, 2) microcontact printing of peptides on polymer surfaces, and 3) selective deactivation of peptides via ultra photodeactivation to create surfaces with peptides in micropatterned coatings.
Results: In this work, we demonstrate that topographical features of increasing amplitudes promote neurite turning to increasing angle turn challenges in a dose response manner. With shallow turns, there isn't much difference in turning efficacy, in greater degree turns only large amplitude channels, 8µm, can promote an SGN neurite to turn somewhat regularly (30% to 23%) to sharp angles (60° - 90°). In related work, we also implicate Rho associated protein kinase (ROCK) and inositol triphosphate (IP3) signaling in the ability of SGNs to align their neurite growth in response to both topographical and biochemical features. Using real time imaging of neurons expressing a genetically encoded calcium indicator, GCaMP, we show real time quantitative assessment of Ca2+ in growth cones sensing and turning in response to these features.
Conclusions: Overall, this research informs the key, basic biological process of how an SGN neurite senses and turns in response to substrate cues in two ways. First, we show that the topographical feature angle and depth determine neurite turning and the efficacy of this turning is limited for sharp turns. Second, our data imply that SGNs use Ca2+, IP3, and ROCK signaling are used by SGNs when sensing and turning in response to both biophysical and biochemical cues. Overall, these findings inform the signaling and engineering required to guide SGN neurite growth towards desired targets, such as CIs and other neural prostheses.

Evaluation of Peripheral Causes for Poor Performance in Cochlear Implant Recipients

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Background: Cochlear implants are the most successful neural prothesis; however, outcomes remain inconsistent, highly variable, and unpredictable. The variability in performance is poorly understood and continues to be a challenge in cochlear implant research. While several pre-clinical factors have been associated with outcomes

such as duration of deafness, age of implantation, and etiology, these only account for 22% of the variance in post-operative outcomes. Although many recipients are afforded significant benefit with a cochlear implant, a subgroup of poor performers exists and are largely overlooked. Further, the best approach to clinical management of poor performance is unclear.

Both peripheral and central factors can impact performance, yet to date, no concrete pre-operative markers have any predictive value on post-operative speech understanding. A better understanding to these factors may improve long-term cochlear implant outcomes. Once such method includes perilymph sampling to create a biological profile, which may aid in understanding disruptions in the transmission of the electric signal through the auditory system. Perilymph sampling allows for collection of inner ear microRNA (miRNA). Historically, miRNA has served as a biomarker for numerous diseases. The objective of this study was to identify miRNA expressions in the inner ear that may serve as a biomarker to predict post-operative word understanding.

Methods: Seventeen adult patients undergoing cochlear implant surgery were enrolled. All were implanted with a fully inserted MED-EL Flex 28 electrode. Perilymph samples were collected peri-operatively at the round window and underwent analysis. Profiles of the miRNA were compared to post-operative word understanding scores at 6 and 18 months post-initial activation. Participants were classified as poor performers if scores were <30% on monosyllabic words.

Results: Seven male and ten female participants were included in the study. No significant relationship was identified between clinical factors and cochlear implant outcome. Average expression of 8 miRNAs was significantly associated with poor performance, with 6 miRNA profiles showing downregulation in this group.

Conclusions: Our results suggest that perilymph sampling may provide understanding of inner ear disease relative to cochlear implant performance. However, further research is needed to understand the interactions between miRNA expressions and other underlying pathophysiologic contributors. The ability to predict post-operative outcomes may drive alternative rehabilitation management for those at high risk for poor performance. Additionally, patient-driven factors should be explored relative to device satisfaction and performance.

Neural Correlates of Effortful Listening With Vocoded Speech

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Background: In challenging listening scenarios, listeners often face cognitive demands which go above and beyond those required when listening to natural speech in quiet. This cognitive load often results in listeners committing additional resources to process the speech signal – often referred to as listening effort. The goal of this study was to better understand the characteristics of auditory processing that relate to changes in listening effort between individuals. While listening effort has been well studied across an array of challenging listening scenarios, our understanding of the profile of those who exert different amounts of listening effort is limited.

Methods: Normal hearing participants (N = 22) were recruited to participate in a delayed visual world paradigm experiment, while both pupillometry and electroencephalography data were collected. On each trial, a fixation cue was given and then followed by either natural speech or 6-channel noise vocoded speech. The type of speech alternated in blocks. After the speech offset, four images appeared, and participants were asked to click on the image that corresponded to the word they heard. Comparisons were planned across the two conditions (natural speech or vocoded speech) as well as across individuals (those showing larger degrees of listening effort or smaller degrees of listening effort).

Results: Averaging across listeners there were differences in the EEG data based on condition. Vocoded speech elicited weaker cortical N1 response than natural speech. Within the noise vocoded condition, listeners who showed greater listening effort (as indexed by larger pupil dilation) exhibited weaker P2 amplitude than those who showed lesser listening effort, indicating that their greater effort is due to weaker engagement of immediate speech-information processing.

Conclusions: These results support previous findings, challenging listening scenarios induce listeners to commit additional effort due to the reduced signal quality and the following poorer encoding. Our novel results comparing high and low effort listeners suggest that those who struggle with extracting information from the signal need to commit additional resources to perceive and interpret the speech signal. Even when all listeners are in a challenging scenario (noise vocoded speech), some listeners are better able to process the acoustic signal, and this is reflected in both the electroencephalography data (reduced P2 amplitude) as well as the pupillometry data (increased pupil size). Data collection with cochlear implant patients in a similar paradigm is ongoing.

Stereo EEG Mapping of Sensorimotor Responses to Self-Generated Speech
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Background: Speech production and perception are frequently dichotomized processes; however, the role of feedback control during production suggests the processes are at least in part interdependent. Interactions between sensory and motor systems during speech production can result in speaker-induced suppression, where sensory responses to one's own speech are suppressed during production. It is theorized that speaker-induced suppression may be the result of feedback control during production, as unpredictable speech (i.e., speech that does not match the feedforward efference copy) is relatively less suppressed during production. Invasive sEEG is beneficial to studying speaker-induced suppression due to the rapid timescale and spatial precision of this phenomenon.
Methods: We used invasive stereo-electroencephalography (sEEG) to examine responses to self-generated speech in a naturalistic, dual perception-production experiment. Five participants undergoing sEEG for surgical treatment of medication-resistant epilepsy overtly produced sentences, then listened to playback of themselves producing those sentences while we recorded neural activity from intracranial electrodes in the temporal lobe, frontal lobe, and insular cortex. Playback was either predictable (immediate playback) or unpredictable (random playback of previous trial). Because playback trials were generated from audio produced during the production component of the experiment, auditory stimuli were identical across conditions. Sentence, word, and phoneme-level timing were transcribed. Multivariate temporal receptive fields were fit to high gamma (70-150 Hz) neural signals to examine phonological feature tuning and effects of production vs. playback and predictability of playback. Specifically, we were interested in how phonological features previously observed during speech perception were differentially encoded during speech production. For example, is speech-induced suppression equal across all phonological categories, or does it differ by category? How does the temporal profile of suppression differ across brain areas?
Results: We found suppression of auditory responses to self-produced speech in posterior superior temporal gyrus (pSTG) and sulcus (pSTS). Phonological tuning was similar during production and playback, but with lower amplitude production responses. In onset regions of pSTG, transient suppression of onsets was observed during production, but the later auditory response was mostly preserved. Onset-selective temporal lobe regions were not strongly modulated by predictability. Inferior frontal cortex responses demonstrated onset-selective encoding of playback, but showed mixed representation of playback predictability. In the insula, we observed sensory responses to speech that were enhanced during production relative to playback and occurred on a faster timescale. Phonological tuning in the insula was seen for a limited number of speech feature categories, primarily vowels. The predictability of playback did not have an effect on responses recorded from the insula.
Conclusions: Changes in high-gamma response between perception and production are not the result of differential encoding of phonological features. Overall, our findings clarify the specific spatiotemporal topographic mapping of speech-induced suppression and enhancement across the human cortex.

Joint, Distributed and Hierarchically Organized Encoding of Linguistic Features in the Human Auditory Cortex
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Background: How the human auditory cortex represents and transforms the sound pressure waveforms produced by a speaker to ultimately enable speech recognition remains unclear. It has been hypothesized that various levels of speech processing occur hierarchically in the human brain. We investigate the encoding of different intermediate linguistic units throughout the auditory cortex and explore where and when they appear.
Methods: We used intracranial neural recordings of HG, PT, and STG from implanted electrodes in the auditory cortex of fifteen neurosurgical patients as they listened to natural speech. We used a multivariate regression framework to predict neural responses from various acoustic and linguistic features of speech and measured the variance explained by each feature. To do so, for each predictor, we fit a single model with the true features and 100 control models with the target feature replaced by a shuffled distribution of itself. We then test the significance of the improvement in prediction accuracy of the neural signal.
Results: We found an explicit and distinct neural encoding of multiple levels of linguistic processing, including the acoustic, phonetic, phonotactic, lexical-phonemic and lexical-semantic features. Grouping neural sites according to their encoded linguistic features revealed several patterns of joint feature encoding, where the higher-level features were simultaneously represented with the lower-level features, forming a type of hierarchy.

Anatomical and functional localization of linguistic encoding showed a gradual emergence of low- to high-level features from primary (i.e., medial HG) to non-primary (i.e., lateral STG) auditory cortical areas. Furthermore, we observed a temporal order in the appearance of these features, where encoding of higher-level features was correlated with neural response latency and the response time-lag for higher-level features was higher than lower-level features across the population.

Conclusions: This joint, anatomically distributed, and temporally ordered appearance of various levels of linguistic features shines a light on what hierarchical processes enable the human auditory cortex to extract meaning from the speech sounds.

Effects of Evaluative Threat on Listening Effort in Online and Laboratory Studies

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Background: Listening effort (LE), a common complaint of individuals with hearing loss, refers to the mental work required to understand speech in challenging listening situations, such as noisy environments. Sustained LE may lead to negative health outcomes such as fatigue and stress. LE is proposed to be moderated by motivation, with greater motivation leading to higher allocation of cognitive resources, resulting in more LE. Here we measured the influence of motivation on LE using evaluative threat, i.e. stress from being judged by others. We predicted greater evaluative threat to lead to greater LE. The study was conducted both online and in a laboratory setting.

Methods: Listening demands were manipulated using tone-vocoded sentences. Evaluative threat was used to influence motivation via mild deception, i.e. informing participants that they must reach a particular 'score' on a listening task for their results to be usable. We measured task performance (correct response rate) as well as behavioural (reaction times (RTs)) and subjective LE outcomes (self-rated work and tiredness). Thirty seven participants with normal hearing (age ranges: 18 – 35) completed the study under laboratory conditions, and another 37 participants with normal hearing (age ranges: 18 – 35) carried out the study online. The sample size was powered to detect a small effect of evaluative threat on performance in the speech recognition task. Results were analysed using linear mixed models.

Results: In the laboratory study, listening demands reduced the correct response rate and increased RTs and self-rated work significantly. No significant effects of evaluative threat were found. In the online study, results showed a significant increase in RTs with greater listening demands and increased self-rated work with evaluative threat.

Conclusions: Most effects of listening demands found in the laboratory study were not replicated online, possibly because stimuli were not presented in a controlled environment. Concerning evaluative threat, we found a small effect on self-rated work in the online study only. Evaluative threat may need to be combined with other motivational factors (e.g. evaluative feedback) to have larger and broader effects on LE outcomes. Future research is needed to directly compare results from online and laboratory studies to understand how these contextual factors influence motivation and LE.

Nonlinear Dynamics in Auditory Cortical Activity Reveal the Neural Basis of Perceptual Warping in Speech Categorization

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Background: Speech perception requires the grouping sounds with similar acoustic information into meaningful phonetic units through the process of categorical perception (CP). Speech categories are influenced by recent memory traces; sounds previously heard can be warped in their representation, providing dynamical shifts to perception.

Methods: The purpose of this study was to determine the neural correlates of such nonlinear dynamics in speech CP. To this end, we measured event related brain potentials (ERPs) as listeners rapidly identified speech along an /u/ to /ɑ/ continuum in three different presentation orders: random, forward (rising F1), and backward (falling F1) conditions.

Results: Behaviorally, we found that sequential stimulus order moved the categorical boundary compared to random token delivery revealing a perceptual warping (biasing) of the heard phonetic category dependent on recent state history of the perceptual system. These behavioral nonlinearities were subject to stark individual

differences. At the neural level, EEG responses localized to middle frontal gyrus differentiated stimulus sequence effects ~300 ms after speech onset.

Conclusions: These findings demonstrate a top-down effect on acoustic-perceptual conversion based on stimulus history.

Validation of the SBAD Tinnitus Detection by Simulated Tinnitus and Designer Receptor Technology

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Background: Subjective tinnitus, the perception of sound in the absence of an external stimulus, is a serious health issue for hundreds of millions of people worldwide. Currently, there are no approved drugs to prevent or treat tinnitus partly due to a lack of reliable detection methods for animal studies. Previously, we developed a behavioral-based paradigm for tinnitus detection in mice called sound-based avoidance detection (SBAD). SBAD is a dual paradigm that uses negative reinforcement (electrical shocks) to infer tinnitus (silent trial) while monitoring potential confounding variables including alertness, motivation, motor functioning, and memory (sound trial). In a previous study, tinnitus detection via SBAD testing was validated by functional assays of the midbrain that demonstrated abnormal increases in neuronal activity in the inferior colliculus in mice that received traumatic noise exposure (Zuo et al., 2017). Here, we aimed to further validate the SBAD method 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.

Methods: Eight adult C57BL/6J mice received bilateral stereotaxic micro-injections of AAV2-CaMKIIa-hM3D(Gq)-mCherry (AAV) at either 50 nL (n = 4) or 150 nL (n = 4) doses in the central nucleus of the IC (stereotaxic coordinates: 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 7 consecutive days of SBAD testing. On every other day of testing starting on the second day, animals received intraperitoneal injections of clozapine-N-oxide dihydrochloride (CNO), a chemogenetic actuator, at a concentration of 5 mg/kg, one hour before testing. On all other days of testing, animals received .5ml injections of .9% saline as a control.

Results: For SBAD silent trials, there was a significant main effect of injectant type (saline vs CNO; p = .013) however there was no significant main effect of AAV dose and no significant interaction between injectant type and AAV dose. Silent trial scores post-CNO injection ranged from approximately 74-100% with five of the eight animals yielding scores that indicated tinnitus.

Conclusions: SBAD scores suggest that selective modulation of the IC in AAV injected mice via post-operative CNO injections can lead to tinnitus percept, however the dose of AAV did not affect SBAD test scores. Future research will assess the three-dimensional localization of AAV injected sites. Because IC hyperactivity is implicated in many tinnitus studies, excitatory DREADD injections of the IC as a reliable experimental model for tinnitus research is well supported.

Chronic Low-Dose Corticosterone Induces Hyperacusis in Rats

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Background: Hyperacusis is a debilitating condition in which everyday sounds are perceived as intolerably loud, irritating, or even painful. Though hyperacusis is often associated with hearing loss, some individuals with clinically normal hearing suffer from hyperacusis, suggesting that other factors may contribute to the emergence of this disorder. Individuals with adrenal cortical insufficiency and a disrupted hypothalamic-pituitary-adrenal (HPA) axis often exhibit abnormal sensitivity and perceptual disturbances to auditory and other sensory stimuli, illustrating stress hormones' role in sensory processing. Therefore, we pharmacologically dysregulated the HPA axis in hearing rats to investigate if this triggered hyperacusis.

Methods: Adult Sprague Dawley rats were treated with a low dose of corticosterone (CORT) (25 µg/ml) in drinking water for 28 days to dysregulate the HPA axis. Two powerful behavioral methods were used to test for different aspects of hyperacusis: (1) active sound avoidance (ASA), which tests for sound aversion and (2) Reaction-Time-Intensity (RT-I) functions, which test for loudness intolerance. Auditory function was assessed by

distortion product otoacoustic emissions (DPOAE) and auditory brain stem responses (ABR). Auditory cortical (ACx) neural sound-evoked responses were assessed by chronically implanted subdural electrodes.

Results: Rats treated with the CORT stress hormone exhibited robust behavioral evidence of sound aversion when evaluated with ASAP and loudness hyperacusis when evaluated by RT-I functions. Importantly, the CORT treated rats also exhibited enhanced sound-evoked cortical neural responses. However, peripheral auditory function assessed by DPOAEs and ABR thresholds remained normal.

Conclusions: Pharmacologic disruption of the HPA axis induced strong evidence of sound aversion and loudness hyperacusis that was associated with enhancing sound-evoked neuronal responses (hyperactivity) in the ACx whereas peripheral function was assessed by DPOAEs and ABR thresholds remained normal. These findings suggest that disruption of the HPA axis plays an important role in the development of hyperacusis. Supported by grants NIH/NIDCD (R01DC014452 and R21DC017813).

An Investigation Into Changes in Neurotransmitter Concentrations Associated With Tinnitus and Hearing Loss

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Background: Cochlear dysfunction, followed by changes in central auditory pathways, is widely assumed to be a causal factor for tinnitus. Numerous animal studies of tinnitus have suggested that imbalance of neurotransmitters in the auditory processing pathway, as a consequence of such cochlear dysfunction, are associated with behavioral markers of tinnitus. Specifically, concentrations of GABA, an inhibitory neurotransmitter which is the most widespread chemical messenger in the auditory pathway, have been seen to be decreased in the primary auditory cortex, medial geniculate body, dorsal cochlear nucleus, and ventral cochlear nucleus. Further, GABA agonists have been seen to be reduced in animals in whom tinnitus has been induced, while intervention studies suggest that the introduction of GABA agonists vigabatrin and taurine appear to reduce behavioral markers of tinnitus in animals. Concentrations of excitatory neurotransmitters such as glutamate are also seen to be impacted in tinnitus. While these promising results have been seen in a range of animal models, this research has been sparsely conducted on human subjects. The only study to investigate tinnitus-related changes in neurotransmitter concentrations has suggested that GABA is reduced in the right auditory cortex of tinnitus sufferers, while choline was seen to be associated with tinnitus severity (Sedley et al., 2015). However, the independent impact of hearing loss, which is seen to be associated with reduced GABA regardless of tinnitus status, has not been investigated.

Methods: In the present study, we employed a volumetric magnetic resonance spectroscopic imaging (MRSI) method to investigate concentration of GABA in six tinnitus sufferers who had accompanying hearing loss, four participants with hearing loss and no tinnitus, and four normal hearing controls. The volume of interest acquired during MRSI was an oblique slab covering bilateral regions of auditory cortex, inferior colliculus, and thalamus. Subject-specific ROIs were defined for each of these three regions in each participant.

Results: Preliminary analyses underscored the feasibility of the study, and demonstrated that we are able to detect GABA and glutamate in the spectrum of molecular weights from regions of interest within the acquired slab. Because of the small sample size, we have focused on data from individual participants and have refrained from conducting group-level analyses yet.

Conclusions: This is an ongoing study, with a goal of recruiting ten participants per group by the end of the study, where we plan to investigate differences in GABA and other neurotransmitters between the three groups. If there are reliable differences in the concentration of inhibitory and excitatory neurotransmitters between the tinnitus and hearing loss groups, it will point to the effect of tinnitus alone; if there are no such differences, it will suggest that hearing loss alone is sufficient to explain changes in neurotransmitters even in individuals with tinnitus.

Simulation and Evaluation of Evoked Compound Action Potentials in a 3D Model of the Human Vestibular System

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Background: The vestibular system is responsible for our sense of balance and spatial orientation. Rotational movements are sensed by the sensory epithelia in the ampullae of the three semi-circular canals whereas linear accelerations are sensed by the two otolith organs. Recent studies have demonstrated the possibility of restoring, even though just in part, the function of the vestibular system by vestibular implants. Computer electrical modeling is a valuable tool to investigate the effects of stimulation parameters and electrode positions and predict stimulation outcomes. One common measure for nerve responses are electrically evoked compound action potentials (eCAPs). In studies on living patients with estimated positions for both active and recording electrodes, eCAPs were recorded to analyze nerve responses. We present an approach for simulating the nerve responses and analyzing the resulting eCAPs in a 3D computer model.
Methods: A semi-automatic modular workflow was employed to transform segmented anatomical data of human vestibular systems from high-resolution μCT scans to realistic electrical computer models. Spherical electrodes with diameter 300 μm were placed in the center of each ampulla and used alternatively as active stimulation electrodes or recording electrodes for eCAPs. The nerve fiber orientation was estimated separately for each nerve branch to consider the anisotropic conductivity of the neural tissues. The extracellular potential distribution was simulated in quasi-static approximation by applying a unit current through the active electrode and scaling it based on the time dependent stimulus waveform. The current flowing through each node of Ranvier was obtained and used to compute the neural response to the electrical stimulation sensed at the recording electrode to simulate the eCAPs. Different stimulus amplitudes were applied at the active electrode to obtain the amplitude growth function (AGF).
Results: The obtained eCAPs waveforms show a comparable shape with respect to experimental recordings in literature. Moreover, the AGF obtained by considering the electrode positioned in the center of the posterior semi-circular canal as active electrode and the recording electrode located in the center of the anterior semi-circular canal is comparable with studies on living patients.
Conclusions: For the first time, eCAP waveforms have been simulated in 3D human vestibular computer model. Preliminary results of simulated eCAPs and AGFs are in good agreement with experimental findings. Our approach will enable improving electrodes placement and yield additional information about stimulation effects. In future work, patient specific anatomy and recorded eCAPs will be compared with the 3D computer model.

Clinical Comparison of Vestibular Evoked Myogenic Potentials Measured With Air- And Bone-Conduction Stimuli in Over 500 Patients

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Background: Vestibular evoked myogenic potentials (VEMPs) are crucial for diagnosis and management of vestibular disorders, because they allow for assessment of the utricule and saccule. VEMPs are elicited by short bursts of sound, and are recorded from the sternocleidomastoid muscle (cVEMP) or the inferior oblique muscle (oVEMP). VEMPs have been used to aid in the diagnosis of superior semicircular canal dehiscence, and have become increasingly common in vestibular assessment. Despite the advantages to VEMP testing, its widespread application is limited to some extent because of significant variability in stimulation and recording parameters. For example, VEMPS elicited by air-conduction are most commonly reported in the literature, but there are a number of theoretical advantages to VEMPS elicited by bone conduction. Specifically, bone-conduction VEMPs will elicit a stronger response in individuals with conductive or mixed hearing losses, and the amplitude of BC-elicited VEMPs are reported to be more reliable than that of AC-elicited VEMPs. Despite the theoretical advantages to BC-elicited VEMPs, there remains very little information on its use in clinical populations, and most reported studies have relatively small number of participants. To address these issues, here we report data from over 500 patients with AC- and BC-elicited VEMPs, and directly compare the responses elicited by each stimuli as a function of hearing loss and auditory pathology.
Methods: AC- and BC-elicited VEMPs were recorded from over 500 patients seen at the Stanford Ear Institute as part of their clinical vestibular assessment. AC-elicited VEMPs were recorded using standard clinical montages, and the following stimulus-response combinations were obtained (AC cVEMP @ 500 Hz; AC oVEMP @ 500 Hz; AC oVEMP @ 4000 Hz). BC oVEMPs were recorded to a click stimulus delivered via the 'Mini-Shaker.' BC cVEMPs were not recorded due to technical limitations. The Mini-Shaker was hand-held by the clinician during testing. Results were analyzed by comparing the amplitude and latency of the VEMPs for patients with varying degrees of hearing loss and auditory pathology.

Results: While preliminary, our results suggest that BC VEMPs elicited to a click stimulus are more reliable in their amplitude than AC VEMPs elicited by any stimulus or recording configuration. Our preliminary analysis suggests that the more reliable nature of the BC VEMP may be useful in diagnosing and interpreting auditory pathology.

Conclusions: Taken together, these preliminary results provide much-needed data comparing AC and BC-elicited VEMPs in clinical populations. Additional analysis is needed to determine whether BC oVEMPs improve the diagnostic accuracy of this procedure across all participants, or whether those benefits are limited to cases in which a patient has a significant conductive component to their hearing loss.

Vestibular Hair Cell Survival and Stereocilia Bundle Morphology in Usher Syndrome Type 1 Patients

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Background: Usher syndrome is a devastating disease characterized by hearing loss, balance deficit, and progressive loss of sight (retinitis pigmentosa). It is classified into three subtypes based on severity. Usher syndrome type 1, is the most severe form. Patients typically have profound hearing loss at birth; experience progressive visual loss before age 10, worsening over several decades; and display developmental delay in balance related behaviors, with balance deficit increasing in severity over time. Given the recent progress in the development of inner ear gene therapy for Usher syndrome, it is critical to better characterize the otopathology in these patients, in order to identify opportunities for therapeutic intervention.

Methods: Here, we utilized the human temporal bone archive at Massachusetts Eye and Ear to investigate the survival of hair cells in the vestibular organs of Usher syndrome type 1 patients. Of the seven Usher cases in the collection, three (aged 64-84) were diagnosed as type 1. Hair cell survival, and stereocilia morphology, in the vestibular organs of these three cases were assessed by differential interference contrast microscopy and confocal microscopy using the endogenous fluorescence of the eosin in the archival stained sections. Data from Usher cases were compared to age matched normal specimens.

Results: In all cases, we saw many surviving vestibular hair cells carrying stereocilia bundles. Thus, vestibular hair cells in Usher syndrome type 1 patients survive for many decades. In one case, hair cells were examined by transmission electron microscopy, enabling a high-resolution assessment of stereocilia bundle morphology. The survival of these cells to later life, even in the most severe cases of Usher syndrome, is strong evidence for the presence of vestibular hair cells in early development, in this number or greater.

Conclusions: These data, obtained in 64–84-year-old patients, provide support for an extended postnatal therapeutic window for gene therapy approaches to rescue vestibular function. This presents the exciting opportunity to rescue the sense of balance in patients with Usher syndrome, providing the potential to significantly increase quality of life in these individuals.

Dopaminergic Inhibition of Sodium Currents in Vestibular Calyces

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Background: Type I vestibular hair cells form synapses with large cup-shaped afferent calyx terminals and efferent terminals make synaptic contact with the outer facets of calyces. Although acetylcholine has been shown to be a major efferent transmitter in the vestibular periphery, other neurotransmitters may also act to adjust transmission of vestibular afferent signals. We have recently shown that dopamine modulates tetrodotoxin-sensitive Na+ currents in vestibular calyx terminals (Meredith and Rennie Frontiers in Neuroscience 15: 710321, 2021) and have begun to further investigate pathways underlying the effect of dopamine on Na+ currents in vestibular afferents.

Methods: Whole cell voltage clamp recordings of Na+ currents were obtained from calyx terminals acutely dissociated along with their accompanying type I hair cells from the cristae of male and female gerbils aged 16-36 days. Patch electrodes contained Cs+-based intracellular solutions and pharmacological agents were applied with whole bath perfusion or included in electrode solution.

Results: Perfusion of 100 uM dopamine in the extracellular solution reversibly reduced peak transient Na+ currents by approximately 20%. Quinpirole (1 uM), a D2 dopamine receptor agonist, produced a similar decrease

in Na+ current amplitude. Application of the D2 receptor antagonist eticlopride (1 uM) significantly reduced the response to dopamine. Na+ channels in other cell types can be modulated through phosphorylation by cAMP-dependent protein kinase (PKA). To investigate involvement of cAMP, a membrane permeant form of cAMP (8-Br-cAMP) was tested. Extracellular application of 500 uM 8-Br-cAMP led to a transient reduction in Na+ current amplitude, whereas co-application of 8-Br-cAMP and the phosphodiesterase inhibitor IBMX (100 uM) produced a sustained reduction in Na+ currents. Additionally, dibutyryl cAMP, a non-hydrolysable form of cAMP, resulted in a progressive reduction of Na+ current amplitude when included in the patch electrode solution. Immunostaining for tyrosine hydroxylase, a marker for dopaminergic fibers, indicated staining of some fibers within vestibular epithelia suggesting that dopamine may be present in efferent neurons.

Conclusions: The reduction in Na+ current amplitude in response to dopamine and quinpirole suggests that efferent signaling through D2 dopaminergic receptors may occur in the peripheral vestibular system. Dopamine may exert its action through a cAMP-dependent mechanism. Dopamine-mediated reduction of Na+ channel activity is expected to produce a decrease in firing in vestibular calyx-bearing neurons. Further investigations will explore the involvement of dopamine receptor subtypes and signaling pathways.

Eccentric Viewing Alters Subjective Visual Vertical Perception

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Background: The vestibular and oculomotor systems are intricately linked. The eye-in-orbit position serves as a reference for space/head orientation and motion perception, thus influencing whole-body postural control. Aging leads to vestibular deficits affecting mobility and are thought to contribute to older adults' visual dependence for perception and postural control. These age-related vestibular changes, combined with eccentric viewing may explain the higher fall risk and mobility issues of individuals with age-related macular degeneration (AMD), who suffer from irreversible central visual field loss and adopt eccentric eye orientations to exploit regions of their peripheral retina.

In this preliminary study, we examined whether eccentric viewing alters one's subjective vertical perception, a basic measure of space representation, often used to assess vestibular function. Considering also visual dependence, we further questioned whether potential eccentricity effects on verticality perception persist with the influence of a misleading visual context. By examining young adults, we could address these questions without the added complexity of aging and visual impairment.

Methods: Nine healthy young adults (4F) made orientation judgements on a briefly flashed rod (16°x1°, flashed for 50ms) tilted to the left or right of the gravitational vertical. The rod appeared with and without a rotating constellation of dots surrounding it (100 dots, 1° diameter appearing concentrically at eccentricities of 12° to 30°, rotating clockwise or counterclockwise at 30°/s). Stimuli were projected on a 193cm x 145cm screen and viewed while seated at a 90cm distance, with the head placed on a chinrest. The task was repeated with observers fixating a target displaced 10° horizontally in the direction of their dominant eye. 100 trials were completed for conditions without a visual context and 280 trials for those with the rotating dots. Rod orientations and rotation direction were randomized within testing blocks and between participants. Wireless eye-tracking goggles were worn to monitor fixation. The data was fit with a probit model, the point of subjective equality (bias) and threshold of detection (sensitivity) were estimated for each participant and condition. We examined the effect of viewing eccentricity by comparing the difference in bias between central and eccentric fixation to 0 and of sensitivity via paired t-tests.

Results: An effect of eccentric viewing was obtained for verticality judgements performed both with and without a visual context, with the bias shifting in the direction opposite fixation. Sensitivity did not differ between viewing conditions. Our data suggest that eccentric viewing may affect one's estimation of vertical regardless of contextual visual information.

Conclusions: The interaction of eye orientation and contextual visual information will be important to consider further in the design of rehabilitation protocols for individuals with AMD who have eccentric fixation and may have increased visual dependence due to aging and associated vestibular loss.

Gender Differences in Concussion-Induced Vestibular Deficits in Mice

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Background: Dizziness or imbalance is a common symptom following a concussion or mild traumatic brain injury. During concussions, the accelerations and decelerations of the head not only impact the central nervous system, but may also affect the peripheral vestibular end organs inside of the bony labyrinth. In a previous study (Huang et al., ARO, 2021), we tested a mouse model of concussion-induced vestibular deficits, in which a closed head impact model of engineered rotational acceleration (CHIMERA) was employed to generate consistent, repeatable concussions in male mice. In this study, we further examined the gender differences in concussion-induced vestibular deficits by examining the vestibulo-ocular reflex (VOR) response and expression of biomarkers for neuroinflammation in the central vestibular nuclei in female mice.

Methods: Young adult male and female mice (C57BL/6J, 8-10 weeks old) were anesthetized with isoflurane and subsequently given three repetitive concussions over the course of three days at an intensity of 0.52J. VORs were measured by an ISCAN eye tracker at 1 day, 5 days, 8 days, 2 weeks, 4 weeks and 8 weeks after the last concussion. Canal function was assessed by measuring steady-state (0.2-4 Hz) and transient VOR to head rotation (rVORs). Otolith function was assessed by measuring steady state responses to translation (tVOR, 0.2-2 Hz). Brain sections were prepared for immunohistochemistry.

Results: In male mice (N=6), while repeated concussions caused substantial reductions in rVOR gains, which lasted over two weeks, they did not induce significant changes in the sensitivity of tVOR. For the female mice (N=6), however, the repeated concussions did not result in significant changes in rVOR gains, but they induced a significant reduction in tVOR gains and a phase lead, which lasted for over two months. Based on morphological characteristics, the female mice demonstrated more robust microglia activation (Iba1+) in the central vestibular nuclei than the male mice. Microglia were activated as early as 1 day following the last concussion and microglia activation was sustained for up to 8 weeks.

Conclusions: The preliminary results indicate a potential gender difference in concussion-induced vestibular deficits in young adult mice. Ongoing studies are necessary to further elucidate these gender differences using behavioral, morphological and neurophysiological biomarkers.

* (JC, TC and MD have contributed equally)

Natural History of Audio-Vestibular Dysfunction in Usher Syndrome Type 1C
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Background: Usher syndrome (Usher) is a group of genetic disorders that cause varying levels of concurrent hearing, balance, and visual loss. Type 1 Usher (USH1) is the most severe form, wherein congenital severe-profound hearing loss and vestibular areflexia are followed by the onset of retinitis pigmentosa (RP) in early adolescence. 6 – 8% of USH1 is caused by mutations in the USH1C gene, but nearly all USH1 cases among the Acadian populations in Canada and Louisiana are caused by the c.216G>A mutation in the USH1C gene (USH1C). While the genetics of Usher has been well studied, the treatment options for these patients are limited. There are no treatments to prevent or slow the progression of Usher. Moreover, the natural clinical history of USH1C is not known. To improve our understanding of the natural progression of vestibular dysfunction, we are conducting natural history studies (NHSs) with USH1C patients at all stages of disease progression.

Methods: USH1C mutational analysis and audio-vestibular phenotype are evaluated via retrospective chart review (n=150) and prospective clinical evaluation (n=50) in USH1C participants of all ages recruited from around the world. A comprehensive evaluation of hearing and vestibular function is performed at 2 clinic visits: at baseline and 6 months later.

Results: Of the 108 individuals enrolled in our retrospective NHS, 73 have genetically confirmed USH1C disease and are aged 2 – 94 years. Of, these, 13 are also enrolled in the prospective NHS. Importantly, 76% (111/146 alleles) of USH1C alleles in this cohort have at least one copy of the Acadian allele. All participants younger than 24 years of age (n=21) use cochlear implants and oral communication; whereas all participants 24 years and older (n=52) use sign language. Preliminary assessment of 5 retrospective (pediatric) and 7 prospective (2 pediatric and 5 adult) USH1C patients showed the absence of VOR via caloric irrigation, sinusoidal harmonic acceleration, and/or video head impulse test. Cervical and ocular vestibular evoked myogenic potentials showed variable results. Surprisingly, similar balance and gait strategies are demonstrated via computerized dynamic posturography, regardless of age.

Conclusions: Understanding the audio-vestibular phenotype – genotype relationship in patients with USH1C will improve clinical management, and the design of future clinical trials of genetic interventions for USH1C patients.

Vestibular Drop Attacks and Meniere's Disease as Results of Otolithic Membrane Damage – a Numerical Model

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Background: Meniere's disease (MD) is a condition of the inner ear with symptoms affecting both vestibular and hearing functions. Some patients with MD experience vestibular drop attacks (VDAs), which are violent falls caused by spurious vestibular signals from the utricle and/or saccule. Recent surgical work has shown that patients who experience VDAs also show disrupted utricular otolithic membranes.
Methods: We use a previously developed numerical model to describe the nonlinear dynamics of an array of active, elastically coupled hair cells. We then reduce the coupling strength of a selected region of the membrane to model the effects of tissue damage.
Results: As we reduce the coupling strength, we observe large and abrupt spikes in hair bundle position. As bundle displacements from the equilibrium position have been shown to lead to depolarization of the hair-cell soma and hence trigger neural activity, this spontaneous activity could elicit false detection of a vestibular signal.
Conclusions: The results of this numerical model suggest that otolithic membrane damage alone may be sufficient to induce VDAs and the vestibular dysfunction seen in patients with MD. Future experimental work is needed to confirm these results in vitro.

A Machine Learning Approach for Pupil and Torsion Tracking to Evaluate the VOR

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Background: The vestibular system is responsible for balancing and the vestibulo-ocular reflex (VOR), which aids in focusing on a target when head movements occur. Scleral coils and video-oculography (VOG) markers have been used in most current VOR measurements. These processes are potentially invasive to the eye and painful. A non-invasive procedure is video-oculography without the use of markers. However, this option loses accuracy in comparison with other traditional measurements due to fewer image features and a clean image boundary. A machine learning approach was explored to see if this gap in functionality could be closed.
Methods: A Machine Learning system was developed by training object-detection models from Tensorflow using 200 images of the eye using a headset fabricated for this project. Eye movements can be broken down into two parts: horizontal/vertical and torsional. The horizontal/vertical movements are measured using the model's bounding box detection of the pupil. The model is given preprocessed images using k-means clustering for increased accuracy. From the bounding box, the center of the pupil can be derived. The location of the pupil center is used to calculate the angular velocity of the eye. The Torsional movements of the eye are measured by identifying iris striations and blood vessels in the sclera. These features are tracked and calibrated to the axial movements and register any movements around the axis perpendicular to the pupil. The iris and sclera are linearly evaluated using polar transformation so that the objects detected can be easily tracked and used for torsion evaluation. A 3D-printed headset was fabricated to test the system using a gyroscope, raspberry pi, infrared light, and camera; then processed in python. The movements are synchronized with the headset's gyroscopic output.
Results: The process created in this study utilizes machine learning for capturing both aspects of eye movement. The rate of error was calculated to be higher than measurements with scleral coils, although a more thoroughly trained model will be able to eliminate error as it is given additional images. The pupil miss rate limits the accuracy but using a higher speed and resolution camera will ameliorate the problem.
Conclusions: A machine learning process was explored for the non-invasive VOR measurements in both 2D and 3D. A low-cost headset was fabricated for testing, validating the process. Additional images for model training will improve the VOR measurements by reducing the error rate.

Developing a Novel Stimulation Protocol for Vestibular Afferent

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Background: Bilateral vestibular deficiency (BVD) causes chronic dizziness, blurry vision during head movement, and postural instability and the vestibular prosthesis is a potential treatment for this disorder. However, the vestibulo-ocular reflex (VOR) gain restored by vestibular prosthesis in human clinical trial is much smaller than what we expected. To improve vestibular stimulation, investigation of how ramping sloe of pulses and intervals has been the focus in our lab recently. We hypothesize that slope of rising ramp over both positive and negative phase and intervals between pulses could influence on recruited vestibular afferent unit number. A novel stimulation protocol with the best slope of rising ramp and interval for pulses was created to increase VOR gain and reduce current spread between individual electrodes.

Methods: Ramped pulses with various slopes induced by change duration time of climbing for zero to the peak were edited in the customized programing. Bench test and computational modeling of electrically evoked compound action potential (ECAP) and current spread were performed to test the recruiting efficacy of stimulus. Interval between pulses were manipulated and carious pulse trains with different intervals were also investigated with both bench test and computational modeling.

Results: Bench test and computational modeling confirm the effects of ramping slope and intervals between pulses on recruiting vestibular afferent. Optimized stimulation pulse train were identified through bench test and current spread modeling. Theoretical framework has been established to support the new stimulation strategy and simulation in computational model suggests it will improve the modulation of vestibular afferent activity by exploiting their sensitivity to the slope of current pulses and interval changes. The theoretical consequence of optimized slope and intervals include reduction in the spread of excitation within the semicircular canals and increase in the neural dynamic range.

Conclusions: A novel protocol to improve vestibular stimulation was developed with optimized pulse ramping slope and interval between pulses. The bench test and computational modeling confirmed the effects of pulse slope and intervals on vestibular afferent recruiting. More animal experiments need to be done in future to confirm finding in this study, and further explore potential of the new stimulation protocol.

Microglial Involvement in Synaptic Pruning of Multisensory Midbrain Maps
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Category: Midbrain: Structure and Function

Background: Network blueprints or neural maps are circuit-level arrangements established during early development that are necessary for complex processing tasks. Primitive maps exhibit an initial excess of connections that are subsequently pruned through a process of refinement. Such modifications are characteristic of early critical periods, when active synapses are maintained, and underutilized contacts are tagged for removal. Microglial cells (MGCs) have been implicated in a variety of unimodal systems in the targeted degradation of selective inputs. Abnormalities in the accurate establishment and subsequent refinement of sensory maps have been linked to certain neurodevelopmental conditions, including autism spectrum disorders. As multisensory integration deficits serve as perhaps the most reliable early indicators and severity predictors of associated cognitive and behavioral symptoms, multimodal map configurations may prove of particular importance. Despite this fact, the mechanisms that govern the sculpting of early multisensory maps remains poorly understood. Thus, the present study examines (1) MGC involvement in shaping segregated multimodal maps in the lateral cortex of the inferior colliculus (LCIC), and (2) whether fractalkine signaling (CX3CL1-CX3CR1) influences MGC engulfment behaviors during an early critical period.

Methods: A living brain preparation was employed to simultaneously label developing auditory and somatosensory inputs to the LCIC in early postnatal CX3CR1-GFP mice (P0, P4, P8, P12, and P20). Tracer placements were made in the ipsilateral central nucleus of the IC (CNIC; 10,000MW dextran AlexaFluor 647 direct conjugate) and somatosensory cortex (biocytin, DyLight 549 streptavidin). GAD67 immunocytochemistry facilitated visualization of the emerging LCIC modular framework with respect to labeled MGCs and multisensory afferent patterns. Acquired confocal z-stacks were imported into ImageJ and Imaris software packages to perform 3-D surface renderings of MGCs, engulfed contacts, and quantification of phagocytosed material as previously described (Schafer et al., 2014; doi: 10.3791/51482).

Results: Auditory and somatosensory inputs are sparse and intermingle in the LCIC at birth, prior to segregating into discrete matrix and modular zones respectively by P12. Significant MGC engulfment of both auditory and somatosensory terminals is evident throughout the peak period of projection shaping (P4-P12), as compared with

that observed after critical period closure (P20). 3-D renderings in heterozygous mice reveal substantial engulfment, including individual microglia with evidence of both phagocytosed auditory and somatosensory terminals. Preliminary findings in homozygous mice with compromised fractalkine receptor signaling show reduced engulfment relative to age-matched controls.

Conclusions: Taken together, these findings implicate MGCs in the selective pruning of emerging multisensory maps in the LCIC and suggest that the mechanisms governing this process are at least in-part fractalkine signaling dependent.

Recordings of the Neural Distortion Response With the ALFIES Method in Cochlear Implant Users: Latest Advances

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Background: We recently developed a method (ALFIES, Carlyon et al, 2021. JARO 22:141–159) to measure the sustained neural response to high-rate electrical stimulation with electroencephalography (EEG). We interleaved two pulse trains modulated at frequencies F1 and F2 and recorded the neural distortion response (NDR) at F2-F1 Hz, a frequency that is unaffected by the electrical artefact. For F2-F1 ~ 40 Hz, we showed a substantial NDR with a group delay consistent with thalamic/cortical sources. The presence of a NDR to interleaved pulse trains implies that at some stage in the auditory pathway and prior to any distortion, smoothing and temporal dependencies undo the interleaving. To investigate the origin of this smoothing, we measured the effect of changing the inter-pulse interval (IPI) between the carriers of the F1 and F2 components on the strength of the NDR. A short time constant may indicate smoothing at an early stage (auditory nerve/brainstem). We also investigated the clinical applicability of the ALFIES method by measuring its level-growth in several device types and in real time.
Methods: Users of Cochlear devices were presented with two 480-pps pulse trains modulated at 80 and 120 Hz. IPIs ranged from 8 to 984 us. When measuring level-growth functions, we fixed the IPI at 8 us and presented the pulse trains at 7 different levels (in 0.5-dB dB steps) at and below most comfortable level (MCL). We also measured level-growth functions with users of Advanced Bionics devices with two interleaved 2160-pps pulse trains modulated at 80 and 120 Hz (similar to Carlyon et al, 2021). The NDR was recorded with a 262-kHz Biosemi amplifier and 8 scalp electrodes. The waveforms were analysed in real time. Recordings were stopped after 3 or 4 minutes if the NDR was stable and above the noise floor for at least 1 minute. If not, the recordings were stopped after 5 minutes.
Results: The NDR was roughly constant for IPIs ranging from 8 to 200-400 us and decreased to be within the neural noise floor at the largest IPI tested (984 us). In the same participants, the NDR for a 173-us IPI was similar to that with the same IPI, but higher-rate (2160-pps) carriers. The level-growth functions were monotonic in all participants, fairly steep (5- to 15-dB reduction when going down 2 dB from MCL), and sometimes plateauing near the MCL.
Conclusions: The time constant of the effect of IPI is consistent with smoothing/temporal dependencies occurring as early as the auditory nerve. The distortion itself may however occur at later stages in the auditory pathway. Reliable measures can be obtained in 3-4 minutes (sometimes less). Finally, level-growth functions can be obtained in two different devices and are steep, boding well for use for objective CI programming.

Neuropathic Damages on the Inner Ear Induced by Plasma Membrane Ca2+-ATPase Mutations

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Background: Using forward genetics approach, we have isolated 4 mouse lines of Pmca2 mutant, each of which has a different missense mutation and shows quite diverse phenotype; there exists a large difference in age-dependent ascending rate of ABR thresholds or period of hair cells/SG cells disappearance. F1 genetic background of C57BL6/J and C3H/HeJ augmented these phenotypic differences from weeks period of onset to years. It remains elusive what precise mechanism underlies human age- dependent hearing loss or presbyacusis onset and development. It also raises controversy that rodent model of age-dependent hearing loss can be applied to human.
Methods: We screened mice by observing the startle responses evoked by a click box and then measuring the auditory brainstem responses (ABR) [2,3]. The isolated mutants were further subjected to comprehensive

phenotypic screen to confirm they were not accompanied by other traits, which is a typical characteristic of human diagnostic-type non-syndromic deafness. To prove possible defects in hair cell calcium-ion exporting activity, Ca-decay assay method on cells expressing the mutant product were employed.

Results: The clear differences in phenotype among the above mutants appear to result from the differences between Ca2+-pump activities of P-type Ca2+-ATPase, the product of mutated Pmca2 genes. Assuming that a biomolecular pathway towards the age-dependent emergence of phenotypes is divided into two axes (functional impairment and survival odds) and clarifying these axes may facilitate discovery of appropriate model of human progressive hearing loss. Ca2+ ion is generally thought to act as a second messenger in multiple signal pathways in very short time range, however it might also act in as-yet-unknown pathway for a life-long period.

Conclusions: To investigate these pathway axes in vitro, we have established cultured cell system in which each mutated protein expression is properly controlled to give equal amount and localization. Molecular analysis of this system is expected to reveal latent bases of the mutant's long-range effects.

Age-Related Changes of Mitochondria Gene Expressions in Cochlea of CBA/CaJ Mice

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Background: Age-related hearing loss (ARHL) is the most common type of hearing loss in the elderly population. So far there is no FDA approved drug therapeutics. Continually emerging evidence suggests that dysfunctional mitochondria results in impaired energy metabolism. Mitochondria associated disorders, that arise from reactive oxygen species (ROS) accumulation, disrupt cellular homeostasis and induce apoptosis, play a key role in the development of ARHL. However, the mechanism is not quite clear. In this study, we investigated the mitochondria gene expressions in cochlea of young and old CBA/CaJ mice and determined whether mitochondria gene expression is associated with aging process in cochlea.

Methods: Nine young adult (4–5-month-old, 4 female, 5 male), eighteen middle-aged (10-15 month, 10 female, 8 male), and fifteen old (21.5-34 month, 8 female, 7 male) CBA/CaJ mice were used. Mice were separated into four groups according to age and degree of hearing loss. Auditory brainstem responses (ABRs) and distortion product otoacoustic emission (DPOAE) amplitudes and thresholds were tested. GeneChip data (Affymetrix) were extracted from entire cochlea tissue samples and transcriptional expression patterns of investigated genes were subjected to one-way ANOVA analysis using GraphPad Prism. Linear regression and correlation tests were used to confirm relations between genotype and phenotype. Candidate genes for screening were selected from the Human Mitochondria Database consisting of all units, subunits, and complexes named with "ATP synthase", "Cytochrome c oxidase", and "NADH dehydrogenase".

Results: Out of 172 mitochondrial-related gene probes analyzed, 10 showed significant changes, of which, 8 genes presented nonmonotonic changes with aging cochlea. Two genes, Cytochrome C Oxidase, subunit VIIc (Cox8a) and NADH Dehydrogenase (ubiqunone) 1 beta subcomplex, 11 (Ndufb11), showed monotonic changes, which are up-regulated in aging cochlea. Overall, the higher significant gene expression changes always occurred in old age group with severe hearing loss compared with young age group normal hearing. These genes encode proteins essential for respiratory process in mitochondrial electron transport chain complexes I and IV, where ROS production is observed abundant. This study will further investigate the protein expression levels of the genes identified, as well as, mitophage markers to understand underlying mechanism for the disruption of mitochondrial gene expressions in aged cochlea.

Conclusions: The data represents age-related mitochondria gene expression changes focused on identifying, targeting, and restoring mitochondria and associated factors for implementation of a preventative strategy in maintaining the cellular respiratory functioning of energy metabolism.

Neural Correlates of Signal-In-Noise Processing is Improved Following Treatment With a Targeted Augmented Acoustic Environment

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Background: A deficit in the ability to understand speech in noisy environments is a notable facet of age-related hearing loss (ARHL) and other auditory disorders stemming from declines in function of the auditory nervous

systems. In addition, signal-in-noise (SiN) detection is a problem in acquired hearing loss and aging. We hypothesized that an augmented acoustic environment (AAE) comprised of tone bursts embedded in background noise of various levels will improve neural coding of sounds in noise via neural plasticity. To test the effect of this novel SiN AAE, old mice were exposed over a 2-month period and then neural responses to a novel signal-in-noise stimuli were compared to those of an unexposed control group of similar ages.

Methods: CBA/CaJ mice, aged 22-24 months, were exposed to SiN AAE for 12 hours per day, for 2 months. Control mice were not exposed to the stimuli. The AAE simulated real-world listening in a noisy environment and consisted of a continuous Gaussian background noise (CBN) at 2 levels: 50 dB SPL for 3 s followed by 65 dB SPL for 6 s. During the 6 s of 65 dB CBN, 16kHz tones lasting 50 ms are presented every 200 ms. The 16 kHz toneburst is presented with a random signal-to-noise ratio of +3, +6, or +12 dB SPL.   Multi-channel recordings were made from the inferior colliculus using Neuronexus 16 channel silicon-iridium vertical electrode arrays. Frequency response areas were acquired using 25 ms tone bursts from 4 to 64 kHz and 0 to 80 dB SPL.   SiN stimuli were WBN with a 1 s period and 25% duty cycle, and recorded at 3 levels: 60, 40, and 0 dB SPL. 50 ms into the on-cycle of the CBN a tone ranging from 8, 12, 16, 20, 24 kHz was presented randomly at 60, 63, 66, 69, 72, 75 dB SPL for 50 ms and each condition was presented for 50 repetitions. Neurons were categorized into onset or sustained responses and in this poster only onset units were used for further analysis. Neural encoding of the SiN was determined by comparing the tone burst and steady state responses to the WBN alone using the Wilcoxon rank sum test.

Results: A total of 425 and 241 onset units were acquired from AAE treated and control mice, respectively. Preliminary results suggest that passive exposure to the SiN AAE improved neural detection of tones in background noise by increasing driven rates to the tone burst. This effect was pronounced for the 40 dB CBN for the 16kHz and 20kHz signals where the 9,12 and 15 dB SNRs showed significantly improved coding.

Conclusions: This novel SiN AAE could potentially be used to improve hearing in noisy environments in listeners with ARHL.

Exposure to a Multi-Frequency Signal-In-Noise Augmented Acoustic Environment Improves Signal-In-Noise Detection in Aged CBA/CaJ Mice

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Background: Growing evidence suggests that neural plasticity induced by training can be an effective treatment for deficits connected with age-related hearing loss (ARHL). An augmented acoustic environment (AAE) is a paradigm first reported by Turner and Willott (2000) in which hearing impaired mice are passively exposed to sounds for a long period with beneficial results. In this study we used a novel AAE consisting of signals embedded in background noise. We tested the effects using pre-pulse modification of the acoustic startle response to measure signal in noise (SiN) processing to determine whether a multi-frequency SiN AAE could improve SiN processing in mice with ARHL.

Methods: Aged CBA/CaJ mice (18 months) were exposed for 2 months to a SiN AAE consisting of a continuous Gaussian wideband noise that varied in intensity from 50- and 65-dB SPL for 12 hours to simulate real-life workplace background noise. Background noise starts at 50 dB for 3 seconds and then increases to 65 dB for 6 seconds and interleaves for 12 hours. During the 6 second period, a 50 msec tone burst at either 8, 16, or 32 kHz was presented every 200 milliseconds, at intensities of +3, +6-, and +12-dB SPL above the background. To assess SiN detection in a behavior paradigm, a 65 dB SPL wide-band background noise was presented for the duration of the protocol. Within each trial, a tonal pre-pulse ranging from 65-77 dB in steps of 3 dB was presented 50 milliseconds before the onset of a startle stimulus. Behavior was tested before, after 1 month, and after 2 months of AAE. There were 180 trials in each frequency protocol, with 30 being control trials and the remaining 150 comprising inhibitory trials. ASR waveforms were classified using an automated machine learning model to identify true startles, removing noisy or low amplitude non-startles. The distributions of startle amplitude ratios of (1-PPI) were compared for trials before and after AAE.

Results: Mice in the AAE group showed statistically significant improvement in detecting the SiN, as evidenced by increases in PPI following 2 months of treatment when compared to baseline. Due to the multi-frequency nature of the AAE, exposed animals demonstrated significant improvements at tonal prepulse frequencies of 8, 16, and 32 kHz, confirming ameliorative effects across the frequency domain. Additionally, response latency, a novel measure of pre-pulse signal salience, will be presented.

Conclusions: Results from this study demonstrates that passive exposure to a multi-frequency SiN AAE improves SiN detection in aged mice and suggests active plasticity in the aged auditory system. These findings suggest that the SiN-ASR may be a useful tool in assessing the efficacy of potential treatment for ARHL.

Sex Differences in Body Composition, Balance Function, and Hearing Function in CBA/CaJ Mice Across the Lifespan

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Background: Numerous studies have reported sex differences in human auditory perception. In general, the results of these studies show that hearing loss is more common among men than in women. In this study, we investigated whether there are sex differences in body composition, balance function, and hearing function in young, middle-aged, and old male and female CBA/CaJ mice.

Methods: The EchoMRI body composition analyses (whole body fat, lean, free water, and total water masses and body weights), balance assessments (rotarod and voluntary wheel running), and auditory brainstem response (ABR) tests (thresholds, wave I latencies, and wave I amplitudes) were performed in male and female CBA/CaJ mice at 6, 16, and 24 months of age.

Results: Young females displayed decreased lean and total water masses and body weights compared to age-matched males. Middle-aged females displayed increased fat masses and decreased total water masses compared to age-matched males. Old females displayed increased fat masses and decreased lean and total water masses compared to age-matched males. Young females also displayed increased wheel running activities compared to age-matched males, while there were no sex differences in wheel running activities at 16 and 24 months of age. There were no sex differences in latencies to fall at 6, 16, and 24 months of age. There were no differences in tone burst ABR thresholds at 4, 8, 16, 32, 48, or 64 kHz between males and females at 6, 16, and 24 months of age. There were no differences in tone burst and click ABR wave I latencies between males and females at 6, 16, and 24 months of age. Young females displayed increased ABR wave I amplitudes at 8 kHz and increased click ABR wave I amplitudes compared to age-matched males. There were no differences in tone burst and click ABR wave I amplitudes between males and females at 16 and 24 months of age.

Conclusions: Our results suggest that there are sex differences in body composition, wheel running activities, and ABR wave amplitudes in CBA/CaJ mice. We are currently investigating whether there are sex differences in voluntary wheel running activities during light and dark cycles in CBA/CaJ mice. We are also investigating whether there are sex differences in age-related cochlear pathologies.

Variability in Early Markers of Sensorineural Hearing Loss With Age

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Background: Age-induced cochlear synaptopathy (CS) was demonstrated in rodent and human temporal bone studies, but it remains unclear whether this pathology is the underlying cause of speech intelligibility declines in the aging population. Studying the causality of this relationship in human is not straightforward, since thus far developed EEG-based diagnostic tools of sensorineural hearing loss (SNHL), do not explicitly quantify the CS component and several SNHL aspects can coexist in age-induced SNHL. To better understand the functional role of different SNHL aspects, we studied how several early markers of SNHL (audiogram or auditory evoked potential (AEP) based) relate to the speech recognition threshold. We complemented this analysis with model simulations to estimate the CS-related decline-rate of AEPs and study its relation to the speech intelligibility performance of the aging cohort.

Methods: A total of 78 Flemish subjects participated in this study and were divided into two groups: (i) a young control group with normal audiograms (n=37, 18-25 years) and (ii) older adults with a normal audiogram, some with complaints of tinnitus or self-reported speech intelligibility problems (n=47, 45-60 years). We adopted a test battery that comprised audiograms measured at standard and extended high frequencies (EHF), envelope following responses (EFR) to a rectangularly amplitude modulated (RAM) pure-tone and speech intelligibility scores (Flemish Matrix sentence test). We calculated the age-decline rate of each metric and compared them to (1) the age-related auditory nerve (AN) fiber population decrement observed in human temporal bones, and (2)

simulated RAM-EFR magnitudes declines for auditory models with different degrees of CS. Moreover, we computed the correlation strength of each metric with speech reception thresholds.

Results: The RAM-EFR magnitude of our aging cohort declined with a rate of 8% per-decade. At the same time, simulated RAM-EFR magnitudes for varying degrees of CS, showed a 7.5% reduction by removing 15% of the AN fibers. Hence, consistent with human temporal bone studies (loss of the 12% of AN fibers per-decade), we predict an approximate 50% loss of the AN fiber population by the age of 40. At that age, the EHF audiogram of our aging cohort had declined with a rate of 16 dB per-decade, whereas the standard audiogram remained within the normal range (below 20 dB-HL).

Conclusions: The data from our aging cohort, together with the model simulations and human temporal bone studies, support the view that CS plays an important role in the RAM-EFR magnitude reduction, and that a timely-diagnosis of speech intelligibility problems can be made on the basis of either EHF thresholds or RAM-EFRs.

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Effects of Age, GABA Receptor Density and Number of Intact Synapses in the Inner Ear on Binaural Detection in the Gerbil

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Background: Supra-threshold hearing deteriorates with age even if a loss of hearing sensitivity is not apparent. This impacts particularly listening in challenging situations, such as understanding speech among babble or localizing and detecting sounds in noisy surroundings. The reasons are not well understood. Changes in central inhibition as well as synaptopathy, i.e. a loss of synapses between inner hair cells and auditory nerve fibers, have been discussed as potential sources for those deficits. Here we investigate supra-threshold hearing in the form of binaural detection in Mongolian gerbils. We relate the animals' behavioral performance with the density of gamma-aminobutyric acid A (GABAA) receptors and the number of intact synapse in the inner ear to allow for conclusions about the contribution of central inhibition and the state of the periphery to deficits in supra-threshold hearing, respectively.

Methods: We determined tone-in-noise detection thresholds and hearing thresholds behaviorally in young, middle-aged, and old Mongolian gerbils using operant conditioning in a go/nogo procedure with food reward. Target tones had a frequency of 700 Hz and were presented monaurally (Sm), diotically (S0) or dichotically (Spi, 180° phase shift). The continuous masking noise (bandwidth 0.25-10 kHz) was presented diotically (N0). BMLDs were derived from detection thresholds. Positron emission tomography (PET) using [18F]flumazenil, a selective GABAA receptor antagonist, was used to determine GABAA receptor density. After the experiments, the animals' cochleae were extracted, immunohistologically processed and the number of synapses between inner hair cells and auditory nerve fibers were counted.

Results: Both the N0Sm-derived BMLD and the N0Spi-derived BMLDs decreased significantly with age. The BMLDs were already reduced in middle-aged animals whose hearing sensitivity was not reduced matching findings in middle-aged humans that show deficits in binaural hearing despite normal hearing sensitivity. [18F]flumazenil binding, indicating GABAA receptor density, also decreased significantly with age and so did the number of intact synapses in the inner ear matching earlier observations in gerbils.

Conclusions: Our data suggest that changes in central inhibition as well as changes in the auditory periphery with age contribute to the age-related decrease of BMLDs. Age-related deficits in other supra-threshold hearing tasks therefore are likely also due to both processes.

Age-Related Loss of Auditory Sensitivity Across the Life Span of CBA/CaJ Mice

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Background: Human ARHL is characterized by high frequency sensorineural hearing loss that progresses to lower frequencies with increasing age. The inbred mouse strain CBA/CaJ is frequently used as a model of age-related hearing loss in humans. While descriptions of age-related hearing loss in the CBA/CaJ mouse have previously been published, each suffers from some drawback, for instance restriction to one sex, limited age-range or infrequent time sampling. A need exists for a better resolution and extended age range of hearing threshold

changes over time in various mouse strains. We analyzed ABR threshold data from two research groups with CBA/CaJ mouse colonies and similar recording techniques, which yielded an extensive database of thresholds over the life span of this mouse strain.

Methods: Mice were bred in-house from CBA/CaJ stock from Jackson Labs, in the respective vivaria of the research groups from University of Rochester and University of South Florida. Auditory Brainstem Responses (ABRs) were measured in conjunction with behavioral and electrophysiological experiments, and only responses from baseline or experimentally naïve animals were included. Both groups used Tucker-Davis Technologies digital signal processing platforms and BioSig acquisition software. The resulting dataset comprised 966 male and 760 female CBA/CaJ mice, with an age range from 28 to 1046 days. Thresholds were grouped into 3-month age groups and collapsed into 3 frequency ranges, Low, Mid and High, and threshold changes were calculated relative to the youngest age grouping.

Results: Thresholds at 3 kHz started at about 50 dB SPL at 1-3 months age and increased to 90 dB by 31-33 months of age. Thresholds at 6, 8, 12 and 16 kHz typically were initially below 20-30 dB, and increased to 70-80 dB by 31-33 months. Thresholds at 24, 32 and 48 kHz began at 30 dB and reached 80-90 dB by 31-33 months. This corresponded to an increase of 50 dB in all frequencies by 31-33 months. Differences in threshold relative to the youngest age grouping became statistically significant for the Low range at 13-15 months, for the Mid-range at 19-21 and for the High range at 16-18 months.

Conclusions: Changes in auditory detection threshold over the life span of the CBA/CaJ mouse strain were evaluated by ABRs in a cross-sectional design using a large data set with extended age range and covering both sexes. Thresholds increased over ages for all frequencies and differences from the youngest age grouping reached significance at different ages at low, middle and high frequencies ranges. The maximum threshold change was 50 dB, which resulted in different final thresholds, depending on frequency. No difference by sex of the mouse was seen. This data provide a framework to establish normative hearing values across the lifespan of the CB/CaJ laboratory mouse.

Identifying Molecular Biomarkers for Augmented Acoustic Environments in Old CBA/CaJ Mice
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Background: Declines in temporal processing due to aging are a hallmark of age-related hearing loss (ARHL-presbycusis). Here, we hypothesize that a long-term exposure to an augmented acoustic environment (AAE) can slow down the progression of ARHL due to induction of beneficial plasticity in the central auditory system. We investigated the effects of AAE on signal processing in aged CBA/CaJ mice and explored the underlying molecular biomarkers.

Methods: 21-22 months old aging CBA/CaJ mice were exposed to a novel signal-in-noise (SiN) AAE stimulus for 12 hours per day for 2 months. Control animals were housed in same conditions without SiN AAE stimulus. The AAE stimulus was a continuous wideband noise at 50 (3 seconds) and 65 (6 seconds) dB SPL. The effects of the AAE were measured behaviorally using pre-pulse inhibition (PPI) of the acoustic startle response to a SiN stimulus paradigm. Signal-in-noise detection was measured using a tonal pre-pulse embedded in 65 dB SPL wide-band background noise presented 50 milliseconds prior to onset of a startle elicitor stimulus. Inhibition calculated relative to trials with background noise but no pre-pulse. At the end of the study, animals were sacrificed and parts of the central auditory system (cochlear nucleus – CN, inferior colliculus – IC, and auditory cortex – AC) were analyzed for biomarkers using western blotting and immunohistochemistry. Specifically, two biomarkers were investigated: FoSB – Gene family forming transcription factor subunit AP-1, and GAD 65/67 – Glutamate decarboxylase isoforms 65/67.

Results: Aged CBA/CaJ mice showed a significant increase in PPI after two months of AAE treatment as compared to the base line, indicating an improvement in SiN detection in background noise. Initial western blotting and immunohistochemistry experiments confirmed that both total FosB and its spliced variant ΔFosB were expressed in the central auditory system of CBA/CaJ mice: IC, CN, AC, and expression was strong in AC and IC compared to CN. There was a decrease in expressions of total FosB due to AAE exposure in CN and in IC. Interestingly, though AAE seems to have no effect on total protein expression of ΔFosB (western blotting) – a molecular mediator for neuro-behavioral plasticity; but preliminary analysis of immunohistochemistry results indicates that there is decrease in the percentage of cells having ΔFosB, specially, in AC and IC. Furthermore, there was an increase in the total number of cells due to AAE exposure (indicated by DAPI nuclei staining).

Similarly, GAD 65/67 proteins also expressed in all three parts of central auditory system. Detailed analysis is ongoing to further understand the effects of AAE on both proteins

Conclusions: This study demonstrate that AAE has potential to mitigate some key aspects of temporal processing deficits of ARHL through the FosB family of genes.

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Detection of Mitochondrial DNA Mutations in the SV-K1 Cell Line and the Cochlea of CBA/CaJ Mice

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Background: Mitochondrial DNA (mtDNA) mutations are associated with aging and age-related diseases. Hearing impairment is common in patients with some mitochondrial disorders, affecting over half of all cases. It has been reported that the accumulation of mtDNA mutations by carrying a mutator allele of the mitochondrial Polg DNA polymerase protein contributes to age-related hearing loss (ARHL) in mice. However, the detailed biological mechanisms of mitochondrial mutations in ARHL are still unknown. Here we used a fast, cost-effective, and reliable method to prepare mtDNA from the cochlea of young adult and old CBA/CaJ mice using a next generation sequencing technique (NGST).

Methods: Young adult (n=3, 3mon) and aged (n=3, 30-34mon) male CBA/CaJ mice served as the auditory animal model. ABRs were recorded to measure hearing changes. SV-K1 cells were studied with two experimental paradigms: vehicle (control, n=3) and hydrogen peroxide (H2O2, 100 nM, n=3) treatments. Cellular DNA from cochlea and cultured cells were extracted using a QIAGEN Miniprep Kit. The digested DNA products were purified using Agencourt AMPure XP (Beckman, Brea, CA). The sequencing was performed in Illumina's HiSeq platform, following the manufacturer's recommendations.

Results: ABR threshold shifts up to 30 dB occurred in all CBA/CaJ old mice vs. young adult mice, suggesting a typical auditory function decay with aging. We screened the mouse mitochondrial genome sequence using mouse nuclear genome references to determine whether or not similar sequences exist in the nuclear genomics. Genebank blast revealed that 21 chromosomes showed the identified sequences of mtDNA. Therefore, to avoid the pitfalls of using two or more sets of primers with the sequencing depth of overlapped regions higher than the non-overlapped regions and nuclear mitochondrial insertion sequences detection, we designed a new PCR-based method using a single primer pair to enrich the entire mitochondrial genome. This yielded uniform coverage, and no interference of nuclear copies of the mitochondrial genome. mtDNA deletion was not found; But A6551 C mutations were observed with a very low copy frequency for H2O2 treatment in SV-K1 cells (0.57%), and the same mutation was observed in the aged cochlea with a relatively high frequency (13.06%). Each cochlea sample also showed at least one missense mutation, with the frequency varying from 0.08% to 66.23%. in total, 7 mutations were identified and all of them did not show homoplasmy. The mutations appeared in coding regions for cytochrome c subunit 1 and cytochrome b, and non-coding regions for tRNA and D-loop.

Conclusions: The catalogue of mutations identified in this study comprise the first evidence of cochlear mtDNA mutations using NGST, and our findings enhance the current knowledge of mtDNA alterations in the mouse cochlea, paving the way for follow-up translational experiments.

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Metabolic-Related Oxidative Stress is a Pathology of the Aging Cochlea

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Background: Age-related hearing loss (presbycusis) highly impacts the daily life for our elderly population. Commonly, it arises from changes in the inner ear and brain as we age. Progressive cochlear hair cell degeneration and loss is a major pathology. However, the mechanisms of inner ear cochlear hair cell damage and death are still not completely known. We have previously demonstrated that hydrogen peroxide (H2O2) stimulation can simulate key aspects of age-related oxidative stress in the cochlea, leading to cell death. In the present study, we investigate

the molecular pathways involving fatty acid oxidation (FAO) and glucose oxidation (GO) and their relationships with oxidative stress-induced cochlear cell death.

Methods: CBA/CaJ mice were divided into two groups: Young adult (2-4 mon) and old (27–30-mon) with equal males/females per group (N=6/group). Auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs) were recorded. In vitro cell treatments using HEI-OC1 cells were conducted with different experimental paradigms. RT-qPCR was used to measure gene expression. Protein biomarker changes were analyzed with Western blots (WB), and confocal laser scanning microscopy with immunohistochemistry.

Results: Auditory function measurements showed age-related impairment: ABR and DPOAE thresholds increased, and DPOAE amplitudes decreased with age. An increase in DNA fragmentation was found in the aged cochlea, with glucose transport 4 (glut4) protein and carnitine palmitoyl transferase I (CPT-1) gene expression (qRT-PCR) down-regulated. In addition, the intensity of peroxisome proliferator-activated receptor gamma (PPAR γ) and Myosin VII α fluorescence signals were decreased in the old cochlea vs. the young cochlea. However, the protein kinase C delta (PKC δ) signal intensity increased with age. Similar changes of PKC δ and PPAR γ protein expressions measured by Western blot was observed in HEI-OC1 cells treated with GW9662 or Phorbol 12Myristate 13Acetate (PMA) vs. control vehicle treatment. Cell death analyzed by DNA laddering was present in cells treated with GW9662 and PMA, but not with vehicle treatment. In addition, we found that H_2O_2 stimulation caused rate-limited enzyme protein expressions; for example, catalase and glutathione peroxidase (GP x) were increased in the aged cochlea vs. young adult cochlea, and in HEI-OC1 cells treated with PMA or GW9662, relative to untreated control cells.

Conclusions: Our results indicate that age-related FAO and GO may trigger endogenous oxidative stress and cell death (apoptosis) pathways in the aging cochlea. For example, age-related PPAR transcriptional activity changes are important factors for this pathway activation, and PKC δ activation is related to aging processes in the cochlea. Taken together, our findings support that PKC δ and PPAR γ pathways promote oxidative stress and subsequent cell death, and are likely key mechanisms involved in age-related hearing loss.

Auditory Cortical Response to Sound Localization Stimuli Differs by the Source Direction, Not by the Hearing Side in Chronic Unilateral Hearing: An fMRI Study

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Background: Neuroplasticity associated with asymmetric auditory input has been examined, but studies on functional plasticity associated with binaural hearing are limited. Behavioral studies reporting high variability in binaural performance, such as auditory localization in single-side deafness, have suggested compensatory plasticity in the central auditory pathway. In this fMRI study, neural activity for auditory localization was explored in patients with unilateral hearing loss (UHL) in comparison to the monaurally stimulated controls.

Methods: A total of 29 patients with severe to profound UHL and 13 normal-hearing controls (NC, n=13, age = 45.9±10.86) participated in the study. The UHL patients were comprised into two groups; the left UHL (LUHL, n=15, age=44.7±15.35) and the right UHL group (RUHL, n=14, age=51.7±7.58). The subjects performed two fMRI sessions of 14 blocks, each with a sound localization task. In each block, four stimuli were presented in one of 5 directions (0◦, ±30◦, ±60◦), and the subject verbally reported the source direction among the left, front, and right sides. The correct response rate and the RMS error were also acquired during the scan. The NC group have conducted the same experiment in three conditions; binaural, right ear-only, and left ear-only.

Results: The UHL groups showed worse sound localization performance than the control group in the binaural condition. However, the UHL groups revealed a higher correct rate and a smaller RMS error on the impaired ear side than controls in monaural conditions. In the fMRI results, the control group with binaural conditions and the UHL groups showed significant brain activations in the bilateral STG to all source directions with dominant activity in the contralateral cortex to the source. Contrarily, the monaural-stimulated controls displayed highly lateralized activity in the auditory cortex contralateral to the stimulated ear, regardless of the source direction. Correlation analyses with clinical variables are underway.

Conclusions: With an extended duration of the asymmetric hearing, contralateral dominance of auditory cortical response was observed with sound localization, as observed in normal controls with binaural hearing. In UHL

groups, the increased ipsilateral activity to the hearing ear seems to be compensatory neuroplasticity, as supported by the improved behavioral performances.

The Functional Organization of Cortical Pitch Processing in the Common Marmoset

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Background: How the brain processes pitch on complex sounds has been one of auditory neuroscience's central questions due to the importance of pitch in music and speech perception. The cortical representation of pitch has been demonstrated by one pitch-sensitive region near the anterolateral border between A1 and R in the common marmoset (Bendor and Wang, 2005). However, it's not clear if there exist other pitch-processing regions in the marmoset brain.

Methods: Here, we performed optical imaging over the entire auditory cortex on the brain surface in awake marmosets. We utilized the intrinsic optical signal in all of our subjects but were also able to image the calcium signal labeled by GCaMP6s throughout the auditory cortex in some of the subjects.

Results: By contrasting responses to harmonic complex sounds with spectrally matched noises, we identified two discrete pitch-sensitive regions. One region is located anterolaterally to the A1 and R border and is consistent with the previously described "pitch-center" by single-unit recording. The second region is newly found at the location more anterior to the "pitch-center" and functionally overlaps with the RT field, referred to as "anterior pitch-region". When tested by synthetic tones comprised of low-numbered harmonics, these two pitch-sensitive regions only appear when the fundamental frequency (F0) is close to or higher than 400Hz, a phenomenon consistent with the estimated harmonic resolvability of the marmoset (Osamanski et al, 2013, Song et al, 2016). The response contrasts in these two pitch-sensitive regions were also robust when tested by more natural sounds such as a female's singing of a-cappella songs (F0 ~300-700Hz). Furthermore, the ratio between the singing contrast and the synthetic tone contrast is higher in the "anterior pitch-region" than in the anterolateral "pitch-center" in all tested subjects.

Conclusions: Together, our results suggest that the cortical pitch processing in marmoset is organized into discrete regions with a functional hierarchy along the anterior direction for natural harmonic sounds.

Relationship Between Physiological Indicators of Cochlear Synaptopathy and Tinnitus in Military Veterans

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Background: The prevalence of tinnitus is higher among Veterans (11.7%) than the general public (5.4%; Folmer et al. 2011). This finding is not explained by age or hearing loss because even young Veterans with clinically normal audiograms show a high prevalence of tinnitus (48% of Veterans in Bramhall et al. 2018). Cochlear synaptopathy, the partial loss of the synapses between inner hair cells and auditory nerve fibers has been proposed as one possible underlying etiology of tinnitus. Animal models and human temporal bone studies indicate that noise exposure is a significant risk factor for synaptopathy. Therefore, synaptopathy resulting from high levels of noise exposure encountered during military service may be contributing to the high rates of noise-induced tinnitus among Veterans. Although synaptopathy cannot be directly evaluated in living humans, animal models indicate that several non-invasive auditory physiological measures are sensitive to synapse loss, including the auditory brainstem response (ABR), the middle ear muscle reflex (MEMR), and the envelope following response (EFR).

Methods: ABR, wideband MEMR, and EFR measurements were obtained in a sample of 99 young military Veterans and non-Veteran controls, aged 19-35 years, with clinically normal audiograms and robust distortion product otoacoustic emissions (DPOAEs). Participants were divided into three groups based on Veteran status and report of tinnitus (Veterans with tinnitus, Veterans without tinnitus, and non-Veteran controls without tinnitus). The effects of tinnitus group on ABR, MEMR, and EFR measurements were independently modeled with Bayesian generalized linear mixed models that included average DPOAE level from 3-8 kHz and sex as predictors. To put the tinnitus group effects into context, these effects were compared with age-related changes in ABR, MEMR, and EFR measurements estimated from other datasets.

Results: Point estimates from statistical models for each physiological indicator suggest a mean reduction in ABR wave I amplitude, EFR magnitude, and MEMR strength for Veterans with tinnitus compared with non-Veteran controls, with the most evident reduction observed for the EFR. When expressed relative to the reduction in

response magnitude associated with aging, the size of the tinnitus group effect varies across physiological indicators, with the smallest relative effect on the ABR and the largest effect on the MEMR.

Conclusions: These findings indicate that tinnitus associated with exposure to high levels of noise during military service may be an indication of cochlear synaptopathy. This suggests that in the future, a test battery of physiological indicators of synaptopathy may allow for identification of a subset of tinnitus patients who might benefit from tinnitus treatments targeting the underlying neuronal deficit.

Reorganization of Afferent Cochlear Synapses on Residual Inner Hair Cells in Mice With Partial Loss of Hair Cells

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Background: While each inner hair cell (IHC) is innervated by multiple spiral ganglion neurons (SGNs), each SGN contacts just a single IHC. While not the initial pattern, this is a consequence of synaptic remodeling and synapse elimination late in cochlear development. The formation and remodeling of synapses between SGNs and IHCs has been described by several laboratories. In a series of experiments intended originally for a different purpose, diphtheria toxin (DT) injected into postnatal day 5 DTR mice was used to eliminate HCs. We found, serendipitously, that some of the mice had only a partial loss of HCs. We quantified synapses on surviving IHCs in mice with partial IHC loss to ask whether a reduced number of IHCs would result in a reduction in synapse elimination during postnatal synaptic remodeling and, consequently, an increased number of synapses on the surviving hair cells.

Methods: 22–26-week-old DTR mice in a CBA/CaJ background were examined and compared to 12~16 week-old wild-type CBA/CaJ mice. DTR mice express a human diphtheria toxin receptor in HCs, making HCs susceptible to loss by DT injection. ABR thresholds and amplitudes were measured at 8, 16 and 32 kHz. Mice were euthanized after the ABR measures and cochlea dissected to prepare cochlear wholemounts for confocal imaging. IHCs, SGN peripheral axons, presynaptic ribbons, postsynaptic densities, and GluA2 AMPAR subunits were immunofluorescently labeled, respectively, with antibodies to Myosin 6/7a, NF200, CtBP2, PSD95, and GluA2. Synapses were functionally defined as colocalized CtBP2 and PSD95 – or CtBP2 and GluA2 – immunofluorescent puncta. Synapses were quantified at 8, 16 and 32 kHz locations, corresponding to the ABR measures, and presented here as synapses/IHC.

Results: ABR thresholds were elevated at 8, 16 and 32 kHz in the DTR mice. ABR amplitudes are also significantly reduced in these mice relative to the wild-type control mice. Consistent with the ABR results, the mice with abnormal ABR showed diffuse IHC and OHC loss over the entire cochlear length. Synapse counts on isolated surviving IHCs increased by 25.8%, 28.7% and 37.4% at 8, 16 and 32 kHz cochlear locations, respectively, relative to the control mice. Synapse counts on the IHCs within patches are similar to those on isolated IHCs, suggesting that the synaptic changes on residual IHCs might involve a large-scale reorganization of synapses rather than local synapse migration from immediately adjacent lost IHCs.

Conclusions: We suggest that after partial loss of hair cells in the DTR mouse model, SGNs do not necessarily lose their peripheral axons or degenerate but can shift their synapses to residual IHCs in a large-scale reorganization of synapses. Because the phenomenon was noticed in older mice, determining the age at which this occurs requires further investigation. (Supported by R01 DC015790)

Mouse Cranial Nerve VIII is Preserved With Haploinsufficiency of Chromatin Remodeler CHD7

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Background: CHARGE syndrome is a multiple anomaly developmental disorder characterized by a variety of sensory deficits, including sensorineural hearing loss. The majority of cases of CHARGE are caused by pathogenic variants in Chromodomain DNA-binding Protein 7 (CHD7), a chromatin remodeler important for the development of neurons and glial cells. The structural substrate for hearing loss is not well understood. Using the Chd7Gt/+ mouse model of CHARGE syndrome, we sought to determine how Chd7 haploinsufficiency affects mature neurons, myelinating Schwann cells, and inner hair cell innervation in the cochlea.

Methods: Auditory Brainstem Responses (ABRs) were recorded in adult Chd7+/+ and Chd7Gt/+ animals. Cochleae were sub-dissected and processed for transmission electron microscopy (TEM). TEM images were

captured in the apical and basal regions of the spiral ganglion. Myelin thickness and axon diameter were measured using ImageJ. Semi-thin sections of spiral ganglia were imaged for further histological analysis, cell counting, and nerve density quantification. Immunohistochemical staining for hair cell synaptic markers CtBP2 and GluA2 was conducted on 4-week-old wild-type and Chd7Gt/+ cochleae, which were imaged by confocal microscopy.

Results: Analysis of ABR recordings in Chd7Gt/+ adult animals show elevated ABR thresholds at 4 kHz and 16 kHz, but not at 32 kHz. ABR Wave I peak latency and amplitude in Chd7Gt/+ mice are not significantly different from wild-type controls. Proportions of neurons and glial cells in the spiral ganglion are not significantly different, nor are densities of nerve projections from the spiral ganglion to the organ of Corti. G-ratio analysis of myelin thickness in peripheral spiral ganglion Type I neuronal projections indicate subtle but statistically significant hypermyelination in Chd7Gt/+ mice. Staining for CtBP2 and GluA2 showed no differences in hair cell synapse formation in Chd7Gt/+ mutant cochleae.

Conclusions: Collectively these results suggest that in the mouse, the inner ear is resilient to haploinsufficiency of CHD7. Previous studies in Chd7Gt/+ embryos showed substantial neuronal loss in the developing spiral ganglion, and here we found that the inner ear is able to compensate for Chd7 loss by adulthood. There may be other peripheral or central auditory components contributing to sensorineural hearing loss in Chd7Gt/+ mice, and middle ear defects may be the primary source of increased ABR thresholds in this mouse model of CHARGE syndrome.

Chronic Electric Stimulation Inhibits Post-Operative Neural Recovery of Electric Thresholds in Aged Guinea Pigs

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Background: Despite advances in cochlear implants (CIs), there remains variability in speech perception outcomes, especially in older patients (e.g. Roberts et al., 2014; Favaretto et al., 2019). One potential contributor may be reduced neural health recovery after CI surgery with older age. The electrically-evoked compound action potential (ECAP) as well as electrically-evoked auditory brainstem response (EABR) can be used to measure electric thresholds and amplitude growth functions (AGFs) as non-invasive measures of neural health. In guinea pigs implanted with CIs, Pfingst et al (2015) demonstrated transient reduction in neural excitability after implantation followed by recovery, i.e. a temporary increase in ECAP thresholds and decrease in AGF slopes followed by recovery toward the original thresholds and slopes over 2-4 weeks (Pfingst et al., 2015). These changes may indicate temporary inflammation that affects neural transmission. However, these animals were not given chronic electric stimulation (ES), and it is not known how ES affects the time course of neural health recovery.

Methods: The goal of this study was to determine if similar patterns of recovery are seen with chronic ES. Aged guinea pigs (9-21 months) were used to better model the aged human CI patient population. Three treatment groups consisted of 1) No-Stimulation (NS; n=6); 2) ES starting at 4 weeks (ES4; n=5); 3) ES starting at 8 weeks (ES8; n=7). All animals were implanted with an 8-channel CI (Cochlear) in the left ear. The ES4 and ES8 group received chronic ES (amplitude-modulated noise, 1800 pps, with threshold and maximum currents determined by EABR thresholds and behavioral programming, respectively) at 40 hours/week up to termination at 24-26 weeks after implantation. EABRs were recorded at 2–4-week intervals over the course of the experiment to assess changes in electric thresholds and AGF slopes. ECAPs were also recorded in the ES8 group.

Results: EABR thresholds improved between 3-24 weeks after implantation in non-stimulated animals (mean change: -50 ± 38 c.u.) but worsened in the ES4 group (mean change: 16 ± 1 c.u.); the difference in threshold change between groups was significant (p=0.04; 2-tailed t-test). Data collection is ongoing for the ES8 group, but preliminary results are intermediate between the NS and ES4 groups (mean change: 0.5 ± 0.02 c.u.).

Conclusions: ES in the weeks after surgery may interfere with neural recovery, at least in aged animals. Delaying stimulation may allow more time for neural recovery and ultimately, lower electric thresholds.

Tympanic Cavity Hypothermic Rinsing Technique as a Tool to Measure Intracochlear Hypothermia Distribution in a Cochlea Hypothermia Model

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Background: Cochlea implants can cause severe trauma leading to intracochlear apoptosis, fibrosis, and eventually to loss of residual hearing. Mild hypothermia has been shown to reduce toxic or mechanical noxious effects, which can result in inflammation and subsequent hearing loss. This paper evaluates the usability of standard surgical otologic rinsing as cooling medium during cochlea implantation as a potential hearing preservation technique.

Methods: Three human temporal bones were prepared following standard mastoidectomy and posterior tympanotomy. Applying a retrocochlear approach leaving the mastoidectomy side intact, temperature probes were placed into the basal turn (n = 4), the middle turn (n = 2), the helicotrema, and the modiolus. Temperature probe positions were visualized by microcomputed tomography (μCT) imaging and manually segmented using Amira® 7.6. Through the posterior tympanotomy, the tympanic cavity was rinsed at 37∘C in the control group, at room temperature (in the range between 22 and 24∘C), and at iced water conditions. Temperature changes were measured in the preheated temporal bone. In each temperature model, rinsing was done for 20min at the pre-specified temperatures measured in 0.5-s intervals. At least five repetitions were performed. Data were statistically analyzed using pairwise t-tests with Bonferroni correction.

Results: Steady-state conditions achieved in all three different temperature ranges were compared in periods between 150 and 300 s. Temperature in the inner ear started dropping within the initial 150 s. Temperature probes placed at basal turn, the helicotrema, and middle turn detected statistically significant fall in temperature levels following body temperature rinses. Irrigation at iced conditions lead to the most significant temperature drops. The curves during all measurements remained stable with 37∘C rinses.

Conclusions: Therapeutic hypothermia is achieved with standard surgical irrigation fluid, and temperature gradients are seen along the cochlea. Rinsing of 120 s duration results in a therapeutic local hypothermia throughout the cochlea. This otoprotective procedure can be easily realized in clinical practice.

Binaural Benefit in Cochlear Implant Users: Relationships Between Quality of Life and Speech Perception

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Background: Previous research has demonstrated a concern for the reflection of clinical outcomes in cochlear implant (CI) users based on quality of life (QoL). Additionally, many previous studies have used a variety of listening configurations (e.g., unilateral CI, bimodal CI and contralateral hearing aid, and bilateral CI) collapsed into one group for such analyses. This study aimed to examine speech perception abilities in relation to self-reported QoL questionnaires in bimodal and bilateral CI users, to better assess binaural advantages in everyday life in this population.

Methods: Twenty-eight post-lingually deafened adult bimodal (i.e., CI and contralateral hearing aid) and bilateral CI users were assessed for speech perception abilities using the AzBio sentence test with CI-only and binaural configurations, in both quiet and noise conditions. Self-reported benefit from CI implantation was measured using the Glasgow Benefit Inventory (GBI), a modified five-factor version of the GBI (i.e., GBI-5F), and the Nijmegen Cochlear Implant questionnaire (NCIQ).

Results: In bimodal and bilateral CI users, AzBio performance in noise was related to the GBI total and general subscore, as well as the GBI-5F QoL, self-confidence, and social involvement scores, with both the CI-only and binaural configurations; no relationships were found with the NCIQ scores. Additionally, there was no relationship between AzBio performance in quiet configurations (CI-only and best aided) and any QoL metric. Dividing participants into two groups based on age revealed that the GBI-5F scoring method explained less of the variance in speech perception abilities in younger participants than older participants.

Conclusions: AzBio speech perception abilities with both CI-only and binaural configurations in noise were related to QoL measures, but generally not speech perception in quiet, which may be less reflective of speech encountered in everyday life. Our work also suggests that that the GBI-5F may be more clinically relevant for clinicians working with older CI users. Future work exploring the utility of the GBI-5F and other QoL questionnaires in comparison to unilateral participants is needed.

Auditory Nerve Stimulation With the Novel Auditory Nerve Implant and a Cochlear Implant – A Computer Model Study

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Background: A novel auditory prosthesis for intracranial electric stimulation of the auditory nerve is currently under development (auditory nerve implant, ANI). The ANI targets the auditory nerve between the cochlea and the brainstem with 3x5 penetrating electrodes. To investigate ANI stimulation prior to the first implantations in human subjects, we developed a computational modeling framework incorporating a 3D model of a human cochlea and auditory nerve. The framework was used to compare neural activation from a conventional cochlear implant (CI) with that from the novel ANI. In this poster, we present new results based on a refined 3D model of the cochlea and the auditory nerve.
Methods: A 3D finite element method (3D-FEM) model of the cochlea and the auditory nerve including auditory nerve fiber (ANF) pathways was created based on histological data. The 3D-FEM model contains a CI electrode array inserted into the scala tympani and an ANI placed in the auditory nerve. The 3D-FEM model was used to simulate the voltage distribution along the ANFs when stimulating with the CI or the ANI. A phenomenological stochastic neuron model was applied to simulate excitation of the ANFs, resulting in excitation profiles that show the activation of the ANFs over their tonotopic frequency. Excitation profiles derived at different stimulation levels were concatenated to spatial tuning curves (STCs). Based on the STCs, we estimated the thresholds, dynamic ranges, and specificity of stimulation with the ANI and the CI.
Results: For the CI, the STCs had a single peak or an additional peak caused by cross-turn stimulation. For the ANI, the STCs varied from single frequency peaks with a narrow spread of activation to multimodal profiles with multiple peak frequencies or very broad excitations. The number of peaks in the STCs were sensitive to the placement of the stimulating electrode and the anatomic and tonotopic organization of ANFs. The peak widths mainly depended on stimulation level and electrical conductivity values of the 3D-FEM model.
Conclusions: A computational modeling framework was developed to simulate the spatial tuning curves of stimulation with a CI and the novel ANI. Thresholds, dynamic ranges, and specificity of the stimulation were estimated based on the neural activation. The results of this project will be used for the future development of speech coding and fitting strategies for the ANI clinical trial.

New Method for Estimation of Artifacts in Intracochlear Pressure Measurements During Bone Conduction Stimulation

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Background: Bone-conduction implants (BCIs) are routinely used as a treatment for conductive and mixed hearing losses. Measurements of intracochlear pressure (ICP) in cadaver heads are considered a promising technique for performing bench tests of BCI hearing level output. Unfortunately, ICP measurements during BC stimulation are prone to contamination by artifacts resulting from the motion of the ICP sensors relative to the specimen. Previous studies have reported on the ability to mitigate these artifacts by applying dental cement to reduce the relative motion between sensors and specimen. We have developed a method to estimate the ICP artifact size, which allows to evaluate the effectiveness of artifact mitigation techniques, most notably by enabling the estimation of the remaining artifacts in ICP recordings.
Methods: Ten fresh frozen human temporal bones were used to perform laser Doppler vibrometry (LDV) and ICP measurements during vibratory stimulation (tests approved by local ethics committee). ICP artifact estimation was made possible by measuring the transfer function from relative motion to ICP while vibrating the ICP sensors from 0.1–10 kHz. LDV and ICP measurements were performed both before and after application of glue (cyanoacrylate gel) around the cochleostomies that were used to insert the sensors into the cochlea. Evaluation of the effectiveness of the glue in the mitigation of artifacts was carried out in the last five of the ten used temporal bones by measuring relative motion and ICP during bone-conduction (BC) stimulation with a modified OSI200 actuator (Cochlear™ Osia® System) after application of glue. The size of ICP artifacts — estimated from the

relative motion and the previously calculated transfer function — were then compared with the total size of the measured ICP.

Results: As expected, application of glue to cochleostomies reduces both relative motion and the ICP artifact measured while vibrating the sensors. Importantly, there seems to be only a minor effect of glue application on the transfer function. At frequencies above approx. 500 Hz, the ICP measured during BC stimulation was clearly above the estimated artifact by 10 to 40 dB.

Conclusions: Our results confirm the efficacy of artifact mitigation at mid-range and high frequencies based on the reduction of relative motion between sensors and the specimen. Moreover, as the transfer function is insensitive to the application of glue, we here describe a new method for artifact estimation that can be applied regardless of having attempted to reduce relative motion. This method represents, therefore, a valuable addition to the toolkit of bench tests of BCI hearing level output.

Reduction of the Distance Between a Bone Conduction Actuator and the Ear Canal Increases the Induced Vibrations at the Level of the Cochlea

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Background: In the past years, several active transcutaneous bone conduction implants have been introduced to the market. These devices not only provide several advantages with respect to the risk of skin infections and aesthetics compared to percutaneous solutions, but they also offer the opportunity to implant the device more closely to the external ear and by extension the cochlea. The presented research investigates the importance of the stimulation location on the induced cochlear promontory vibration and differential intracochlear pressure for the Cochlear™ Osia® OSI200 actuator.

Methods: Five human cadaveric heads were implanted with a transcutaneous OSI200 actuator at three different implant locations: (1) the recommended surgical position, (2) the recommended position for single-sided deafness and (3) the standard position for percutaneous solutions. These distances are taken slightly larger than the recommended values to accommodate the enlarged tympanotomy required to access the middle ear cavity. At each location, a bone screw was implanted to couple the actuator to the anatomy.

In each specimen, the vibration of the promontory was measured simultaneous with the intracochlear sound pressure level when applying a stimulation level of 60 dB HL with the actuator at 8 different frequencies. Signals were acquired using lock-in amplifiers.

To investigate a potential correlation between the device location and the induced vibration or pressure, a Spearman correlation was calculated.

Results: Valid results could be obtained in four out of the five heads. Promontory velocities were obtained ranging between 10^{-3} - 10^{-1} mm/s and differential pressures between 80-120 dB rel. 20 µPa depending on the signal frequency and stimulation location.

The promontory velocity decreased in function of the implant distance to the external ear canal and showed a significant correlation in 3 of the 4 heads and on the group level. For the fourth head a p-value of 0.059 was obtained. Spearman correlation factors between 0.34 and 0.58 were obtained, indicating a moderate to strong correlation between both factors. For the differential pressure, similar observations could be made, yet no significant correlation coefficients could be obtained.

Despite the limited sample size used in the reported experiment, a significant correlation could be obtained on the group level for the promontory vibration, underlining the importance the device location. As an additional measure, the differential intracochlear sound pressure was obtained, which showed similar trends. Prior studies have indicated that increased promontory vibration and differential pressure would lead to a higher sound perception.

Conclusions: The promontory velocity induced by a bone conduction actuator is negatively correlated with the device's location with respect to the external ear. Results show that when the distance between the device and the external ear canal is increased, the vibrational amplitude reduces, both at the individual level and the group level.

An Absorbing Film can Enhance Optoacoustically Induced Auditory Brainstem Responses in the Mouse Model

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Background: Optoacoustic stimulation offers a great opportunity as an alternative stimulation strategy for the hearing organ. To be used for a novel auditory prosthesis, the optoacoustic stimulation must be safe regarding biocompatibility and at the same time economic regarding energy consumption. Strategies enhancing the efficiency of optical stimulation are therefore needed to be explored in suited animal models. Previously we have reported that mice are a better-suited animal model than guinea pigs for studies regarding biocompatibility due to the better availability of antibodies for this animal model compared to bigger mammals. We herein sought to assess if a self-adhesive absorbing film applied on the TM before the optoacoustic stimulation could enhance oABRs response in a mouse model.
Methods: Optoacoustically induced auditory brainstem responses (oABR) were recorded during irradiation of the tympanic membrane (TM) with pulsed laser light. Measurements were performed, before and after the application of a film consisting of self-adhesive absorbing silicone elastomers applied on the TM in 14 female, 6-12 weeks old, anesthetized CBA/J mice. The results were compared additionally with the ABRs induced through acoustic click stimulation.
Results: The oABR wave I demonstrated on average 5 times enhanced amplitudes when using an absorbing film on the TM during optical stimulation in comparison with the stimulation of the bare TM. Using our stimulation strategy with 79 mW laser power, we induced oABR waves in the 50-60% range of the acoustical stimulation reached with 80 dB SPL click stimuli.
Conclusions: We herein confirm that the mouse model can be used for certain work packages needed during research for a new generation of auditory prostheses. Using absorbing films on the TM during optical stimulation considerably enhances oABR wave I amplitude. Future studies regarding the stimulation strategy are needed to further enhance the efficiency of optoacoustic stimulation while reducing energy input within biocompatibility margins.

Contextual Plasticity in Sound Localization Vs. Source Separation
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Background: Contextual plasticity (CP) is a localization aftereffect occurring on the time scale of seconds to minutes. It has been observed as a bias in horizontal sound localization of click target stimuli presented alone, when interleaved with contextual adaptor-target trials in which the adaptor was at a fixed location while the target location varied. The observed bias is always away from the contextual adaptor location, even though the adaptor is not present on the experimental trials. In a previous study [Linkova et al. (2021) ARO Abstract #W30], two experiments examined whether this phenomenon is dependent on engagement of the subject in an active localization task on the contextual trials (Exp. 1), and whether CP is also observed in virtual environments, both reverberant and anechoic (Exp. 2). Here, we examine two hypotheses: 1) that CP is a consequence of the auditory spatial representation adapting to improve source separation at the cost of introducing biases in localization, and 2) that CP is caused by adaptation that aims to utilize the whole spatial representation range for localization when stimuli are presented from a limited range.
Methods: In both experiments, the target stimulus was a 2-ms noise burst (click), while the adaptor was a click train consisting of 12 such clicks. Six target locations were used, ±33, ±22, $\pm11°$ in Exp 1 and ±30, ±20, $\pm10°$ in Exp. 2. Adaptor locations were fixed across block at 0, ±45, or $\pm90°$ in Exp. 1 and 0 or $\pm50°$ in Exp. 2. In addition, baseline blocks contained no adaptors. Subjects responded by using a numerical keypad while seated with their heads supported by a headrest. Virtual environments in Exp. 2 were simulated by using non-individualized HRTFs and BRIRs. For hypothesis #1, response variance and stimulus-response correlation were analyzed. For hypothesis #2, the direction and size of drifts in responses over time were correlated with stimulus distribution.
Results: Variances tended to increase near the adaptor location, not consistent with hypothesis #1. Drifts in responses depended not only on the distribution of the stimuli, but also on the relative location of individual targets re. the adaptor, partially consistent with hypothesis #2.
Conclusions: These results suggest that CP adaptation is caused by a mechanism aimed more at improving source localization than improving source separation.

Differing Bilateral Benefits for Spatial Release From Masking and Sound Localization Accuracy Using Bone Conduction Devices
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Background: Normal binaural hearing facilitates spatial hearing and therefore many everyday listening tasks, such as understanding speech against a backdrop of competing sounds originating from various locations, and localization of sounds. For bone conduction (BC) stimulation, used to alleviate conductive hearing losses, limited transcranial attenuation results in cross-stimulation so that both cochleae are stimulated from the position of the bone conduction transducer and thus may compromise the binaural processing. The aim of this study was to compare spatial hearing by unilateral and bilateral BC stimulation in normal-hearing listeners with simulated bilateral conductive hearing loss.

Methods: Bilateral conductive hearing loss was induced in 25 subjects (mean age = 28.5 years) with air conduction (AC) and BC pure-tone averages across 0.5, 1, 2, and 4 kHz (PTA4) < 5 dB HL. Subjects participated in a speech-in-speech task and a horizontal sound localization task in a within-subject repeated measures design (unilateral and bilateral bone conduction stimulation) using Baha 5 clinical sound processors on a softband. For the speech-in-speech task, the main outcome measure was the threshold for 40% correct speech recognition in co-located (0°) and spatially and symmetrically separated (±30° and ±150°) competing speech conditions. Spatial release from masking was quantified as the difference between co-located and separated thresholds. For the localization task, the main outcome measure was the overall variance in localization accuracy quantified as an Error Index (EI) (0.0 = perfect performance; 1.0 = random performance). Four stimuli providing various spatial cues were used in the sound localization task.

Results: Repeated measures analysis of variance showed a small (mean=0.7 dB) but statistically significant bilateral BC benefit for recognition thresholds of speech in competing speech for both spatial conditions. Spatial release from masking was identical (mean=2.3 dB) for unilateral and bilateral conditions, and significantly different from zero (p < 0.001). Sound localization by unilateral BC was poor across stimuli, and a distinct bilateral BC sound localization benefit existed (p<0.0001) but varied in magnitude across stimuli. The smallest benefit occurred for a stimulus mainly allowing access to interaural time differences (octave-filtered noise, CF=0.5 kHz, mean EI-benefit = 0.21), and the largest benefit occurred for a stimulus mainly allowing access to interaural level cues (octave-filtered noise, CF=4.0 kHz, mean EI-benefit = 0.42).

Conclusions: Results suggest differing bilateral BC benefits for spatial release from masking and horizontal sound localization. Results further suggest that patients with bilateral conductive hearing loss and BC thresholds within the normal range may benefit from a bilateral fitting of BCD, particularly for horizontal localization of sounds.

The Scent of a Female: Female Olfactory Cues Modulate ABR Amplitudes in Male Mice.
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Background: Non-auditory sensory cues can influence auditory brainstem activity through identified neural pathways. Despite the interaction of olfactory and acoustic signals at the level of behavior, there is little evidence that olfactory cues influence auditory activity.

Methods: To investigate this interaction, we measured the auditory brainstem response (ABR) in anesthetized male mice in response to 5 ms harmonic tone bursts with 1 ms rise and fall times and a frequency spacing reflecting female squeaks. ABRs were measured during presentation of the odors of female urine and cat fur through an olfactometer, with a control consisting of constant air flow over distilled water. To account for baseline changes in amplitude over time, we compared the change in amplitude of Waves I-IV between the two ABR measurements immediately prior to odor presentation, to the change in amplitude between the measurements just before and just after odor presentation.

Results: General linear models show that ABR amplitude significantly increased in response to the odor of female urine, but not cat fur. Increases were significant for Wave I, Wave III, and Wave IV, but not Wave II, which was the smallest wave in amplitude. ABR latencies were not significantly altered by either female urine or cat fur.

Conclusions: These findings suggest that social odor cues can alter auditory processing at early stages. Given the lack of support for direct projections from olfactory to auditory regions, these findings further suggest the possibility that odor cues may exert their influence through top-down pathways.

Molecular and Morphological Heterogeneity of Lateral Olivocochlear Neurons

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Background: Olivocochlear neurons (OCNs) provide direct efferent feedback from the superior olivary complex, where their cell bodies reside, to the cochlea. Medial olivocochlear neurons (MOCs) innervate outer hair cells (OHCs) and modulate cochlear gain control. Lateral olivocochlear neurons (LOCs) innervate the peripheral fibers of Type I spiral ganglion neurons (SGNs) below the inner hair cells (IHCs). Collectively, OCNs have been implicated in various auditory functions, including protection against acoustic trauma, attentional modulation, and separation of speech sounds from noise. However, our knowledge of efferent wiring logic in the cochlea remains limited. Clarifying the molecular and anatomical logic of OCNs is necessary in order to better understand how efferents modulate hearing and how they may shape cochlear circuitry during development.

Methods: In order to expand our understanding of OCN development and function, we performed droplet-based single-nucleus RNA-sequencing of ChatCre;Sun1-GFP brainstems from 61 mice at two pre-hearing (P1, P5) and one mature (P26-28) time point. Of the total 45,828 nuclei captured, 1,674 are OCNs, 589 of which are from 32 P26-28 animals. To probe the molecular basis of LOC heterogeneity, we sub-clustered the nuclei from the mature time point, which revealed two distinct subpopulations of LOCs (LOC1 and LOC2).

Results: Relative expression of neuropeptides, like NPY, CGRP, and Ucn, is the main distinguishing factor between the two LOC sub-clusters. Immunohistochemical analysis showed that all of these peptides are expressed in a medial-high to lateral-low gradient in LOCs in the lateral superior olive (LSO), in partially overlapping patterns (e.g., many but not all NPY+ LOCs are also Ucn+). The developmental timing of each peptide's expression pattern in the LSO also differs, indicating a variety of peptidergic identities amongst LOCs.

In order to assess whether LOCs from the LOC1 and LOC2 subpopulations have distinct innervation patterns in the cochlea, we analyzed sparsely-labelled OCN axons in mature cochleae. Low doses of tamoxifen administered at E16.5-E17.5 to RetCreER;Ai140 mice labeled between 1 to ~12 OCNs in a given animal. Cochleae were immunostained for NPY (to indicate LOC subtype identity), synaptophysin, Calb2 (to visualize IHCs and Type I SGN subtypes), and GFP; terminal axons of GFP+ LOCs were reconstructed in Imaris. We identified a wide array of LOC axonal morphologies but found little correlation between NPY levels and anatomical features (e.g., branch number, terminal number, synaptophysin puncta number and colocalization with Calb2+ and Calb2- fibers). Additionally, we found that NPY is distributed heterogeneously within individual LOCs, which extend fibers that often make synaptophysin+ contacts with both Calb2+ and Calb2- SGN fibers.

Conclusions: The apparent lack of selectivity even among cells with diverse morphologies and peptide profiles suggests that LOC innervation of the cochlea is opportunistic, which might allow them to have diverse and wide-ranging effects on cochlear function.

Presynaptic Rac1 is a Key Regulator of Synaptic Transmission in the Mammalian Auditory Brainstem

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Background: Fundamental to hearing is the ability to localize sound sources and detect temporal features of sound. This requires reliable, rapid, and precise synaptic transmission from the initial auditory processing stations to the higher auditory centers. Actin cytoskeleton dynamics are implicated in synaptic transmission and plasticity by regulation multiple stages of the synaptic vesicle (SV) life cycle. Dysregulation of actin cytoskeleton dynamics is linked to neurodevelopmental and neuropsychiatric disorders. Rac1 is a critical regulator of actin signaling cascades. Human Mutations in Rac1 are associated with intellectual disability. Despite the importance of this molecule, its regulation of the temporal dynamics of SV release and replenishment to enable the encoding of sound information is poorly understood. In mammals, the connection between the globular bushy cells (GBCs)

and the medial nucleus of the trapezoid body neurons (MNTB) is critical for encoding sound localization and temporal features of music and communication sounds. The GBC axon forms the calyx of Held, a glutamatergic presynaptic terminal that is the sole input driving AP spiking in the MNTB. The calyx uses fast SV release kinetics to relay the patterns of afferent AP spikes in the cochlear nucleus to the MNTB. This, in turn, results in rapid and precise inhibition of key mono- and binaural cell groups. Thus, understanding the molecular mechanisms regulating synaptic transmission at the calyx of Held is critical to understanding the initial stages of auditory processing

Methods: We used Rac1 flox/flox mice, and injected Helper Dependent Ad vector expressing Cre recombinase into the cochlear nucleus at P14 mice to bypass the developmental stages. Rac1 was conditionally and exclusively deleted in the presynaptic calyx of Held terminal. We utilized electrophysiology, imaging, and transmission electron microscopy (TEM)) at P28-P33, calyces of Held/MNTB synapses.

Results: We found that deletion of Rac1 enhanced the size of EPSCs and increased the release probability with no change in the gross morphology of calyx of Held terminal. Preliminary ultrastructural analysis of active zone morphology indicates that the docked SVs in the active zone were more in number in Knock-out (KO) calyces in comparison to Wild-Type (WT).

Conclusions: Our findings show that the deletion of presynaptic Rac1 changes synaptic strength and plasticity. This suggests that Rac1 plays a critical role in controlling the synaptic transmission and is a critical regulator of actin dynamics that regulate synaptic transmission at high frequency AP firing synapses. Our findings suggest that human mutations in Rac1 may lead to auditory deficits.

Endbulb Morphology in the Cochlear Nucleus of the Naked Mole Rat

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Background: Extensive research using applied sensory deprivation shows that auditory input shapes circuitry in the central auditory system during development. In cats and mice with induced hearing loss, changes in morphology already manifest in the endbulbs of Held in the cochlear nucleus. These changes include flattening of postsynaptic densities (PSD) and increase of PSD area. Exactly how auditory input changes subsequent brain regions is unclear. This project takes advantage of a unique model species, the naked mole-rat (Heterocephalus glaber), which exhibit naturally exhibit elevated auditory thresholds, poor frequency selectivity, and limited ability to localize sound, to examine the influence that auditory input imposes on brain circuitry and specifically the endbulbs of Held in the cochlear nucleus.

Methods: Serial section transmission electron micrographs were used to 3D-reconstruct endbulbs of Held in the cochlear nucleus of naked mole-rats. A total of 54 endbulb profiles from 3 animals were reconstructed. Mitochondria volume fraction and features of synaptic architecture, including number and shape of PSD and number of docked vesicles, were also quantified.

Results: Naked mole-rat endbulbs were similar in size to values reported in other species, including mouse and cat, reaffirming the high degree of evolutionary conservation of these structures. Size and shape of PSDs were not indicative of deafness-associated changes previously reported in cat. A correlation was found between endbulb profile size and number of PSDs. Further work is ongoing to determine how these parameters are related to each other.

Conclusions: Current findings suggest that naked mole-rats, despite their elevated auditory thresholds, do not exhibit changes in synaptic ultrastructure characteristic of congenitally deaf animals. These findings suggest that reduced auditory input has a diminished impact in the cochlear nucleus of naked mole-rats or that central mechanisms compensate for the loss of auditory input. These findings could help identify potential mechanisms in the naked mole-rat that could be used to prevent neuroanatomical changes triggered by reduced auditory input in other animals including humans.

Are There Differentially Expressed miRNAs in Sudden Sensorineural Hearing Loss Patients Stable Over Time?

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Background: Sudden sensorineural hearing loss (SSNHL) is an acute unexplained hearing loss that develops over less than 72-hours. Serum levels of some small, non-coding RNAs, microRNAs (miRNAs) are altered within 28 days of the onset of SSNHL compared to normal hearing individuals. This study determines if these changes persist by comparing the miRNA expression profile in the serum of SSNHL patients within 1 month of hearing loss onset with that of patients 3-12 months after hearing loss onset.

Methods: Serum was collected from consenting adult SSNHL patients at presentation or during subsequent clinical follow-up. Patient samples drawn 3-12 months after the onset of hearing loss were matched by age and sex to samples drawn from patients presenting within 28 days of hearing loss onset. Total RNA including miRNAs was extracted from 200μl aliquots of pooled sera of all groups using miRNeasy Mini Kit (Qiagen, Toronto, ON, Canada). After total RNA extraction, Reverse transcription (RT) was undertaken with a TaqMan cDNA synthesis kit utilizing a preamplification step followed by TaqMan™ MicroRNA quantitative real-time PCR. miRNA expression level was measured using threshold cycle values normalized to a global mean ($2-\Delta\Delta Ct$) at a cut off level<32. miRNAs with fold changes >2.0 or< 0.5 compared to normal hearing controls based on previous work were studied. Inter-group mean expression levels of the miRNAs of interest were statistically compared by student's t-test using SPSS version 26.

Results: 4 Serum drawn from 4 (3 male) SSNHL patients' (mean age 70 years, Std. deviation 16.99) 3-12 months after hearing loss onset (delayed group) and that drawn from 8 (6 male) SSNHL patient (mean age 54 years, Std. deviation 20.46) within 28 dates of hearing loss onset (immediate group) were analyzed for miRNA expression levels. The mean expression levels of miR -132-3p, -18-5p and -128-3p 3.825, 0.255 and 2.297 in the delayed group and 4.830, 3.539 and 2.465 in the immediate group were similar.

Conclusions: There was no evidence of a change in serum mean miRNA expression profiles of SSNHL over time.

Chronic Traumatic Encephalopathy: Histopathological Findings in the Central Auditory Pathway

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Background: Chronic traumatic encephalopathy (CTE) is a neurodegenerative disease associated with repeated exposure to concussive head trauma. Auditory symptoms have been anecdotally associated with CTE; however, the precise pathophysiology remains unknown. Herein, we aim to investigate if neuropathological changes occur along the central auditory pathway (CAP) in postmortem patients with confirmed diagnosis of CTE.

Methods: Specimens were obtained from Boston University Brain Bank. A total of 38 brains from patients with a confirmed neuropathological diagnosis of CTE (fourteen early stage and twelve late stage) were evaluated by light microscopy. Twelve additional brains from cognitively intact individuals without history of mild repetitive head injury were used as controls. Specimens were immunohistochemically assessed for presence and quantification of hyperphosphorylated tau (p-tau) density and/or neurofibrillary tangles (NFTs) in the auditory cortex and inferior colliculus (IC).

Results: Neuropathological changes were found along the CAP in patients with CTE. NFT density in the gray matter of the auditory cortex and Heschl gyrus was greater in patients with late stage CTE than controls ($p<.001$ and $p<.001$, respectively). NFT density in the gray matter of the auditory cortex was also greater in later stages ($p=.023$). Late stage CTE patients presented the greatest NFT density in the IC, with a significant difference compared to controls and early-stage patients ($p=.015$ and $p=.027$, respectively). There was a significant positive correlation between NFT density and stage of CTE in the gray matter of the auditory cortex, Heschl gyrus and IC ($r= .799$, $p<0.001$, $r= .721$, $p< .001$ and $r= .476$, $p= .003$, respectively).

Conclusions: This is the first histopathological study to examine the CAP in individuals with CTE. Neuropathological changes in the CAP were found in patients with CTE, with later stages of CTE appearing to have increased deposition of tau compared to earlier stages. Further studies are warranted with a larger number of specimens to better characterize neuropathologic change at each stage.

Analysis of Clinical Issues and Outcomes in Medical Device Reporting in Implantable Bone Conduction Hearing Aids

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Background: Implantable bone conduction hearing aids (BCHA) are surgically implanted devices worn by individuals with single-sided deafness or chronic conductive/mixed hearing loss. These devices bypass the outer and middle ear and stimulate the cochlea directly through bone conduction. Unlike typical hearing aids, implantable BCHAs have either a percutaneous or transcutaneous surgically implanted component. After a post-surgical period of osseointegration, an external hearing aid is attached to a titanium post or magnet. Due to their relatively simple surgery and effectiveness in restoring hearing sensation, these devices have become increasingly common in both children and adults.

Methods: FDA receives device-related issues from different sources, including mandatory reporting entities such as device manufacturers, importers and user facilities, and voluntary reporters such as clinicians and patients. Medical device report (MDR) analysis may reveal device associated trends that reflect potential safety signals. MDRs provide information including, but not limited to patient age, patient sex, type of implant, event description of the presenting clinical issue and outcome and actions taken by the clinician or manufacturer (if any). MDR analysis was conducted to categorize the type and range of clinical issues and outcomes. MDRs for implantable BCHAs (excluding active implantable BCHAs) submitted between January 1st, 2017, and September 8th, 2021 (~4 years) were retrieved from the Manufacturer and User Facility Device Experience (MAUDE) database for analysis. Duplicate reports were removed.

Results: Bacterial infections and skin issues (irritation, swelling, erosion) are common clinical issues observed in implantable bone conduction hearing aid device users. Other clinical issues observed include trauma, pain, and lack of benefit. Patient age ranged from 4 months to 94 years (Mean = 44.17 years, SD =23.77 years). Several "clinical issue" and "outcome" categories were created based on the event description. Of these 2903 MDRs, 1497 (52%) referenced infection (e.g., bacterial, abscess, cellulitis), 422 referred to skin issues (14.54%), 224 reported pain (7.72%), and 116 (4%) referred to loss of osseointegration. For the outcome category, there were 2277 (78.44%) explants, 309 (10.64%) revision surgeries, 240 (8.27%) cases resolved with medical treatment (e.g., oral/topical antibiotics, steroids, or both), 29 ongoing/unresolved cases (1%), 48 (1.65%) with an unknown outcome, and 16 patients became non-users due to lack of benefit.

Conclusions: Bacterial infection resulting in ex-plantation and skin issues resulting in revision surgeries were the most common trends observed. These issues may occur due to the abutment or magnet extrusion, or poor management and/or tissue weakness at the implant site. Knowledge about the type and frequency of MDRs may help clinicians counsel their patients on benefits and risks of implantable BCHAs as a treatment option. In particular, caregivers of young children should be clearly instructed on the management of the post-surgical site to help address the above clinical issues associated with implantable BCHAs.

Histopathology of the Tongue in a Hamster Model of COVID-19

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Background: SARS-CoV-2 infection in humans causes anosmia and ageusia with incidence rates of 38.2% and 36.6% respectively. The ACE2 receptor, the primary point of entry for SARS-CoV-2, resides in the tongue's epithelium and in type 2 taste cells. Patients often lose bitter and sweet tastes, which are sensed by type 2 taste cells and are identified with foods such as chocolate and coffee. Receptors for bitter taste are primarily located in the posterior tongue whereas the receptors for sweet taste are primarily located in the anterior tongue. Our objective was to investigate the location of the SARS-CoV-2 virus within the tongue over the course of the infection.

Methods: Golden Syrian hamsters were inoculated intranasally with SARS-CoV-2 virus or vehicle. Whole tongues were collected at 2, 3, 5, 8, 17, 21, 35, and 42 days post infection (dpi) for analysis. After fixation with 10% formaldehyde, tongues were embedded in paraffin, thin sectioned and stained for H and E or labeled with SARS-CoV-2 NP antibody.

Results: There was no significant change in gross appearance of the tongue at any time point. Consistent and marked labeling of the SARS-CoV-2 antigen was present in the circumvallate papillae taste buds from dpi 2 - dpi42 and autonomic ganglia from dpi2 - dpi42. Weaker labeling was observed in the serous microsalivary glands of the posterior tongue from dpi3 - dpi35.

Conclusions: Our findings suggest that the SARS-CoV-2 virus preferentially infects the circumvallate papillae taste buds, potentially acting as a mechanism for causing loss of taste. This effect could be enhanced by a diminished secretion of saliva caused by infection of the serous microsalivary glands and the autonomic ganglia which innervate them.

Localization of Human Thyroid Stem/Progenitor Cells
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Background: Induced pluripotent stem cells and embryonic stem cells have become prominent in medical research. Specifically, studies have reported the use of induced pluripotent stem cells and embryonic stem cells in thyroid regeneration. However, there are only few reports on thyroid regeneration focusing on adult stem cells that exist in the organ itself. Recently, we examined genetically modified mice with fast turnover of thyroid follicular cells and found a cluster of cells positive for NKX2-1, weakly positive for PAX8, and negative for E-cadherin in the thyroid gland, whose properties indicate their role as stem/progenitor cells that inhabit the thyroid tissue. The clustered cells found in this genetically modified mouse are characterized by their localization and are present in a thin layer lining the tracheal cartilage and muscle. Total laryngectomy or pharyngolaryngectomy is a procedure performed in cases of advanced stage laryngeal or hypopharyngeal cancer. During these surgical treatments, the thyroid gland on the affected side is often removed concurrently; that is, the normal thyroid gland is excised with the surrounding laryngeal cartilage and tracheal cartilage. In this study, we examined surgical samples, including thyroid glands, in detail to determine the presence of stem/progenitor cells as previously reported in mice.
Methods: Sixty-nine surgical samples were obtained from total laryngectomy or pharyngolaryngectomy performed as treatment for laryngeal and hypopharyngeal cancers at the Department of Otorhinolaryngology/Head and Neck Surgery, Hamamatsu University School of Medicine (Hamamatsu, Shizuoka, Japan) between 2016 and 2019.
The appropriate pathological paraffin blocks were selected that were suitable for analysis based on the hematoxylin and eosin (HE)-stained sections, and continuous sections were created. Immunostaining was performed in the order of E-cadherin, NKX2-1, and PAX8, and the presence of clustered cells with the following characteristics was examined: NKX2-1, positive; E-cadherin, negative; and PAX8, weakly positive.
Results: Of the 69 cases examined, continuous sections were generated from 73 paraffin blocks for a total of 51 cases that contained thyroid gland and tracheal and laryngeal cartilage, whereupon immunostaining was performed. After detailed analysis, one case was found to present a cluster of cells without follicle formation that was similar to those found in the genetically modified mice, which was NKX2-1-positive, weakly PAX8-positive, and E-cadherin-negative.
Conclusions: We found the presence of such stem/progenitor cells in one examined case. The results could facilitate the progression of human thyroid adult stem cell research. It may also be possible to regenerate thyroid tissue from patients' cells in situ, or by extracting these clustered cells, culturing them, and putting them back to the patient. This type of treatment would greatly improve the quality of life for patients who currently require life-long thyroid drug treatment owing to total thyroidectomy or hypothyroidism.

Coordinated Neuron and Astrocyte Activity within Isofrequency Zones Promotes Cellular Maturation in the Developing Auditory System
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Background: Neurons within isofrequency lamina of the developing auditory system fire correlated bursts of action potentials prior to hearing onset. This robust, intrinsically generated activity is important for both neuron survival and circuit refinement. Astrocytes secrete factors that promote synapse maturation, are critical for synaptic function, and mature in parallel with neurons in the developing brain; however, little is known about the mechanisms that coordinate the maturation of astrocytes and neurons during this developmental period.
Methods: We used in vivo widefield and two-photon imaging of the inferior colliculus and auditory cortex of awake pups before the onset of hearing, which expressed genetically encoded calcium indicators in astrocytes and/or neurons.

Results: Using in vivo calcium imaging in awake neonatal mice, we show that bursts of neuronal activity passing through sound processing networks in the midbrain and cortex induce calcium transients in astrocytes before the onset of hearing. Astrocyte transients were dependent on high levels of neuronal activity and were constrained to regions near active synapses, ensuring close spatial and temporal coordination of neuron and astrocyte activity. Using in vivo pharmacology and astrocyte-specific genetic manipulations, we determined that astrocyte responses were induced by the synergistic activation of two metabotropic glutamate receptors, mGluR5 and mGluR3, which promoted IP3R2-dependent calcium release from intracellular stores. This spatially and temporally coordinated neuron-astrocyte activity was restricted to the pre-hearing period, as sound induced activation of neurons in auditory cortex did not elicit calcium increases in nearby astrocytes, consistent with the developmental decline in expression of mGluR5 by astrocytes. Single-nucleus RNA sequencing of the auditory midbrain isolated from astrocyte-specific mGluR5 knockout mice just prior to hearing onset revealed that selective suppression of astrocyte calcium signaling led to a decline in expression of genes involved in morphogenesis and functional maturation of both neurons and astrocytes.

Conclusions: Together, these results indicate that periodic increases in astrocyte calcium induced by neuronal burst firing during the pre-hearing period promotes the structural and functional maturation of sound processing circuits.

Using Ear Transplantations as a Novel Therapeutic Approach
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Background: Loss of inner ear hair cells and/or inner ear afferents results in hearing and balance defects. Current attempts at hearing restoration focus mostly on hair cells and their innervation, with less emphasis on how to reestablish correct central connections of regenerated neurons. As a novel approach toward restoration of lost central connections, we focus on connecting inner ear auditory and vestibular afferents of transplanted otic vesicles with their respective brainstem nuclei. Our previous studies in frogs and chickens demonstrated that inner ear afferents from transplanted ears can navigate along existing nerves to reach the hindbrain and reroute to innervate appropriate target nuclei. However, whether transplanted ears can develop and send axons centrally in mammals has not yet been determined.

Methods: Otic vesicles were transplanted from embryonic day 9.5-10.5 Thy1-YFP mice into early postnatal wild type mice (P7-P10). Otic vesicles were transplanted either adjacent to the facial nerve or within the vestibule. Mice were sacrificed approximately 1.5 to 3.5 weeks post-surgery. Antibodies were used to label neurons (tubulin, neurofilament) and hair cells (myoVI, myoVIIa) to determine the degree of neurosensory development. In addition, the ability for sensory afferents to grow along host nerves was assessed by Thy1-YFP fluorescence from the transplanted ears.

Results: Ears transplanted adjacent to the facial nerve or within the vestibule developed beyond the otocyst stage; however, ears transplanted adjacent to the facial nerve were typically more developed than those transplanted within the vestibule. Transplanted ears were encapsulated in cartilage and developed various structures, such as canals and ducts. Using antibodies to label neurons and hair cells, we demonstrated patches of neurosensory epithelia in these ears. Hair cells developed within these patches of sensory epithelia and were innervated by sensory neurons. Furthermore, YFP-positive neurons from transplanted ears projected along the facial nerve.

Conclusions: Our findings demonstrate that otic vesicle transplantation into a mouse results in a viable ear that develops hair cells and neurons. Though afferents from the transplanted ears can project along host cranial nerves, further work is needed to assess the ability of these afferents to reach the cochlear and vestibular nuclei, as shown previously in frogs and chicken. Collectively these data suggest that ear replacement strategies may indeed function as a novel therapeutic approach in mammals.

Dio3-Cre and Dio2-Cre Knockin Alleles Identify Deiodinase-Expressing Cell Types That Control Thyroid Hormone Levels in Cochlear Development
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Background: Thyroid hormone is required for cochlear maturation and auditory development. In addition to adequate thyroid hormone in the circulation, we have previously shown that the cochlea auto-regulates levels of the active form of thyroid hormone (T3, triiodothyronine) by the metabolic action of type 3 (Dio3) and type 2

(Dio2) deiodinases that deplete and amplify T3, respectively. Loss of either Dio3 or Dio2 results in deafness and phenotypes of premature and retarded development of the organ of Corti, respectively. Thus, cell types that express Dio3 and Dio2 are critical for control of cochlear development but are poorly defined because of the transient, low-level expression of the enzymes and lack of reliable antibodies.

Methods: We derived knockin mouse strains that express Cre recombinase from the endogenous Dio2 and Dio3 genes. Deiodinase-positive cells are sensitively detected by Cre-mediated activation of a readily-visualized fluorescent marker when crossed to Ai6[GFP] reporter mice.

Results: Dio3 and Dio2 were expressed in inverse developmental patterns with Dio3 expressed at embryonic and neonatal stages before Dio2 is induced in the second postnatal week before onset of hearing. Dio3 was detected in several immature tissues whereas Dio2 was expressed in highly vascularized tissues in fibrocytes in the spiral ligament and in the spiral limbus. Dio2 was also prominent in osteoblasts in the otic capsule but not in the organ of Corti, suggesting that supporting tissues amplify and release T3 internally.

Conclusions: Dio3-Cre and Dio2-Cre models overcome technical obstacles to allow specific detection of deiodinase-expressing cell types in cochlear development. The enzymes are expressed in inverse developmental patterns in distinct cell types, suggesting a dynamic and strict paracrine-like control over T3 levels within the cochlea. The early expression of Dio3 suggests a need to limit exposure of immature tissues to T3.

Follistatin Cooperates With Sonic Hedgehog Signaling to Specify the Apical Cochlea Responsible for Low Frequency Hearing

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Background: The tonotopy is a special topographical arrangement of the auditory system that facilitates sound frequency discrimination. Various morphological and physiological characteristics of sensory and non-sensory components of the cochlea have been reported to be associated with tonotopy. However, how tonotopy is established is just beginning to be understood. Sonic hedgehog (SHH) signaling gradient has been shown to initiate the establishment of tonotopy by specifying regional identities along the embryonic cochlea in both birds and mammals. Although BMP7 and retinoic acid have been identified as downstream effectors mediating SHH function in tonotopic organization in birds, how SHH exerts its role in mammals remains elusive.

Methods: To test the hypothesis that follistatin (FST), an antagonist of TGFβ signaling, mediates SHH signaling to establish tonotopy, the cochleae of loss (Fst-/- or Pax2-Cre; Fstlox/lox) or gain (R26-FST) of FST function mutants along with loss (Smolox/lox) or gain (SmoM2) of SHH function mutant mice were analyzed for gene expression pattern, cell lineage, hair cell morphology, and hearing function.

Results: In Fst-/- cochlea, Msx1 expression at the apex is normal at E11.75 but is completely downregulated by E15.5. Other apical cochlear markers such as Slitrk3 and Efnb2 but not basal markers are also downregulated in Fst-/- cochlea. In addition, apical identity cannot be induced by constitutive SHH activation in the absence of FST in E14.5 Pax2-Cre; SmoM2; Fstlox/lox cochlea. However, overexpression of FST (R26-FST) is unable to promote apical identity, and removal of late SHH signaling (Emx2-Cre; Smolox/lox) fails to maintain apical identity although Fst expression is maintained. These results suggest that FST alone is not sufficient but cooperates with SHH signaling to induce and maintain apical identity. To follow the lineage of apical cells in Fst-/- cochlea, we fate-mapped Msx1-positive cells using Msx1CreERT2/+; R26-tdTomato with or without Fst-/- allele. Apical cochlear cell population was greatly expanded in control but was failed to expand in Fst-/- cochlea. Consistent with abnormal apical specification in the embryonic cochlea, analyses of the global gene expression profiles and various tonotopic features of hair cell morphology reveals that apical cochlear region is absent in the mature cochlea of Pax2-Cre; Fstlox/lox mice at 4 weeks. These molecular and morphological changes in the apical cochlea results in low-frequency specific hearing loss.

Conclusions: Taken together, our results indicate that FST cooperates with SHH to establish tonotopy by specifying and maintaining the apical cochlea responsible for low-frequency hearing in mammals.

The Role of Shank2 in the Establishment of Cell-Intrinsic Polarity in Cochlear Hair Cells

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Background: Auditory hair cells of the mammalian cochlea have a staircase arrangement of the stereocilia filled with actin filament on the apical surface. During development, kinocilium, the primary cilium of hair cell, moves from the center to the lateral pole, and the V-shaped hair bundles of outer hair cells have a vertex pointing to the kinocilium. This machinery is referred to as cell-intrinsic polarity. The establishment of the intrinsic polarity has been shown to be regulated by GPSM2 and Gαi, which are located the lateral domains of the apical surface of hair cells. However, the potential players in the medial domain of hair cell surface remain largely unknown. In addition, the role of intrinsic polarity in hearing function is difficult to determine because proteins regulating intrinsic polarity also play other roles such as stereocilia elongation. SHANK2 is a multidomain-scaffolding protein implicated in the structural and functional coordination of multiprotein complexes at excitatory postsynaptic density in the brain. Recently, SHANK2 has been identified to interact with aPKC and play a critical role in the tight junction formation in epithelial cells.

Methods: Temporal and spatial expression patterns of Shank2 and several genes and proteins related to intrinsic polarity were analyzed using in situ hybridization and immunofluorescence. Hair bundle morphology and hair cell orientation were determined using scanning electron microscopy (SEM) and immunofluorescence. Hearing function is assessed by auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE).

Results: Shank2 mRNAs are expressed in the differentiating hair cells and spiral ganglion neurons. In the hair cells, SHANK2 proteins are restricted to the medial apical surface. Shank2-/- mice have disorganized hair bundles with normal kinociliary localization at the lateral pole, indicating defective intrinsic but not tissue-level polarity. Shank2-/- mice suffer from progressive hearing loss especially at mid-high frequencies. To analyze the cause of hearing loss in Shank2-/- mice, we compared auditory phenotypes of Shank2-/- with hair cell-specific (Gfi1-Cre; Shank2lox/lox) or spiral ganglion neuron (SGN)-specific (Bhlhe22-Cre; Shank2lox/lox) conditional knockout mice. We observed that Gfi1-Cre; Shank2lox/lox mice exhibited almost identical phenotypes of hearing loss and hair bundle defects as Shank2-/- mice. In contrast, stereociliary morphologies and hearing function were unaffected in Bhlhe22-Cre; Shank2lox/lox mice. We are currently investigating the molecular mechanisms and interacting partners of SHANK2 to contribute to intrinsic polarity.

Conclusions: These results suggest that SHANK2 expressed in the medial apical surface of hair cells plays a crucial role in the establishment of intrinsic polarity essential for normal stereociliary organization and hearing function.

Regulation of Cochlear Supporting Cell Fate by FGF/MAPK Signaling
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Background: We showed previously that mice modeling Muenke syndrome (Fgfr3P244R/+) have hearing loss associated with supporting cell (SC) fate transformations of two Deiters' cells (DCs) to two outer pillar cells (OPCs), which convert sequentially between E17.5 and P3. Removing one copy of the gene encoding the FGFR2b/1b ligand, FGF10, which does not normally interact with FGFR3, but which is expressed in lateral Kolliker's organ and spiral ganglion neurons, restores both SC fates and hearing. This occurs because the mutation changes FGFR3 binding specificity, allowing activation by FGF10. However, removing the copy of Fgf10 does not block the SC fate change, which occurs on schedule in Fgfr3P244R/+;Fgf10-/+ mice, but rather, it allows subsequent reversion of incorrect fates to normal, an interesting example of cochlear cell fate plasticity.

Methods: We asked whether initiation of Fgfr3P244R/+ phenotypes could be blocked by inhibiting FGF10 function. Since FGF10 has many critical functions in the developing otocyst and embryo, we used doxycycline-induced ubiquitous expression of a secreted dominant-negative FGFR2b ectodomain (dnFGFR2b) that acts rapidly and reversibly as an extracellular ligand trap to sequester FGF10 and block signaling through its receptors.

Results: By modulating the timing of ubiquitous dnFGFR2b induction we found that the Fgfr3P244R/+ SC fate transformations could be completely blocked, and that there is a critical period (E17.5-E18.5) that is most effective. To reduce off-target effects of dnFGFR2b, we limited its induction domain using several CRE drivers. We found that epithelial-restricted induction of dnFGFR2b blocked the SC fate transformations, suggesting that ganglion-expressed FGFR2b ligands have little contribution to activation of FGFR3P244R. However, blocking the Fgfr3P244R/+ SC fate transformations did not restore hearing. Indeed, Fgfr3+/+ mice expressing dnFGFR2b also had hearing loss, which correlated with loss of the outer sulcus.

Since Fgfr3P244R/+ cochleae have elevated expression of FGF/RAS/MAPK target genes during the period of the SC fate transformations, we asked whether ectopic activation of the RAS/MAPK pathway in wildtype SCs could induce DC-to-OPC fate transformations. Thus, we generated and analyzed mice compound heterozygous for Tg(Fgfr3-icre/ERT2), which drives CreERT2 in DCs and PCs, or Sox2CreERT2, which drives CreERT2 in a broader domain of SCs, and Tg(cLGLKrasG12V), allowing CRE-dependent activation of highly active KRAS. Early postnatal induction of either CreERT2 driver activated MAPK targets in CRE-expressing cells, and both drivers induced SC fate transformations, with Sox2CreERT much more effective than Tg(Fgfr3-icre/ERT2). The former phenotype was very similar to that of Fgfr3P244R/+ mice, but did not include induction of ectopic OHCs, suggesting that this Fgfr3P244R/+ phenotype requires earlier activation of FGFR3 signaling.

Conclusions: In summary, the levels, timing and location of FGF/RAS/MAPK signaling all contribute to SC differentiation, and these details should guide therapeutic approaches to Muenke syndrome hearing loss or other desired SC fate manipulations.

FGFR2b/1b Ligands Are Required for Both Cochlear Epithelial and Ganglion Development During Mid-Gestation

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Background: The genes encoding FGF3 and FGF10, Fibroblast Growth Factor ligands that signal through FGF receptor tyrosine kinases FGFR2b and FGFR1b, are expressed dynamically throughout otic morphogenesis in both epithelial and ganglion domains. They are required individually for normal otic morphogenesis, but until recently, their early and redundant roles in otic placode induction complicated investigation of subsequent combinatorial functions in cochlear epithelial morphogenesis and gangliogenesis.

Methods: To interrogate Fgf3 and Fgf10 functions after otic placode induction we use doxycycline-induced ubiquitous expression of a secreted dominant-negative FGFR2b ectodomain (dnFGFR2b) that acts rapidly as an extracellular ligand trap to sequester FGF3 and FGF10 and block signaling through both FGFR2b and FGFR1b. Our previous studies of temporally regulated dnFGFR2b induction showed that FGFR2b/1b signaling is required for early otocyst epithelial proliferation and continuously for gross otic morphogenesis. However, the roles of this signaling at later stages, and how and from which tissues the signaling impacts cochlear epithelial and ganglion development after initial otocyst formation and patterning are unknown.

Results: We now find that inducing dnFGFR2b globally from E11.5-E18.5 blocks specification of two cochlear non-sensory domains, Reissner's membrane and the outer sulcus, similarly to mutating Fgf10 alone. Unexpectedly, and in contrast to Fgf10 null mutants, we find that the cochlear sensory epithelium and ganglion are also profoundly disrupted; hair cells are missing, delayed in differentiation and in some cases misoriented. Multiple inner pillar cells may be seen, but outer pillar cells are absent. The spiral ganglion is reduced and composed of dispersed clumps. This suggests important new roles for both Fgf10 and Fgf3 in regulating cochlear sensory and neural development during mid-gestation.

To localize the signaling tissues responsible for the various effects of dnFGFR2b, we used CRE drivers to limit its expression. We find that restricting dnFGFR2b induction to the Tg(Pax2-cre) lineage (epithelium and ganglion) is less disruptive than inducing it globally, but still blocks differentiation of the non-sensory tissues, affects supporting cells and reduces the cochlear ganglion. Given that global inhibition of FGFR2b ligands is more severe, we suggest a novel role for mesenchymal FGFR2b/1b signaling. Indeed, restricting dnFGFR2b induction to the mesenchyme using Mesp1Cre has strong effects on both supporting cells and the ganglion. Surprisingly, activating dnFGFR2b separately in either the Emx2Cre (epithelial) or Bhlhb5Cre (ganglion) domains mainly affects cochlear non-sensory epithelial differentiation, but not the ganglion itself.

Conclusions: We are in the process of probing the timing and mechansims of the effects of FGFR2b/1b ligands on shaping cochlear development and will present our latest findings.

SMAD4-Dependent Pathway Patterns the Lateral Axis of Sensory and Non-Sensory Domains in the Developing Cochlea

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Background: The cochlear duct is patterned and divided into five distinct regions along the medial-lateral (radial) axis: greater epithelial ridge (GER), sensory domain, lesser epithelial ridge (LER) on the floor of the cochlear duct

and Reissner's membrane (RM), stria vascularis (SV) on the roof of the cochlear duct. It was previously suggested that WNT and BMP signals present in the medial and lateral regions, respectively, play a role in the radial patterning during early stages of cochlear development. Loss of the BMP target gene (Id genes) or absence of the BMP receptor (BMPR1A) resulted in an expansion of the medial domain at the expense of the lateral domain. However, the intracellular mediators of the BMP signaling pathway required for cochlear development are unknown. In this study, we tested the hypothesis that BMP signaling in the radial cochlear patterning is mediated by SMAD-dependent canonical TGFβ/BMP pathway.

Methods: To investigate the role of SMAD4 in cochlear radial patterning, we analyzed the developing cochlea of Emx2Cre/+; Smad4f/f mice. Specification of the radial axis and formation of the five distinct regions were determined using in-situ hybridization and whole mount immunostaining.

Results: In the cochlea of Emx2Cre/+; Smad4f/f embryos, expression of Bmp4 (LER) in the lateral cochlear floor and Esrrb, Trp2 and Meis1 (SV) in the lateral cochlear roof were abolished, indicating that the lateral cochlear domain is lost in the absence of SMAD4 function. In contrast, expression of Fgf10 (GER) in the medial floor and Otx2 (RM) in the medial roof were expanded to the lateral domain or ectopically upregulated. These results indicate that the medial domain is induced in the lateral domain in the absence of SMAD4. In addition, the number of cells in the outer sensory compartment labeled with BCL11B (OHC), PROX1 (IPC, OPC, and DC), P75NTR (IPC) were significantly reduced. Conversely, the inner sensory compartment labeled with VGLUT3 (IHC) was greatly increased. These results suggest that SMAD4 plays a role in proper balancing between inner and outer sensory compartments. Interestingly, mirror images of the organ of Corti were appeared in the basal and middle turns of the cochlea. When we examined the location of ARL13b-labeled kinocilium, the hair cells in the ectopic organ of Corti were oriented in the medial direction, as opposed to normal hair cells, which are laterally oriented.

Conclusions: Our results demonstrate that SMAD4-dependent pathway plays a crucial role in the lateral patterning of sensory and non-sensory compartments in both the floor and roof of the cochlear duct.

MyD88 Regulates Expression of TLR4 and TRPV1 at the Plasma Membrane

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Background: Aminoglycosides remain clinically necessary to treat bacterial infections that elicit severe systemic inflammation. Unfortunately, parenteral administration of these drugs can cause permanent hearing loss following entry into mammalian sensory hair cells via non-selective cation channels, such as TMC1, TRPA1 and TRPV1. Bacteriogenic-induced activation of TLR4 (via lipopolysaccharides, LPS) upregulates and sensitizes TRPV1 channels increasing cellular uptake of aminoglycosides and thereby exacerbating hearing loss (Koo et al., 2015; Jiang et al., 2019). LPS binding of TLR4 activates an intracellular signaling cascade via the adaptor protein, MyD88, while the loss of TLR4, TRPV1 or MyD88 activity ameliorates severe inflammation and drug-induced hearing loss. (Koo et al., 2015; Jiang et al., 2019; unpublished data). We hypothesize that MyD88 facilitates internalization of, and/or a conformational change, in plasmalemmal TRPV1 to increase cellular uptake of aminoglycosides.

Methods: Cultured MDCT cells were transfected with MyD88 siRNA, TLR4 siRNA, or with scrambled control siRNA, 24 hours prior to treatment with 1μg/ml LPS. Cells were then: lysed for detection of TLR4, MyD88 and TRPV1 levels by qPCR (n=11) and immunoblot (n=5); treated with fluorescently-tagged gentamicin (GTTR) for 1 minute prior to washing, fixation and assessment of cellular GTTR uptake by confocal microscopy (n=3); or proximal ligation assays (PLA) to assess cellular location of protein-protein interactions between TLR4, MyD88 and TRPV1 (n=3).

Results: The TRPV1 agonist, capsaicin, enhances, while the TRPV1 antagonist capsazepine attenuates, cellular uptake of GTTR, with or without MyD88 or TLR4 knockdown, suggesting that plasmalemmal TRPV1 is functional independent of MyD88 or TLR4 expression. However, after MyD88 knockdown, cells (with or without LPS treatment) had reduced uptake of GTTR compared to their control cells. MyD88 knockdown increased TRPV1 expression that is further elevated by LPS exposure suggesting that MyD88 is required for TRPV1 internalization and degradation. Also, increased MyD88 and TRPV1 protein expression after LPS treatment is attenuated after TLR4 knockdown. In PLA experiments, MyD88-TRPV1 and TLR4-TPRV1 interactions are increased after LPS treatment. Knockdown of MyD88 attenuated LPS-induced degradation of TLR4 expression observed in MyD88+ cells in immunoblots. This suggests that LPS-increased internalization of TLR4 is dependent on MyD88, as previously demonstrated.

Conclusions: Our data suggest that MyD88 traffics a TLR4-TRPV1 complex away from the membrane, while knockdown of MyD88 prevents this internalization - 'trapping' these proteins at the plasma membrane. These results implicate that MyD88 activity is required for inflammation-exacerbated aminoglycoside-induced cytotoxicity and hearing loss.

Hearing in Generation Genome: Comprehensive Newborn Hearing Screening Through Seqaboo (SEQuencing a Baby for an Optimal Outcome)

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Background: Early detection and evaluation of hearing loss in children is clearly associated with improved speech and language outcome. The current newborn hearing screening program (NBHS), while widely implemented, is hampered by an inability to detect certain types of hearing loss as well as a low positive predictive value. Comprehensive NBHS, which incorporates physiologic, genetic, and cCMV screening, has been proposed to improve detection, reduce loss to follow-up, and provide etiologic information sooner to deaf and hard-of-hearing (DHH) newborns' families. Recently, compelling data have shown that incorporating genetic screening in NBHS may positively impact clinical outcomes. SEQaBOO is a program for assessing DHH newborns and evaluating parental attitudes concerning genomic medicine.

Methods: Parents of newborns may enroll in SEQaBOO following a positive NBHS or at confirmatory diagnostic audiometry. The family may enroll in one of two arms: (1) comprehensive genome sequencing and variant interpretation for hearing loss genes in the baby, and optional ACMG secondary findings for parents, as well as cCMV detection, plus annual surveys, or (2) annual SEQaBOO surveys only. Annual surveys collect data on family medical history, health information and evolving attitudes on genomic medicine. Sequencing data and interpretation are possible prior to diagnostic audiometry and have influenced standard-of-care follow up. Confirmatory clinical genetic testing of positive results is required per IRB protocol.

Results: Of 203 families approached, 73% (n=149) enrolled with >50% choosing the genome sequencing arm of the study. Most NBHS referrals are unilateral and pass diagnostic audiometry with negative genetic results. Among SEQaBOO babies ultimately diagnosed as DHH (n=28), a genetic etiology of GJB2 and SLC26A4 variants was reported in six babies. Genome wide copy number variant analysis revealed STRC and OTOA deletions from read count analyses. For 13 babies, genetic diagnoses were inconclusive with only one pathogenic/likely pathogenic variant identified in a recessive gene with or without a second variant classified as a variant of uncertain significance (VUS) or a VUS in a gene associated with dominant DHH. cCMV screening of babies' cord blood confirmed two known cases of cCMV (n=2). Additionally, our platform detected the incidental finding of a chromosomal translocation in one parent and child and can identify absence of heterozygosity. Nine cases have known non-genetic etiologies of the child's DHH. Three ACMG pathogenic secondary findings in adults were disclosed (COL3A1, NF2 and PKP2).

Feedback from parental surveys was positive with 61.5% of parents acknowledging the benefits of receiving genome sequencing results on themselves, 67.6% commenting on benefits to both themselves and their child and 62.6% stating that the genetic sequencing results helped them understand their child's DHH.

Conclusions: Newborn screening has long been driven by technology and can now embrace integration of genome sequencing to provide life-altering treatments and management to DHH infants.

Development of Exon Skipping Strategy to Treat Hearing Loss in USH2A Patients

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Background: Usher syndrome (USH) is the leading cause of inherited deafness and 75% of USH2 are caused by mutations in the USH2A for which no pharmacological treatment is available. We evaluated strategies of genome editing to skip the mutation enriched exon 13 with the production of in-frame shortened USH2A transcripts as

potential editing therapy. We generated human inner ear organoids to derive hair cells from USH2A patient iPSCs and evaluated delivery strategies of lipid nanoparticles (LNPs) and AAVs for editing complex. The combinatory approach will likely provide an avenue to develop therapy for hearing loss in USH2A and other genetic hearing loss.

Methods: CRISPR/Cas9 was used to induce exon skipping in USH2A transcripts. Paired sgRNAs were designed and tested for efficiency by NGS and RT-PCR. The human inner ear organoids were generated by mimicking in vivo ear development. Expression of marker genes was assessed by immunohistochemistry/RT-PCR. Inner ear organoids were tested for delivery by LNP-mRNA/sgRNA and by dual AAVs that carried SpCas9 and sgRNAs.

Results: 1.   Using mouse auditory HEI-OC1 cells, we designed and tested sgRNA pairs for skipping of exon 12 (human USH2A exon 13 equivalent), either flanked the entire exon 12 or the splicing acceptor site. The acceptor targeting sgRNAs showed improved editing efficiency of 60% vs. 23% by exon targeting sgRNAs, resulting in the production of the in-frame Ush2a transcripts in OC1 cells.

2.   Using human WERI-RB1 cells, we designed and tested sgRNA pairs that either flanked USH2A exon 13 or the splicing acceptor site by nucleofection. Acceptor site targeting gRNAs led to exon skipping efficiency of 84%, and 73% in-frame exon 13 skipped USH2A transcripts.

3.   We performed exon skipping in human USH2A patient iPSCs with homozygous c.2299delG mutation using the acceptor site targeting sgRNA pair and obtained an efficiency of 75% exon skipping. By synergistic activation mediator (SAM), we activated USH2A in the iPS cells after exon skipping and detected skipping in 71% of USH2A transcripts.

4.   We derived inner ear organoids from the USH2A patient and control iPSCs with the expression of otic markers. Hair cells were similarly produced from the patient and control iPSCs. Auditory neurons connected to the hair cells were identified in the human inner ear organoids.

Conclusions: Our study identified sgRNA pairs that induce efficient skipping of mouse Ush2a exon 12, and human USH2A exon 13 that harbors the most frequent mutations in USH2A patients. We have established USH2A patient inner ear organoids with the production of hair cells. Our study is an important step towards the development of exon skipping therapy in USH2A patients, which lays a foundation for modelling inner ear diseases by human inner ear organoids and developing editing-based treatments.

Heritability and Genetic Risk Loci in Tinnitus in the Lifelines Cohort Study

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Background: Tinnitus, a disorder in which the phantom perception of sound is heard without an external stimulus, has a prevalence between 5 and 43%. The most important risk factor for tinnitus is hearing loss, but tinnitus is also associated with psychiatric disorders, functional syndromes, and cardiovascular diseases. Studies showed that tinnitus has a moderate heritable component of 6 to 43%. More recently, genome-wide associations studies (GWAS) revealed multiple genetic risk loci for tinnitus and showed that tinnitus was genetically correlated with hearing loss and neuropsychiatric disorders. This study aimed to assess more accurate narrow sense heritability estimates and identify novel risk loci for tinnitus in a large cohort from the Netherlands.

Methods: This study used cross-sectional baseline data from the Lifelines Cohort Study, a multi-disciplinary prospective population-based cohort study examining the health and health-related behaviors of 167,729 persons across three generations living in the northern Netherlands. Data on health-related parameters, including tinnitus, were acquired via self-report questionnaire. Narrow sense heritability estimates (h2) were calculated based on pedigree data using the residual maximum likelihood-based variance decomposition method implemented in the ASreml-R package (v4). In Lifelines approximately 38,500 participants were genotyped using the Infinium GSA MultiEthnic Disease V1 chip. Standard quality control was performed, and genetic imputation was done through the Sanger imputation service. A genome-wide association analysis was performed with the SAIGEgds package (version 1.4.0) that used a mixed model approach to account for sample relatedness. For the h2 estimation we included hearing problems, age, sex as covariates and for the GWAS we also included the top 5 principal components to account for population stratification. Single nucleotide polymorphisms (SNPs) with a minor allele frequency <1% were excluded. Genome-wide significance was set to $P<5e-8$.

Results: Data on tinnitus were available for 122,884 participants, of which 39,128 reported 'any' form of tinnitus (AT) and a subset of the AT group (N= 7,965) reported 'constant' tinnitus (CT). Compared to controls,

participants reporting tinnitus were generally older (43.8 SD 12.6 vs. 47.7 (12.8) years) and were more often male (N= 32637 (39.0%) vs. N= 17385 (44.4%)). The h2 estimates were 8.7% for AT and 8.0% for CT. When not correcting for hearing problems, estimates were 10.3% for AT and 9.5% for CT. The GWAS yielded one genome-wide significant SNP for CT, namely rs75468282, which falls in a coding region of the ARHGAP12 gene. The GWAS for AT revealed one SNP, namely rs10740832, which is in the regulatory region of the SVILP1 gene. **Conclusions:** Using a large cohort not previously used for GWAS to investigate tinnitus, this study found similar h2 estimates for tinnitus as reported previously for other cohorts. Moreover, this study identified two novel risk loci for tinnitus. These results should be replicated using other cohort studies.

Genotype-Phenotype Correlations of Pathogenic Coch Variants in DFNA9: A Huge Systematic Review and Audiometric Meta-Analysis

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Background: Pathogenic missense variants in COCH are associated with DFNA9, an autosomal dominantly inherited type of progressive sensorineural hearing loss with or without vestibular dysfunction. This work presents a systematic review and meta-analysis of all known DFNA9-associated COCH variants and their associated phenotypes.
Methods: We applied PRISMA and HuGENet guidelines in this systematic review. The literature search yielded 48 studies describing the audiovestibular phenotypes of 27 DFNA9-associated variants in COCH. A meta-analysis of audiometric data was performed, and age-related typical audiograms were constructed. We further performed more in-depth analyses on the age of onset and progression of hearing loss.
Results: We present a detailed overview of genotype-phenotype correlations of all currently known pathogenic COCH variants associated with DFNA9. Significant differences were found between the calculated ages of onset and progression of the audiovestibular phenotypes of subjects with pathogenic variants affecting the LCCL domain of cochlin and the vWFA2 and Ivd1 domains.
Conclusions: The audiovestibular phenotypes associated with DFNA9 are highly variable. Variants affecting the LCCL domain of cochlin generally lead to a faster progression of hearing loss when compared to variants affecting the other domains. Take-away: this review serves as a reference for prospective natural history studies in preparation for future therapeutic interventions.

Molecular Diagnosis for Patients Affected by Pendred Syndrome: A New Genotype-Phenotype Correlation and a Novel Candidate Gene

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Background: Pendred syndrome (PDS) is the most common form of syndromic Hearing Loss (HL) and is characterized by elevated clinical heterogeneity and low causative variants detection rates. PDS patients usually manifest sensorineural HL and enlarged vestibular aqueduct (EVA), although other inner ear malformations (e.g. Mondini dysplasia) and goitre, with or without hypothyroidism, can also be present. The syndrome is inherited with an autosomal recessive pattern and the major gene involved is SLC26A4. However, the presence of the "CEVA haplotype" in trans with a pathogenic variant of SLC26A4 or, mutations in FOXI1 or KCNJI0, have also been described. Interestingly, for 25-50% of the patients presumably affected by PDS, only one causative mutation can be identified, suggesting that other genetic contributions remain hidden from the routinely performed diagnostic assays.

Our work aims to: 1) define a molecular diagnosis for a cohort of PDS patients. 2) asses the symptoms consistently present in all the subjects considered 3) identify new candidate gene possibly causative of PDS.
Methods: A carefully selected cohort of 24 patients underwent a detailed phenotypical evaluation, including thyroid functions assessments, radiological and audiological examinations. Subsequently, Whole-Exome Sequencing (WES) was performed, first focusing on genes associated with PDS, but also on other genes causative of HL. Further, we assessed the presence of the twelve variants of the CEVA haplotype and performed Multiplex ligation-dependent probe amplification analyses (MLPA), seeking for insertion/deletion within SLC26A4.
Results: For five patients out of 24 the molecular diagnosis was defined by identifying homozygous/compound heterozygous mutations in SLC26A4 or pathogenic mutation in trans with the CEVA haplotype. On the other hand, five individuals resulted heterozygous for a single mutation. Interestingly, the audiograms of these ten patients presented a characteristic slope at the medium and high frequencies, suggesting an interesting genotype-phenotype correlation. Finally, a compelling homozygous ultra-rare missense variant in MYO5C has been identified in a patient negative for SLC26A4 mutations.
Conclusions: In conclusion, the overall variants detection rate was 41.7%, and a molecular diagnosis was defined for 20.8% of the PDS patients. Further, from our study an interesting genotype-phenotype correlation has emerged providing new insight into PDS. Finally, we propose that MYO5C should be evaluated in other PDS-like subjects to reinforce the hypothesis of a novel candidate gene for PDS.

Single-Cell Long-Read Rna Sequencing of the Inner Ear

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Background: Mammalian genes typically produce multiple transcripts, contributing to transcriptomic and proteomic diversity. However, alternative mRNA isoforms in the inner ear remains unexplored, impeding the exploration of auditory functional diversity and disease process. It is conceivable that the combined existence of multiple splicing isoforms and their dynamic change over time and environmental stress could be the underlying pathophysiological mechanisms that were largely overlooked due to the technical limitations. Thus, the effective identification of these isoforms will provide valuable clues for basic and clinical research on auditory systems.
Methods: To address this need, we integrated 10X Genomics short-read and PacBio long-read RNA sequencing (ScISOr-Seq) techniques and revealed landscape of full-length transcriptome in the cochlea. We then further analyzed the cooccurring alternative splicing events in the newly found novel transcripts, followed by RT-PCR and Sanger sequencing validation. To validate whether protein isoforms were generated from novel transcripts, we performed Mass Spectrometry-based proteomics. Lastly, we investigated the function of this novel isoform using the CRISPR-mediated knockout approach, followed by electrophysiological recording, immunohistochemistry, and transmission electron microscopy.
Results: We identified 173,313 previously unannotated transcripts absent in the current annotation and found 222,580 AS events in the novel transcripts. In addition, we observed that 85.9% of the total novel transcripts had coding ability and further verified their products by using mass spectrometry (MS)-based proteomics, confirming the complexity and diversity of spliced isoforms in the inner ear. To demonstrate the role of splicing isoforms in auditory functions, we analyzed one gene, Otoferlin (Otof), in detail. We generated an otoferlin canonical isoform deletion (Otof-ΔC) animal model to demonstrate that the novel transcript could be translated to a functional protein isoform that participates in the synaptic transmission of IHCs. In addition, we observed that the proportion of the otoferlin isoforms is tightly associated with the synaptopathic hearing impairment, such as hidden hearing loss, noise-induced, and age-related cochlear synaptopathy.
Conclusions: Our work presented a comprehensive view of transcript isoform expression in the inner ear that enabled better understanding of the complex molecular mechanisms underlying auditory function in both physiological and pathogenic states.

Whole Exome Sequencing Shed the Light on the Possibility of Dual Molecular Diagnoses for Unusual Hearing Loss Cases

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Background: Hearing loss (HL) is one of the pathologies that benefited the most from the introduction of Whole Exome Sequencing (WES) into the diagnostic routine. Indeed, HL is not only the most prevalent sensory impairment, but it is also characterized by a high clinical and genetic heterogeneity. To date, ~124 genes have been reported as causative of Non-Syndromic HL (NSHL), and more than 400 syndromes are associated with HL (SHL).
Here we report the definition of dual molecular diagnoses for a series of cases affected by NSHL and other Mendelian conditions caused by mutations at two different loci segregating independently.
Methods: During the last year, we employed a multi-step approach to characterize 102 patients affected by HL. In particular, a deep clinical evaluation was followed by a first round of genetic testing, such as the analysis of GJB2 and the evaluation of STRC copy number variations. Finally, WES was carried out in patients negative to the first analyses.
Results: Our approach allowed the identification of a dual molecular diagnosis in seven families affected by HL. Briefly, both the clinical and genetic findings led to patients' classification into two groups: 1) subjects with distinct phenotypes that can be ascribed to mutations at two loci (Family 1-5), and 2) individuals with two genes involved in the same phenotype (Family 6,7).
For example, Family 1, classified in group 1, displayed Marfan syndrome due to mutation in FBN1, together with distal renal tubular acidosis and HL, caused by two pathogenic mutations in ATP6V1B1.
On the other hand, subjects of Family 6 belong to class two, indeed they manifested HL due to mutations in both USH2A (causative of Usher syndrome) and EYA4 (causative of NSHL).
Conclusions: These results highlight the complex molecular architecture of HL suggesting, for the first time, the importance of considering an alternative scenario, the one of "dual molecular diagnosis". In fact, in our cohort we identified a high percentage of patients who received a conclusive dual molecular diagnosis. Thus, these results suggest that this scenario should be taken into account for HL patients, especially for those presenting with clinical features that do not fit into a known syndrome, or families with elevated intra-familial phenotypic variability.

Optimized Rapid CRISPR/Cas9-Based Method for Modeling Hearing and Vestibular Disorders

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Background: Low-cost next generation sequencing technologies have facilitated many genome-wide association studies (GWAS) and exome sequencing projects that have identified hundreds of variants and genes associated with hearing loss. There are currently more than 150 loci and over 100 genes associated with non-syndromic hearing loss. Human geneticists now face the immense challenge of functionally test these candidate disease genes to understand disease pathophysiology. Identifying which candidate genes or loci are pathologically relevant represents a critical barrier that limits our ability to generate relevant human disease models. Our long-term goal is to understand how gene function contributes to hearing loss in humans.
Methods: Our objective is to functionally validate the candidate genes using zebrafish as a model system. Model organisms have revolutionized our understanding of the human disease; inactivation of candidate human disease genes in animals (i.e. creating gene "knockouts") often triggers analogous phenotypes and provides a valuable disease model. Zebrafish provide an ideal model organism to study hearing loss because of their external embryonic development, transparent body, accessible inner ear, and the presence of lateral line neuromasts (functionally analogous to mechanoreceptors of the mammalian inner ear).
CRISPR/Cas9 based methods are being used to screen for phenotypes in F0 (the founding generation) by generating biallelic mutations; we further optimized this strategy to maximize phenotype penetrance and use this method in a high-throughput manner. We combined this optimized mutagenesis approach with morphological and behavioral phenotyping strategies to identify and genes involved in hearing loss as in inner ear development.
Results: We screened over 100 genes and identified ~30 genes involved in hearing loss and inner ear development.

Conclusions: Our research established a method to screen for genes involved in hearing with limited or no functional information and bridge the gap between discovery of disease gene and their functional validation. Further, we established morphological and behavioral assay that can employed in phenotyping multiple mutants on a large-scale. Our study helps us to understand the function of the target gene and associated disease pathology.

Hearing Features and Cochlear Implantation Outcomes in Patients With Pathogenic MYO15A Variants: A Multi-Center Observational Study

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Background: Recessive variants in the MYO15A gene constitute one of the leading cause of hereditary hearing loss. However, the clinical features of MYO15A-related sensorineural hearing impairment (SNHI) have not been systemically investigated. This study aimed to delineate the hearing features and outcomes in patients with pathogenic MYO15A variants.
Methods: This retrospective study recruited 40 patients with biallelic MYO15A variants from 31 unrelated families. The patients were grouped based on the presence of N-terminal domain variants (N variants). The longitudinal audiological data and for those undergoing cochlear implantation, the auditory and speech performance with cochlear implants, were ascertained and compared between patients with different genotypes.
Results: At the first audiometric examination, 32 patients (80.0%) presented with severe-to-profound SNHI. Patients with at least one allele of the N variant (Group A) exhibited significantly better hearing levels than those with biallelic non-N variants (Group B) (78.2 ± 23.9 dBHL and 94.7 ± 22.8 dBHL, respectively) (p = 0.033). The proportion of patients exhibiting low-frequency residual hearing in group A (64.7%) was significantly higher than that in group B (26.1%) (p = 0.024). Progressive SNHI was observed in 82.4% of patients with non-profound SNHI, in whom the average progression rate of hearing loss was 6.3 ± 4.8 dBHL/year irrespective of the genotypes. Most of the 25 patients who underwent cochlear implantation exhibited favorable auditory and speech performances post-implantation.
Conclusions: The hearing features of patients with biallelic pathogenic MYO15A variants are characterized by severe-to-profound SNHI, rapid hearing progression, and favorable outcomes with cochlear implants. Periodic auditory monitoring is warranted for these patients to enable early intervention.

A Missense Variant in COMT Associated With Hearing Loss Among Young Adults: The National Longitudinal Study of Adolescent to Adult Health (Add Health)

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Background: Hearing loss is a major public problem with heritability of 0.36 and great individual variation in age of onset. Catechol-O-methyltransferase (COMT) encodes an enzyme that is involved in the inactivation of catecholamine neurotransmitters and is highly expressed in sensory hair cells of the inner ear. COMT SNP rs4680 is nonsynonymous with a guanine (G) to adenine (A) substitution in the DNA nucleotide sequence resulting in a valine (Val) to methionine (Met) amino acid substitution. rs4680 G > A influences COMT enzyme activity (AA with low, AG with medium, GG with higher enzyme activity) which catalyzes the transfer of a methyl group from S-adenosylmethionine to catecholamines that play a key role in auditory function. A genetic variant of rs9332377, an intronic variant in COMT, has been associated with cisplatin-induced hearing loss in children. Both SNPs rs4680 and rs9332377 are in linkage disequilibrium with a D-prime score of 0.84 in the 1000 Genomes Project. Association between rs4680 and hearing loss has not been reported previously in population studies.

Methods: Add Health is an ongoing longitudinal study of a nationally-representative cohort of U.S. students originally sampled in Grades 7–12 (1994–1995). Genotypes of candidate genes for cardiovascular disease were available in Wave IV. Hearing loss was assessed by asking "Which statement best describes your hearing without a hearing aid or other assistive device?" (excellent, good, a little/moderate/a lot of trouble, or deaf). The quality control of genetic variants was implemented as minor allele frequency > 0.01 and Hardy-Weinberg Equilibrium p-value $> 1 \times 10^{-6}$. Allele distribution by hearing loss status was evaluated using χ^2 tests. The allelic and genotypic association was evaluated using a regression model adjusted for age and sex by racial/ethnic groups. The inverse variance-weighted effect magnitude was estimated using a genetic meta-analysis model.

Results: We report an association analysis based on 13,403 individuals enrolled in Add Health from four U.S. racial/ethnic groups (57.8% Non-Hispanic (NH) white/Caucasian, 20.6% NH black/African American, 5.7% NH Asian, and 16.1% Hispanic) in Wave IV. The "A" allele frequency of rs4680 (a missense variant in COMT) was 0.44. The prevalence of hearing loss was 7.9% for individuals with "A" allele and 6.5% with "G" allele. The "A" allele was significantly associated with increased hearing loss (Z-score=2.61, p-value=0.0089). The prevalence of hearing loss was 6.0%, 7.2%, and 8.7% for individuals with GG, AG, and AA genotypes, respectively, which was consistent with a genetic additive model. The genotypic association model showed rs4680 was significantly associated with increased hearing loss (Z-score=2.83, p-value=0.0047).

Conclusions: A missense variant of rs4680 in COMT was significantly associated with increased hearing loss among young adults in a multi-racial/ethnic U.S. population-based cohort.

Probing the Common Vs. Distinct Molecular Mechanisms in SLC26 Family of Proteins
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Background: The mammalian solute carrier 26 (SLC26)/sulfate permease (SulP) family consists mostly of membrane-based anion transporters with diverse physiological roles. Dysfunction of many family members are associated with various human diseases, including syndromic and non-syndromic deafness (SLC26A4, pendrin, and A5, prestin) and bronchiectasis (SLC26A9). Recent structural studies revealed similarities between prestin, a voltage-driven motor, and SLC26A9, an anion transporter with channel-like properties, indicating a general architectural arrangement of polypeptides among the SLC26 family members. In this study, we sought to investigate whether the pathological mechanisms are also shared among SLC26 family proteins with distinct physiological functions. To this end, we focused on several disease-associated missense variants found within the N- and C-terminal cytosolic domains of pendrin and compared their functional consequences in prestin and SLC26A9 by artificially changing the corresponding residues.

Methods: Standard site-directed mutagenesis was used to generate p.Y20H and p. H723R, D, or Y in human pendrin, p.Y16H and p.H707R in mouse prestin, and p.Y8H and p.H731R in human SLC26A9. All variants were placed with a C-terminal mTurquoise2 tag in a pSBtet-Pur vector (Addgene), which allows doxycycline-dependent expression. Stable cell lines that express these variants were established using HEK293T cells as described before (Kuwabara et al., 2018). Using these stable cell lines, functional assays were performed for each variant along with corresponding wild-type (WT) controls. For pendrin anion transport function, previously established in vitro plate reader-based assays were performed (Wasano et al., 2020). For prestin function, nonlinear capacitance (NLC), a proxy of electromotility, was measured. For SLC26A9 anion transport function, both electrophysiological and plate reader-based assays were performed. Expression and plasma membrane (PM) targeting was confirmed by fluorescence microscopy for each variant.

Results: Pendrin disease-associated variants Y20H and H723R, D, and Y all exhibited reduced transport activities with normal PM targeting, indicating that they are likely pathogenic. Similarly, in SLC26A9, artificially made Y8H and H731R variants exhibited impaired transport functions in both electrophysiological and plate reader-based assays while targeting to the PM. Interestingly, corresponding artificial variants in prestin, Y16H and H707R, exhibited WT-like NLC.

Conclusions: Although SLC26 family of proteins share similar overall molecular architecture, underlying molecular mechanisms may not be exactly the same. Our effort in identifying the common versus distinct functional consequences of disease-associated variants within the SLC26 family members will greatly facilitates the understanding of molecular mechanisms that support diverse physiological roles of the SLC26 proteins. This work is supported by NIDCD (DC017482 to KH) and the Knowles Hearing Center.

Distinguishing Morphological Characteristics of Inner Ear Mitochondria

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Background: The purpose of this research is to study the structure of mitochondria in inner ear sensory and vestibular hair cells. The main hypothesis of the study is that mitochondria are structurally heterogeneous, depending on hair cell type and location of mitochondria in proximity to other organelles within a hair cell, which may affect their function. One such structural difference is polarization of the mitochondrial crista junctions (CJs), which are openings of the inner folds of the mitochondria into the intermembrane space that may be functionally related to ATP production or transport.

Methods: Inner ear vestibular tissue originally obtained from Long-Evans rats under an approved UIC IACUC procedure was used for electron microscope tomography. Serial tomograms were joined using etomo (IMOD) and data were processed with the IMOD (v. 3.13.6) software package (Kremer et al., 1996). Segmentation was performed by manual tracing in the planes of highest resolution with the program 3dmod (IMOD) to obtain the final models. Reconstructions were visualized using 3dmod (IMOD); this program allows stepping through slices of the reconstruction in any orientation (SLICER option) and tracking or modeling features of interest in any of the three dimensions. Several drawing tools (Sculpt, Join, Warp, Interpolator) and a proximity analysis feature (Mtk) were also used.

Results: Quantitative analyses were performed to test our hypothesis, including counts of total mitochondria within the two different types of hair cells, efferent boutons, and afferent calyces, counts of CJs on either side of a mitochondria in relation to other cellular organelles, analyzing the polarization ratios (side toward vs side away) vs distances, bioenergetic calculations of ATP production, and a proximity analysis test (Mtk) using CJ randomization as a control. Preliminary results support the hypothesis of structural heterogeneity of mitochondria as CJs are polarized toward specific organelles within the cell and appear to be non-randomly distributed. In addition, several sizes of mitochondria were found depending on cell and afferent or efferent bouton type.

Conclusions: These initial results suggest functional causes for the structural differences. The CJ polarization toward organelles that use energy may be present to transport ATP more efficiently to that organelle. For example, the cuticular plate is an organelle that functions to return stereocilia rootlets back to their original position. The results indicate a non-random polarization of mitochondrial crista junctions toward the cuticular plate. Synaptic ribbons, of course, send signals to the brain, an energy-requiring process. These structural differences in mitochondria may provide key information to better understand their function and to address mitochondrial deafness and dizziness disorders.

Examining the Role of Gata3 in the Development of Vestibular Hair Cells

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Background: Vestibular hair cells (VHCs) are mechanosensory receptors in the inner ear that allow animals to coordinate movements and orient their position in space by detecting velocity, gravity and body position, and angular acceleration. Around 69 million Americans experience episodic vestibular dysfunction at least once, while 8 million Americans suffer from chronic vestibular disorders. Some vestibular disorders are caused by the loss of VHCs. In mammals, VHCs die with age and do not regenerate.

Methods: We utilized both Gata3 conditional knock-out (CKO) and over-expresser mouse lines in order to demonstrate the loss-of-function and gain-of-function of Gata3 within the entire population of VHC in comparison to a sub-population of Fgf8-positive VHCs in the utricle at several prenatal and postnatal time points. We were able to examine Gata3 expression in specific cell populations of the utricle using in situ hybridization and RNAScope. We performed immunohistochemistry to characterize the histology and orientation of the VHCs and found that neither Gata3 CKO nor over-expression impacts orientation.

Results: Additionally, we analyzed the total VHC number and the size of the sensory cells within different utricular zones. Using immunohistochemistry and in situ hybridization, we also determined if over-expression of Gata3 could force a Type I VHC phenotype within the Type II VHCs since previous RNA-sequencing data suggests that levels of Gata3 can determine VHC type.

Conclusions: We show here the unique function of Gata3 in vestibular hair cells in partial VHC type specification and compare it to our previous data on the role of Gata3 cochlear hair cells at the same time points.

HIC1 Represses ATOH1 Transcription and Hair Cell Differentiation in the Cochlea

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Background: Across species, expression of the basic helix-loop-helix transcription factor ATOH1 promotes differentiation of cochlear supporting cells to sensory hair cells required for hearing. In mammals, this process is limited to development, whereas nonmammalian vertebrates can also regenerate hair cells after injury. The mechanistic basis for this difference is not fully understood. Hypermethylated in cancer 1 (HIC1) is a transcriptional repressor known to inhibit Atoh1 in the cerebellum. We therefore investigated its potential role in cochlear hair cell differentiation. We hypothesized that HIC1 could contribute to Atoh1 repression in the cochlea through transcriptional regulation and interaction with Wnt.

Methods: Hic1 expression was assessed in the mammalian inner ear using qRT-PCR and in situ hybridization specific to Hic1. We use a murine cochlear organoid system comprising expanded postnatal LGR5+ progenitor cells to study the effect of HIC1 transcriptional silencing or overexpression on activation of the Atoh1 autoregulatory enhancer, expression of hair cell genes, and hair cell differentiation. Using a luciferase reporter system to study the mechanism of HIC1-mediated repression of Atoh1, we demonstrate that wild-type HIC1, but not the zinc-finger DNA-binding mutant C521S, is able to inhibit Atoh1 and Wnt reporter activation.

Results: We find that Hic1 is expressed throughout the postnatal murine cochlear sensory epithelium. In cochlear organoids, Hic1 knockdown induces Atoh1 expression and promotes hair cell differentiation, while Hic1 overexpression hinders differentiation. Wild-type HIC1, but not the DNA-binding mutant C521S, suppresses activity of the Atoh1 autoregulatory enhancer and blocks its responsiveness to β-catenin activation.

Conclusions: Our findings suggest an important role for HIC1 in mediating Atoh1 repression, at least in part through repression of TCF/b-catenin signaling, and that its targeted inhibition may potentiate hair cell regeneration in the mammalian cochlea.

Effects of Activity on the Ultrastructure of Inner Hair Cell Ribbon Synapse Upon Disruption of Otoferlin

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Background: Cochlear inner hair cells (IHCs) accurately transmit auditory information over potential prolonged time through the so-called ribbon synapse. To this end, the electron-dense synaptic ribbon tethers synaptic vesicles (SV) to act as a reservoir for replenishing the readily releasable pool of SVs at the active zone (AZ). Upon depolarisation of IHCs, Ca2+ influx induces SV release, likely from the membrane proximal (MP) pool. Tethering to the presynaptic density (PD) and the AZ membrane is proposed to mediate SV recruitment and release (Chakrabarti et al., 2018). For sustained signal transmission, endocytosis must happen subsequently in the forms of bulk endocytosis and clathrin-mediated endocytosis at IHC ribbon synapses (Neef et al., 2009). Otoferlin (Otof) is proposed to organise Ca2+ channel localisation (Heydrich et al., 2009), form tethers at the AZ membrane (Vogl et al., 2015) and finely coupling stimulation to SV release at IHCs (Takago et al., 2019) by acting as Ca2+ sensor promoting SV fusion (Roux et al. 2006). In mice, lack of Otof (OtofKO/KO) results in almost abolished of exocytosis and, therefore, deafness (Roux et al., 2006). Supporting its diverse functions, Otof has been identified in the plasma membrane, SVs and endosomal compartment (Roux et al., 2006; Dulon et al., 2009; Strenzke et al., 2016). Due to its interaction with endocytic proteins (Duncker et al., 2013; Jung et al., 2015; Kroll et al., 2019), we hypothesise that lack of Otof alters the proportion of endocytic structures upon prolonged activity.

Methods: To test our hypothesis, we analysed murine IHC ribbon synapse ultrastructure by means of electron microscopy at postnatal days 16-21. The apical turn of organs of Corti were dissected and incubated in a stimulatory (high K+), resting or inhibitory solutions. Then, they were instantly immobilised by high-pressure freezing, followed by freeze substitution and embedding in epoxy resin. We employed electron tomography to resolve endocytic structures, as well as SVs of ribbon synapses. Tomograms were acquired from semi-thin sections in which different SV pools and endocytic structures were analysed.

Results: We present here the ultrastructural characterisation of exocytic and endocytic structures comparing OtofKO/KO to wild-type ribbon synapse under different activity states. We quantified endocytic structures up to 400 nm from the ribbon surface and SV pools, emphasising on the MP-SVs. Preliminary results indicate that upon stimulation of OtofKO/KO IHCs there is an increase in formation of endocytic structures, which neither affects the ribbon associated nor the MP-SVs. A deeper analysis of the MP subpools seems to point towards an increase of tethered SVs on the detriment of docked SVs.

Conclusions: We conclude that deficiency of Otof in IHCs leads to alterations in endocytic structures, which supports the proposed roles for Otof.

Consequences of Early-Onset Mild Hearing Loss on Brain and Behavior in Rats

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Background: Extensive research indicates that children with hearing loss show intellectual and social interaction disabilities. However, the underlying neurobiological mechanisms are largely unknown. To identify possible mechanisms, a rat model of mild (noise-induced) hearing loss was developed and behaviorally phenotyped for cognitive and social functioning. To link the behavioral output to potentially underlying neurobiological changes, brain regions involved in cognitive and social functioning were immunologically and histologically investigated.

Methods: Four-week-old Wistar rats were exposed to white noise at 120 dB SPL for 2 hours. To quantify hearing loss, auditory brainstem responses (ABR) were measured. Social behavior was assessed when rats were 5 weeks old using a social play test, and again when they were 10 weeks old using a social interaction test. Cognitive performance was tested when the rats were 8 weeks old using the novel object recognition (NOR) task. Thickness of cortical regions involved in cognitive and social behaviors, and the size of the hippocampus, were analyzed using cresyl violet staining. Neurogenesis in the hippocampus was analyzed using a marker for DCX.

Results: Elevated auditory thresholds (approximately 15 dB) were found at 16 and 32 kHz, 2 weeks after noise exposure. During the NOR assessment, animals with mild hearing loss explored both the novel and familiar objects equally, suggesting reduced cognitive function compared to control animals. No differences were found in social and play behavior after hearing loss at either juvenile or adult ages. The cresyl violet staining showed that hearing loss did not affect the thickness of cortical regions of interest or the size of the hippocampus. Additionally, no difference was found in neurogenesis in the hippocampus after mild hearing loss in the young rats. Further neurobiological assessments on the hippocampus and the cortices and more extensive analyses on the ABR data are still under investigation.

Conclusions: Mild hearing loss in young rats induced cognitive impairment but had no effect on social behavior. No overt neurobiological changes were found in cortical thickness or hippocampal size after mild hearing loss. Despite the reduction in recognition memory, this model did not show alterations in the levels of neurogenesis in the hippocampus. Additional analyses will explore other neurobiological factors contributing to altered cognitive function.

The Multiple Roles for Gata3 During Inner Ear Development

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Background: The inner ear is a complex labyrinth that forms throughout embryonic development. Many different genes work together and contribute to the formation of each sensory epithelium. One important gene is Gata3, a zinc finger transcription factor that begins expression in the otic placode and becomes restricted to the proneurosensory regions with continued expression postnatally. Previous studies have shown that early embryonic deletion of Gata3 result in loss of hair cells (HCs), supporting cells (SCs), and spiral ganglion neurons (SGNs).

Methods: We specifically deleted Gata3 at E11.5 using Sox2-creERT2 to investigate Gata3's requirement in proneurosensory cells. Additionally, deletion using Fgf10-creERT2 allowed us to investigate the requirement of

Gata3 in nonsensory greater epithelia ridge (GER) cells. Finally, Atoh1-cre and Fgf8-cre lines were utilized to explore the requirement of Gata3 in all HCs or and inner hair cells (IHCs) specifically.

Results: We found that later deletion of Gata3 using the Sox2-creERT2 line resulted in a basal to apical loss of proneurosensory cells, while deletion of Gata3 from all HCs or just IHCs resulted in a disorganized OC early postnatally. However, using the Fgf8-cre line we found that Gata3 is needed for long term maintenance of IHCs as aged mice show greatly diminished ABRs thresholds and loss of IHCs.

Conclusions: Taken together, these results show the widespread need for Gata3 during inner ear development and its various roles in regulating transcriptional networks.

Selective Loss-Of-Function of GATA3 Within Spiral Ganglion Neurons at Later Stages in Neuronal Development Demonstrates Role in Axonal Development

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Background: Hearing loss is the most common sensory disorder, yet current treatment options are largely restricted to hearing aids and cochlear implants. Hearing aids require some of the hair cells (HCs) of the organ of Corti (OC), while cochlear implants are ideal for patients who have lost most of their HCs. Both treatment options require present and properly organized spiral ganglion neurons (SGNs) to work. Several major transcription factors have been shown to be important in proper development of SGNs, such as Gata3, a zinc-finger factor protein. Previous studies have looked at the elimination of Gata3 from SGNs but have been limited by the timing of Gata3 deletion. Therefore, the role of Gata3 in post-mitotic developing SGNs remains understudied.

Methods: We combined a Gata3 floxed mouse line with a Pou4f1-creERT2 line, which allows for cell-type specific and time-dependent deletion of Gata3 from SGNs. Using immunohistochemistry, we have examined the phenotype of peripheral neurites from mice in which Gata3 was conditionally deleted from SGNs at later embryonic developmental timepoints. To visualize central projections in these mutants, we used lipophilic dye tracing to label neurites as they reach the cochlear nucleus. In addition, we have used qPCR to quantify changes in expression of other genes in response to Gata3 elimination.

Results: Our preliminary data indicates that loss of Gata3 from developing neurons at post-mitotic stages results in aberrant peripheral projections and changes in expression levels of other genes.

Conclusions: Based upon our preliminary findings, we hope to further the knowledge of the role of Gata3 in the gene regulatory network and uncover other gene relationships involved in inner ear development.

Improved Resolution of Synchrotron-Radiation Phase-Contrast Imaging of the Human Cochlea: One Step Closer to Histology

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Background: High-resolution 3D imaging of cochlear ultrastructure is challenging because of its complex anatomy. Despite the unparalleled resolution and contrast of histology, it is a destructive technique and there is a potential for artifacts; moreover, specimen preparation is lengthy. X-ray based imaging modalities such as micro-computed tomography (micro-CT) are limited as the high density of temporal bone surrounding the cochlea limits X-ray penetration depth. Our group has demonstrated the successful application of synchrotron-based imaging techniques as a non-destructive alternative in visualizing cochlear soft tissues. Both implanted and intact cochlear samples have been visualized using synchrotron-radiation phase-contrast imaging (SR-PCI) at a resolution of 9 µm. The results of these studies have allowed us to visualize cochlear anatomy with high spatial resolution and contrast, and these images have been used to improve cochlear implant frequency mapping. The next step towards improving SR-PCI results is to increase imaging resolution in order to visualize at cellular level. Increasing the resolution of SR-PCI is challenging given current hardware limitations, such as the small field-of-view (FOV) of higher resolution detectors, which only allow approximately 1/20 of a human cochlea to be imaged. The objective of this work is to investigate the application of the SR-PCI technique in imaging an intact human cochlea at a higher resolution.

Methods: A novel technique was used to image one embalmed human temporal bone using two detectors: a low- and a high-resolution detector. The low-resolution detector had a 13 µm voxel size and a large FOV and was placed at a distance of 2 m from the sample. The high-resolution detector had a 1.4 µm voxel size and a smaller FOV and was placed at 0.6 m from the sample. The low-resolution, large-FOV detector was used to accurately

locate the cochlea within the temporal bone and provided the details needed for the high-resolution detector. Using the CT image obtained from the low-resolution detector, an imaging trajectory was chosen to capture 27 small regions of the cochlea using the high-resolution detector. These scans were then stitched together to make a 3D image of the entire cochlea at voxel size of 1.4 µm.

Results: The images obtained provided a high-level of detail of the cochlear soft tissues and vascularization that was previously only attainable through histology. The ducts and channels located in the thin walls between the cochlear turn were fully visible and could be traced from the hook region to the cochlear apex.

Conclusions: To our best knowledge, this is the first in-line dual-detector application of an SR-PCI technique to scan the human cochlea. Further studies are required to analyze the cell types and the anatomical features visualized by this technique.

Independent Vestibular Labyrinthine Function After Major Surgical Trauma to the Human Cochlea

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Background: The receptors for hearing and balance are housed together in the labyrinth of the inner ear and share the same fluids. Surgical damage to either receptor system was widely believed to cause certain permanent loss of the receptor function of the other. That principle, however, has been called into question because there have been anecdotal reports in individual patients of at least partial preservation of cochlear function after major surgical damage to the vestibular division and vice versa.

Methods: We performed specific objective vestibular function tests before and after surgical trauma (subtotal cochlear removal) for treatment of intracochlear tumors in 27 consecutive patients in a tertiary referral center. Vestibular function was assessed by calorics (low frequency response of the lateral semicircular canal), vestibulo-ocular reflex by video head impulse test (vHIT) of the three semicircular canals, cervical and ocular vestibular evoked myogenic potentials (cVEMP, saccule and 36 oVEMP, utricle). Preoperative and postoperative distributions were compared with paired t-tests.

Results: Here we show that there was no significant difference between pre- and post-operative measures for all tests of the five vestibular organs, and that after major surgical cochlear trauma, the vestibular receptors continue to function independently.

Conclusions: These surprising observations have important implications for our understanding of the function and the surgery of the peripheral auditory and vestibular system in general and open up new possibilities for the development, construction and evaluation of neural interfaces for electrical or optical stimulation of the peripheral auditory and vestibular nervous system

Non-Linear and Pharmacologically Linearized Basilar Membrane Responses in the Guinea Pig Cochlear Apex

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Background: The mechanisms behind transduction of low frequencies, such as those used in speech and music, are not fully characterized. Reticular lamina vibration has been described and exhibits reasonably broad band, and even low pass tuning characteristics. Recently our displacement and phase measurements of the guinea pig apical organ of Corti suggested that apical frequency processing may rely upon unique principles not reflected in the cochlear base. It remains to be seen how apical guinea pig basilar membrane behavior interacts with the organ of Corti, from a tuning and timing perspective.

Methods: Using spectral domain optical coherence tomography vibrometry, we present displacement and phase measurements from three distinct basilar membrane locations in the apical and third turn of the guinea pig cochlea, spaced approximately 1.5 turns, or 4.3mm apart. These recordings were made in our intact, minimally invasive cochlear preparation, before and during the presence of the loop diuretic furosemide – a drug which abolishes the endocochlear potential, thereby minimizing outer hair cell force production.

Results: As previously reported, basilar membrane vibration was smaller than organ of Corti vibration at the three locations recorded, although the magnitude difference between the two structures diminished as recording location approached the helicotrema. We also found that some tuning characteristics of the basilar membrane were qualitatively similar to the organ of Corti: the most apical location tended toward band pass tuning, and the middle

location more low pass. Indeed, the band-pass characteristics of the basal location were also present – although likely induced by the use of multi-tone stimuli. This effect was confirmed for higher sound pressure levels, where tuning became more band-pass for all stimuli.

Crucially, there was a distinct but small difference in the cutoff frequencies of the three locations, broadly mirroring our findings in the organ of Corti. This difference in CF is far smaller than tonotopic maps predict for this 1.5 turn span of the cochlea. These small shifts in frequency tuning support our previous findings that showed diminished frequency selectivity in the guinea pig apical organ of Corti when compared to the base.

Conclusions: Effects of furosemide on displacement and group delay were compared across and within locations and structures. Overall, the results offer context and insight into the interaction of the apical organ of Corti and basilar membrane when stimulated at speech frequencies.

Full-Wave Sound Analysis of Straight Cochlea Model With Compressible Perilymph

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Background: After the proposal of Békésy's traveling wave theory, simulation techniques of auditory systems have been advanced largely. However, in most cases, perilymph in cochlea is treated as incompressible medium. In this presentation, we design a new cochlear model in which perilymph is assumed as a compressible medium, and explain why this assumption is so important.

Methods: A 35mm-length straight cochlea model is designed by stacking three types of tapered blocks of scala vestibuli, scala media, and scala tympani. The 0.1mm-height block of scala media is symmetrically sandwiched by the blocks of scala vestibuli and scala tympani (1.2mm-1.2mm at base and 0.7mm-0.7mm at apex). A 34mm-length fan-shaped basilar membrane (30μm-100μm at base and 10μm-500μm at apex) is embedded in the scala media. A 0.65mm-diameter helicotrema is given at the apical end of the scala media to connect scala vestibuli and scala tympani. A fixed-end reflection condition is given to the round window. Scala media, scala tympani and helicotrema are filled with "compressible perilymph" to allow compression waves travel in there. COMSOL Multiphysics 5.3 is used for simulations.

Results: Sound pressures in the scala vestibule and scala tympani, and displacement of the basilar membrane are calculated along the cochlea when the oval window is excited by 1Pa sound wave. Looking at the section from the base to the place where the maximum displacement of the basilar membrane occurs, the sound pressure levels in the scala vestibuli and scala tympani are largely different. However, these sound pressure difference is decreased gradually, and instead traveling wave is growing rapidly. Contrary, at the section from the place where the maximum displacement of the basilar membrane is obtained to the apex, the sound pressure levels in the scala vestibuli and scala tympani are completely identical. These results indicate that the sound wave excited at the oval window is expressed by the sum of even and odd symmetric sound waves, and only the odd-mode sound contributes to generate the traveling wave on the basilar membrane. Also, it should be noted that the even-mode sound wave is excited in the cochlea and reflected at the apex with free-end-reflection condition. Therefore, against the incoming sound waves, input impedance of the cochlea is determined by the total contribution of the even and odd mode sound waves in the cochlea. In other words, if the even-mode sound wave is ignored, performance of the cochlea will be incorrectly evaluated at the higher frequencies.

Conclusions: To avoid such miss-evaluation, "compressible perilymph" should be applied to the scala vestibule and scala tympani in simulations. In the presentation, we will demonstrate that this approach is quite useful to explain auditory physiology and auditory diseases.

αII Spectrin is Required for Coupling Prestin's Gating Charge Movement to Electromotility in OHCs

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Background: Outer hair cells (OHCs) amplify sound perception by electromechanical transduction, whereby stereociliar driven changes in transmembrane voltage drives cellular length changes. Recent data has suggested that the spectrin gene family plays a critical role in OHC-enhanced hearing. Typically, the ubiquitous form αII spectrin pairs with a β subunit to form heterotetramers that can bestow membrane stability and elasticity and serve as a signaling hub. The goal of this study was to determine the impact of spectrin loss from the OHC on hearing function and to determine if the spectrin scaffold is critical to the electromotile properties of the OHC.

Methods: We have undertaken a targeted genetic approach to specifically remove αII spectrin from OHCs in mice, by using tamoxifen incudible Cre-mediated gene recombination. Auditory brainstem response (ABR), distortion product otoacoustic emissions (DPOAE) were recorded in tamoxifen induced mice. OHC electromotility (eM) and nonlinear capacitance (NLC) were tested by whole-cell patch clamp.

Results: αII spectrin is highly expressed in the OHC actin-rich cuticular plate where it forms ring structures at the base of the stereocilia rootlets. αII spectrin is also expressed along the OHC lateral membrane, which together with β spectrin, crosslinks with actin filaments to form the cortical lattice (cytoskeleton) that underlies the plasma membrane. We find that the depletion of αII spectrin from OHCs causes a significant reduction in hearing sensitivity, as evidenced by increased ABR thresholds, and OHC function, as evidenced by decreased DPOAE responses and eM. Furthermore, we find that αII spectrin knockout mice have markedly reduced OHC size as confirmed by linear capacitance measurements. These data are consistent with a potential role for spectrin's involvement in OHC eM.

Conclusions: Taken together, it seems plausible that αII spectrin has a dual role in OHC performance by anchoring stereocilia in the cuticular plate rootlets and participating in electromechanical transduction via the cortical lattice.

The Relationship Between ABO Blood Group and Otoacoustic Emissions—Findings From a Large-Scale Study

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Background: A growing number of studies have shown that individuals with different ABO blood group are at dissimilar risk for many health disorders. For human auditory function, some studies have found that participants with O blood group have a higher prevalence of noise-induced hearing loss (NIHL) than participants with non-O blood group. Several small-scale studies have examined the effect of ABO blood group on otoacoustic emissions (OAEs), which are important indicators of cochlear function in humans and can also relate to functional hearing status. These studies found that normal hearing females with O blood group had lower prevalence of spontaneous OAEs (SOAEs) and a lower distortion product OAE (DPOAE) amplitude at some frequencies compared to participants with non-O blood groups. Males with O blood group were also found to have lower DPOAE amplitudes at some frequencies, compared to males with non-O blood group, and blood group O participants showed reduced SOAE prevalence. One study also suggested that individuals with O blood group may have poorer speech discrimination ability in noise than non-O blood group individuals. These findings point to possible differences in auditory function across different ABO blood groups.

Methods: In this study, a larger sample of normal hearing adults (n=463) was used to investigate the effect of ABO blood group on OAEs. Participants were Han Chinese, both male and female, with an age range of 20-59 years. To determine auditory status, the current study used a full range of OAEs measures—TEOAE amplitude, DPOAE amplitude, DPOAE input output (I/O) functions, and SOAE prevalence. Examiners are blind to blood group status. The study hypothesis was that participants with blood type O would consistently demonstrate reduced auditory status over the range of tests.

Results: TEOAE and DPOAE results found that the O blood group did not show statistically significant differences from the A, B and AB blood groups. Similarly, for I/O function and SOAE results, no significant differences across blood groups were noted. Consistently with some earlier studies, gender differences in TEOAE amplitude were found.

Conclusions: In this large sample size study, O blood group participants were not found to have significantly poorer correlates of auditory function. The hypothesis that people with different blood groups have different levels of cochlear function could not be confirmed in the current study. The discrepancy between these findings and previous studies suggests that any effect of blood group on auditory function in normal hearing individuals is doubtful. In order to further explore the possible relationship between ABO blood group and human auditory function, attention should be focused on NIHL and its potential differential effects of blood group, as this is another area where larger scale studies are yet to be carried out.

Intracellular Bioimaging of Lipid Droplet Analysis Using Label-Free Three-Dimensional Tomography and Association with Ferroptosis in Cisplatin-Induced Ototoxicity

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Background: Optical diffraction tomography (ODT) is a 3D-quantitative phase imaging technique that can obtain high-resolution images from living cells without specific staining. The intracellular lipid droplets (LDs) observed under OCT accumulate during cell damage and inflammatory process, and may be associated with ferroptosis, a recently highlighted iron-dependent cell death. Herein, we analyzed the characteristics of LDs in auditory hair cells for cisplatin-induced ototoxicity and its protective condition through high-resolution 3D quantitative-phase imaging and reconstructing the refractive index (RI) distribution.
Methods: The cells were pre-treated with α-Lipoic acid (LA) with concentration of 500 μM for 24h, before treated with the cisplatin with concentration of 15 μM for 48h. Cell viability was evaluated with a Cell Counting Kit-8 (CCK-8). The primary antibodies such as anti-PARP, anti-LC3-B, anti-P62, anti-GPX4, and anti-xCT were confirmed by western blot. The cells were subcultured in petri dishes, and a coverslip was attached on top of dishes before tomography. The 3D images of HEI-OC1 cells were scanned by tomography. The volume, mass, and number of LDs in HEI-OC1 cells were analyzed by Lipid Analysis Program using specific RI.
Results: In HEI-OC1 cells, pre-treated with LA showed high viability comparable to the control group. In 3D image RI analysis, the number of intracellular LDs treated with cisplatin was significantly increased compared to the control group and significantly decreased in pre-treatment with LA compared to the cisplatin group. In addition, the volume and mass of LDs also showed the same results as the number of LDs among three groups. LA-treated cells showed high expression of p62, LC3-II and were decreased apoptosis by PARP cleavage. Expression of GPX4 and xCT significantly decreased in cisplatin group and LA alleviated cisplatin-induced ototoxicity by inhibiting ferroptosis.
Conclusions: Our results demonstrated that cisplatin-induced ototoxicity in HEI-OC1 cells lead to ferroptosis, LA showed the potential of an oto-protective agent to mitigate ferroptosis. Investigation of LDs of various cells in the organ of Corti using ODT can provide biologic evidence for inner ear ferroptosis and is expected to be an opportunity to approach the molecular pathway of ferroptosis.

Hearing Restoration After the Establishment of Noise-Induced Permanent Hearing Loss
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Background: Noise trauma is the most common cause of hearing loss in adults. Studies from our laboratory has shown that immediate and chronic elevations in reactive oxygen species (ROS), TRPV1 and TNF-α (cochlear inflammation) lead to permanent threshold shift (PTS). Recent data from our laboratory show that it is possible to restore hearing after the establishment of noise-induced PTS. We show that pretreatment with a combination of drugs, etanercept (TNF-α antagonist) and capsaicin, prevented noise-induced PTS when administered systemically. We hypothesize that this combined treatment could enable restoration of homeostasis even following PTS.
Methods: The Wistar rat model was used for noise induced PTS. Pre-treatment and noise exposure (NE) (122 dB OBN, centered at 16kHz, 1 hr), or treatment administered at 1- 4 weeks post NE. ABR threshold shifts, Wave I amplitudes and latencies were calculated at 8, 16 and 32 kHz. The cochleae collected were used for studies outlined below.
Results: Dose response studies: Combination of oral capsaicin (3 - 10mg/kg, oral gavage) and ETA (1mg/kg, sc) provided maximum protection at 5mg/kg capsaicin + ETA (1mg/kg) dose from PTS. NE caused permanent ABR threshold shifts of 27, 35 and 45 dB over the 8, 16 and 32 kHz at 28 days post NE. these were significantly reduced by capsaicin 3-10mg/kg+ETA. Time course studies performed with capsaicin (5mg/kg) + ETA (1mg/kg) when administered at 1, 2, 3 or at 4 weeks after PTS indicate significant protection/restorations beginning at or after 2 weeks. In these studies, NE produced PTS of 30, 25 and 35 dB at 7 weeks (post NE) at 8, 16 and 32 kHz frequencies. Significant protection was seen when treatment was started at 2- or 3-weeks post NE. The last set of treatments performed 4 weeks following establishment of PTS showed restoration of ABR thresholds to 5, 8 and 12 dB over 8, 16 and 32 kHz respectively. In addition to changes in threshold shifts, Wave I amplitudes were also significantly restored by the combined drug treatments. Immunohistochemical and molecular studies indicate significant endogenous increases in neurotrophic factor neurotrophin-3 (NT-3), STAT3, and Arginase-1 but decreased STAT-1 transcription factor with the drug combination. Moreover, ribbon synapses determined in

whole mount preparations show significant loss of paired synapses with PTS compared to control at 9 weeks post NE which were reduced by capsaicin and ETA treatment.

Conclusions: It appears possible to restore hearing after noise-induced PTS has been established. Systemic combined desensitization of TRPV1 and TNF-α by capsaicin and Etanercept after PTS appears integral for restore ABR thresholds and normal ribbon synaptic functions. Most significantly, this combination treatment enables the re-engineering of cochlear microenvironment towards homeostasis.

Induction of Different Isoforms of Regulators of G Protein Signaling (RGS) in the Cochlea by Cisplatin

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Background: Regulator of G protein signaling (RGS) protein accelerate the GTPase activity of heterotrimeric G proteins, thereby terminating the signals generated by G protein-coupled receptors (GPCRs). In a previous study, we showed that the chemotherapeutic agent, cisplatin, induces the expression of RGS17 in the rat cochlea, which promotes ototoxicity by suppressing the tonic protective actions of cannabinoid receptor 2 (CB2) in the cochlea. Furthermore, knockdown of RGS17 reduced cisplatin-mediated ototoxicity. We have now expanded this study to determine the effect of cisplatin on the expression of other RGS proteins and CB2 in the cochlea. In addition, we examine the otoprotective agent, epigallocatechin gallate, EGCG, could counter the effect of cisplatin on these targets.

Methods: For these studies, male Wistar rats were administered cisplatin (11 mg/kg) by intraperitoneal injections and their cochleae were harvested at 3 days post treatment. Other groups were pretreated with phosphate buffered saline or EGCG (100 mg/kg) alone administered prior to cisplatin treatment., followed by three daily treatment with EGCG. Hearing was assessed by auditory brainstem responses (ABRs). Gene expression was assessed by quantitative real time PCR while protein levels were determined by immunohistochemistry of cochlear sections.

Results: Cisplatin also produced significant loss in body weight which averaged $17.6 \pm 4.2\%$ when assessed by day 3. This treatment resulted in significant increases in auditory brainstem response (ABR) thresholds, averaging 13, 22 and 25 dB at 8, 16 and 32 kHz, respectively. We observed significant increases in the expression of RGS4, RGS7, RGS10, RGS16, RGS17 and RGS19 in the cochlea, ranging from 2.0 - 3.5 fold, following cisplatin administration. Co-administration of oral EGCG with cisplatin, which we have previously shown attenuated cisplatin-induced weight loss, hearing loss and suppressed the expression of the different RGS isoforms. EGCG also significantly increased the expression of the CB2 receptor by ~3.5 fold. Increases in RGS4, RGS17 and CB2 receptor expression were associated with focal increases in immunoreactivity of these proteins in cochlear sections. Cisplatin treatment also increased early inflammatory cell markers in the cochlea, such as CXCL1 chemokine and its receptor CXCR2. These increases were also abrogated by co-administration of EGCG.

Conclusions: Overall, these data highlight RGS proteins as potential mediators of cisplatin toxicity and suggest the participation of multiple RGS proteins in this regard. Furthermore, these data further underscore the close interactions between RGS proteins, inflammation and the CB2 receptor in the cochlea which could serve as potential targets for treating hearing loss.

Toll-Like Receptor 9 Agonist CpG Oligodeoxynucleotides Potentiates Aminoglycoside Ototoxicity

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Background: Toll-like receptor (TLR) signaling pathway is the key regulator of the innate immune system in response to systemic infection. The influence of virus-associated TLR9 signaling cascades on the cochlea is not clear. This study aims to investigate the auditory effects of systemic TLR9 agonist during chronic kanamycin treatment.

Methods: We treated CBA/CaJ mice with the TLR9 agonist CpG oligodeoxynucleotides (ODN) one day before kanamycin injection and on the 5th and 10th days during a 14-day course of kanamycin treatment. Auditory brainstem response (ABR) thresholds were evaluated before and after the treatment.

Results: Systemic CpG ODN alone did not affect the baseline ABR threshold. Three weeks after kanamycin treatment, CpG ODN significantly increased kanamycin-induced ABR threshold shifts and outer hair cell loss. CpG ODN also elevated cochlear Irf-7, Tnf-α, Il-1, and Il-6 transcript levels during kanamycin treatment.

Conclusions: This study implies that patients with underlying virus infection may experience more severe aminoglycoside-induced hearing loss if it occurs. It is crucial to consider the systemic effect on the auditory system during animal ototoxicity studies and the clinical usage of aminoglycoside antibiotics.

Avenanthramide-C Reduces the Generation of Oxygen Reactive Species While Protecting Auditory Hair Cells From Oxidative Damage in Cisplatin-Induced Ototoxicity

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Background: Cisplatin (CP) is a useful medicine used to treat a variety of cancers; but, because of its ototoxic potential, cancer patients who are exposed to it are at risk of hearing loss, which can further degrade a patient's quality of life. Avenanthramide-C (AVN-C) offers protection to a wide range of cell types. Since cisplatin-induced hearing loss has been thoroughly investigated and recorded, we wanted to see if the antioxidant AVN-C could protect mammalian HCs from oxidative stress induced by cisplatin.
Methods: In this investigation, normal adult C57Bl/6 mice were employed. Three study groups (control, CP, and AVN-C+CP) had their auditory brainstem responses examined. The outer hair cells (OHCs) were examined using immunohistochemistry. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was used to check cell viability, and 2,7-dichlorodihydrofluorescein diacetate assays were performed to assess ROS generation, and the expression levels of TNF, IL1, iNOS, and COX2 were measured to scrutinize the inflammatory
Results: Hearing thresholds were increased by CP, but reduced by AVN-C. The OHCs were seriously harmed by CP, but they were salvaged by AVN-C. In HEI-OC1 cells, CP lowered cell viability and raised the quantity of reactive oxygen species (ROS), but AVN-C decreased ROS. In CP-treated HEI-OC1 cells, all the examined primers were considerably elevated, but they were dramatically downregulated in AVN-C-treated cells.
Conclusions: The antioxidant AVN-C was found to have a strong protective effect against cisplatin-induced ototoxicity by protecting the auditory hair cells from cisplatin damage. Because AVN-C works by inhibiting ROS, it is a promising candidate for future therapy aimed at preventing sensorineural hearing loss.

Avenanthramide-C Rescues High-Dose Methotrexate-Induced Ototoxicity in Mice

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Background: Methotrexate (MTX) is effective in treating various types of cancer. However, it can also cause damage to the normal organs and cell types. Avenanthramide-C (AVN-C) protects a variety of cell types. Since hearing loss caused by MTX treatment has not been well studied, we aimed to investigate the effect of MTX on hearing and provide insights into the possible mechanism involved in MTX-induced hearing loss as well as the prevention of MTX ototoxicity using AVN-C.
Methods: Normal adult C57Bl/6 mice were used in this study. The serum and perilymph levels of MTX were evaluated using liquid chromatography-mass spectrometry. The auditory brainstem response and wave I amplitude were assessed in five study groups (control, MTX, MTX+FA (Folinic Acid), MTX+AVN-C, and MTX+FA+AVN-C). Scanning electron microscopy was performed to check the outer hair cells (OHCs). C-terminal binding protein 2 staining and anti-neurofilament 200 staining were performed to assess the cochlear synapses and neuronal integrity. Using House Ear Institute-Organ of Corti 1 (HEI-OC1) cells, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and 2′,7′-dichlorodihydrofluorescein diacetate assays were performed and the expression levels of TNFα, IL1β, IL6, BAX, and HRK were measured.
Results: The levels of MTX in the serum and perilymph increased 30 minutes after administration. MTX increased the hearing threshold, whereas AVN-C and FA reduced it. MTX decreased the wave I amplitude, but AVN-C and FA maintained it. MTX severely damaged the cochlear synapses and neuronal integrity, but FA and AVN-C rescued them. MTX decreased the cell viability; MTX increased the reactive oxygen species (ROS) level

in HEI-OC1 cells, whereas FA and AVN-C reduced the ROS level. All tested primers were significantly upregulated in MTX-treated HEI-OC1 cells, whereas they were downregulated in AVN-C- and FA-treated cells. **Conclusions:** We showed that MTX can cause severe hearing loss. It can cross the blood-labyrinth barrier and cause damage to the cochlear neurons and OHCs. The antioxidant AVN-C exerted a strong protective effect against MTX-induced ototoxicity and preserved the inner ear structures (synapses, neurons, and OHCs) from the damage caused by MTX. The particular mechanism of AVN-C against MTX suggests that ROS is involved in the MTX-induced ototoxicity.

Mitigation of Blast-Induced Hearing Damage Associated With Mild-TBI in Chinchillas Using Liraglutide
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Background: Sensorineural hearing loss is one of the most prevalent types of blast-induced hearing damage among Service members, even with the use of hearing protection devices (HPDs). Epidemiology studies revealed that this hearing damage is associated with traumatic brain injury (TBI), in which liraglutide, a GLP-1 receptor agonist, has been reported as a potential treatment for. Our preliminary studies have indicated that liraglutide mitigates hearing damage resulting from multiple low-level blast exposures (3-5 psi). This study aims to investigate the therapeutic function of liraglutide to mitigate the progression of auditory damage after multiple exposures to higher-level blasts (15-20 psi) which can cause mild TBI.
Methods: Three groups of chinchillas were included in this study: blast control, pre-blast liraglutide (drug) treatment, and post-blast drug treatment. Animals in all groups were exposed to 3 blasts at the blast overpressure level equivalent to mild TBI (15–20 psi or 103–138 kPa) on Day 1 with ears protected by HPDs (e.g., earplugs). The blast control group only experienced the blast exposures without receiving liraglutide treatment. For animals in the pre-blast drug group, the 7-day long liraglutide treatment began 2 days prior to Day 1, and for animals in the post-blast drug group the treatment began after blast exposure on Day 1. Chinchillas were then observed for 14 or 28 days with auditory brainstem response (ABR), distortion product otoacoustic emission (DPOAE), and middle latency response (MLR) recorded pre- and post-blast on Day 1, and on days 4, 7, 14, and 28. Upon the completion of the experiment on Day 14 or Day 28, the cochlea and brain tissues of chinchillas were collected for immunofluorescence studies.
Results: Changes of the ABR threshold after the blast exposure and liraglutide treatment indicated that the post-blast hearing function was ameliorated by the drug. ABR waveform analysis, DPOAE levels, MLR measurements, together with the immunofluorescence results indicated the therapeutic function of the liraglutide treatment which could be caused by changes in both the peripheral and central auditory systems.
Conclusions: Preliminary study on the therapeutic function of GLP-1R agonist (Liraglutide) for hearing restoration after blast exposure suggests that the blast-induced hearing damage associated with mild-TBI can be mitigated through the 7-day drug treatment. The improvement of hearing function showed consistencies with histopathological results which might suggest that multiple structures in the auditory system were affected by the blast exposure and liraglutide treatment.

IGF-1-Deficient Mouse Neuroblastoma Cells Show Altered Response to Ototoxics
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Background: Human deficiency of insulin-like growth factor type 1 (IGF-1) causes a rare disorder (OMIM608747; ORPHA73272), which leads to growth retardation, microcephaly, sensorineural hearing loss (SNHL) and neurological disorders [1,2]. The Igf1-deficient mouse replicates this syndrome neurological phenotype, and shows impaired neuronal differentiation and increased apoptosis of auditory neurons [3,4]. IGF-1 is a pleotropic hormone affecting multiple cellular functions, including decreasing neuroinflammation and promoting cellular senescence, though molecular mechanisms involved are still poorly understood [5].
Methods: A cellular model of the human disease was generated in the murine neuroblastoma brain cell line Neuro-2a (N2a) by using CRISPR/Cas9 technology, to study IGF-1 deficiency and understand the alterations linked to neuronal loss. For gene editing, the crRNA:tracrRNA:Cas9 complex was transfected as a ribonucleoprotein and its incorporation into cells was verified by detecting fluorescent-labeled tracrRNA. By the surveyor assay we assessed the presence of mutations and mutated cells from the selected pools were isolated

using limiting dilution. XTT and B-Gal staining were used to evaluate cell proliferation and senescence. RT-qPCR and Western Blotting were used to assess gene expression and protein levels, respectively.

Results: Igf1 gene editing was confirmed by Sanger sequencing and the allelic multiplicity generated after gene editing was analyzed by next-generation sequencing. Two mutated cell lines with a mutation frequency above 90%, 4A10 and 2G3, were selected for the study. 4A10 and 2G3 showed differential expression of the IGF system factors and pro-inflammatory cytokines, as well as of neurotrophins and their receptors. Igf1-deficient N2a lines showed lower proliferative and basal senescence rates than wild type N2a. Interestingly, deletion of Igf1 makes them more resistant to cisplatin treatment, suggesting that ablation of the Igf1 gene impairs cell cycle progression and appears to increase their sensitivity to oxidative stress.

Conclusions: Chronic IGF-1 deficiency reduces N2a growth and survival. Igf1-deficient cell lines have decreased trophic support and increased inflammation. These new cell lines are an opportunity to unravel new molecular mechanisms of neuronal injury associated with chronic IGF-1 deficiency.

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Kv4.2 Knockout Increases Suprathreshold Auditory Brainstem Responses but Confers Greater Susceptibility to Noise-Induced Damage

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Background: Type I auditory neurons (ANs) transmit acoustic information from the cochlear inner hair cells (IHC) to the auditory brainstem. Recent work has identified three subtypes of type I ANs based on distinct transcriptomic profiles (Shrestha et al., 2018, Petitpre et al., 2018). These transcriptomic differences may underly differences in spontaneous firing rates (SR) of type I AN (Liberman, 1978) and the greater sensitivity of the low-SR fibers to insult. Among these transcriptomic differences, the three subtypes of type I ANs differ in their expression of A-type voltage-gated potassium channels, with kcnd2 (encoding the Kv4.2 ion channel) being the only member of the A-type potassium ion channels restricted to middle- and low-SR fibers (Shrestha et al., 2018). We set out to determine the influence of Kv4.2 on hearing and susceptibility to noise-induced hearing loss using measurements of auditory brainstem responses in kcnd2 knockout (KO) and wildtype (WT) animals.

Methods: Auditory brainstem responses were collected from C57BL/6 and Kcnd2-/- animals at baseline (6 weeks old) and one week following acoustic overexposure (8-16 kHz octave band noise for 2 hours at 110 dB SPL). Organs of Corti of unexposed and exposed KO and WT animals were collected for immunofluorescent assessment.

Results: Immunofluorescent examination confirmed expression of Kv4.2 in the auditory neurons of WT mice and no changes in the expression of other Kv channels in KO animals. KO animals showed no differences in ABR thresholds compared to WT animals, except at 32 kHz, where their thresholds were significantly lower than those of WT animals. However, KO animals showed greater wave I amplitude input/output slopes at most tested frequencies, with differences becoming more apparent with increasing frequencies. Following acoustic overexposure, KO animals lost these advantages and showed no difference in either ABR thresholds or wave I amplitude input/output slopes from WT animals. Preliminary synapse count data do not indicate differences in paired synapse numbers between KO and WT animals in baseline conditions; however, KO animals appear to have greater loss of paired synapses in the range corresponding to the frequencies of the noise exposure.

Conclusions: Consistent with Kv4.2 not being expressed in high SR (low threshold) fibers, KO mice showed no difference in auditory thresholds. The lack of Kv4.2 resulted in increased ABR amplitudes, without necessitating increased number of synapses. However, the increased excitability rendered the low-SR fibers in KO animals more susceptible to noise-induced damage. We will further determine whether the observed frequency-dependent differences in ABR suprathreshold responses are due to greater expression of Kv4.2 and/or its interaction partners

in the high frequency regions of the cochlea by performing immunostaining experiments. We will additionally investigate the perceptual consequences of knocking out Kv4.2 using the acoustic startle response.

Prevention of Acquired Sensorineural Hearing Loss in Mice by in Vivo Htra2 Gene Editing

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Background: Aging, noise, infection, and ototoxic drugs are the major causes of human acquired sensorineural hearing loss, but treatment options are limited. CRISPR/Cas9 technology has tremendous potential to become a new therapeutic modality for acquired non-inherited sensorineural hearing loss. This study was designed to investigate the possibility of preventing ototoxic deafness, one of the most common types of acquired non-inherited sensorineural hearing loss, via disrupting an apoptosis-related gene with CRISPR/Cas9 technology.
Methods: We selected Htra2 as the target gene and developed two CRISPR/Cas9 systems, SpCas9 and SaCas9. The indel frequency of each gRNA for SpCas9 or SaCas9 system was measured in HEI-OC1 cells by high-throughput sequencing (HTS). AAV2/Anc80L65 was used to deliver CRISPR/Cas9 systems in vitro and in vivo. To identify whether disrupting the Htra2 gene could protect cochlear hair cells against neomycin-induced ototoxicity, we performed the in vitro experiment in the cochlear explants firstly, measuring the hair cell survival and apoptosis by immunohistochemistry and confocal microscopy. The AAV-CRISPR/Cas9 systems were injected into the inner ear of wild type mice at P1, and the editing efficiency in vivo was evaluated by HTS. The protective effect in vivo was evaluated using immunohistochemical analysis and measurement of ABR thresholds, peak amplitudes, and latencies of ABR wave 1. Non-injected ears served as controls. Ears of both sides were randomly assigned to injected and non-injected groups, and wild type mice were randomized into neomycin-treated and neomycin-untreated experiments without regard to gender.
Results: The results indicate that adeno-associated virus (AAV)-mediated delivery of CRISPR/SpCas9 system ameliorates neomycin-induced apoptosis, promotes hair cell survival, and significantly improves hearing function in neomycin-treated mice. The protective effect of the AAV–CRISPR/Cas9 system in vivo is sustained up to 8 weeks after neomycin exposure. For more efficient delivery of the whole CRISPR/Cas9 system, we also explore the AAV–CRISPR/SaCas9 system to prevent neomycin-induced deafness. The in vivo editing efficiency of the SaCas9 system is 1.73% on average. We observed significant improvement in auditory brainstem response thresholds in the injected ears compared with the non-injected ears. At 4 weeks after neomycin exposure, the protective effect of the AAV–CRISPR/SaCas9 system is still obvious, with the improvement in auditory brainstem response threshold up to 50 dB at 8 kHz.
Conclusions: These findings demonstrate the safe and effective prevention of aminoglycoside-induced deafness via Htra2 gene editing and support further development of the CRISPR/Cas9 technology in the treatment of non-inherited hearing loss as well as other non-inherited diseases.

Identification and Characterization of a Novel Therapeutic Class of Otoprotectants for the Prevention of Cisplatin-Induced Hearing Loss

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Background: Among the effective chemotherapeutics used in oncology, cisplatin remains the first line of treatment for various cancer types, despite its deleterious effects on the peripheral auditory system. Several human and animal studies have demonstrated that cisplatin causes damage to the sensory hair cells (HCs), spiral ganglion neurons (SGNs) and cells of the stria vascularis (SV) in the inner ear, leading to permanent and bilateral sensorineural hearing loss. Here we report on the identification and characterization of a novel class of molecules with otoprotective properties against cisplatin toxicity.
Methods: Using a diverse range of techniques, including in-vitro cisplatin-binding assays, otoprotection in neonatal cochlear explants, pharmacokinetic and efficacy studies in animal models, a variety of potentially otoprotective molecules representing different mechanism of action modalities were screened, and their activities were confirmed and characterized.

Results: Approximately 100 molecules were screened at multiple concentrations in rat cochlear explant assays to evaluate otoprotection in HCs, SGNs and SV cells against cisplatin-induced damage. This led to the identification of a novel therapeutic class of molecules with consistently greater effects in preventing cisplatin-induced damage to HCs, SGNs and SV cells than other otoprotective molecules reported in the literature and in clinical studies, such as Sodium ThioSulfate (STS). Selected molecules from this novel class were further tested for their cisplatin binding ability at equimolar concentrations in vitro using HPLC. Top performing candidates were then formulated to optimize local delivery and their pharmacokinetic properties were evaluated. Intratympanic delivery of the lead molecule OMY-150 in an optimized formulation (OTO-510) provided therapeutic levels of OMY-150 to the inner ear compartment with minimal systemic exposure. To evaluate its in vivo efficacy, OTO-510 was administered to rats via the intratympanic route prior to systemic cisplatin dosing, and the degree of otoprotection was assessed functionally (Auditory Brainstem Responses, ABRs) and histologically (cochlear evaluation). OTO-510 prevented deleterious hearing threshold shifts and preserved all inner ear structures in vivo.

Conclusions: OMY-150 and its structural analogs represent a novel therapeutic class of compounds with potent and effective otoprotective properties against cisplatin-induced damage. The pharmacokinetic profile of intra-tympanically administered OTO-510 combines inner ear exposure at otoprotective levels with minimal systemic exposure that does not compromise the anti-tumor efficacy of intravenously administered cisplatin. In all, these findings support the continued development of OTO-510 for the prevention of hearing loss caused by cisplatin administration in cancer patients.

Assessment of Immune System Cells After Cochlear Implantation

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Background: The cellular immune system encompasses numerous cell types (e.g., macrophages and other myeloid cells, and fibroblasts) that are key agents in the normal response to injury, including wound repair and the foreign body response. In the cochlea, these cells respond to tissue damage caused by acoustic trauma and ototoxic reagents. They also have been implicated in the excessive tissue growth that often occurs around cochlear implants and the corrosion of the implant that may contribute to loss of implant function over time. The activities of the immune cells suggest they may also contribute to transient changes in function that occur in the near term after implant insertion. Firm evidence in support of these hypotheses is lacking due to the challenges of evaluating the activity of cellular immune system in implanted subjects. Here we attempted to label and quantify CD45-positive cells in cochlear tissue from implanted guinea pigs.

Methods: Guinea pigs were implanted with single-channel, platinum-iridium ball-electrode cochlear implants via a cochleostomy, using standard surgical protocols and post-surgical care. Electrically-evoked compound action potential (ECAP) data were collected daily. Subjects were euthanized and systemically perfused at 7 or 10 days after implantation, when functional responses typically reached a minimum and macrophage numbers are expected to peak. Cochleae were dissected and decalcified, then CD45 antibody staining with DAB (3,3′-Diaminobenzidine) labeling was performed, using extended incubation times (overnight for most steps) to allow greater penetration of reagents into fibrous tissue that had developed in scala tympani. Tissues where then embedded in JB-4 resin and sectioned in the mid-modiolar plane. Sections were lightly stained with toluidine blue, cover-slipped, and examined by light microscopy.

Results: DAB reaction product could be seen labeling CD45-positive cells in fibrous tissue developing within the scala tympani of implanted ears in some subjects. In others, reaction product could not be detected, but macrophages, polymorphonuclear leukocytes and fibrocytes could be identified by their distinctive morphologies. These cells were not detected within the organ of Corti.

Conclusions: These results suggest that macrophages and other immune system cells are present in the fibrotic tissue forming in scala tympani after cochlear implant insertion. The changes over time in numbers and location of these cells can be assessed even when dense tissues interfere with immunocytochemical labeling. Thus, it is feasible to use these techniques to test hypotheses about the possible relationship between immune system activity and changes in implant function over time. These results are a step toward understanding and managing the roles of myeloid cells in processes that are essential to maintaining the health of the implanted subject but also are potentially detrimental to implant function.

A Longitudinal Transcriptomic Study of Noise-Induced Hearing Loss Reveals the Role of Unfolded Protein Response and the Therapeutic Target of Hearing Recovery

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Background: Exposure to damaging degree of loud noise leads to permanent hearing loss, but noise we encounter in our daily life causes temporary hearing loss. The mechanism by which hearing is restored after temporary hearing loss has not yet been elucidated.
Methods: We investigate the time-dependent transcriptomic changes after transient threshold shift (TTS) and permanent threshold shift (PTS) noises stimulation in cochlea.
Results: Based on the results of differential expressed genes and gene ontology analysis, noise exposure generates endoplasmic reticulum (ER) stress. And we found that genes involved in unfolded protein response (UPR) were upregulated initially upon noise exposure and remained activated during TTS at 2wk, the time of hearing recovery, while those were deactivated during PTS. As a result of western blot analysis in cochlea, among the three branches of UPR, genes related PERK pathway were significantly induced by noise exposure. To further elucidate the role of PERK branch in hearing recovery, we investigated the effects PERK inhibitor and activator on TTS and PTS. Interestingly, PERK inhibitor treatment inhibited the hearing recovery in TTS, suggesting that UPR is required for hearing recovery in TTS. In contrast, PERK activator treatment partially rescued hearing shift in PTS, but did not further accelerate the hearing recovery in TTS.
Conclusions: Our findings indicate that UPR plays a role in protecting the cochlea from cellular damage by noise and modulation of UPR can be utilized to prevent NIHL.

Cochlear Explant Pinning as a Method to Study Synaptopathy and Synaptic Regeneration In Vitro

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Background: Cochlear ribbon synapses between inner hair cells (IHCs) and spiral ganglion neurons (SGNs) appear to be the most sensitive element to noise damage and aging. Loss of these synapses may result in difficulty in hearing in background noise and may be associated with other bothersome symptoms, including tinnitus and hyperacusis. An in vitro model for cochlear synaptopathy using kainic acid (KA) to induce glutamatergic excitotoxicity is well-established. However, prior methods have relied on cochlear explant adhesion to glass coverslips for drug treatment and culture, which typically does not allow for subsequent manipulation and analysis of the tissue. While cochlear explant pinning has been previously used for ototoxicity models, it has not previously been described as an in vitro model of synaptopathy and regeneration.
Methods: Cochleae from CBA/CAJ mice at postnatal day 4 (P4) were dissected and a stainless steel minutien pin (0.02 tip diameter, 10 mm length; Fine Science Tools, Foster City, CA, USA) was placed through the modiolus to secure the explant to individual plastic tissue chambers. Control cochleae were cultured for 30 hours, KA-treated cochleae were treated with 0.5 mM of KA for 1 hour then cultured for 29 hours to destroy synapses, and neurotrophin-3 (NT-3)-treated cochleae were treated with 0.5 mM of KA for 1 hour, then treated with 0.6 μM of NT-3 for 29 hours to assess for synaptic regeneration. The explants were then fixed and underwent immunostaining for a pre- and post-synaptic markers CtBP2 and PSD95, respectively. Confocal images were taken at multiple locations along the mid-portion of the cochlea. Image analysis was then performed blinded to treatment identities using Amira Imaging Software.
Results: Control explants had 11.6 ± 2.3 synapses per IHC, KA explants had 3.3 ± 2.2 synapses per IHC, and KA plus NT-3 explants had 9.4 ± 3.4 synapses per IHC. The synaptic counts between control and KA cultures were significantly different ($p < 0.001$, t-test), as were the counts between NT-3 and KA cultures ($p = 0.007$, t-test). The counts between control and NT-3 cultures were not significantly different ($p = 0.2$, t-test).
Conclusions: Cochlear explant pinning is an effective method to study in vitro synaptopathy and regeneration. By avoiding adhesion to a plate, this method may provide advantages for sample manipulation and analysis following treatment.

Early Degeneration of Spiral Ganglion Neurons in a Mouse Model of Noise-Induced Hearing Loss

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Background: Noise-induced hearing loss (NIHL) is one of the most frequent recognized occupational diseases and is suspected to be responsible for audiological symptoms like tinnitus or hyperacusis and is frequently followed by emotional and cognitive psychic disorders. Although a lot of research has focused on the physiological and anatomical effects of NIHL, the time course of the involved pathologies and its underlying mechanisms are still under investigation. Only a few experiments were investigating the time-dependent degeneration of (type I) spiral ganglion neurons (SGN), representing the primary neurons in the auditory system, and it is still unclear if the observations are due to an early, direct impact of overexcitation or related to deprivation-induced neuroplasticity.

Methods: It was the aim of the present study to investigate the time course of SGN degeneration within a 14-days period after single or repeated traumatic noise exposure starting immediately after trauma. Normal hearing mice (NMRI strain) were noise exposed for 3 h with a broad-band white noise (5-20 kHz) at 115 dB SPL under anesthesia. Mean density of spiral ganglion neurons was analyzed in the different experimental groups (noise-exposed once or twice and investigated at different time points within 2 weeks post-exposure) and statistically compared to an unexposed, normal hearing control group.

Results: Our results show a significant reduction of SGN densities after noise exposure, starting immediately after the applied trauma and further progressing within a few hours post-exposure. The effect is present in the high-frequency basal cochlear turn as well as in the low-frequency apical part, whereby it is more pronounced in the apical region. Interestingly, a repeated noise exposure does not significantly alter SGN densities in addition.

Conclusions: Recent work by our group was able to demonstrate an early effect of acoustic trauma on neuronal structures, showing acute apoptosis-related degeneration in several structures of the ascending auditory system, particularly located in the auditory brainstem (Gröschel et al., 2010; Coordes et al., 2012). With the results of the current experiments, we can extend this assumption towards the peripheral auditory neurons. These findings are of great significance for the therapeutic treatment after acoustic overexposure. This is of particular importance regarding the timing of clinical intervention after traumatic noise injury since the results of the present study indicate only a short time window to reduce the observed neuropathologies in the auditory periphery. The results are also of clinical relevance from a prosthetic point of view since the loss of spiral ganglion neurons and their peripheral neurites would largely influence the outcome of cochlear implant supply.

The Effect of a Viral-Induced Inflammation Model on Cochlear Uptake of Aminoglycosides

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Background: There are intense efforts to repurpose existing FDA-approved drugs to treat emerging COVID-19 infections. Yet, the toxic side-effects of several repurposed drugs include ototoxicity. SARS-CoV-2 is a single-stranded RNA (ssRNA) virus that is primarily detected by Toll-like receptor 7 (TLR7). This study tests whether a TLR7 agonist (gardiquimod) induces COVID-19-like inflammation and modulates cochlear and serum levels of aminoglycosides, as a model of ototoxic drugs used for treating COVID-19. We tested three gardiquimod delivery routes: intraperitoneal (i.p.), intravenous (i.v.), and subcutaneous (s.c.) injections to compare their efficacy in inducing inflammation. We will also test different doses of gardiquimod to determine if specific doses increase cochlear uptake of gentamicin, an ototoxic aminoglycoside, without modulating serum levels of gentamicin.

Methods: C57BL/6 mice received DPBS (control) or gardiquimod (5mg/kg) through i.v., i.p., and s.c. injection (N=4-6 per group). Three and twenty-four hours later, blood and cochlear tissues were collected for analysis of cytokine expression levels via qRT-PCR or Luminex multiplex ELISA assays. These cytokines include: IFNα, IFNβ, IFNγ, MCP1, MIP1α, NFκB, TNFα, IP10, IL1α, IL1β, IL2, IL4, IL5, IL6, IL8, IL10, IL12α, and IL12β. C57BL/6 mice will receive DPBS (control) or gardiquimod (up to 20 mg/kg) s.c. (N=6 per group), and 24 hours later, mice will receive gentamicin for 1 hour via i.p. injection. Blood and cochlear samples will be collected to evaluate gentamicin levels using a gentamicin ELISA kit.

Results: All three delivery routes generated inflammatory responses after gardiquimod treatment, with increased serum and cochlear levels of cytokines associated with COVID-19 infections in humans compared to DPBS groups, e.g., IP10, MCP1, MIP1α, IL6, TNFα. However, 24 hours after treatment, the s.c. group had higher fold expression of many cytokines compared to i.p or i.v. groups. Determination of gardiquimod dosing that increased cochlear levels, but not serum levels, of gentamicin is in progress.

Conclusions: The pilot data show that s.c. injection of gardiquimod induced a more robust and sustained inflammatory response that mimics viral infection-induced inflammation in humans. Furthermore, we will identify the highest gardiquimod dose that increased cochlear, but not serum, levels of gentamicin for future studies.

Safety and Efficacy of Intratympanic Histamine Injection as an Adjuvant Agent of Dexamethosone: An Experimental Study in Noise-Induced Murine Model

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Background: Dexamethasone (DEX) is widely used for inner ear disease and intratympanic (IT) injection has the advantage of reducing systemic side effects. In this study, we investigated the safety and efficacy of IT histamine (HIS) injection as an adjuvant to increase the inner ear penetration rate of DEX.
Methods: IT injections were performed in C57BL/6J (B6) mice following the control, DEX-only, 1% HIS+DEX and 4% HIS+DEX groups. The safety of adjuvant HIS was assessed through behaviour monitoring, oto-endoscopic examination of the tympanic membrane, middle ear assessment using light microscopy, and cytotoxicity evaluation of organ of Corti (OC) explant and HEI-OC1 culture treatment. Perilymph concentrations of DEX were measured by UHPLC and DEX receptor expression was assessed by immunofluorescence assay. Changes in the structure of the round window membrane (RWM) was observed by transmission electron microscopy to elucidate the mechanism of HIS. The efficacy of adjuvant HIS was investigated by OC morphology and hearing tests of auditory brainstem response and distortion product otoacoustic emissions.
Results: No systemic allergic reaction was observed, and inflammatory reaction of the eardrum and middle ear mucosa was observed only in the 4% HIS+DEX group 3-week after the injection. No cytotoxic effects on OC were shown at the perilymph concentrations of penetrated HIS. Perilymph concentration of DEX was measured to be about 2 times higher for 1% HIS+DEX group and about 6 times higher for 4% HIS+DEX group compared to DEX-only group. HIS temporarily damaged the structure of RWM leading increase of permeability and the damaged structure recovered after 3 weeks. In OC morphology and hearing tests, the 1% and 4% HIS group showed significant recovery compared to the control group but did not show a significant improvement compared to the DEX-only group.
Conclusions: HIS temporarily impairs the structure of the RWM, significantly increasing the inner ear penetration rate of DEX and increasing the expression of DEX receptors. HIS at a concentration of 1% was found to be relatively safe for the eardrum and middle ear mucosa and showed an effect of increasing the perilymph DEX concentration by about 2 times. However, a high perilymph concentration of DEX does not seem to guarantee significant improvement in OC morphology or hearing.

Efficiency of a Dexamethasone Nanosuspension as an Intratympanic Injection for Acute Hearing Loss

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Background: In this study, we investigated whether dexamethasone, a hydrophobic drug, can be made into a stable nanosuspension solution, and whether this dexamethasone nanosuspension solution has a higher drug delivery efficiency to cochlea than the hydrophilic dexamethasone sodium phosphate (Dex-SP).
Methods: Nanocrystals of dexamethasone were produced by the NUFSTM (Nanoparticulation using fat and solid lipid) method, a patented method of Biosynectics, inc. Excipients were added to keep nanocrystals suspended in water. Three kinds of nanosuspensions (NUFS A, NUFS B, and NUFS C) were made by varying the composition of this excipient. We examined the safety and efficacy of nanosuspensions in in vitro and in vivo experiments.
Results: The size of dexamethasone nanocrystals in three kinds of nanosuspensions were approximately between 250 and 350 nm. When observed at room temperature for up to 8 hours, all three solutions apparently maintained a suspension state, but the particle size of NUFS C increased to about 1000 nm over time. In the in vitro toxicity assessment, cytotoxicity was not observed when three solutions were treated in HEI-OC1 cell line up to 100 ug/ml. The concentrations of dexamethasone in the perilymph after middle ear drug injection, were examined up to 24 hours, and three groups of nanosuspensions showed significantly higher drug concentration than that of Dex-

SP in the result at 6 hours. In addition, interestingly, the concentration of dexamethasone in the tissue of cochlea of NUFS group, was 26-fold higher than that of Dex-SP at 6 hours. In the evaluation of drug efficacy, NUFS B showed better phosphorylation of glucocorticoid receptors than Dex-SP in both in vitro and in vivo, and, in the ototoxic animal model, it showed significantly better hearing protection effect than Dex-SP against ototoxic drugs. In safety evaluation, it showed no toxicity at concentrations up to 20 mg/mL in an in vivo test.

Conclusions: A nanosuspension of dexamethasone was able to deliver dexamethasone to the cochlea very safely and efficiently and showed potential as a formula for intratympanic injection. In addition, it can be applied in studies on the delivery of various hydrophobic antioxidants to treat acute hearing loss.

Interaction of SLC26A4 With Adaptor Protein (AP-2) Complex in Mitochondria-Rich Cells of the Endolymphatic Sac

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Background: Pathogenic variants in SLC26A4 are a common cause of sensorineural hearing loss associated with enlargement of the vestibular aqueduct (EVA). SLC26A4 encodes pendrin, which is specifically expressed in epithelial cells of the inner ear, kidney, thyroid, and a few other tissues. Pendrin is a chloride-bicarbonate anion exchanger expressed in or near the apical plasma membrane of mitochondria-rich cells of the endolymphatic sac, where it is necessary for endolymph reabsorption and inner ear morphogenesis (Honda et al., eLife, 2017). We have proposed a model in which EVA is caused by disruption of NaCl and water absorption in mitochondria-rich cells.

Methods: To gain further insights into the components and regulation of this pathway, we used yeast two-hybrid screening to define the pendrin interactome. The intracellular amino- and carboxy-terminal regions of mouse pendrin were used as bait to screen prey libraries constructed with mRNA from E16.5 mouse endolymphatic sac and adult kidney (Hybrigenics Services, Gard, France). The screen identified 189 potential interacting proteins.

Results: One of the candidates identified using the carboxy-terminal domain of pendrin as bait was the μ2 subunit of the adaptor protein 2 (AP-2) complex. AP-2, one of the key components required for clathrin-mediated endocytosis, binds to the cytosolic tails of transmembrane cargo proteins for internalization. We prioritized AP-2 for evaluation as a candidate interacting protein since regulation of pendrin expression at the plasma membrane by clathrin-mediated endocytosis could be a therapeutic target for EVA.

Confocal microscopy demonstrated that both pendrin and AP-2 immunolabeling are concentrated and colocalized in the apical region of the endolymphatic sac epithelium. Immunogold electron microscopy showed that pendrin is localized along the microvilli of the apical-luminal surface of mitochondria-rich cells, and is also associated with clathrin-coated vesicles near the basal regions of these microvilli.

The interaction between the carboxy-terminal domain of pendrin and the μ2 subunit of AP-2 was further validated in yeast cells using one-by-one yeast two-hybrid assays, and in HeLa cells using nanoscale pull-down assays. Using both yeast- and HeLa cell-based assays and computational structural modeling, we identified amino acid residues in pendrin and the μ2 subunit that are required for this interaction to occur.

Conclusions: We are currently optimizing AAV infection of endolymphatic sac epithelium to further characterize this interaction at the functional level and how it influences pendrin expression and function. We will also test the hypothesis that decreasing μ2 function can increase SLC26A4 retention at the plasma membrane and rescue endolymph absorption in mice segregating hypofunctional missense alleles of Slc26a4. This would be a novel approach to treatment of hearing loss causes by Slc26a4 insufficiency.

Increased Expression of Group II mGluRs in the Inferior Colliculus of Aged Compared to Young Adult Mice

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Background: Metabotropic glutamate receptors (mGluRs) are thoroughly investigated because ligands targeting them have a potential for clinical development in several psychiatric and neurological disorders. These receptors are expressed throughout the auditory system but knowledge regarding their roles is very limited.
MGluRs are classified into three groups: group I includes receptor subtypes 1 and 5, group II – subtypes 2 and 3, and group III – subtypes 4, 6, 7, and 8. The objective of this project is to characterize group II mGluR (mGluR2/3) expression in the mouse inferior colliculus (IC). This interest arises from our recent results showing that behavioral signs of tinnitus in mice were suppressed by intraperitoneal administration of mGluR2/3 agonist LY354740 (Galazyuk et al., 2019). Furthermore, LY354740 reduced spontaneous activity in the IC. Our most recent work using extracellular single cell recordings revealed that iontophoretic and topical mGluR2/3 pharmacological activation in the IC enhanced sound-evoked and spontaneous firing (Kristaponyte et al., 2020). Work from other brain areas demonstrated that group II mGluR expression is age dependent. Thus, we compared mGluR2/3 expression between young adult and aged mice. We investigated receptor expression levels across four IC regions: dorsal (ICd) and lateral (IClc) cortices, and dorsal and ventral parts of the central nucleus (ICcd and ICcv, respectively).
Methods: Brains from six adult (84-85 days old) and six aged (20-21 months old) CBA/CaJ mice were used. Anti-mGluR2/3 antibody (Millipore AB1553) was labeled with a biotinylated anti-rabbit antibody and visualized via Ni-DAB reaction. Optical density analysis was performed by collecting single focal plane images using constant illumination and exposure settings. Overall optical density measures were taken for the entire frame, and neuropil-only or cell body-only optical density measures were collected by specifying an ROI within each image.
Results: We found increased DAB staining in aged animals in every IC region tested, suggesting that mGluR2/3 expression increases with age. This between-group difference was present in the neuropil but not in cell bodies. Furthermore, in both young adult and aged mice, group II mGluR staining was not uniform across the IC. We observed a gradient in staining across the dorsal-ventral IC axis: the darkest staining was present in the dorsal cortex, lighter staining in the dorsal part of the central nucleus, and even lighter staining in the ventral part of the central nucleus. The lateral cortex was lightly stained, similarly to the ICcv.
Conclusions: Our results suggest that group II mGluR expression changes over the lifespan of CBA/CaJ mice. Higher expression levels in older animals indicate that mGluR2/3 modulation of IC neuronal activity plays a larger role in aged animals. Furthermore, this modulation is stronger in the dorsal IC irrespective of the age.
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Integration of Visual and Auditory Inputs is Shaped by Morphology and Synaptic Properties in Midbrain Neurons

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Background: Concurrent presentation of visual stimuli with auditory cues can lead to lower detection threshold, enhanced responses and faster response latency compared to unimodal stimuli. These phenomena have been studied on the neuronal level for multimodal structures in the midbrain, like the avian optic tectum. However, the exact cellular mechanisms of multisensory enhancement are less well described. A multimodal neuron type in the avian optic tectum, the Shepherds' crook neuron (SCN), receives visual and auditory inputs on opposing dendritic structures. Thus, SCN are an ideal model to study the physiology of multimodal integration on a cellular level.
Methods: We performed whole-cell recordings from SCN in acute brain slices of the avian optic tectum. Modality-specific activation of inputs was achieved by localized electric stimulation of axonal input fibers. We then created a compartment model of SCN using NEURON, constrained both by our own in-vitro results and published data. In this model we tested the hypothesis that SCN show enhanced responses to in-vivo like inputs and that the cellular peculiarities of SCN have a crucial influence on multimodal integration.
Results: In slice recordings we found a modality specific difference in the efficacy of synaptic inputs. This was caused by slower EPSC decay kinetics of visual inputs. Blocking NMDA-type glutamate receptors abolished these differences. The SCN compartment model replicated the in-vitro findings. Simultaneous activation of visual and auditory inputs showed lower conductance and action potential thresholds compared to unimodal stimuli. When

we simulated in-vivo like stochastic activation of inputs, superadditive multimodal enhancement demonstrating the principle of inverse effectiveness was evident. Furthermore, the slower dynamics of visual responses caused a temporal window of best multimodal integration that was shifted towards auditory stimuli following visual stimuli. Multimodal stimuli from a virtual source closer than 30-50m caused stronger and consistently more rapid responses in SCN. Next, we found that the position of the axon initial segment and the difference in synaptic kinetics had complementary effects on multimodal enhancement. These features systematically influenced the input rate (i.e. simulated stimulus strength) at which best multimodal enhancement could occur.
Conclusions: Thus, our model predicts that SCN responses benefit from multimodal stimuli originating within a specific distance from the animal, which will lead to quicker and more robust selection of relevant salient stimuli. Moreover, the unique morphology and modality-specific synaptic properties allow tuning of multimodal integration to specific input features and will help to exploit multimodal enhancement over a wide range of stimulus properties.

Measurement of Eardrum Impedance Using Optical Coherence Tomography
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Background: Ear drum impedance is useful, in combination with other measures, to assess conductive hearing loss. The current standards for equipment used to measure eardrum impedance are outlined in IEC 60645-5. Complexities of the ear canal geometry, the shape of the eardrum, and lack of real-time guidance for positioning a probe make it challenging to accurately determine the impedance of the eardrum. In this study, a new method is introduced to measure ear drum impedance using Spectral Domain Optical Coherence Tomography (SDOCT).
Methods: We recently demonstrated a method for simultaneous measurement of the pressure and vibration using SDOCT (Ramdas et al., Applied Acoustics, 169, 2020). The method involves the design and use of a miniature optical sensor consisting of a thin diaphragm attached to a microscale air-filled cavity. The pre-calibrated sensor is placed close to (within 1-2 mm of) a vibrating surface of interest, and SDOCT is used to simultaneously measure the velocity of the sensor diaphragm as well as the vibrating surface of interest as a function of frequency. Using the pre-calibration of the sensor, the sensor vibrations are converted to pressure. Hence, the acoustic impedance of the vibrating surface is determined as the ratio of pressure in vicinity of the vibrating surface and its volume-velocity. The current study aims to demonstrate the OCT-based simultaneous velocity-pressure measurement to measure impedance at the eardrum.
The GRAS 43AG7 ear simulator, which mimics the impedance at the human eardrum, is used as a test subject to perform the impedance measurement. The pinna is removed, and a thin flexible diaphragm is attached to the 'ear canal' portion of the simulator. The sensor-diaphragm is brought close to within 1 mm of the flexible diaphragm. The acoustic impedance at the thin diaphragm is measured using the OCT-based method, and then used to determine the impedance at the eardrum. The obtained results are benchmarked with the eardrum impedance reported in Rosowski, JASA 90(1), 1991.
Results: Eardrum impedance measured on ear simulator using the proposed method matches reasonably well with the human eardrum impedance reported in Rosowski, JASA 90(1), 1991.
Conclusions: An OCT-based method is introduced to measure eardrum impedance and demonstrated in an ear simulator. The next step is to demonstrate the method in the human ear.
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Towards High-Precision Myringotomy Blades: A Finite-Element Based Approach
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Background: The current surgical treatment of otitis media with effusion (OME) is myringotomy and tympanostomy tube placement. In myringotomy, a small incision is created in the tympanic membrane (TM) using a surgical blade. Tympanostomy tube is placed inside this incision. Tympanostomy is among the most prevalent pediatric surgeries and is cited as the most prevalent pediatric ambulatory surgery in the United States. Like lots of surgical procedures, myringotomy might have some risks including infection, bleeding, chronic scarring, injury to other ear structures, incision failure, and the need for repeating the surgery. Using minimally invasive surgical technologies like microsurgical blades with more precise and efficient designs can decrease the treatment drawbacks and lead to better outcomes.

Methods: We studied the cutting process of the human TM via the insertion of microsurgical scalpel blades. To this end, a combination of 2D and 3D finite-element models were developed to investigate TM cutting and deformation due to blade external force. In the 2D model, a limited section of the TM was considered and cohesive elements were exploited for modeling crack propagation during the insertion process in a displacement-controlled insertion mode. The blade reaction force obtained from the 2D model was then adopted to study the whole TM deformation in the 3D model using a force-controlled indentation mode.

Results: The effects of microsurgical blade geometry and TM thickness on the cutting process and force-displacement plot were investigated. The results showed that thinner and sharper blades had a more efficient cutting performance with lower insertion force. Also, thicker TMs showed larger cutting force and smaller indentation deformation in 2D and 3D models, respectively. Furthermore, the TM deformation using the external force was explored on different parts of the TM in various quadrants. The deformation results revealed that the effect of changing the cutting zone radially is more significant than changing its location circumferentially. In all quadrants, a unique qualitative pattern was observed on the TM deformation in different radii: by increasing the distance of the indentation location from the umbo the tissue deformation increased until a specific distance beyond which as the indentation location got closer to the TM annulus the deformation decreased.

Conclusions: The reaction force of the surgical blade and TM deformation during the cutting process strongly depends on the blade's geometrical design, TM thickness, and cutting location on the TM. The force-displacement plots obtained using finite element models can be used to design myringotomy micro-surgical blades with more efficient geometry or to improve the fidelity of haptic devices in virtual-reality surgical simulators.

Improved Bone Conduction Hearing After Middle Ear Surgery - Investigation of the Mechanism of the Improvement

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Background: In clinical practice, a decrease in bone conduction (BC) hearing (an increased BC threshold) is regarded as a sensorineural impairment. Therefore, BC hearing improvement is generally not expected after middle ear surgery. However, we have often observed BC hearing improvement after surgeries. The purposes of the study are to evaluate the extent and characteristics of the BC hearing improvement after middle ear surgeries and suggest its mechanism.

Methods: We reviewed the data of all patients (583 subjects) who underwent middle ear surgeries (ossiculoplasty or stapes surgery) to improve hearing between 2012 and 2020 in the Department of Otolaryngology, Ajou University Hospital, Suwon, Republic of Korea. "BC improvement" was defined as a BC threshold decrease >15 dB at two or more frequencies. Surgeries they received were classified into staged ossiculoplasty after canal wall up mastoidectomy (Group A) (n=171), staged ossiculoplasty after canal wall down mastoidectomy (Group B) (n=209), only ossiculoplasty without any previous mastoidectomies (Group C) (n=165), or stapes surgery (Group D) (n=37). Then, we created a hypothetical circuit model to explain this phenomenon.

Results: BC improvement was detected in 12.8% of Group A, 9.1% of Group B, and 8.5% of Group C. The improvement was more pronounced in Group D; 27.0% of them. With weak correlation, the larger the AC hearing, the greater the BC improvement (Pearson's r =.395 in Group A, P<.001; r =.375 in Group B, P<.001; r =.296 in Group C, P<.001; r =.422 in Group D, P=.009). In Group D, patients with otosclerosis, postoperative BC improvement was as large as 10 dB, 30.3 [3.2] (mean [SE]) to 20.3 [3.2] dB at 1 kHz. Also at 2 kHz, the improvement was 9.2 dB, which was from 37.8 [2.6] dB to 28.6 [3.1] dB. According to the "third window theory", as the number of windows increases, BC hearing improves, and as the number decreases, it falls. Particularly in otosclerosis, a sclerotic oval window (OW) did not function as a window. The patient had only one functional window (round window). However, after stapes footplate mobilization by surgery, the OW gain functions as a window (the patient now has two windows), improving BC hearing.

Conclusions: BC hearing improvement after middle ear surgery is not rare; the hearing may be underestimated before surgery. Such improvement is generally associated with larger postoperative AC hearing gains. In addition, the improvement was more evident after stapes surgery, which may be associated with a renewed ability of the OW to function as a window.

Transplantation of Human iPS Cell-Derived Airway Epithelial Cells Sheet Into Rat Middle Ear

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Background: Middle ear mucosa is one of the airway epithelia with ciliated cells. The middle ear mucosa contributes to maintaining aeration in the middle ear cavity through gas exchange in the upper tympanic cavity and mastoid, and the mucociliary clearance in the lower tympanic cavity and around the Eustachian tube. Extensive mucosal defects in the mastoid cavity and tympanic cavity that can occur during surgery for intractable otitis media are thought to be the cause of postoperative middle ear lesions, including adhesions of the tympanic membrane, fibrosis, and recurrent cholesteatoma. Our previous report showed the effectiveness of transplanting autologous tissue-derived "nasal mucosal cell sheet," which is a different type of airway epithelium, into the middle ear to achieve early postoperative mucosal regeneration (Yamamoto K et al., 2015). However, the nasal mucosal cell sheet requires the excision of the nasal mucosa. Therefore, to manage intractable otitis media, we focused on human induced pluripotent stem cell (hiPSC)-derived airway epithelial cells (AECs), which have been used in upper airway mucosal regeneration and transplantation therapy. If the human leukocyte antigen (HLA)-matched iPSCs are available from a clinical cell stock in the future, it might be possible to achieve a similar treatment without the excision of patient tissues. As a proof of concept study, we transplanted hiPSC-derived AECs into the middle ear of immunodeficient rats.
Methods: hiPSC was induced into AECs, which included ciliated cells, as previously reported (Konishi et al., 2015). The bilateral middle ear mucosa of X-Linked Severe Combined Immunodeficiency (X-SCID) rats was scraped, and the hiPSC-derived AECs sheets were transplanted in the ears unilaterally. We examined the survival rate of transplanted epithelial cells in the middle ear at the 1-week (n=6) and 2-week (n=8) after transplantation by immunofluorescent staining.
Results: Human nuclear antigen (HNA)-positive cells were observed in the transplanted side of the middle ear cavity surface in five of six rats in the 1-week postoperative group and three of eight rats in the 2-week postoperative group. Besides, the HNA-positive ciliated cells were observed in three of six rats in the 1-week group and three of eight rats in the 2-week postoperative group. No HNA-positive cells were found on the control side. The transplanted AECs contained cytokeratin 5- and mucin 5AC-positive cells, indicating that the transplanted cells included basal cells and goblet cells as well as ciliated cells, which are components of the middle ear epithelia.
Conclusions: We developed a fundamental method for the transplantation of hiPSC-derived AECs into the middle ear mucosa. Furthermore, we confirmed that hiPSC-derived AECs survived on the surface of the middle ear cavity of X-SCID rats. This study suggested the possibility of novel transplantation therapy for chronic otitis media.

The Influence of Preoperative Anemia on Blood Transfusion Outcomes in Major Head and Neck Surgery: A Systematic Review and Meta-Analysis

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Background: Head and neck cancers (HNCs) encompass various malignancies in the upper aerodigestive tract affecting structures vital for swallowing and breathing. In most cases, resection of the tumor is the mainstay of treatment. In major head and neck surgery (HNS), free flaps can be used to help restore function and enhance postoperative quality of life. However, these complex and lengthy operations are often associated with complications such as the need for perioperative blood transfusion (PBT). Exposure to PBT has been associated with lower overall survival and tumor recurrence due to its immunomodulatory effects. Low preoperative hemoglobin (Hgb) is a modifiable risk factor that predisposes patients to PBT and can be an important marker for exposure to PBT in preoperative planning. As such, the purpose of this meta-analysis was to summarize the high-risk preoperative Hgb at which patients are at increased risk for PBT.
Methods: Electronic databases were systematically searched from inception using key terms and synonyms for HNC, HNC surgeries, Hgb and PBT in MEDLINE, Embase, CINAHL and the Cochrane databases. Studies with adult patients (at least 18 years of age) undergoing major HNS were eligible if both the exposure (preoperative Hgb, hematocrit or anemia) and outcome variable (PBT) were included in analysis. Major HNS is defined as tumor resection with free flap reconstruction. Reviews, responses, perspectives, editorials, case reports and case series (n<20) were excluded. Thyroid, parathyroid, and upper esophageal neoplasms were also excluded. Two independent, blinded reviewers used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) for screening and data abstraction. An a-priori, random effects model was used to pool estimates.

Results: Our search yielded 2164 studies, with 144 reviewed in full-text and 7 included in meta-analysis. Transfusion practices varied considerably between centers (19-85%). We found significantly more PBT in patients with lower preoperative Hgb at 47.62% (CI=41.19-54.06) compared to normal preoperative Hgb at 13.92% (CI=10.19-17.65). This difference was not explained by other variables (age, sex, flap type and flap site) in subgroup analysis. When pooled by PBT exposure, mean preoperative Hgb was lower in the PBT group (12.05g/dL) compared to the no PBT group (13.58g/dL), although this was not statistically significant.
Conclusions: There was significant heterogeneity in transfusion practices and some centers transfused up to 85% of their patients. While a specific Hgb threshold is unclear, low preoperative Hgb can predispose patients undergoing major HNC surgery to PBT. As many patients with HNC present with anemia, this review can alert clinicians to undergo the appropriate preoperative intervention such as intravenous iron therapy prior to surgery to reduce unnecessary PBT.

Front-Back Auditory Spatial Attention
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Background: The ability to detect and attend to sounds in panoramic 3-D space is fundamental, yet little is known about the behavioral and neural properties when attending to the back hemispace.
Methods: Here, we compared EEG neuronal oscillations while subjects (n=25 young adults) in a spatial attention task in the front vs. back hemispace. We focused on oscillations in the alpha and theta bands that are implicated in attention control and modulation of sensory processing, respectively. Subjects distinguished amplitude-modulated (AM) white noise (25 or 75 Hz) from 5 speakers at 45° intervals in the front or back 180° hemispace (in separate blocks). Most stimuli were presented from an attended midline location ("standard", p=.84), with occasional stimuli from one of the other 4 speakers ("shift", p=.04/location). EEG power as a function of speaker location was examined in theta (4-7 Hz) and alpha (8-12 Hz) bands relative to pre-stimulus baseline.
Results: Reaction times to sounds slowed to shifts away from the standard in the front (p<.004), but not back (p=.11), hemispace. Stimulus onset at standard and shift locations decreased early alpha power (80-130 ms) at right ventral-frontal and bilateral posterior-parietal sites, with greater reductions in the front hemispace (p<.01). Later alpha reduction was greater at the front vs. back standard locations (160-230ms) over both frontal and parietal sites (p<.001). At parietal sites alpha power reductions were greatest at standard locations and gradually decrease with distance at shift locations only in the front hemispace (p<.05). At longer latencies, reductions in alpha power were much greater for front vs. back standards (280-350ms, p<.001), but were comparable at shift locations. Midfrontal theta power increased with distance from the standard over a wide time range (20-230ms, p<.001), was comparable for front vs. back, and equalized across locations at longer latencies.
Conclusions: Taken together, the behavioral and EEG findings suggest that the allocation of endogenous attention over space was more focal in the front vs. back hemispace.

Risk of Thyroid Carcinoma in Patients Treated Surgically With Assumed Benign Cytology in Riyadh, Saudi Arabia
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Background: Fine needle aspiration (FNA) is a well-established technique in the diagnosis, staging and follow-up of thyroid nodules. FNA results are routinely reported with the Bethesda System for Reporting Thyroid Cytopathology. This study aimed to report the rate of thyroid malignancy in cases of benign fine-needle aspiration (Bethesda category II) at King Abdulaziz Medical City and to evaluate the factors that affect the false-negative outcomes of FNA.
Methods: In this retrospective study, all patients referred for thyroid surgery from 2009 to 2019 were reviewed (n = 1968). For the study, only patients with a benign FNA, corresponding to the Bethesda category II, were included (n = 384). Information related to age, gender, body mass index (BMI), serum thyroid stimulating hormone, type of surgery, and histopathological outcomes were retrieved.
Results: Of the sample (n=384) with an initial benign FNA, 63 patients had a malignancy on the postoperative pathological examination, yielding an overall false-negative rate of 16.4%. The most frequently reported histotype was papillary thyroid microcarcinomas (n=52). For the false negative group, the mean age was 43.8 years (range

21-70 years) with a 84.1% female predominance. The surgical choice for 74% (n=46) of the cases was total thyroidectomy. Age, gender, thyroid function, and BMI did not affect the false negative rate of benign FNA (p>0.05).

Conclusions: This study indicated a higher risk of malignancy compared to literature related to benign FNA. The risk of malignancy should be considered, even with benign FNA.

Prevalence of Smell and Taste Disturbances in Pediatric Patients With COVID-19 Versus Other Upper Respiratory Tract Infections: A Case-Control Study in Riyadh, Saudi Arabia

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Background: Coronavirus disease (COVID-19) and upper respiratory tract infections (URTI) might mimic each other in their mild clinical presentation in children, making their differentiation difficult. Therefore, we aim to explore the differences between COVID-19 and URTI in pediatric population, with a great emphasis on smell and taste disturbances.

Methods: The medical records of 1200 patients were retrospectively reviewed in a tertiary hospital during 2020; to identify those diagnosed with Upper Respiratory Tract Infections (URTI group) – as a control group – and those with COVID-19 (COVID group) -as a case group. Patients with bacterial, chronic, lower tract respiratory infections, and speech delays were excluded. Both groups were matched by age, gender, and being symptomatic using propensity score matching with 1:1 ratio. SAS 9.2 was used for data analysis, and P < 0.05 was declared as statistically significant.

Results: After the matching, 468 patients were included (234 for each). The overall mean age was 9.90±2.34 years with 239 males (51.1%). Age, gender, and headache were not different between the groups (P>0.05). Anosmia (20.1% vs. 10.3%; P=0.003), and ageusia (24.4% vs. 6.4%; P<0.0001) were higher in COVID. Cough (34.2% vs. 52.1%; P<0.0001), fever (65.0% vs. 81.6%; P<.0001), and dyspnea (12.4% vs.35.0%; P<.0001) were higher in URTI.

Conclusions: The patients with COVID-19 had a higher prevalence of anosmia and ageusia, while cough, fever, and dyspnea were higher in URTI in pediatric population. Therefore, taking these differences into consideration might aid physicians in their differential diagnosis and treatment during the pandemic

AAV-Anc80L65 Mediated Gene Transfer in the Mouse Cochlea

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Background: Adeno-associated virus (AAV) is a useful vector for inner ear gene therapy because of its high efficiency transduction in hair cells (HCs), especially inner HCs. Another advantage of AAV is that, unlike adenovirus vectors, a perilymphatic injection can result in transduction of the auditory epithelium and therefore it can be used to deliver transgenes into HCs for treating diseases caused by HC mutations. However, some prevalent diseases require gene transfer into supporting cells (SCs). Two clear examples are HC regeneration therapy based on transdifferentiation of SCs to new HCs, and treating SC-altering mutations such as GJB2. Here we report results obtained by injecting AAV-Anc80L65-CMV-eGFP into inner ears of mature animals, the likely and most practical target of gene therapy for deafness.

Methods: AAV-Anc80L65-CMV-eGFP was inoculated into adult inner ears of wild type mice or diphtheria toxin (DT) treated Pou4f3(+/DTR) mice, via a round window (RW) injection with a vent (perforation) in the posterior semicircular canal. Auditory brainstem response (ABR) test was performed 2 weeks later and then inner ears were prepared for immunofluorescence staining. Because vestibular SC transfection is also of interest, we assessed transduction of the AAV in SCs in both cochlea and utricle.

Results: Anc80L65 transduced 22.36%±14.21% of pillar cells (PCs), 52.49%±13.81% of SCs medial to PCs (MSCs) and 48.01%±12.27% of SCs lateral to PCs (LSCs) in normal cochlea (average transduction from apex to basal turn). In DT injured cochlea, average transduction of SCs were 35.84%±13.69%, 68.76%±10.26%, 57.72%±9.73% in PCs, MSCs, and LSCs, respectively. Anc80L65 also transduced 54.86%±19.74% of SCs in normal utricle and 86.32%±4.72% of SCs in injured utricle. Statistical analysis revealed that Anc80L65 transduced significantly more cochlear MSCs and utricular SCs in traumatized inner ear than in normal ones. ABR

thresholds of Anc80L65-injected normal ears were significantly higher at all tested frequencies compared with age-matched controls (no surgery), but remained comparable with the normal saline injected ears.
Conclusions: AAV-Anc80L65 is capable of efficient transduction in SCs of both normal and traumatized adult mice inner ears, with no vector-related injury on hearing thresholds. AAV may therefore be useful for SC gene therapy for HC regeneration as well as treatment of hereditary hearing loss caused by SC genes.

Elevated Levels of IL-6 and Other Cytokines After Cochlear Implant Insertion in Mice

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Background: Insertion of a cochlear implant (CI) into the scala tympani causes cochlear trauma and a foreign body response which can influence functional outcomes. Short-term changes involve participation of the immune system, which evolved for beneficial body defense activity, but may over-react leading to detrimental effects. Following CI insertion, such responses to trauma and the presence of a foreign body often lead to fibrosis and transient functional deficits. Better understanding of signaling molecules mediating responses to trauma by the immune system should help in controlling the duration and extent of those events. The availability of molecular tools and assays for the murine system make it useful for studies aimed at detecting immunological responses to CI insertion. Here we assayed systemic changes in levels of immune signaling molecules in mice after CI insertion.
Methods: Ten CBA/J wild-type, young, mature mice of both genders were equally divided in 2 groups, one receiving a CI, and the other a control. For CI insertion, mice were anesthetized with isoflurane, given ketoprofen (5 mg/kg, SC) for pain and glycopyrrolate (0.02mg/kg SC) to decrease salivation. An incision was made behind the left pinna and the bulla was exposed and opened with a hand drill. Bone was removed to create a defect large enough to visualize the round window membrane, which was punctured with a pointed absorptive pledget. A platinum-iridium single-ball CI electrode was inserted into the scala tympani. The bulla was sealed with carboxylate cement and the incision sutured. Five days later, all mice were anesthetized, blood withdrawn by cardiac puncture and serum harvested and immediately frozen. Sera were assayed with a MILLIPLEX Mouse Cytokine/Chemokine Magnetic Bead Panel to measure the levels of inflammatory markers in each sample.
Results: Differences between implanted and control cochleae in the levels of several immune molecules were detected in serum samples, indicating systemic changes in immune system activity. Especially notable, implanted ears had significantly higher levels of IL-6 and MIP-2, and CXCL1 also was substantially elevated, but not significantly. These data point to a systemic immune challenge in the implanted ears. These molecules are associated with the acute phase of injury response, which includes fibrosis and bone formation in other systems, and our findings confirm their activity in the response to implant insertion into the cochlea.
Conclusions: These data are useful for targeting inhibitory measures for preventing excessive fibrosis and bone formation that may occlude the normally fluid-filled scalae. We predict that performing the same assay on perilymph (in addition to serum) could provide more details about the local reaction in the cochlea. Such assays, when performed using fluids obtained from cochlear implant patients during surgery, may yield information useful for moderating the immune response.

Group Vestibular Rehabilitation Programme as a Cost-Effective Outpatient Management Option for Dizzy Patients

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Background: The current options for outpatient vestibular rehabilitation include self-administered exercises at home or costly customized personal sessions. In the consideration of optimising vestibular rehabilitation results

balanced against finite healthcare resources, this study was performed to evaluate the effectiveness of outpatient Group Vestibular Rehabilitation Therapy (G-VRT) program in patients presenting to a tertiary institute's ENT outpatient clinic for dizziness.

Methods: Medical records of 77 patients who presented between December 2019 and July 2020 with persistent dizziness and were treated with G-VRT were retrospectively analyzed. All patients received a modified version of Dr. Goto's G-VRT(1hr) consisting of vestibulo-ocular training, static and dynamic training and a short lecture in small groups of 3 to 10 patients, both administered by an ENT specialist. All patients were instructed to perform the vestibular exercises taught in the G-VRT program 3 times a day for first 3 months with a daily recording of their performance in a diary provided. Outcome measures evaluated at 2,4,8 and 12 weeks post G-VRT included compliance to the G-VRT program, Functional Level Scales (FLS), the Dizziness Handicap Inventory (DHI) and the Visual Analog Scale (VAS) score.

Results: All outcome measure scores were significantly improved after G-VRT; DHI scores improved from 25.92 $\pm$ 2.51 to 16.42 $\pm$ 2.39 (P=0.000), VAS for dizziness improved from 3.81 $\pm$ 0.27 to 2.52 $\pm$0.33 (P=0.000) and FLS improved from 2.80 $\pm$ 0.13 to 2.21 $\pm$ 0.14 (P=0.000). A larger therapeutic effect was seen in younger patients and patients with the higher DHI scores at presentation (P< 0.01).

Conclusions: In view of the good therapeutic response observed with G-VRT, we propose that it is a useful cost-effect management modality for the rehabilitation dizzy patients in an outpatient setting. Larger case-control studies comparing G-VRT with the current practice of generic or customized vestibular exercise will be useful to validate the clinical value of G-VRT.

Can a Calcitonin Gene-Related Peptide (CGRP) Receptor Antagonist Mitigate Neuroinflammatory, Hyper-Immune, and Nausea-Like Responses to SARS-Cov-2 Infection in Preclinical Mouse Models?
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Background: In December 2019, the coronavirus disease (COVID-19) caused by SARS CoV-2 was identified. COVID-19 causes a respiratory illness like the flu with symptoms such as fever, cough, headache, chills, and nausea. The FDA has approved Biohaven Pharmaceuticals to proceed to a clinical trial of its CGRP-receptor antagonist to treat patients with severe COVID-19, suggesting that the neuroinflammatory reaction that is initiated by CGRP in response to SARS-CoV-2 could be a therapeutic target for treating severe COVID-19.   We were interested in testing if a CGRP receptor antagonist (olcegepant) would mitigate COVID-19 symptoms in mice.

Methods: As a readout of SARS-CoV-2 infection symptoms, we have assessed weight loss, O2 saturation, temperature in young and old mouse models with the CGRP receptor antagonized by olcegepant (2 mg/kg/day/SQ). In ongoing experiments, we will be also monitoring the presence of a nausea-like state by assessing hypothermic responses to provocative motion.

Results: To date, we have determined that CGRP receptor antagonism is only protective in older C57B6 and older 129Sv mice, as there was no significant difference between CGRP receptor antagonism and placebo controls in younger mice.   Ongoing studies will determine if CGRP antagonism is similarly protective against provocative motion-induced nausea - like symptoms.

Conclusions: Information gained from these studies will provide a direct assessment of whether a CGRP-receptor antagonist can mitigate both mild and severe symptoms associated with SARS-CoV-2 infection. This research is supported by a COVID19 research supplement to NIH R01 DC017261 (AL). We would also like to thank Dr. Ralph Baric (UNC) for the MA-10 SARS CoV-2 virus stock, and Dr. Steve Dewhurst (UR) for MA-10 viral expansion.

Combined Analysis of the Test-Retest Reliability of Distortion-Product Thresholds Based on DPOAE Level Maps and Short-Pulse DPOAE Amplitudes of the Nonlinear-Distortion Component
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Background: When referred to baseline measures, serial monitoring of pure-tone behavioral thresholds and distortion-product otoacoustic emissions (DPOAEs) can be used to detect the progression of cochlear damage. Estimated distortion-product thresholds based on short-pulse DPOAE level maps (EDPT LM) provide a direct quantitative estimate of hearing loss due to cochlear-amplifier degradation. They combine a short-pulse stimulus paradigm optimized to reduce DPOAE interference effects and an individually optimum stimulus-intensity

paradigm, which when combined allow objective, accurate, and reliable threshold estimates. However, EDPTLM as a stand-alone measure show in a normal hearing population, similar to pure-tone-audiometry in about 3 – 5% of the cases threshold shifts of more than 10 dB (Bader et. al, 2021). Here we demonstrate the effect of combined analysis of EDPTLM threshold and short-pulse DPOAE amplitudes of the nonlinear-distortion component (POD) level shifts.

Methods: The EDPTLM were recorded seven times within three months at 14 frequencies with f2 = 1–14 kHz in 20 ears from ten subjects with normal hearing (4PTA 0.5–4kHz < 20 dB HL). To obtain EDPTLM, short-pulse DPOAEs were recorded using 21 L1,L2 pairs. Reconstruction of DPOAE growth behavior as a function of L1 and L2 using nonlinear curve fitting enabled the derivation of EDPTLM for each frequency. Test-retest reliability was determined using average threshold differences between each possible test-session (N=21) for all POD when the corresponding EDPTLM was computable and accepted.

Results: Applying a 10 dB critical threshold increase criterion for EDPTLM and a 10 dB critical level decrease criterion for POD to the matched data set of threshold and level differences for f2 = 1 – 14 kHz (N= 32323), 3.54% (N = 1145) of EDPTLM differences between visits or 1.45% (N = 470) of POD level differences were critical. When we calculated the percentage of visit-to-visit differences at which both – EDPTLM and POD – differences, exceeded their criterion, 0.41% (N = 134) of differences breached the criterion.

Conclusions: In future, to increase the sensitivity for detecting true pathologic threshold shifts, the observation of simultaneous increases of EDPT derived from DPOAE level maps and decreases of DPOAE amplitudes may be more sensitive and specific as compared to analysis of DPOAE amplitudes alone, at least in cases of sufficient cochlear amplifier gain to detect a DPOAE at baseline.

Comparing Upper and Lower Sideband Distortion Product Otoacoustic Emissions in Mice With Tectorial Membrane Defects
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Background: Otoacoustic emissions are clinically important as they serve to separate cochlear from retro-cochlear disorders. Hence, they can assist in the differential diagnosis of sensory changes associated with the active processes linked to outer hair cells (OHCs). In these experiments, mice with genetic deletions/mutations affecting the tectorial membrane (TM) were used to evaluate changes in upper and lower sideband (USB, LSB) distortion product otoacoustic emissions (DPOAEs). These DPOAE components were studied in mutants with near-normal and partial loss of cochlear amplification.

Methods: Mice with loss of or mutations in genes that affect the structure and physical properties of the TM were tested using DPOAEs. Magnitudes of 2f1-f2 (LSB) and 2f2-f1 (USB) were compared to evaluate the effect of hearing loss on these two intermodulation distortion products in mutants with and without spontaneous otoacoustic emissions (SOAEs). Both iso-input and input-output or growth functions were collected at various f2 frequencies. The latter were used to determine DPOAE thresholds, the level of f1 that produced a 2f1-f2 or a 2f2-f1 of 0 dB SPL. The frequency ratio, f2/f1, was 1.2 for all measurements.

Results: In wild-type (WT) mice, the DPOAE at 2f2-f1 (USB) is small compared to the spectral component at 2f1-f2 (LSB). In fact, the threshold for 2f2-f1 is 35-40 dB higher than for 2f1-f2, similar to threshold shifts associated with loss of amplification. In addition, TM mutants with partial loss of gain tend to produce WT-like 2f2-f1 responses despite reductions in 2f1-f2. Although some mice with TM defects showed larger 2f2-f1 responses relative to controls, these enhancements were only observed in frequency regions where mutants generated SOAEs. The latter emissions are observed in a majority of TM mouse models when the loss of sensitivity is less than ~25 dB SPL.

Conclusions: In control animals, USB DPOAEs at 2f2-f1 are much smaller than those at 2f1-f2. In addition, mice with reduced sensitivity, as indicated by reductions in 2f1-f2, generate near-normal DPOAEs at 2f2-f1 consistent with the idea that the USB DPOAE is not receiving significant cochlear amplification. This observation suggests that the USB DPOAEs may originate near the DP place (Martin et al., 1998; Knight and Kemp, 2001). In this case, OHC generators basal to f2, and with best frequencies (BF) near 2f2-f1, would facilitate feedback of 2f2-f1. Hence, it is likely that OHC sources in the region of overlap between the excitation patterns of the two primaries are generating 2f2-f1 before they reach their respective BF locations and where the generators are responding more linearly on the tails of their tuning functions.

Secondary Interactions Among Two-Tone Distortion Products Investigated Using a Nonlinear Mechano-Electro-Acoustical Model of the Mammalian Cochlea

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Background: Two-tone distortion products (DP) are generated inside the cochlea due to nonlinearities when excited by two simultaneous pure tones (f1, and f2 > f1). They are known to influence our sound perception, as well as lead to sound emission out of the ear called distortion product otoacoustic emissions (DPOAE). The DP frequencies are an algebraic combination of f1 and f2, in which cubic terms (2f1-f2) and (2f2-f1) have the highest magnitude compared to other DPs (such as 3f1-2f2, 4f1-3f2,3f2-2f1,4f2-3f1). The DPs are thought to be generated due to cochlear nonlinearities between the f2 and f1 best places, travel from that generation site, and possibly peak at the DP best place. This study introduces and tests a new hypothesis that secondary interactions among a pair of lower-order DPs could be a significant generator of higher-order DPs (e.g., 2f1-f2 and 3f1-2f2 could interact to generate 4f1-3f2).

Methods: A nonlinear mechano-electro-acoustic (MEA) model of the cochlea is developed by including mechano-electrical transduction nonlinearity in the quasi-linear MEA model (Ramamoorthy et al, JASA 2007; Agarwal and Ramamoorthy, Journal of Applied Physics 2020) and combined with a middle ear model. The DP and DPOAE predicted by the nonlinear MEA model are benchmarked with Meaud, U.Mich dissertation, 2010 and Wen et al, Hearing Research, 2018, respectively.

In order to predict the relative strength of secondary interaction among DPs compared to that generated directly in the f2 and f1 interaction region, two simulations are performed. In the first simulation, bipolar currents across scala vestibuli and scala tympani are applied at the f2 and f1 best places respectively thus generating several DPs such as 2f1-f2, 2f2-f1, 3f1-2f2, 3f2-2f1, 4f1-3f2, 4f2-3f1, etc. In the second simulation, bipolar currents are applied at the 2f1-f2 and 3f1-2f2 best places respectively. Note that in the second simulation, 2(3f1-2f2) - (2f1-f2) = 4f1-3f2 is the 'cubic DP', whereas in the first simulation, 4f1-3f2 is a higher-order DP. The 4f1-3f2 response in the basilar membrane vibrations predicted from both simulations are compared to determine the relative contribution from secondary DP interactions.

Results: The results show that 4f1-3f2 generated by the secondary interactions among 2f1-f2 and 3f1-2f2 are close to that generated by the f1 and f2 interaction. This result supports the proposed hypothesis that secondary interactions among lower-order DPs (2f1-f2 and 3f1-2f2) may be primary generators of higher-order DPs (4f1-3f2).

Conclusions: High level of secondary interaction among DPs suggests that the generation site of higher-order DPs may be broader than the f1 and f2 interaction region.

Auditory Hypersensitivity and Processing Deficits in a Rat Model of Fragile X Syndrome

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Background: Abnormal sensory processing is a hallmark of autism spectrum disorders (ASD) and related neurodevelopmental disorders like Fragile X syndrome (FX), most notably manifesting as extreme sensitivity to sound (i.e. hyperacusis). Auditory hypersensitivity is not only a pressing clinical problem in FX and ASD, but it also likely reflects fundamental circuit defects that extend to more complex but less accessible features of the disorders, such as impaired communication and language development. The goal of this study was therefore to develop a quantitative behavioral read-out of auditory processing deficits in FX that could serve as a clinically translatable platform for screening potential therapies in animal models.

Methods: We characterized auditory hypersensitivity in a Fmr1 knockout (KO) transgenic rat model of FX using a Go/No-Go operant conditioning task to assess sound detection thresholds and suprathreshold auditory reaction time-intensity (RT-I) functions, a reliable psychoacoustic measure of loudness growth, at a variety of stimulus frequencies, bandwidths and durations.

Results: Male Fmr1 KO and littermate WT rats learn the sound detection task at the same rate and reach the same level of peak performance but Fmr1 KO animals exhibited faster auditory RTs over a broad range of intensities and steeper RT-I slopes compared to WT controls, consistent with perceptual evidence of excessive loudness growth in FX. Fmr1 KO animals also displayed altered perceptual integration of sound duration and bandwidth compared to WT controls, suggesting that faster RTs in these animals is evidence of aberrant auditory processing rather than generalized hyperactivity or altered motor responses.

Conclusions: This novel symptoms-to-circuit approach has the potential to uncover fundamental deficits at the core of FX and ASD pathophysiology while also having direct clinical implications for one of the most disruptive features of these disorders.

Comodulation Masking Release in Young and Old Mongolian Gerbils

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Background: Elderly human subjects often experience perceptual difficulties processing signals in background noise with a complex acoustic spectro-temporal structure due to multiple sources being active at the same time. By exploiting the spectro-temporal structure of masking noise, the auditory system can considerably improve its sensitivity for detecting tones in such a complex acoustic environment. A well-established paradigm in human psychoacoustics in which young and old human subjects differ in perceptual performance, is the comodulation masking release (CMR) paradigm. It compares tone-detection thresholds in maskers with uncorrelated envelopes in different frequency bands with thresholds in maskers with correlated envelopes in different frequency bands. The threshold difference between those two conditions has been termed CMR. Here we report the CMR in seven Mongolian gerbils with an age ranging from 8 to 40 months that were obtained in the "flanking-band paradigm" (see Schooneveldt and Moore 1987, doi: 10.1121/1.395639).
Methods: Normal-hearing gerbils of different age were trained in a Go/NoGo procedure to report a 400-ms tone being presented in a noise masker. Tone frequency was either 700 Hz or 4000 Hz. The noise masker with an overall level of 57 dB SPL was composed of two 25-Hz wide bands, one centered on the tone frequency (On-Frequency Band, OFB) and one on a frequency differing by -400, -100, 0, 100, or 400 Hz from the tone frequency (Flanking Band, FB). Masked thresholds were determined for a condition in which the two masker bands had uncorrelated envelopes (UN) and a condition in which the two masker bands had identical (correlated CO) envelopes. CMR was calculated as the difference between UN and CO thresholds.
Results: In the flanking-band paradigm gerbils showed a large CMR ranging between 10 and 22 dB (mean values) which is similar to the human CMR. Signal frequency did not affect CMR suggesting that the possibility to encode the temporal fine structure in the frequency range around 700 Hz does not provide for a larger CMR than in the frequency range around 4000 Hz in which hardly any phase locking to the temporal fine structure is possible. This suggests that temporal fine structure perception is less relevant for the CMR effect and that the CMR effect in this stimulus paradigm is likely due to the processing of the signal envelope. There was no effect of the gerbils' age on CMR, consistent with the hypothesis that CMR does not rely on the perception of temporal fine structure that is compromised in old gerbils compared to young gerbils (Oetjen et al. 2020, ARO Midwinter Meeting).
Conclusions: Since Mongolian gerbils show a similar CMR as human subjects, they constitute a suitable animal model for investigating the underlying mechanisms.
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Temporal Dynamics of Auditory Bistable Perception and Baseline Pupil Size

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Background: Pupillary responses (pupil size changes) are indexed to changes of the neural activities in the locus coeruleus (LC) via Norepinephrine (NE) release (LC-NE system), which has been shown to reflect a broad range of cognitive processes. For example, Einhauser et al. (2008) have reported that transient pupil dilation as a consequence of the reconciliation with the neural network in bistable perception precedes the perceptual alternation, suggesting that NE releases as an outcome of a phasic arousal/attention change. However, whether the effect can only occur at the timing of perceptual alternation or whether the arousal-related pupil size changes can further explain the occurrence pattern of perceptual alternation in a sustained longer time range than single trial remains unclear. Here, we found that baseline pupil size as an index of temporal fluctuation of arousal level over a longer range of timescales than the transient phasic changes relate to the frequency of perceptual alternation in auditory bistability.
Methods: We observed pupil size while participants listened to repeating triplets (ABA_ABA_ABA_..., where A and B are tone bursts differing in frequency, and _ indicates blank), which produce switching percepts, typically a single galloping rhythm (ABA_...) or two streams with different rhythms (A_A_... and B___B____...). The auditory sequence was displayed for 180 s in one session, segregated by a visual response cue every 5–7 s to ask for

answering the number of perceptual alternations in Experiment 1 (0, 1, 2, 3, 4, or more than 5), or responding whether the perceptual alternation occurred in Experiment 2 (yes/no). Baseline pupil size was defined as the mean of pupil diameter -1 to 0 s reference to the response cue.

Results: Baseline pupil size as the mean pupil diameter over a period of 1 s prior to the task response monotonically increased with increasing number of perceptual alternations (Experiment 1) or became larger with the higher probability of perceptual alternations occurrence (Experiment 2). The finding suggests that the higher the baseline pupil size-linked arousal is, the more frequent the reconstruction of the dynamic neural network shifts, presumably indexed by the number of perceptual switches. Furthermore, a cross-correlation analysis indicates that baseline pupil size predicted perceptual alternation at least 35 s before the behavioral response and that the overall correspondence between pupil size and perceptual alternation was maintained over a sustained time window of 45 s at minimum.

Conclusions: The overall results suggest that variability of baseline pupil size reflects the stochastic dynamics of arousal fluctuation in the brain related to bistable perception.

High-Resolution Transcriptional Analysis of Avian Auditory Epithelia in an Explant Culture Model for Hair Cell Regeneration

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Background: Unlike mammalian cochleae, the avian basilar papilla (BP) has the robust capacity for hair cell (HC) regeneration. Although the major process of HC regeneration in avian BPs has been revealed, precise molecular mechanisms for the activation of supporting cells (SCs) toward HC regeneration are still unclear. Aiming to elucidate molecular events during SC activation for HC regeneration, we performed single-cell RNA sequencing of chick BPs during the process of HC regeneration using an explant culture model that we previously established (Matsunaga 2020).

Methods: BPs of the post-hatched one-day chicks were provided for explant cultures. Exposure to streptomycin (SM) for 48 h was used to destroy HCs. Capture of single cells dissociated from chick BPs at different stages during HC regeneration and cDNA synthesis was performed by C1 Single-Cell Auto Prep system. The raw sequencing data were converted into FASTQ files using the Illumina bcl2fastq software and mapped to the chicken reference genome GRCg6a.

Results: A total of 1,054 cells from chick BPs were analyzed. Unbiased clustering identified 12 clusters. According to the expression patterns of SC marker genes, five clusters were annotated as SC clusters from 12 clusters. The data of five SC clusters were extracted and re-clustered. In consequence, eight SC-clusters were obtained. Based on the expression patterns of ATOH1 and HC differentiation genes including GFI1 and LHX3, two SC-clusters were annotated clusters containing SCs during transdifferentiation into HCs. One of the clusters that consisted of non-sensory cell populations were identified by differentially expressed genes. Finally, we extracted four SC-clusters, namely quiescent SC, primed SC, activated SC and converting HC clusters and performed a pseudotime trajectory analysis to illustrate key genes for inducing SC activation. A pseudotime trajectory analysis revealed the importance of EDNRB2 for activation of SCs and induction of subsequent HC differentiation. Ednrb2 was identified as being expressed in activated SCs in BP explants damaged by SM.

Conclusions: The present results provide new insights regarding SC transdifferentiation into HCs in chick auditory epithelia and indicate possible roles of endothelin B signaling in induction of SC transdifferentiation.

Single Cell RNA-Sequencing Analysis of Small Molecule A2CE Inhibition of p27Kip1 in Adult Mouse Cochleae

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Background: Adult mammals lack the ability to regenerate hair cells in the cochlea following damage, resulting in permanent hearing loss. Previous studies have shown genetic manipulation of key pro-regeneration genes was sufficient in producing new but immature hair cells from the supporting cell population in adult mouse cochleae, specifically Atoh1 overexpression combined with p27Kip1 (p27) inhibition (Liu et al, 2012; Cox et al, 2014; Walters et al, 2017). Alsterpaullone 2-cyanoethyl (A2CE), a previously identified p27 transcriptional inhibitor, is a

candidate for a small molecule-based drug therapy to recapitulate these results without genetic manipulations, which is much more clinically translatable. Here, we utilize a combination of RT-PCR and single cell RNA sequencing at several time points to characterize the effect A2CE has on adult mice following local transtympanic delivery of the small molecule.

Methods: Adult FVB strain mice (P28) of both sexes were given 5mM A2CE via transtympanic injections to the left ears. After waiting either 4 hours or 24 hours, mice were euthanized and the inner ear organs of Corti were collected and harvested for RT-PCR. p27 mRNA was normalized to a housekeeping gene. Basilar membranes were isolated and all steps for cDNA library were performed following 10x Genomics protocols, with Chromium Next GEM Single Cell 3' Reagents Kit v3.1. Illumina Nextseq and raw counts were processed using Cell Ranger (v6.0.1). Single-cell data was analyzed in R studio and clustered with Seurat (v4.0.4).

Results: In vivo transtympanic administration of 5 mM A2CE at the 4-hour time point showed no significant reduction of p27 in RT-PCR analysis of cochlear sensory epithelia, while 24-hour time point produced a significant reduction. Single Cell RNA-sequencing at the 4-hour time point following injections resulted in clear clustering of the supporting cell (SC) and hair cell (HC) populations (IHC: 189 cells, OHC: 468 cells) with sensory epithelial populations revealed using differentially expressed genes. A total number of 26118 samples were collected across both treatments, with 19,201 genes detected, after manual filtering of raw counts based on nCount and nFeature with mitochondria percentage. No significant reduction was detected in p27 between the vehicle (15% DMSO) control and A2CE administration in HC and SC clusters, recapitulating the 4-hour RT-PCR results.

Conclusions: RT-PCR data showed that transcriptional inhibition of p27 by A2CE takes at least 24 hours for the effect to be significant, and the single cell data at the 4-hour time point corroborates this. Further work will include a single cell RNA-sequencing of the 24-hour time point to evaluate the pathways and effects of the drug A2CE on the cochlear sensory epithelial cells.

DTR Mice Receiving Combinatorial Atoh1 and Gfi1 Gene Transfer 4 Months After DT Exhibit New Cochlear Hair Cells

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Background: Hair cell regeneration in the adult mammalian cochlea is limited even after Atoh1 induction. Gfi1 is a transcription factor necessary for hair cell differentiation and survival. It was reported to increase hair cell differentiation in embryonic stem cells along with Atoh1 and Pou4f3 induction. We have previously shown that combinatorial Adenovirus-mediated Atoh1 and Gfi1 over-expression can enhance hair cell regeneration in adult mature cochlea when the virus injection was done at the same time as diphtheria toxin injection. In most future clinical cases requiring hair cell regeneration treatment, months to years will likely pass between hair cell loss and clinical therapy. Here we tested whether injecting Gfi1-Atoh1 adenovirus several months after DT can lead to appearance of new hair cells.

Methods: Adult Pou4f3-DTR mice (Golub et al. 2012) were deafened by DT injection between the ages of 32 and 50 days. Adenovirus carrying tdTomato reporter gene with Gfi1 + Atoh1 was injected into the left ear scala media, between 18 and 21 weeks after DT. The right-side cochlea was used as control. Animals were euthanized and prepared for histology 4 weeks later. Tissues were processed for whole-mount immunohistochemistry to visualize Myosin VIIa and the reporter gene, then analyzed by epi-fluorescence.

Results: Of the 8 left ears in this study, 6 displayed Myosin VIIa positive cells. These cells appeared to be in and around the area of the organ of Corti, but location could not be easily confirmed due to cell location shifts in long term deaf ears. Some of the Myosin VIIa positive cells were negative for the reporter. Myosin VIIa positive cells were seen in both apical and basal turns of the left ears. Right (control) ears displayed no Myosin VIIa positive cells.

Conclusions: Adenovirus-mediated gene delivery by scala media injection several months after hair cell elimination with DT resulted in the appearance of new hair cell-like cells, based on Myosin VIIa expression. Some of these new hair cells did not express the reporter gene, suggesting that a wave of transdifferentiation may proceed even without viral mediated forced expression of GFI-1 and Atoh1 in every cell. The data demonstrate a first success in regenerating hair cells months after deafening, validate the usefulness of the DTR mouse for

regeneration studies and reinforces the need for viral vectors that can incorporate several transcription factors and have high affinity to supporting cells.

Speech Perception by Distantly-Presented Bone-Conducted Ultrasound: Assessments by a Monosyllable Articulation Test and Phonetic-Feature Transmission Analyses
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Background: Bone-conducted ultrasound (BCU) is perceived even by the profoundly sensorineural deaf. A novel hearing aid using the perception of amplitude-modulated BCU (BCU hearing aid: BCUHA) has been developed. In BCUHA, the vibrator is usually pressed against a part of the cranial bone behind the ear (mastoid process); however, BCU presented to parts of the body distant from the head can be perceived. In this study, to evaluate this "distantly-presented BCU" hearing, Japanese monosyllable articulation tests were conducted. Additionally, sequential information transfer analyses (SINFA) were conducted to determine what type of articulatory features were well transmitted.
Methods: 10 Japanese adults (males, 22-26 years), with normal-hearing participated in the monosyllable articulation test. 100-Japanese monosyllables recorded in a female voice were used as modulator signals. 30-kHz ultrasonic carrier was amplitude-modulated (AM) by the monosyllables and presented using a vibrator to following three locations: (a) mastoid process of the temporal bone, (b) sternocleidomastoid muscle (muscle of the neck), and (c) clavicle. The vibrator was fixed using an over-the-head-type steel band (mastoid), an elastic band (sternocleidomastoid muscle) or a shoulder supporter band (clavicle) with presentation pressure of 2 N (sternocleidomastoid muscle) or 5 N (mastoid and clavicle). Additionally, air-conducted (AC) monosyllables, i.e., the modulator signal itself, was presented to the same side as the BCU stimuli for comparison.
Results: In the monosyllable articulation test, the score for AC condition was higher than that of BCU condition. Confusion (miss-recognition of monosyllable) was more prevalent in the BC than in the AC condition. Differences in the percent correct between AC and BCU were significant in the clavicle for vowels and in all locations for consonants. Additionally, the phonetic features transfer ratios of vowels varied depending on the stimulus placements. Moreover, the "contracted sound" and "nasality" phonetic features of consonants were well transmitted when the stimuli were presented onto the sternocleidomastoid.
Conclusions: The results obtained suggested that a practical speech transmission, comparable to ordinary BCUs, presented onto the head, can be obtained by distantly-presented BCU. Additionally, it is also suggested that the feature information transmission may depend on the characteristics in the biological tissues such as attenuation rate and frequency characteristics.

Speech Sound Discrimination by Young and Old Mongolian Gerbils
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Background: Many elderly listeners have difficulties in speech perception, even if auditory thresholds in quiet are normal. Especially under noisy conditions, elderly have difficulties in speech comprehension. However, the underlying mechanisms resulting in compromised speech reception with age are still unknown. This study investigates the discrimination of speech sounds in Mongolian gerbils as a model organism. Gerbils show a good low-frequency hearing with a similar sensitivity as humans for the frequency range of human speech and they show characteristics of strial and neural presbycusis, which makes them a suitable model for investigating discrimination of human speech sounds and age-related hearing loss. Here, we report data on the discrimination of logatomes (CVCs - consonant-vowel-consonant combinations, VCVs - vowel-consonant-vowel combinations) by young and old gerbils in order to reveal possible age-related changes in the perception of speech sounds.
Methods: Four young (8 to 21 months) and five old (34 to 45 months) Mongolian gerbils were trained to perform an oddball target detection paradigm in which they discriminated a deviant CVC or VCV in a sequence of CVC or VCV standards, respectively. In CVCs, the central vowels /a/, /aː/, /ɛ/, /eː/, /ɪ/, /iː/, /ɔ/, /oː/, /ʊ/ and /uː/ were combined with the outer consonants /b/, /d/, /s/ and /t/. In VCVs, central consonants were /b/, /d/, /f/, /g/, /k/, /l/, /m/, /n/, /p/, /s/, /t/ and /v/, together with the outer vowels /a/, /ɪ/ and /ʊ/. The experiments were performed with an ICRA-1-noise-masker with speech-like spectral properties, and logatomes of multiple speakers were presented at various signal-to-noise ratios. Response latencies served to generate perceptual maps employing multidimensional

scaling reflecting the gerbils' internal representations of the sounds. To evaluate which features of vowels and consonants are most important for discrimination, the dimensions of the perceptual maps were correlated with different features of the speech sounds, and it was investigated how the discrimination differs between young and old gerbils.

Results: The perceptual representations of vowels in multidimensional scaling were very similar in young and old gerbils, and perceptual distances in young and old gerbils were highly correlated. Both age groups discriminated vowels predominantly based on differences between spectral features of formants, particularly the frequency of the first and second formant being determined by tongue height and position. Although showing similar patterns in the perceptual maps, the perceptual distances of consonants by young and old gerbils were only weakly correlated. The discrimination of consonants mostly depended on combinations of their articulatory features. However, the relative importance of the different articulatory features for consonant discrimination changed with age.

Conclusions: In contrast to the known age-related deficits in speech sound discrimination in humans, gerbils showed no decrease in their ability to discriminate logatomes with increasing age.

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Perceptual-Neural Association in Noise: Varies Across Bilinguals' First and Second Language

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Background: While perceptual measures show a weakened trend on speech in noise perception among bilinguals compared to monolinguals, the literature points out good consequences of bilingualism for the neural architecture. The current study aimed to understand the association between perceptual and physiological outcomes in bilingual listeners and test whether this association varies across bilinguals' two languages (first [L1] and second [L2] languages).

Methods: Thirty Arabic-Hebrew bilinguals and 29 Hebrew monolinguals between the ages of 20 and 35 years participated in the study. Auditory brainstem responses evoked by the speech syllable /da/ were collected in quiet and noise, and the perception of speech stimuli under the same listening conditions was examined. The perceptual performance among bilinguals was tested in their two languages (Arabic [L1] and Hebrew [L2]). The perceptual-physiological relationship was also examined.

Results: The results showed that increased brainstem resistance was related to better perceptual abilities among bilingual participants. This relationship varied across bilinguals' two languages. Better speech in noise performance in L1 was correlated to fewer changes in F0 representations, and higher perceptual accuracy in L2 was linked to minor shifts in auditory neural timing.

Conclusions: This set of findings prompts the use of neural brainstem responses to explain (even partially) inter-subject variability in bilinguals' performance in challenging listening conditions. The perceptual-physiological associations demonstrate that different neural responses may determine which bilingual individuals are expected to be more prone to the detrimental effect of noise, even in their L1. The current findings can aid the development of assistance programs (for example, in academic settings) to help individuals predicted to encounter more difficulties in such conditions.

Tinnitus Detection in CBA/CaJ Mice Using Active Avoidance Shuttle Box Test Model

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Background: Tinnitus is the maladaptive perception of sound in an absence of external stimulus. In many tinnitus sufferers, these symptoms are associated with negative quality of life. The condition disproportionately impacts individuals with risky occupational or avocational noise exposure. Patients and animal models of tinnitus exhibit altered central auditory system neural activity that is presumed to support the tinnitus percept. An effective method of evaluating tinnitus in rodents would improve the ability to develop a treatment, but existing tinnitus detection methods are cumbersome or controversial. This study used an active avoidance (AA) procedure to assess tinnitus by testing the ability of mice to detect and avoid tones across a wide frequency range

Methods: 14 CBA/CaJ mice between 3 and 4 months of age were trained daily in a shuttle-box and conditioned to associate a tone with a foot shock (0.2 mA). Mice could avoid the shock after tone onset by moving to the other side of the shuttle box. Tones ranged in frequency from 9.51 to 36.4 kHz in 32 steps and were presented at 70dB SPL. Tones were played in a randomized order during a 40-minute session of 70-85 trials per session. Mice that

failed to avoid by moving to the safe side within 5 seconds received a shock for a maximum of 15 seconds. Mice that demonstrated 3 days of stable performance with an avoidance rate of >70% across all frequencies were then exposed to 1 hour acoustic trauma of 113 dB narrowband (1kHz) noise, centered at 16 kHz. Post-exposure AA was assessed at 5, 6, 11, and 12 weeks and the presence of tinnitus was inferred by a statistically significant decrease in the conditioned response of 2 standard deviations or more. Baseline and post-acoustic trauma ABRs quantified the degree of temporary threshold shift.

Results: 5 out of 14 mice were found to have behavioral AA evidence of tinnitus demonstrated by a notch in AA response rate at a frequency near or greater than the trauma frequency. The remaining mice had flat AA profiles which did not show evidence of tinnitus. Reaction time or time to crossing was also analyzed and will be discussed as an additional measure of the presence of hyperacusis. Finally, the 5 mice showing evidence of tinnitus had a significantly higher ABR threshold shift (>35 dB) than mice that did not have tinnitus.

Conclusions: The results from this study show that an AA shuttle box procedure which tests the salience of a wide range of tones shows promise in identifying tinnitus in a mouse model. Future studies are planned that will compare this technique to the GPIAS paradigm using pre-pulse inhibition of the acoustic startle response.
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Can High-Frequency Brain Oscillations and Blood Oxygen Levels Reflect Tinnitus and Tinnitus With Co-Occurring Hyperacusis?

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Background: Tinnitus (a constant phantom humming or ringing) and hyperacusis (the noisy or even painful perception of moderate sounds) are major health impairments with a prevalence of 10% to 20% in the population. These impairments in everyday life can worsen or even cause the condition of psychiatric disorders such as depression and anxiety. Currently, conflicting views on the neural correlate of tinnitus (Knipper et al., 2020) hinder the development of effective diagnosis and therapy for tinnitus. Although hyperacusis often co-occurs with tinnitus, it is until now considered neither in clinical diagnosis nor for targeted, individualized therapies. Successful individualized therapy of both sub-entities (tinnitus with or without hyperacusis) requires differentiation, identification and classification of hearing disorders by objective tools.

Methods: Based on previous observations that link reduced and delayed auditory processing and reduced evoked and resting state BOLD fMRI responses to tinnitus (Hofmeier et al. 2021, Refat et al. 2021), we currently investigate pre-defined frequency bands of neural oscillations in combined NIRS/EEG recordings.

Results: Ongoing and task-related activity in selected EEG frequency bands [Hz] delta [1-3] theta [4-7] alpha [8-13] beta [14-20] low gamma [20-40] gamma [40-60] high gamma [60-120] very high gamma [120-200] is discussed as a promising objective measure to identify specific tinnitus sub-entities (tinnitus with or without hyperacusis).

Conclusions: These objective differences could enable individualized therapeutic intervention strategies in patient groups suffering from the above-mentioned sub-entities and thus could increase the urgently needed specificity of future tinnitus intervention.

The Impact of Tinnitus in Adult Cochlear Implant Recipients: A Qualitative Study

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Background: Tinnitus is an important but under-research problem with cochlear implantation (CI). Early results indicate that a quarter of CI recipients experience a moderate to severe tinnitus handicap (Andersson et al., 2009). There is some evidence that CI can result in tinnitus reduction, and in few cases tinnitus increase, besides hearing restoration (Mertens et al., 2016; Ramos Macías et al., 2018, Quaranta et al. 2004). Surprisingly, little is known about the real-life impact that tinnitus actually has on those with CIs. In this study, we aim to explore the

difficulties caused by tinnitus on adult CI recipients using a qualitative approach. In addition, we aimed to explore how CI recipients managed their tinnitus.

Methods: A forum was conducted on an online platform, named Cochlear Conversation. In February 2021, Cochlear Conversation members were invited to voluntarily participate to the forum. Participants were adults who had been implanted with a cochlear implant (Cochlear Ltd) and experienced tinnitus. The forum opened the discussion on three main pre-defined topics: impact of tinnitus on everyday life, impact of cochlear implant on tinnitus and management strategies used to manage. A thematic analysis of the forum discussion was used to develop key themes surrounding these topics (Braun and Clarke, 2006).

Results: One hundred and thirty-six participants participated to the forum. Three key themes were identified from the thematic analysis of the forum: situations impacting tinnitus, difficulties caused by tinnitus and tinnitus management. Each one included sub-themes, such as sound environment, emotional and physical state, sound processor use and time of the day, related to the key theme 'situations impacting tinnitus'. Tinnitus can cause difficulties affecting the social life, the hearing performance, the quality of sleep, the emotions, the concentration and exacerbating some comorbidities. The 'tinnitus management' theme showed that different strategies were used depending on the time of the day, during the day or at night. Tinnitus management strategies varied between self-administrated practices, such as stress management or distraction activities, to therapies provided by health care professionals.

Conclusions: The difficulties associated with tinnitus in CI users were anxiety, stress, fatigue, sleep disorders, social isolation, concentration and hearing difficulties. Tinnitus has been managed differently among CI recipients, ranging from self-administrated activities, to distract from tinnitus or to regulate tinnitus-related stress, to therapies provided by health care professionals. Management strategies can differ between day and night. The themes and sub-themes identified in this study will be used to develop a survey assessing the difficulties caused by tinnitus in CI users.

Developmental Time Determines the Topography, Function, and Circuit Wiring of the Gaze Stabilization Circuit in the Larval Zebrafish

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Background: Brainstem reflex circuits mediate all vital behaviors, but the developmental principles that govern their functional assembly have been challenging to define. Here, we use the larval zebrafish gaze stabilization reflex as a simple model to understand and perturb balance circuit assembly. Vertebrates stabilize gaze using just three populations (sensory afferents, central vestibular brainstem neurons, and motoneurons) to transform vertical (up/down) head movement direction into compensatory eye rotations. The organizational logic that governs circuit assembly is unresolved, though previous work has suggested a role for spatiotemporal cues.

Methods: Here, we examined how gaze-stabilizing central vestibular neurons develop in space and time. We designed a vertical translation paradigm to specifically probe the nose-up or nose-down identity of central vestibular neurons. We then combined calcium imaging of up/down subtypes with anatomical birthdating to link spatiotemporal development with mature function and circuit wiring.

Results: First, we discovered that gaze stabilization circuitry is organized as a micro-topographical map with respect to head tilt direction. Temporal patters of neurogenesis predicted the topography, functional subtype properties, and circuit wiring of gaze-stabilizing central vestibular neurons. Second, genetic loss-of-function experiments eliminated ocular motoneurons as a candidate source of cues that govern topography and circuit wiring.

Conclusions: These results provide a novel developmental organizational framework with which to understand the assembly of the gaze stabilization circuit and suggest a key mechanism: spatiotemporally-available molecular cues. We propose that such cues are released independently of ocular motoneurons.

Rotarod and Acoustic Startle Reflex Performance of Two Mouse Models for Meniere's Disease

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Background: Meniere's disease (MD) is an inner ear disorder of idiopathic origin, characterized by chronic episodes of vertigo, tinnitus, pressure in the ear, and sensorineural hearing loss. Endolymphatic hydrops is believed to be a hallmark of MD pathology. However, 5-15% of MD cases have been identified as familial (Lopez-Escamez et al., 2015). Whole-genome sequencing studies of individuals with familial MD have identified DTNA and FAM136A as candidate genes for autosomal dominant inheritance of MD (Requena et al., 2015). Although the exact functions of these genes have not been identified, FAM136A encodes a mitochondrial protein, and DTNA encodes a cytoskeletal protein involved in the formation and maintenance of synapses, as well as being linked to aquaporin.

Methods: In this project, we tested vestibular and auditory function in DTNA and FAM136A knockout mice models using balance and hearing tests. The RotaRod test, which involves measuring latency-to-fall times for mice placed on an accelerating rotating rod, was used to evaluate balance; dark conditions were used to present a vestibular challenge (performance being monitored with an infrared camera). In addition, a startle reflex-based clicker test was used to evaluate hearing. Balance and hearing data for each gene in both sexes at different ages were obtained and analyzed with Microsoft Excel and GraphPad Prism using descriptive statistics, Student's t-tests, 3-way ANOVA and MANOVA tests. Optical coherence tomography (OCT) was also done on skulls from WT and KO mice.

Results: Three-factor ANOVA results indicated that sex, age, and genotype were significantly correlated with reduced mean latencies for male DTNA KO mice in the RotaRod test (WT, 22.94 sec vs KO, 17.4 sec, n = 484, $p < 1.09*E-12$), while only age had an expected significant correlation with mean latencies for FAM136A KO mice. FAM136A KO mice lost hearing, however, several months before WTs (9-11 vs 15-20 months of age). In male DTNA KO mice, divergence in mean latencies was first evident at early stages (4 months of age), and performance of older males was even more affected with a greater decrease in mean latencies. Head tilts and circling were not generally observed in these mice, although there may be some differences in the endolymphatic compartments, based on OCT observations.

Conclusions: Our results indicate that the mutations in the FAM136A gene generate problems with hearing, while defects in the DTNA gene produce balance deficits. In the initial human study, both genes were studied in the same family, so it was difficult to tease out specific deficits. Future directions will include generating a double knockout mouse model for both genes in order to better understand the hearing loss and balance related symptoms associated with Meniere's Disease.

Habituation of Velocity Storage Provides Effective Treatment for Mal De Débarquement Syndrome (MdDS)
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Background: The velocity storage mechanism of the central vestibular system contributes to the perception of self-motion. We hypothesize that the oscillatory vertigo in MdDS is a consequence of oscillatory signals that are continuously regenerated by a maladapted velocity storage. The premise that a maladapted velocity storage may be corrected by re-adapting the vestibulo-ocular reflex (VOR) has been successfully translated into the development of a first effective treatment method for MdDS, with which hundreds of patients have had improved symptoms to date. To further improve the long-term outcomes of MdDS, we examined the utility of a complementary treatment method. Specifically, we hypothesized that if MdDS is caused by dysregulated velocity storage, minimizing its contribution may reduce the symptoms of MdDS. Habituation of the VOR, i.e. reduction velocity storage capacity, is an effect known to last for a long time, possibly permanently.

Methods: Thirty-eight patients with motion-triggered MdDS (32 female, 6 male, age 47±14 yo, ranging 22-78) were randomly assigned to two treatment groups. Twenty-one patients in Group 1 and 17 patients in Group 2 underwent VOR habituation and re-adaptation, respectively. Success of a treatment was defined as a minimum of 50% symptom improvement assessed with an 11-point Likert scale (0-no symptoms, 10 worst symptoms), which when met indicates a considerable strength of the treatment. The strength of velocity storage was quantified with the time constant (Tc) associated with the decay of the slow phase velocity of the VOR nystagmus during rotational tests.

Results: The overall average Tc of patients was 17±3 s, slightly longer than the average Tc, 15.5±2.7 s, of 18 healthy people with similar ages who were previously tested in our laboratory. In Group 1, the Tc was reduced after the treatment by 18% on average (p=0.007), and the treatment was deemed immediately successful in 50% of the patients. In Group 2, the Tc was reduced only by 5% on average (n.s.), and the treatment was deemed immediately successful in 71% of the patients. A 6-month follow-up is still in progress (68% complete). However, results thus far suggest better long-term outcomes for Group 1 (64%) than Group 2 (50%). The success rates assessed immediately and 6 months after the treatment in Group 2 are consistent with our previous longitudinal study on the re-adaptation treatment.

Conclusions: Data indicate that the initial effectiveness of the habituation method is maintained for longer terms or may even improve over a period of time. On the other hand, the initial effectiveness of the re-adaptation method may be reversed when symptoms are re-triggered by inevitable re-exposure to motion. Thus, habituation of velocity storage shows promise as an effective permanent treatment for MdDS that can complement VOR re-adaptation.

Progression of Vestibular Dysfunction in Ush1c Mice

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Background: Vestibular dysfunction and the loss of hearing and vision are the sensory impairments that characterize Usher syndrome (Usher). While the basic genetic alterations underpinning Usher are well described, the clinical natural course and mechanisms of the vestibular dysfunction – when symptoms begin and how quickly they progress, and which end-organs are affected – are not known. The goal of this study was to analyze the progression of vestibular dysfunction over time in an Ush1c mouse model with the most common type 1 Usher mutation in the Acadian populations of Louisiana and Canada (Ush1c c.216G>A).

Methods: Vestibular function was assessed in Wild-type mice (WT) and Ush1c mice of different ages (1, 6 and 12 months). Eye position and pupil orientation with respect to orbit were measured using an ISCAN eye tracker. Vestibulo-ocular reflexes (VOR) were measured during head rotation (0.2, 0.5, 1, 2, 4Hz) and head translation (0.2, 0.5, 1 and 2 Hz). Static ocular counter roll (OCR) responses were measured after 45-degree head downward tilt. Using single unit recordings, we examined vestibular afferent spontaneous discharge and their sensitivities to head rotation and translation. We further performed immunohistochemistry analysis to examine the morphology of the vestibular end-organs in Ush1c mice over time.

Results: Ush1c mice exhibited much lower rotational VOR responses than WT mice at 1 month and 6 months of age, indicating severe canal function deficits. Importantly, at 4 Hz, Ush1c mice exhibited higher rotational VOR gains at 1-month of age than at 6-months, indicating progression of the canal function deficit. Different from the rotational VOR, Ush1c mice exhibited similar translational VOR gains to WT mice at 1- and 6-months of age. However, Ush1c mice exhibited lower OCR gains at both ages than WT mice, indicating otolith deficits. In Ush1c mice, OCR gains at 6 months were lower than that at 1 month, indicating progression of otolith dysfunction over time. Preliminary morphological studies showed sensory hair cells were still present in all vestibular tissues at one month of age in Ush1c mice. By one year of age, semicircular canals and utricles did not show significant changes. However, severe hair cell loss was observed in the saccule. The preliminary results of single unit recordings at 6 months of age showed that the Ush1c mice had substantially reduced spontaneous firing rates of vestibular afferents (42.2+/-3.5 spike/s vs. 72.3+/-3 spike/s, P<0.001), reduced the number of regular afferents (14.5% vs. 45%), and reduced sensitivities to head rotation or translation.

Conclusions: These functional and morphological findings suggest that Ush1c -related vestibular dysfunction progresses over time and is end-organ specific. Ongoing studies are to further elucidate the progression of vestibular dysfunction in Ush1c mice to provide insight into understanding the mechanisms of disease and developing effective clinical treatments.

Pupillary Light Responses of Mice Lacking TMC1 and TMC2

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Background: Transmembrane channel-like proteins 1 and 2 (TMC1 and TMC2) are required for sensory transduction in vestibular hair cells. As a result, mice lacking Tmc1 and Tmc2 exhibit little vestibulo-ocular reflex (VOR) responses to head rotation and translation. Since the vestibular nuclei have been suggested to have direct projections to the preganglionic Edinger–Westphal (EWpg) nucleus that control pupil constriction, we examined pupillary light responses of mice lacking Tmc1 and Tmc2. Our goal was to investigate whether pupil diameter and pupillary light responses can be used as biomarkers for mice lacking Tmc1 and Tmc2.

Methods: Twenty-two wild-type mice (WT), six mice lacking Tmc1 and Tmc2 (Tmc1-/- Tmc2-/-) and five mice lacking Tmc1 but expressing Tmc2 (Gfi1Cre inducible expression of Tm[Tmc2] in absence of Tmc1: Tm[Tmc2];Tmc1-/-, Asai et al. 2018) were used in the study. Eye position and pupil diameter were measured using an ISCAN eye tracker. The optokinetic responses (OKR) were measured to evaluate visual function in the mice. In addition to behavioral studies, we also studied the spontaneous discharge rates of vestibular afferents in these animals.

Results: Under light conditions, Tmc1-/-Tmc2-/- mice exhibited significantly smaller pupil diameters than WT mice (0.63+/-0.04 mm vs. 1.12+/-0.03 mm, P<0.001). While Tm[Tmc2];Tmc1-/- mice exhibited smaller pupil diameters (0.78+/-0.05 mm) than WT mice, their pupil diameters were larger than Tmc1-/- Tmc2-/- mice. Under dark conditions, however, the three groups of mice exhibited similar pupil diameters (WT: 2.35+/-0.07 mm; Tmc1-/-Tmc2-/-: 2.16+/-0.11 mm; Tm[Tmc2]Tmc1-/-: 2.18+/-0.04 mm), suggesting that they have the same baseline pupillary drive. In response to light-on and off, Tmc1-/- Tmc2-/- mice exhibited the highest rate of pupil diameter change, although this did not reach statistical significance from WT mice. OKR responses were similar among the three groups of mice. We further measured the spontaneous discharge rates of vestibular afferents in WT mice (72.97+/-2.75 spike/s, N=208), Tmc1-/-Tmc2-/- mice (41.76+/-3.13, N=76) and Tm[Tmc2];Tmc1-/- mice (58.58+/-3.64 spike/s, N=139). While Tm[Tmc2];Tmc1-/- mice exhibited higher spontaneous firing rates than Tmc1-/-Tmc2-/- mice, their spontaneous firing rates were still lower than WT mice.

Conclusions: The results indicate that mice lacking both Tmc1 and Tmc2 exhibit significantly smaller pupil diameters than WT mice and mice only lacking Tmc1. Since OKR responses were similar in the three groups of mice, these differences may not result from differences in visual function. Based on the significant differences in the spontaneous firing rates of vestibular afferents in the three groups of mice, we hypothesized that the lower baseline vestibular afferent activity might lead to reduced inhibition of EWpg motoneurons, which would increase excitation of ciliary ganglion neurons and result in smaller pupil sizes. Ongoing studies will further investigate pupillary responses of mice with genetic vestibular deficits to develop a novel biomarker for vestibular dysfunction in animals and in humans.

Age-Related Decline in Otolith and Canal Afferent Functions in C57BL/6J Mice
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Background: Decline in peripheral vestibular function is a key contributor to dizziness and imbalance among older people. In this study, we employed a single unit recording technique to examine the effects of aging on canal and otolith afferent functions in C57BL/6J mice. Due to a mutation in the ahl gene, the C57BL/6J mice exhibited accelerated age-related hearing loss. Early studies suggest that the auditory and vestibular system of the strain exhibit different aging processes given that it showed a minimal and very low rate of decline in vestibular sensory evoked potential (VsEPs) responses over the lifetime. Although the VsEP measurement can provide valuable information about the summed activity of the irregular saccule afferents, the effects of aging on the canal afferent function and otolith regular afferent function remain to be elucidated. The present study investigated changes in vestibular afferent spontaneous discharge and dynamic responses to head movement during aging. Angular and translational vestibulo-ocular reflexes (VORs) were also measured to test canal and otolith function, respectively.

Methods: Single unit recordings of vestibular afferents were made from male C57BL/6J mice age 4-20 months. A craniotomy was performed to allow access to the 8th nerve by a microelectrode. Vestibular afferent spontaneous firing rates, regularity and sensitivity to head rotation and translation were analyzed. To measure the angular and translational VORs, the animals were subjected to sinusoidal head rotation (0.2-4Hz) and translation (0.2-2Hz) while their eye movements were recorded by an eye tracker.

Results: A total of 781 vestibular afferents were recorded from 20 animals. The vestibular afferents exhibited a significant decline in spontaneous firing rate after 9 months of age. The irregular afferents showed a larger reduction in firing rates than the regular afferents. While the canal afferents did not exhibit changes in spontaneous

firing regularity (CV*) during aging, the otolith afferents of old mice (20 months) had less irregular spontaneous firing than those of young mice (4 months). The otolith afferents of the old mice further exhibited significantly lower sensitivities to head linear acceleration (1Hz) in comparison to the young mice. The canal afferents, however, did not exhibit significant changes in gains to head rotation during aging. We did not find significant changes in the gains and phases of both angular and translational VORs during aging.

Conclusions: Since vestibular afferents carry the final output signals of the vestibular sensory transduction and synaptic transmission to the CNS, single unit recording of vestibular afferents provides a direct assay of the vestibular peripheral function. The preliminary analysis suggests that aging impaired vestibular function by reducing the static and dynamic signals sent to the central vestibular system. The otolith afferents and the irregular canal afferents may be more vulnerable to aging.

Characteristics of the Mammalian Vestibular Short-Latency Evoked Potential

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Background: The Vestibular short-latency Evoked Potential (VsEP) has been used as an objective measure of vestibular nerve function and represents the synchronous firing of vestibular primary afferents to the onset of motion. It was first termed the short latency vestibular evoked potential by Elidan et al. in 1982 due to its similarity to the auditory brain stem evoked potential. Since then, the VsEP has been measured in various vertebrate models and preparations; the most common being a non-invasive, vertex recording to linear-jerk pulses. This approach is attractive for several reasons, such as the ability to measure vestibular nerve function in chronic recovery animal models without disturbance of the labyrinth. However, with the cochlea intact, this approach is prone to auditory contamination, making interpretation difficult. Recently, however, it has been argued that a 1-2ms linear-jerk pulse is sufficient to activate vestibular sensory receptors, without cochlear activation; whereas responses evoked by brief (<0.5ms) jerk pulses are subject to acoustic forward masking, highlighting their cochlear contribution. This finding has potential clinical implications, as there may be techniques to stimulate the vestibular portion of the labyrinth in isolation of the cochlea. Given the increased popularity of the VsEP in recent years and its potential utility, it is important to validate key features of the response for future interpretation and use.

Methods: This work aimed to characterise the near-field VsEP recorded close to the vestibular afferents from facial nerve canal in anaesthetized guinea pigs. Linear-jerk pulses of varying widths (~0.1-3ms) were used to evoke the VsEP and were compared to responses with the cochlea intact, before and after controlled experimental manipulations, such as acoustic masking, changes in stimulation rate, and cochlear ablation. Responses from the facial nerve canal were also compared to the vertex.

Results: Responses evoked by a 2ms jerk pulse were not suppressed by acoustic forward-masking but were significantly masked using continuous broadband noise. Changing the stimulation rate was used to characterise differences in cochlear and vestibular neuronal forward masking. With the cochlea intact, 50% of the response was forward-masked with a stimulation rate of 60Hz, whereas the response after cochlear ablation did not forward-mask at this rate. Overall, surgical ablation of the cochlea revealed significant cochlear contribution of the response across all linear-jerk pulse widths. An iso-acceleration, iso-jerk, and iso-VsEP paradigm was used to re-examine the result that the VsEP is sensitive to kinematic jerk. Surprisingly, results indicate that the VsEP scales with linear acceleration of the earbar, rather than kinematic jerk. Interestingly, responses with the cochlea intact from both the facial nerve canal and vertex, scaled with kinematic jerk, rather than acceleration.

Conclusions: This work reveals new findings that call into question the original interpretation of the sensitivity and stimulation characteristics of the mammalian VsEP.

What Induces Endolymph Formation in the Developing Inner Ear

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Background: The development of the murine inner ear begins with an ectodermal invagination that forms an otocyst at embryonic day(E) 9.5. The otocyst is initially filled with amniotic fluid of plasma-like composition. When and how the developing epithelia change the composition of the luminal fluid is currently unknown. Between E10 and E10.5, two protrusions begin to extend from the otocyst; one forms the cochlear, and the other

forms the endolymphatic sac. While the protrusions elongated and the cochlear coils, the center of the otocyst reorganizes into the vestibular labyrinth. The lumen of the endolymphatic sac opens at E10.5 and the lumen of the cochlear protrusion opens at E14.5. Lumen formation depends on fluid secretion in the vestibular labyrinth and fluid absorption in the endolymphatic sac. Fluid secretion is most likely driven by ion transport; however, which ions are transported to promote lumen formation during embryonic development is currently unknown.

Methods: We collected extra-sensory epithelium of vestibule at the age of E16.5, E18.5, and P5, and divided it according to the presence of dark cells as follows: utricular roof epithelium and common crus which contained dark cells and semicircular canals which did not contain dark cells. RNAseq was performed with those samples to analyze the changes of ion channels according to the development. After selecting candidate genes for endolymph formation, we measured endolymphatic volume changes using confocal 3D live imaging with the application of candidate ion channel inhibitors for functional study. The localization of the candidate ion channels was examined by immunohistochemistry

Results: A total of 48440 known genes were identified by RNAseq analysis. The genes in each sample formed close clusters according to the cell types and development period. The majority of genes are related to ion activities such as ion transport, membrane transport. Four major ions thought to be involved in the endolymph formation with high probability were sodium, chloride, calcium, and potassium ions. A functional study using 3D volume change with the application of chloride-free solution at E16.5 showed blockage of endolymphatic fluid secretion. Intracellular calcium activity is also important by means of endolymph formation. The functional study showed decreased endolymph secretion later stage. Potassium only worked at P5, which was confirmed with XE991(KCNQ inhibitor) treatment in a functional study.

Conclusions: During inner ear development, sodium and chloride ion is strongly likely to be associated with endolymphatic fluid secretion, not the potassium ion. This finding may contribute to elucidating the mechanism of inner ear formation, congenital disorders.

Vestibular Function and Vestibulo-Visual Sensory Integration in People With Mild-Moderate Severity Parkinson's Disease: A Comparison With Age-Matched Controls

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Background: Vestibular dysfunction may be a consequence of the common neurodegenerative disorder, Parkinson's disease (PD). Neuropathological studies support this hypothesis, but clinical studies of vestibular reflex testing have produced conflicting findings, with some studies using sub-optimal testing protocols. Modern tests, the head impulse test (HIMP), the suppression head impulse test (SHIMP) and bone-conducted ocular and cervical vestibular-evoked myogenic potentials (oVEMPs and cVEMPs), have scarcely been reported in PD. Virtual reality (VR) assessment tools, adjuncts to vestibular test batteries, have not been applied to PD. Knowledge of vestibular dysfunction in PD will help guide targeted rehabilitation.

Methods: This prospective observational study compared vestibular function in 40 people with PD to 40 age-matched controls (HC). The study analysed dynamic vestibular reflex function: i) semi-circular canal mediated vestibulo-ocular reflex (VOR) function and saccades with HIMP and SHIMP; ii) otolith mediated VOR with oVEMPs, and iii) otolith mediated vestibulo-collic reflex (VCR) with cVEMPs. VEMPs were induced by both air-conducted sound (clicks) and bone-conducted vibration (forehead taps). Static otolithic function and verticality perception was assessed via a VR subjective visual vertical (SVV) test. Vestibulo-visual integration was determined through a VR standing balance protocol.

Results: In mild to moderate PD, VOR gains measured with HIMP and SHIMP were not significantly different from HC (p>.05). However, in PD, SHIMP peak saccade velocity was reduced (p<.001) and latency prolonged (p=.003). Bone conducted (tap) oVEMPs were more robust than air-conducted clicks in both groups, though PD had smaller tap oVEMP peak-to-peak amplitudes than HC (p=.03). PD had significantly more absent cVEMP responses to both clicks (p=.03) and taps (p=.002) and more abnormal SVV responses (p=.01) with greater variability (p<.001). PD failed at significantly lower levels of VR visual perturbation on both firm (p=.01) and foam surfaces (p=.001) than HC. Increasing age, impaired lower limb proprioception, impaired SVV, abnormal HIMP and cVEMP scores (for foam only) were associated with worse balance performance, but PD group was not.

Conclusions: In people with mild to moderate PD, the impulsive VOR remains largely unaffected. However, saccadic dysfunction is evident with SHIMP. The dynamic VCR, verticality perception and vestibulo-visual

integration are affected by PD. Thus, vestibular and balance rehabilitation programmes for this cohort should prioritise sensory integration, gait and posture interventions over gaze stability exercises.

Head Movement in Response to On- And Off-Axis Angular Whole-Body Rotations in Rats Exposed to Intense Noise or Intratympanic Injection of Sodium Arsanilate

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Background: Vestibular short latency evoked potential (VsEP) waveforms are delayed and attenuated in rats exposed to intense noise (Stewart et al. 2020) or intratympanic injection of sodium arsanilate. Attenuated VsEP responses in noise exposed rats are associated with reduced calretinin staining of sacculus calyx-only afferents (Stewart et al. 2020). Sodium arsanilate induces hair cell loss throughout the vestibular sensorium (Vignaux et al 2012). To characterize vestibular deficits, we designed experiments to quantitatively evaluate head orientation and neck reflexes in rats free to move their heads but subjected to whole body angular rotation.
Methods: Long-Evan rats were prepared for vestibular experiments by attaching a small headpost to their skulls designed to hold a motion sensor (Yost labs, 3-space LX Embedded). Rats were then habituated to a whole-body restraint device that allowed them to move their heads freely. Restrained rats were placed on a servo-controlled rotator either with the head located at the center of rotation or located 10 cm in front of the axis of rotation. The rotator was programmed to generate abrupt velocity steps (25 to 100 deg/sec), clockwise or counterclockwise in a dark environment. Step timing, amplitude and direction were randomized. Turntable motion was measured by an identical Yost sensor mounted on the rotator. A search coil on the animal's head post was used to determine yaw head position. Synchronized data from the sensors and search coil were analyzed by custom MatLab scripts.
Results: Sensor data were transformed from the head to a lab-based coordinate frame to determine head position and velocity relative to the fixed body and turntable. Head orientation (yaw, pitch and roll) relative to the body was determined before each step. Prior to treatment (noise or arsanilate), animals exhibited similar head orientations; pitched down <40 deg with small roll or yaw angles (<20 deg) relative to the body. After treatment, increases in head pitch (50~70 deg) and/or roll (~20 deg) were seen in most animals with variable changes about the yaw axis. Head movements in response to on-axis velocity steps were qualitatively comparable to those reported for guinea pigs (Shanidze et al. 2010); yaw head velocity in the direction of body rotation characterized by an overshoot and damped oscillation. In addition, the head rolled during the acceleration phase of the velocity step. Off-axis responses were larger in yaw and roll directions.
Conclusions: We used wavelet analysis to quantify head velocity and determine how responses were altered by the arsanilate or noise exposure treatments. Since head movement velocity was dependent on the orientation of the head prior to the step, the analysis was designed to account for this confound and for changes in head orientation as animals recovered from the peripheral lesions induced by treatment.

Autoimmune Biomarkers in the Pathogenesis of Sudden Sensorineural Hearing Loss

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Background: Sudden sensorineural hearing loss (SSNHL) is defined as an acute unilateral drop in hearing over the course of 24-72 hours. Most cases are thought to be idiopathic. SSNHL incidence increases with age, reaching up to 77 per 100,000 in patients over 65 years old. Although the pathophysiology of SSNHL is still unclear, multiple lines of evidence clearly implicate autoimmune processes in the disease process. Previous studies from several laboratories have presented compelling evidence of the presence of autoantibodies in the serum of patients with SSNHL and that injection of these antigens induce hearing loss in a murine model of disease. Our hypothesis is that autoantibodies against specific antigens are diagnostic of the disease, predict response to treatment, and can assist in the design of therapeutic interventions.
Methods: Analyze serum antibodies of SSNHL patients and controls for binding to known inner ear antigens and identify novel self-proteins by mass spectroscopy and Rapid Exoproteome Antigen Profiling (REAP). With REAP, patient samples are applied to a genetically barcoded library containing 2,688 unique members of the human exoproteome displayed on the surface of yeast.
Results: We conducted a pilot analysis, evaluating 8 patients' sera with SSNHL with REAP and compared them to normal controls with no known sensorineural hearing loss (5) and genetic hearing loss from infancy (1). While

the SSNHL patients were noted to cluster together, the private/non overlapping nature of the reactivities in these patients is similar to what we have encountered previously with other autoimmune diseases, for example SLE. Importantly, many of the proteins that were recognized by antibodies in the sera that were unique to SSNHL included OTOL1, an inner ear protein expressed in LFNG+ve supporting cells, and a plethora of proteins expressed in spiral ganglion neurons (SGN) including two sodium channel genes, SCN4A and SCNA3A, the transporter proteins SLC9A3, SLC17A7, SLC31A1, SLC4A4, SLC6A6, and a host of proteins with variable functions.

Conclusions: SSNHL remains an illusive disease on both it's pathogenesis and response to treatment. REAP affords the potential to analyze a large number of samples and detect the presence of autoantibodies against multiple different antigens, all in a quantitative manner. We were able to
identify multiple novel antigens recognized by SSNHL patient serum autoantibodies to several proteins expressed in two populations of spiral ganglion neurons and LFNG+ve supporting cells in the sensory epithelium in patients with SSNHL which were not present in control patients. These support an autoimmune pathogenesis of SSNHL. Further analysis on a larger cohort will allow us to correlate these to clinical presentation and outcome. Future development of a diagnostic test for the autoimmune pathogenesis of SSNHL will allow to direct our treatment accordingly, improving patient care.

A Plasma Metabolomic Signature of Moderate or Severe Hearing Loss

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Background: Metabolomics investigations are a comprehensive assessment of an individual's metabolic processes that can provide insight into biological pathways underpinning neurodegenerative conditions, including age-related hearing loss. In animal models, changes in metabolite profiles were demonstrated following acoustic trauma, but the association of plasma metabolite profiles and hearing status in humans has not previously been studied.

Methods: We conducted a cross-sectional investigation of the association of plasma metabolite profiles and self-reported moderate or severe hearing loss among 3609 women who were participants in the Nurses' Health Study (NHS). Information on hearing status at the time of the blood collection and on relevant demographic, medical, diet and lifestyle factors was collected on biennial questionnaires and metabolic profiling was conducted by liquid chromatography-mass spectrometry. The association of each metabolite with hearing loss was assessed in logistic regression models adjusted for age and potential confounders. The false discovery rate was controlled at 5% through the q value approach. Metabolite Set Enrichment Analysis (MSEA) was conducted to identify metabolite classes that are enriched for concordant associations with hearing loss.

Results: In the NHS, 46 out of 602 measured metabolites were significantly associated with moderate or severe hearing loss in multivariable-adjusted models (q value < 0.05). These findings included positive associations for 2 carnitines, 29 diacylglycerols (DAGs)/triacylglycerols (TAGs), 6 glycerolipids (glycerophosphoethanolamines (LPE), glycerophosphocholines (PC and LPC)), 3 nucleoside derivatives, 2 carboxylic acid derivatives and uric acid, with multivariable-adjusted odds ratios (MVORs, 95% CI) ranging from 1.21 (1.02, 1.43) for C3-carnitine to 3.07 (1.60, 5.87) for C36:4 PC. Inverse associations were observed for 2 amino acids, glycine [MVOR = 0.47 (0.31, 0.71)] and serine [MVOR = 0.76 (0.63, 0.93)], and for C18:2 LPC [MVOR = 0.65 (0.47 to 0.88)]. As a metabolite class, the set of 56 TAGs were enriched for positive associations with hearing loss (P-value = 1.61×10^{-9}), while the set of 11 steroid esters were enriched for inverse associations with hearing loss (P-value = 1.71×10^{-5}). A metabolomic score for hearing loss was derived as a weighted combination of the 46 metabolites in a Lasso regression. The MVOR for moderate or severe hearing loss was 1.38 (1.24 to 1.54)(P-value = 6.86×10^{-9}) per standard deviation increase in metabolomic score.

Conclusions: In this first large population-based investigation of a metabolomic signature of hearing loss, we identified plasma metabolites significantly associated with moderate or severe hearing loss. Metabolite Set Enrichment Analysis (MSEA) demonstrated TAG levels were positively associated, while steroid esters were inversely associated with hearing loss. The metabolite panel identified in this study provides new insight into pathoetiologic processes underlying adult onset hearing loss.

Oticon Medical's Virtual Reality Platform for Ecologically Valid Hearing Research

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Background: The pursuit of ecological validity in hearing research is key if we are to understand the real-life challenges of hearing device users and for ensuring the design of systems and interventions that can truly benefit these users in their daily life. Virtual Reality (VR) can, together with audio virtualization, be used to create realistic and engaging audio-visual scenes for behavioral and cognitive hearing research while providing a high level of control of experimental conditions: VR is a key enabler of ecological validity in hearing research.
Methods: The Oticon Medical Virtual Reality (OMVR) experiment building platform fast-tracks the creation or adaptation of hearing research experiments templates to be used for investigating themes such as spatial hearing, fusion of visual and acoustic modalities, cognitive strategies of multi-modal cues processing, cross-modal reorganization, etc. The OMVR offers several pre-built templates that can be easily configured to the researcher's needs, while new templates can be created and integrated if needed. The VR engine ensures accurate audio virtualization for spatialized stereo playback on headphones, on an arbitrary number of loudspeakers (ideal for bi-modal users), or via real-time binaural streaming to Oticon Medical cochlear implant (CI) users. Real-time CI vocoding can also be applied to the spatialized binaural stereo output for a simulated CI experience. Biometric data issued from an embedded eye-tracker and intrinsic VR head-tracking are synchronized during experiment runs and aggregated with behavioral data such as reaction time, localization scores, etc. Additional data collection modalities such as fNIRS or EEG can easily be included in the paradigms thanks to the Lab Streaming Layer-based (LSL) synchronization protocol.
Results: We present the architecture and characterization data for the OMVR platform as well as examples of experimental paradigms implemented and executed with OMVR including a virtual versusvirtual versus real sound localization paradigm, and the exploration of cognitive resource allocation during a localization task using pupillometry.
Conclusions: The OMVR platform lowers the barrier to entry to harnessing VR for ecologically valid experimental hearing research. The auralization method – binaural or loudspeaker-based- must be chosen carefully depending on the question to be addressed.

Perceptual Plasticity Induced by Home-Based Auditory Training and Hearing Aids
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Background: Older adults with age-related hearing loss generally use hearing aids to compensate for their loss. Certain challenges in speech perception, especially in noise, still exist, despite today's high technology of hearing aids. Auditory training has been considered as an additional rehabilitation option to the hearing aid use. However, due to the time limitations of audiologists with such training, the value of auditory training may be underestimated. In addition, due to the "new life" of telemedicine as a result of COVID-19, the current study came to develop a home-based auditory training program to be used with the adaptation process of the hearing aid use.
Methods: 53 middle aged adults with age-related symmetrical sensorineural hearing loss participated in the study. The participants were divided into three different groups depending on the status of the use of HAs. Group 1: novel hearing aid users (participants who were fit with bilateral hearing aids for the first time). Group 2: experienced hearing aid users (participants who use bilateral hearing aids for at least two years). Group 3: non-hearing aid users. These three groups underwent auditory training for three weeks. The training was set from easy to more difficult tasks, in quiet and under background noise conditions. All participants filled out self-assessment questionnaires before and after training and underwent cognitive tests.
Results: Overall, auditory training on speech in noise enhanced speech perception through the course of the training sessions in all three groups. Even participants who were non-rehabilitated (non users of hearing aids) showed perceptual learning. However, differences were still seen between users and non-users across sessions. In sessions, where stimuli were presented in quiet, similar improvements were observed in novel and experienced users. In noise conditions, experienced users showed higher speech in noise scores than novel users. Self-assessment improvements in hearing ability were observed across the three groups. Novel users showed rapid adaptation to hearing aids with the auditory training. Finally, the results revealed a relationship between cognitive measures and the time of hearing aid use.

Conclusions: These results illustrate the potential of perceptual learning to improve perceptual skills across a range of perceptual abilities and even for older adults with hearing impairments. Combining hearing aid adaptation with training induce improvements that can be a marker of plasticity. A longer period of hearing aid use is important to reflect working memory enhancement. Home-based training induced subjective benefits and a positive experience. Follow up and retention should be further assessed.

Validation of Four-Digitally Recorded Hmong Monosyllable Recognition Tests

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Background: There is currently no available validated word recognition test for the Hmong language. The purpose of this study is to validate four digitally recorded lists of 50 Hmong words each (N=200 phonetically balanced monosyllabic Hmong white dialect words) for clinical and research applications. Female and male Hmong talkers were selected and evaluated by bilingual Hmong respondents with normal hearing to record the four-digitally recorded Hmong word lists. The aims are to: (1) identify and exclude problematic words using statistical and expert validation techniques, (2) compare the performance of the Hmong word list to the standard English word list, and (3) produce a standardized recording for dissemination.

Methods: A randomized, incomplete block design is used to assign each respondent to listen to and repeat four unique lists of phonetically balanced Hmong words (n=50 words for each list), delivered by female and male talkers. Respondents are also tested with an English word list (one list of 50 words)—List 1A of the Northwestern University – 6 (NU-6) list. A standard sound booth is utilized. Words presented at a level of 50 dB HL and 30 dB of contralateral masking. Final balanced word lists are compiled after the words are scored for accurate response by a Hmong speaker, reviewed by an expert panel, and evaluated with descriptive statistics. Words with less than 80% correct aggregate response were reviewed for non-inclusion. A nonparametric non-inferiority test (Mann-Whitney) was used to compare the average percent correct verbalizations of the four Hmong word lists to the English word list.

Results: Seventy normal hearing bilingual Hmong respondents participated (n = 35 female, n =35 male; mean age of 29.50, SD ±7.1). 93.5 % (187/200) of words met validation criteria for ≥92% correct response and 6% (12/200) of words met validation criteria for ≥80% correct response. 0.5% (1/200) words were problematic and did not meet the validation criteria for 80%, but after expert review, the word is deemed adequate and included to maintain four unique balanced word lists. No words are excluded from the four unique Hmong word lists by study validation criteria. All four Hmong word lists were not inferior to the English word list (p-value < 0.001).

Conclusions: This is the first study to develop and validate four 50 unique phonetically balanced Hmong word lists (N=200 words). These lists are determined to be useful for assessing Hmong speakers' word recognition ability for clinical and research applications.

Prognostic Determinants of Hearing Outcomes in Children With Congenital Cytomegalovirus Infection

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Background: Congenital cytomegalovirus (cCMV) infection is the most prevalent cause of acquired sensorineural hearing loss in children. While some were asymptomatic at birth, hearing loss could evolve in both symptomatic and asymptomatic cCMV infection children. However, the prognostic determinants of sensorineural hearing loss remain unclear. The aim of this study was to explore the prognostic factors of hearing outcome in children with cCMV infection.

Methods: Thirty-nine children with cCMV infection in a tertiary hospital were enrolled. The presence of cCMV-related symptoms at birth, the results of the newborn hearing screening, and the blood viral loads were ascertained. The children were categorized into three groups according to their symptoms at birth: symptomatic, asymptomatic with isolated hearing loss, and asymptomatic with normal hearing. The results of serial audiologic tests and initial blood viral loads were compared among different groups.

Results: A total of 16 children developed sensorineural hearing loss in our cohort. Sensorineural hearing loss developed in 60% of children who were initially symptomatic, and in 34.5% of those who were initially asymptomatic with normal hearing or isolated hearing loss, respectively. All children who failed newborn hearing screening were confirmed to have sensorineural hearing loss, while 11.5% of those who passed newborn hearing screening developed sensorineural hearing loss during the follow-up period. The initial viral loads were higher in children who were symptomatic at birth, those who failed newborn hearing screening, and those who developed sensorineural hearing loss. Of note, we observed hearing loss deterioration in a patient after serum CMV suppression was achieved, and in another patient with the flare-up of viral load.

Conclusions: The presence of cCMV-related symptoms at birth, failure in newborn hearing screening, and viral activity are the prognostic factors for hearing outcomes. Besides, regular audiologic examinations are necessary even after serum viral suppression, especially for children with serum CMV load flare-up due to certain immunological causes.

Simulation Study of a Novel Auditory Nerve Implant: Model and Expected Percepts

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Background: An intracranial auditory prosthesis that targets the auditory nerve between the cochlea and brainstem (Auditory Nerve Implant, ANI) is currently being developed across five institutions and two medical device companies (MED-EL and Blackrock Neurotech). The ANI consists of a MED-EL stimulator attached to a Utah Slanted Electrode Array (USEA; developed by Blackrock Neurotech) with 15 electrodes (12 active) in a 3x5 configuration. The ANI will be directly implanted into the auditory nerve with the aim of achieving more focused activation, greater transmission of auditory information to the brain, and improve hearing performance compared to current hearing treatment options.

Methods: A spatial model of the auditory nerve cross-section was created to mimic the anatomical organization of the fibers, overall shape, and diameter of the auditory nerve. Two tonotopic frequency organizations were tested: an ideal 2.25 turn spiral like the tonotopy observed in the cochlea and a randomized tonotopy where the frequencies from the ideal model were jittered. These 2D models of the auditory nerve fibers were translated into 3D. The tips of the USEA were placed within these fibers based on the expected positioning of the ANI in future patients. A computational model of the neuronal activation was employed by applying an electrical current at the electrode tips to simulate excitation profiles of the auditory nerve fibers. These excitation profiles were converted into vocoded stimuli to test the expected percepts of the ANI in normal-hearing listeners. The main outcomes measured with these stimuli were threshold, dynamic range, loudness balancing, and pitch ordering.

Results: The excitation profiles showed a variety of shapes ranging from single frequency peak with a narrow spread of activation to multiple peak frequencies with broader spread of activation. The model with randomized tonotopy showed broader spread of activation. Threshold and loudness balancing levels were determined for each of the 12 simulated electrodes for each of the listeners. The loudness-balanced stimuli were used in the pitch-ordering task under two conditions: no level roving and 2 dB level roving. The results of the pitch-ordering tasks indicated that it was possible to map the simulated electrodes to the appropriate frequency bands, even with the multiple frequency peaks observed on some electrodes.

Conclusions: A computational model of the ANI was created to simulate the expected effects of directly stimulating the auditory nerve fibers with a penetrating USEA. The results of these studies will aid in the future development of perceptual tasks for the ANI clinical trial.

Listeners With No More Than "Slight" Hearing Loss who Exhibit Binaural Deficits Also Appear to Exhibit Reduced Amounts of Binaural Interference

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Background: We have published several studies demonstrating that groups of listeners having hearing levels (HL) at 4 kHz that exceed 7.5 dB also exhibit poorer performance in binaural tasks as compared to groups of listeners having hearing levels at 4 kHz $\leq$7.5 dB HL. The poorer binaural performance appeared to stem from

increased levels of stimulus-dependent, additive internal noise. Importantly, all of the listeners in the two groups were classified, audiometrically, as having no more than "slight" hearing loss. The purpose of this study was to assess whether listeners having absolute thresholds at 4 kHz >7.5 dB HL, and who exhibit binaural deficits, also exhibit greater susceptibility to what has been termed "binaural interference." Binaural interference refers to a degradation in binaural processing that occurs when a target stimulus conveying binaural information in one frequency region is accompanied by a second, simultaneously-gated, stimulus in a frequency region remote from the target and which conveys conflicting binaural information vis a vis the target. Empirically, the question was whether the magnitude of binaural interference observed would be similar for the two groups (i.e., ≤7.5 dB or > 7.5 dB) or would be dependent on group membership.

Methods: Detection thresholds were measured in the NoSo and NoSπ configurations for both "conventional" and "transposed" tonal signals and maskers centered at 4 kHz. The conventional stimuli consisted of tonal signals and 100-Hz-wide Gaussian noise maskers centered at 4 kHz. The transposed stimuli were generated by transposing to 4 kHz, tonal signals and 100-Hz-wide Gaussian noise maskers centered at 125 Hz. For both the 4-kHz-centered conventional and transposed stimuli, NoSo and NoSπ detection thresholds were also measured in the presence of simultaneously-gated 400-Hz-wide Gaussian noise centered at 500 Hz (the interferer). In separate conditions, the interferer was diotic or was interaurally uncorrelated.

Results: Listeners exhibiting elevated NoSπ thresholds (typical of those in ">7.5 dB groups"), actually exhibited less binaural interference than did those exhibiting lower NoSπ thresholds typical of those in "≤7.5 dB HL" groups. That outcome cannot be explained by a "ceiling effect" stemming from interferer-induced loss of the ability to utilize binaural cues to detect the signal.

Conclusions: We speculate that the relatively smaller amounts of binaural interference exhibited by listeners with relatively elevated NoSπ thresholds could be quite important for binaural processing in everyday listening environments because it leaves them with a substantially reduced "pool" of potentially available binaural cues.

Epidemiology of Sports-Related Facial Fractures in the United States: NEDS Study
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Background: Sports-related injuries account for up to 25% of all injuries annually, contributing to the growing healthcare costs in the United States. Of those approximately 1 in 5 results in a facial fracture(s). Many factors including the inability to interview the patient or perform a physical exam, co-presentation of multiple trauma, and pronounce facial swelling can make the management of facial injuries challenging to emergency physicians and trauma surgeons. Therefore, it is crucial to study the epidemiology of sports-related facial fractures and their development over time in the US.

Methods: Data were obtained from the Nationwide Emergency Department Sample (NEDS) 2008-2017 databases. ICD-9 and -10 codes were used to filter sports-related facial fractures cases and identify the sport involved. Data analysis and manipulation were performed using SPSS v27 (IBM, Armonk, NY) software, while GraphPad Prism 9 (GraphPad Software, San Diego, CA) was used for data visualization. Bivariate chi-squared analyses and binary logistic regressions were performed to describe the epidemiology of sports-related facial fractures.

Results: During the study period 454,372 individuals (mean age, 25.9; 354,263 (78%) are males, 100,019 (22%) are female) presented to the ED with sports-related facial fracture, which was the primary diagnosis in 337,594 patients. Most common injuries in males were because of Cycling (40,085 (15.3%), p<0.001, V: 0.100), Baseball (15,587 (5.9%)) and Volleyball (9,037 (3.4%)). While in females they were because of Cycling (11,608 (15.4%)), Baseball (4,660 (6.2%)) and Dancing/Yoga/Gymnastics (4,299 (5.7%)). Majority of injuries were among young patients (45.9% were 10-19 years old, p<0.001, V: 0.052) and the main injury type was nose bone fracture (60.2% in males and 68.3% in females, p<0.001, V: 0.042).

Conclusions: In our study, 454,372 individuals presented to ED in the United States between 2008 and 2017 with sports-related facial fractures; in more than 74% of these cases, facial fractures were the primary diagnosis. Cycling is the largest contributor to sports-related facial fractures in the ED followed by Baseball in both males and females and nose bone fractures are the main injury type. Further studies to identify the outcome of these injuries are warranted.

Effects of Age and Antibiotic Treatment on the Survival of Spiral Ganglion Neurons on the Whole Rat Cochlea

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Background: Spiral ganglion neurons (SGNs) build the bridge between the peripheral and the central auditory system by transferring sensory input from the hair cells (HCs) in the cochlea to the cochlear nucleus. Factors such as aging, pharmacological treatment (e.g. by antibiotics), and noise trauma can induce HC degeneration followed by SGN loss. Previous studies investigated SGN degeneration only in high-frequency regions of the cochlea for different animal models. We here studied how age and pharmacological treatment affect the degeneration of SGNs not only in the basal, high-frequency area of the cochlea, but also in the lower middle, upper middle, and apical turns of the rat cochlea.

Methods: The density of SGNs over all cochlea turns of normal hearing (NH) and neonatally deafened (ND) Wistar rats of different age groups was quantified. Profound hearing loss was induced by kanamycin treatment between postnatal day (P) 10 to P20, respectively, resulting in a rapid and permanent rise of hearing threshold (>90 dB). SGN density was quantified by immunofluorescence staining using the neuronal marker HuC/HuD. In addition, alterations in the composition of SGN subpopulations were investigated by using fluorescence staining of the calcium binding proteins (CBPs) calbindin and calretinin, which may have cell protective effects. SGN degeneration was studied at four different points in time (P18, P30, P77, and P252).

Results: Following pharmacologically induced destruction of HCs, SGNs degenerated significantly over all four cochlea turns within nine months (P252) with the decrease being lowest in the apical turn with only 55%. In detail, SGN degeneration was significantly stronger in the higher frequency (basal/lower middle) areas compared to the lower frequency (apical/upper middle) areas of the rat cochlea. In the apical turn, a mean density of 1.29 SGNs/1000 μm2 (≙44.7%) was still present, compared to mean densities of only 0.47 SGNs/1000 μm2 (≙16.1%), 0.23 SGNs/1000 μm2 (≙8.0%), and 0.18 SGNs/1000 μm2 (≙9.4%) in the upper middle, lower middle and basal turns, respectively. Simultaneously, the percentage of CBP-positive cells of all surviving SGNs increased in ND animals with the duration of deafness; however, it only reached significance in the upper middle and apical turns. In comparison, NH animals did not show any significant SGN degeneration or change in the percentage of CBP-positive cells of all SGNs.

Conclusions: Overall, our data suggest that although SGNs degenerate rapidly throughout the cochleae in ND animals, neurons in the apical, low-frequency region, are more resistant to input loss from HCs, resulting in significantly more surviving SGNs. An increasing percentage of all surviving SGNs expressing CBPs could indicate a protective effect of these proteins for the survival of SGNs after deafness.

Tropism of Novel AAV Capsid Variants in the Inner Ear

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Background: Usher syndrome (Usher) is the leading genetic cause of combined deaf-blindness. Of the three clinical types, type 1 (USH1) is the most severe with congenital sensorineural hearing impairment and vestibular dysfunction, and adolescent onset of retinitis pigmentosa. While the genetics of Usher is well understood, the treatment options for these patients are limited. To fill this gap, we created a mouse model of Type 1C Usher (USH1C) that contains the splice site mutation (USH1C c.216G>A) responsible for USH1C in patients. USH1C mice have hearing, balance, and visual dysfunction similar to patients. Additionally, the expression of harmonin protein, encoded by the USH1C gene, is significantly reduced in hair cells in the inner ear and photoreceptors in the retinas of USH1C mice. Our long-term goals are to develop an AAV-based gene replacement therapy for the deafness, imbalance, and vision loss in USH1C. The objective of this study is to determine the cell specificity of newly engineered AAV capsids in the inner ear.

Methods: Wild-type (WT) mice were treated with 1.9x10E12 gc/ml of AAV2.P2-V1(Y-F+T-V)-CBA-GFP, AAV2.P2-V3-CBA-GFP, or saline at postnatal (P) day 1-2 by semicircular canal (SCC) injection. For a preliminary assessment of treatment safety, hearing was measured by auditory brainstem response (ABR) analysis at 1 month-of-age. Temporal bones were then harvested, and the cochlear and vestibular end organs were micro-dissected to assess viral transduction using immunohistochemistry analysis.

Results: Preliminary results of mice treated with AAV2.P2-V1(Y-F+T-V)-CBA-GFP virus show GFP signal in cochlear and vestibular hair cell supporting cells. GFP signal was not detected in cochlear or vestibular cells in

mice treated with AAV2.P2-V3-CBA-GFP virus. Mice treated by SCC injection with AAVs or saline had ABR thresholds similar to untreated WT control mice.

Conclusions: Our results show that injection of novel AAV vectors via the SCC leads to safe and effective transduction of cochlear and vestibular cells in mice. Studies are ongoing to evaluate additional novel capsids to further expand the toolbox for effectively treating diseases of the inner ear.

Long-Term Survival of LGR5+ Supporting Cells After Ototoxic Trauma in the Adult Mouse Cochlea

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Background: Sensorineural hearing loss is mainly caused by irreversible damage to sensory hair cells (HCs). A subgroup of supporting cells (SCs) in the cochlea express the leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5), a marker for tissue-resident stem cells. These LGR5+ SCs could potentially be used as an endogenous source of stem cells for regeneration of HCs to treat hearing loss and deafness. We have recently described that LGR5+ SCs survive one week after ototoxic trauma (Smith-Cortinez et al., 2021, Front. Mol. Neurosci., 10.3389/fnmol.2021.729625). However, it is still unknown whether LGR5+ SCs from the deafened mouse cochlea retain regenerative potential or if they are present in the long term after deafening. Here, we will evaluate long-term survival of LGR5+ SCs in adult deafened cochleas of Lgr5GFP transgenic mice and determine the regenerative potential of these LGR5+ SCs.

Methods: Adult (postnatal day 30-50) normal-hearing Lgr5-eGFP-IRES-creERT2 heterozygous (Lgr5GFP) and deafened Lgr5GFP mice will be used. Animals will be deafened with a single dose of furosemide (100 mg/kg i.v.) and kanamycin (males: 700 mg/kg s.c. and females: 900 mg/kg). Seven and 28 days after deafening, auditory brainstem responses (ABRs) will be recorded. Cochleas will be harvested to characterize mature hair cells and LGR5+ SCs by immunofluorescence microscopy, quantitative real time PCR (q-RT-PCR), and FACS. In addition, we will sort LGR5+SCs from cochleas of normal-hearing and deafened mice and culture them as 3D cochlear organoids to analyse their regenerative capacity.

Results: As previously described we found survival of LGR5+ SC in the third row of Deiters' cells one week after deafening in adult Lgr5GFP mice (Smith-Cortinez et al., 2021, Front. Mol. Neurosci., 10.3389/fnmol.2021.729625). The q-RT-PCR expression profile showed up-regulation of Lgr5 in the deafened cochlea, compared to the normal-hearing cochlea. Animal experiments evaluating long-term survival are currently ongoing and we will present conclusive results. Preliminary data showed that sorted LGR5+SCs from adult normal-hearing mice develop cochlear organoids. The initial results from the cochlear organoids derived from deafened mice will be presented.

Conclusions: The presence of LGR5+ cells in the adult mouse cochlea demonstrates potential endogenous cochlear stem cells with regenerative capacities in adulthood. This is confirmed by the development of organoids from these cells. Furthermore, these LGR5+ SCs do survive an ototoxic trauma. To our knowledge, this is the first study showing increased Lgr5 expression after deafening in the adult mouse cochlea. This might be a result of ototoxicity-induced LGR5+ cell proliferation, and will be further explored and objectified in a future study.

Correlation of Neurophysiological, Behavioral, and Performance Thresholds in Cochlear Implanted Rats

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Background: In cochlear implant (CI) patients, a variety of markers are available to measure and evaluate the response of the auditory system to electrical intracochlear stimulation. This includes objective markers, such as measurements of electrically evoked compound action potentials (eCAPs), stapedius reflex or electrically evoked auditory brainstem responses (eABR). Furthermore, in human CI users, assessment of subjective hearing thresholds and loudness scaling is a key step in the fitting procedure, which ensures the dynamic range of the implants is well matched to each individual patient's sensitivity. However, determining the optimal stimulation intensity can be challenging, especially for small infants or in animal models, and it remains open which objective marker provides the most stable reference point for choosing appropriate stimulation intensities. This project

investigates the correlation between neurophysiological and behavioral threshold markers and their relation to in fact behaviorally visible good hearing performance.

Methods: To answer this question, neonatally deafened Wistar rats were supplied with bilateral CIs in young adulthood. EABRs were recorded at different current levels immediately after CI implantation as well as repeatedly over months and thresholds were determined. After recovery from surgery, we trained all animals on a 2-alternative forced choice sound lateralization task. Biphasic, bipolar pulses were presented at a pulse rate of 900 pps. Interaural time differences (ITDs) of +/- 80 µs served as spatial cues. The animals received several training sessions with different stimulation intensities in a range of -2 to 6 dB. The stimulation intensity or auditory binaural level (ABL) at which the animals performed with ≥75% correct was defined as intensity threshold of good ITD performance. In addition, behavioral thresholds were determined regularly in response to a single pulse presented at increasing ABLs. A response was considered positive if the CI rat showed sudden behavioral changes at stimulus onset like looking up, turning its head, or twitching out of calm.

Results: The lowest threshold across all three markers was identified for the behavioral response at an average of -10.5 dB (0 dB=100 µA). In comparison, the mean eABR threshold was -0.6 dB and thus 9.9 dB above the behavioral threshold. The intensity threshold of good ITD performance was found at a mean of 1.9 dB ABL and was thus only 2.5 dB above the eABR threshold but 12.4 dB above the behavioral threshold of our CI animals.

Conclusions: Our results show that neurophysiological (eABR) thresholds can be likely to overestimate behavioral thresholds but range closer to the intensity thresholds of good ITD performance. Therefore, we propose a stimulation intensity of at least 2-3 dB above eABR threshold to achieve good hearing performance. Overall, we could demonstrate the importance of correlating different objective markers to obtain an impression of the wide perceptual range under CI stimulation.

L-Ergothioneine (EGT) Treatment in Old CBA/CaJ Male Mice Slows the Progression of Age-Related Hearing Loss

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Background: The naturally occurring amino acid, L-ergothioneine (EGT), has immense potential as a therapeutic and has shown promise in the treatment of other disease models, including neurological disorders. EGT is naturally uptaken into cells via its specific receptor, OCTN1, suggesting that it is highly conserved. EGT is then utilized by cells as an antioxidant and anti-inflammatory, particularly under stressed conditions. In our current study, EGT was administered over 6 months to CBA/CaJ mice as a possible treatment for age-related hearing loss (ARHL), since presbycusis has been linked to higher levels of cochlear oxidative stress and chronic inflammation. Mice were evaluated for hearing function using auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs).

Methods: Testing was begun at 25-26 months of age for male and female CBA/CaJ mice. Both males and females were divided into three groups: control, low-dose, and high-dose. Dosing schema for these groups was: Control; 0.1mL saline for the first 7 days, 0.2mL once/week until testing end date; Low-dose; 35mg/kg for the first 7 days, 70mg/kg once a week until testing end date; High-dose:70mg/kg for the first 7 days, 140mg/kg once/week until testing end date. Baseline hearing measures were established prior to beginning treatment. Hearing measures were then obtained at the 1st month, 2nd month, 4th month, and 6th month timepoints from the start of the treatments.

Results: Results showed a distinct sex difference for the response to the treatments, for ABRs and DPOAEs. Males exhibited improvements from baseline testing in hearing for both DPOAEs and ABRs with some effects lasting throughout the entire test period. Notably, intragroup hearing measure comparisons for both male treatment groups (low-dose and high-dose) showed a significant slowing down of the progression of ARHL, with only the high-dose males showing significant threshold elevations from baseline at the 6th month timepoint. This is in stark contrast to the male control group which displayed significant ARHL threshold increases for both DPOAEs and ABRs by the 4th month. Conversely, females did not show improvements with either treatment dose, and may even have been negatively impacted when compared to controls for the same time period.

Conclusions: These findings suggest that EGT has a future as a naturally derived therapeutic for slowing down the progression of ARHL. Specifically, EGT, while seemingly effective in the treatment of presbycusis in aging males, it could be modified to be a general prophylaxis for other age-related disorders. In addition, treatment

protocols could include eating a larger proportion of EGT-rich foods. Based on these promising initial results, sex differences, whole blood EGT levels and molecular markers are being further investigated until project completion.

Inner Hair Cell Cav1.3 Calcium Channels Are Required for Preservation of BK Channel Expression Even After the Onset of Hearing

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Category: Hair Cells: Anatomy and Physiology
Background: Cav1.3 Ca2+ channels fulfill multiple functions in inner hair cell (IHCs). In mature mice, they mediate sound-induced Ca2+ influx for transmitter release at ribbon synapses. Before the onset of hearing at postnatal day 12 (P12) in mice, Cav1.3 channels are required in IHCs for the generation of Ca2+ action potentials. Mice with a systemic (Cav1.3–/–) or an embryonic cochlea-specific ablation of Cav1.3 channels are deaf due to the loss of transmitter release. Moreover, lack of Ca2+ action potentials before the onset of hearing disrupts the terminal differentiation of IHCs [Brandt et al. (2003) J Neurosci 23:10832; Nemzou et al. (2006) Neurosci 141:1849; Eckrich et al. (2019) Front Cell Neurosci 13:225]. To isolate multiple roles of Cav1.3 channels in mature IHCs therefore requires an alternative mouse model with Cav1.3 channel ablation after IHCs have reached maturity.
Methods: We used Cav1.3-flex mice with Cre-dependent ablation of Cav1.3 coupled to eGFP expression [Satheesh et al. (2012) Hum Mol Gen 21:3896]. These mice were crossbred with Prestin::Cre mice with hair-cell specific Cre expression starting around hearing onset [Tian et al. (2004) Dev Dyn 231:199]. We analyzed Ba2+ currents (IBa) through Cav1.3 channels using whole-cell patch clamp recordings of IHCs. Additionally, the expression of IHC proteins was assessed using whole-mount immunolabeling including BK K+ channels, which are a hallmark of mature IHCs. Auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE) were recorded to assess hearing function.
Results: The onset of Cre expression in Prestin::Cre;Cav1.3–/flex mice did not simultaneously excise Cav1.3 in all IHCs, thereby inducing phenotypes ranging from Cav1.3–/––like to nearly wildtype-like IHCs at young ages (P14 - P35) and residual hearing function. The phenotype was progressing with age resulting in profound hearing loss and absence of BK channels in 4-month-old animals.
Conclusions: We thus conclude that Cav1.3 expression is required to maintain the general IHC phenotype after the onset of hearing.

Novel Topical Therapeutics Against Hearing Loss

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Category: Inner Ear: Drug Delivery
Background: Introduction: Regardless of the etiology of hearing loss, mechanistically, inflammation and oxidative stress are major players in inner ear structures damage. Therefore, the development of treatments targeting reactive oxygen species and inflammation represent a viable approach for the prevention of noise induced hearing loss. Our group has synthesized several antioxidant-macromolecular carrier conjugates that show promise in accessing the inner ear structures and protecting inner ear cells against oxidative damage.
Methods: Experimental methods: Hyaluronan-antioxidant conjugates (HAO) were prepared by chemical conjugation of HA and antioxidants. The conjugates were characterized via 1H-NMR and HPLC. The materials were tested for cytocompatibility with mouse inner ear cells with an MTS colorimetric assay. Cell proliferation rates of conjugate-treated cells were determined via CyQUANT assay. The oxidative protection properties of HAO were assessed using a fluorescent reagent (CM-H2DCFDA). The mechanism of oxidative protection of the conjugates was interrogated microscopically and via NADP/NADPH assays. Additionally, the drug permeation properties of the conjugates via round window membrane (RWM)-mimicking tissue models were assessed using HPLC, LC-MS and an enzymatic antioxidant assay.

Results: Results and discussions: HAO conjugates were successfully synthesized with different antioxidant percentages. When HEI-OC1 and SV-k1 cells were treated with HAOs, the metabolic activity in both cell types significantly increased. Interestingly, this observation did not correlate with an increase in cell numbers, suggesting that HAOs directly impact the mitochondrial activity of the cells. As mitochondrial overdrive is one of the issues leading to hearing loss, we next investigated if HAO treatment increases the reactive oxygen species (ROS) content of the treated cells; however, HAO treated cells displayed similar ROS levels with the untreated control cells. In contrast, when cells were pretreated with HAO then oxidatively stressed with hydrogen peroxide, the conjugates seem to have protective effects on both HEI-OC1 and SV-k1 cells. Our additional data indicate that the macromolecule carrier (hyaluronan) helps with the RWM penetration and subsequent inner ear cell internalization of the antioxidant thus providing protective effects.

Conclusions: Conclusions: Our data so far highlights the practicality of chemically conjugating HA with an antioxidant. We anticipate that our ongoing experiments will support our hypothesis that HA conjugation with antioxidants can protect inner ear cells from oxidative damage and can enhance the drug's permeability across the round window membrane.

Phospholipid PIP2 Mediates Slow Adaptation in Cochlear and Vestibular Hair Cells

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Background: The mechano-electrical transduction (MET) process allows the transduction of mechanical information from sound and head movements into electrical signals, and it is a fundamental step in auditory and vestibular system function. MET takes place at the level of the hair bundle and is triggered by stereocilia deflection. During a sustained displacement, the receptor current peaks then decays, indicating a gradual decrease in MET channel open probability. This particular process is called "adaptation", it shifts the operating range of the MET process and might be important in preserving the sensitivity of the system and in filtering (Crawford et al., 1989; Eatock et al., 1987, Ricci et al. 2005). The slow adaptation process operates with a time constant on the order of 10 ms or more and requires Ca2+ entry through the MET channels and the activity of myosin motors. Although the myosin motor involved is still unknown in the cochlea, it is known that Myosin1c (Myo1c) is a regulator of adaptation in the vestibular system (Holt et al., 2002; Yamoah and Gillespie, 1996; Caprara et al. 2020). Recently, we demonstrated that the mechanism of slow adaptation does not involve the upper tip-link insertion movement as hypothesized by the motor model (Caprara et al. 2020), questioning the molecular mechanism of the adaptation process.

Methods: Using electrophysiological recording in mouse vestibular and cochlear hair cells, we tested a new hypothesis that involves the activity of myosin motors and membrane phospholipids like PIP2 in the regulation of slow adaptation. In particular, we hypothesized that PIP2 is the major player in the slow adaptation process, and myosins at the tip of the shorter stereocilia are responsible for transporting PIP2 to the MET channel proximity to mediate adaptation.

Results: First, using a pharmacological approach, we tested if PIP2 plays a role in slow adaptation in cochlear and vestibular hair cells, and then we tested its interplay with Myo1c in vestibular hair cells. Our results showed that PIP2 is necessary to regulate slow adaptation in both auditory and vestibular systems. In vestibular hair cells, the addition of exogenous PIP2 rescues slow adaptation when Myo1c is inhibited, indicating that also, when the activity of Myo1c is inhibited, exogenous PIP2 is sufficient to preserve slow adaptation

Conclusions: These results support our hypothesis of PIP2 being a more direct mediator of slow adaptation and functions downstream of the Myo1c role, likely responsible for transporting and concentrating PIP2 near MET channels. These data are the first step in determining the underlying molecular mechanism of slow adaptation in mammals, the key process that preserves the sensitivity of the system and allows us to detect a wide range of sound intensities with extremely high precision.

A Review of Ototoxicity From Non-Platinum-Based Chemo- And Immuno-Therapies

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Background: Platinum-based chemotherapeutics have known ototoxic effects. There has been an increase in the development of cancer chemo- and immuno- therapies, and many of these emerging agents have been associated

with ototoxicity. Increased awareness of the ototoxic effects of these newer agents is essential for the care of cancer patients due to the profound negative impact of hearing loss on patient quality of life. Improved understanding of emerging cancer therapies that have ototoxic effects has potential to motivate standardization of ototoxicity monitoring protocols and optimize care for cancer patients. The goals of this review are to provide a review investigating, evaluating, and summarizing published evidence of ototoxicity associated with non-platinum-based cancer therapies in order to increase clinician and patient awareness of these emerging ototoxic agents.

Methods: A structured search of the published literature (up to and including June, 2021) was conducted using the databases PubMed, EMBASE, and Web of Science. Search terms included ototoxicity, hearing loss, and a range of therapeutic drug classes. Search results were initially screened by title and abstract, followed by in-depth full text evaluation for inclusion and exclusion criteria.

Results: A total of 51 publications were identified for inclusion in this review including 36 case reports and 15 clinical trials. 21/36 (58%) of the case reports and 3/15 (20%) of the clinical trials were published within the last 10 years. Pre-treatment hearing status was documented in 20/51 (39%) of the case reports and in 14/15 (93%) of the clinical trials. Permanent ototoxicity was documented in 26/36 (72%) of the case reports and in 7/15 (47%) of the clinical trials. However, hearing outcomes were not reported unanimously.

Conclusions: There are a growing number of reports linking non-platinum-based cancer therapies and ototoxicity. Nearly half of these reports were published within the last 10 years (47%). The ototoxic effects are often irreversible (64%) and pre-treatment hearing was not often reported (39%), particularly with case studies. As the number of novel cancer therapeutic agents grow, these results highlight the need for increased awareness of ototoxicity as a potential side-effect and standardization of ototoxicity monitoring protocols to improve recognition of ototoxicity.

Drosophila Dyb Mutants, Unravelling the Role of Dystrophin Glycoprotein Complex in Meniere Disease

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Category: Vestibular: Basic Research and Clinical

Background: Meniere's disease (MD) is an inner ear disorder characterised by recurrent vertigo attacks, sensorineural hearing loss and tinnitus. Previous studies linked DTNA, a Dystrophin Glycoprotein Complex (DGC) molecule, with MD in familial and sporadic cases. In Drosophila, the Johnston's organ (JO), known as the fly's 'inner ear', is a chordotonal organ localised in the 2nd antennal segment, which mediates the sensation of hearing, gravity and wind. In Drosophila, climbing and hearing assays in DTNA orthologue Dystobrevin (Dyb) knockout have demonstrated that Dyb plays a role in hearing and proprioception, and the ablation causes a mild MD-like phenotype.

Methods: In order to investigate the cells and molecular defects involved in the MD-like phenotype observed, we generate tissue-specific knockdown using the UAS/Gal4 systems and RNAi lines. In addition, we perform immunocytochemistry at the pupal stage of the Drosophila antenna in different Drosophila fluorescent lines. To detect the Dyb location, we generate a Dyb GFP-enhancer transgenic reporter line. Also, we use Dys tagged with EGFP-FlAsH-StrepII-TEV-3xFlag (Dys-GFP) line to identify the intracellular location of DGC. The knockout line Dyb11 and the control Oregon-R (OR) were used to study the effect produced by the Dyb ablation.

Results: Dyb knockout mutants showed an MD-like reduction in hearing and proprioception. Tissue-specific knockdown using RNAi suggested that the cells involved in the MD-phenotype are neurons and their specialised supporting cells. Fluorescent markers showed morphological changes. The Dyb GFP-enhancer transgenic reporter line shows the expression of Dyb in the 'ligament' supporting cell, whereas Dys is located in the scolopale and ligament cells according to the Dys-GFP line. The counting of neurons and ligament cells showed a slight reduction in the Dyb11 ligament cells compared with the control, but no significant differences were found. Dyb11 showed that sensory cilium differs from the control and the cilia length is significantly shorter than the control. In addition, NompC, a transient receptor potential (TRP) channel, presents a diffuse location. In contrast, the inactive channel (Iav) distribution in the Dyb11 line presented similar distribution to the control.

Conclusions: Our results show that Dyb disruption in sensory neuron and ligament cells generates a mild MD-like phenotype, only displayed in the dark (therefore lacking visual stimuli). Therefore both cell types are involved in the MD-like phenotype. Immunostaining confirmed the location of Dyb in the ligaments cells, but the Dys-GFP line suggests that the DGC location is scolopale and ligament cells. The function of Dyb, Dys and the DGC in the

JO remains unknown, but Dyb absence affects the systems' stability, thereby generating sensory cilium and NompC channel distribution changes.

The Role of Cochlear Vasculature in the Pathogenesis of Norrie Disease

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Category: Genetics B: General

Background: Norrie disease is an X-linked recessive condition, caused by mutation of the gene NDP, in which boys are born severely visually impaired with disrupted retinal vasculature. The majority develop progressive hearing loss and a proportion present with additional neurological features and developmental delay. The hearing loss in addition to blindness severely reduces quality of life, however, the later onset provides a window of opportunity for treatment.

NDP encodes a secreted signalling molecule Norrin, an atypical WNT which binds to coreceptors FZD4, LRP5 and TSPAN12 on the surface of target cells. Activation of the canonical WNT signalling pathway stabilizes cytoplasmic β-catenin, which in turn translocates to the nucleus and modulates the expression of downstream genes. Mice with a loss-of function mutation in Ndp show cochlea microvasculature abnormalities and loss of hair cells, but the primary target cells of Norrin signalling in the cochlea remain unresolved. This study tested whether increased activation of the canonical WNT pathway mediator, β-catenin, in endothelial cells, prevents hair cell death.

Methods: We used mice carrying a tamoxifen-inducible Cdh5CreERT2 driver transgene and the Ctnnb1 flex3 allele to activate β-catenin constitutively in vascular endothelial cells, effectively by-passing the need for Norrin signalling in these cells. We evaluated the effect of this intervention on the progression of the cochlear pathology in mice hemizygous for the Ndp-KO allele (Ndptm1Wbrg/Y) compared to untreated Ndp-KO mice using histological, molecular and functional analyses.

Results: Stabilization of β-catenin signalling in the cochlea vasculature of Ndp-KO mice at postnatal day 10 rescued microvascular morphological abnormalities of the lateral wall. It prevented regression of the stria vascularis capillaries, restored expression of the tight junction protein CLDN5 and cochlear vessel barrier function. Most importantly, it prevented the death of outer hair cells, essential for functional hearing. Later β-catenin stabilization in the vasculature partially rescued OHC loss.

Conclusions: Increased activation of the canonical WNT pathway mediator, β-catenin, in endothelial cells, prevents hair cell death in Ndp-KO mice. This study suggests that hair cell survival is not dependent upon secreted Norrin acting on hair cells, rather that sustained activation of the WNT signalling pathway in cochlea endothelial cells is sufficient to ameliorate hair cell loss. These results suggest that maintaining vasculature integrity is an important function of Norrin signalling in the cochlea which has implications for the design and targeting, and timing of novel therapies to treat hearing loss in Norrie disease.

Effect of Middle Ear Muscle Reflex on Forward and Reverse Transmission in Middle Ear Model

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Category: Middle and External Ear

Background: Middle-ear muscle reflex (MEMR) involving the stapedius was thought to be activated by loud sounds (aprox. 80 dB SPL). However, recent findings indicate that the threshold may be much lower (Boothalingam and Goodman, (2021); Keefe et al., 2017). Here, we present analysis of forward and reverse transmission of the analog circuit model of middle ear (Pascal et al., 1998) in which the MEMR can be simulated by increasing the stiffness of the stapedius.

Methods: The middle-ear model was taken from literature. The model was coupled with a lumped element model of the ear canal simulating the measurement setup in which a probe is sealed in the ear canal. We focus on the effect of MEMR on the pressure at the place of the probe.

Results: MEMR activation due to the increase of stapedius stiffness attenuates low frequency (<800 Hz) pressure transmitted to the cochlea, whereas the pressure transfer at higher frequencies (>800 Hz) is slightly increased. In the case of reverse transmission from the cochlea to the ear canal, up to about 1.4 kHz MEMR attenuates the transfer function level. At high frequencies (>1.4 kHz), MEMR increases the transfer function level.

For the sound source in the ear canal (forward direction), MEMR decreases the pressure for frequencies up to about 800 Hz. In contrast, at higher frequencies up to about 2 kHz MEMR slightly increases the pressure. If the pressure source is inside the cochlea, e.g. otoacoustic emission, MEMR may have an even larger effect: by about 2 times larger than the MEMR effect on the pressure in the case of forward direction. For frequencies up to 1.4 kHz, MEMR attenuates the ear canal pressure. For frequencies above 1.4 kHz, MEMR increases the ear canal pressure.
Conclusions: The middle ear model showed that MEMR simulated by stiffness changes in the stapedius part of the model has near 1 kHz about two-times higher effect on the ear canal pressure evoked with a sound source emitted from within the cochlea than a sound source in the ear canal. This result have an important outcome for criteria used to detect the MEMR effect on otoacoustic emissions, e.g. the assumption that MEMR was not involved if the residual calculated as a difference between stimulus-frequency otoacoustic emission with and without contralateral suppression has rotating phase (Guinan et al., 2003).

Mechanical Modeling of Changes in Auditory Cortical Morphology Due to Hearing Loss
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Category: Auditory Cortex and Thalamus: Structure and Function
Background: Hearing loss can be caused by a variety of genetic, environmental and age-related factors. These factors in turn may result in the loss of cochlear hair cells or signal conduction through peripheral auditory pathways. Imaging studies also suggest that auditory deprivation during childhood result in observable morphological changes within the central nervous system (Manno et al., NeuroImage, 2021). This implies that hearing loss should not be understood solely as an auditory problem but as an issue with widespread impacts on brain structures during development, including non-auditory areas. Therefore identifying mechanical parameters of gray and white matter that affect morphological changes due to hearing loss is of crucial importance. In this work we quantitatively measure auditory cortical changes in neuroimaging using a feline model of congenital hearing loss. We then apply a mechanical model to estimate which alterations in cortical physical properties may have produced the changes observed in imaging.
Methods: The brains of seven cats, three with normal hearing, three with bilateral hearing loss and one with left-side hearing loss were imaged using structural MRI. Automatic gray-white matter image segmentation was done using the alternating kernel method before manual correction. Smooth closed surfaces of the auditory cortex were generated using Delaunay triangulation before separation into inner (gray-white boundary) and outer (pial) surfaces. Three parameters characterizing morphology of the cerebral cortex were evaluated: cortical thickness (defined as the distance traveled by vertices during diffeomorphic inner to outer surface registration), local folding index (defined as the estimated space reduction due to gyrification), and mean curvature (the average of cortical principal curvatures). Next, changes in these metrics were reproduced by altering the shear and bulk moduli of the gray and white matter in a computational model of gyrification mechanics (Tallinen et al., Nature, 2016).
Results: Decreases in curvature, folding index and increases in cortical thickness were observed in cats with hearing loss as compared to normal hearing controls. Adjustments in the shear and bulk moduli revealed specific combinations which replicate the observed structural changes. Slight increases in shear modulus resulted in decreases in folding index and increases in cortical thickness reproducing the results seen in deaf cats.
Conclusions: Quantitative changes in cortical thickness, curvature and folding index were evaluated in neuroimaging data of deaf versus hearing cats. Specific adjustments in mechanical properties of gray and white matter in the computational model of cortical development were found to replicate our MRI observations. Further experimentation could potentially reveal biologically plausible sources of changes in these mechanical properties.

Three-Dimensional Visualization of the Membranous Labyrinth: The Ductus Reuniens
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Category: Inner Ear: Anatomy and Physiology
Background: The labyrinth of the inner ear is a complex three-dimensional structure comprised of a membranous otic labyrinth (derived from ectoderm) and various soft tissues of mesodermal origin occupying the space between

the otic labyrinth and bony otic capsule. The ductus reuniens (DR) joins the inferior portion of the saccule to the scala media providing the only endolymphatic connection between the vestibular and auditory end organs of the ear. The DR, however, remains poorly understood in both its morphology and spatial relationships to surrounding anatomy. In turn, its (patho)physiological role in the healthy and the diseased ear remains unclear. Recent studies are beginning to address this deficiency. For example, recent evidence from a large case series in 27 patients shows that vestibular receptor function can be preserved after surgical ablation of the cochlea highlighting that endolymph flow from the cochlea to the vestibular labyrinth via the DR is not necessary for normal function of the human peripheral vestibular system (Plontke et al 2021).

Methods: This study serves to elucidate the 3D morphology of the DR and its relationship to surrounding bony and membranous anatomy, providing a detailed, quantitative description of its morphology. Temporal bones from three humans and two guinea pigs were fixed using Karnovsky's fixative and then soaked in 2 % Osmium tetroxide. This process allowed better visualization of the membranous labyrinth. The samples were then scanned by micro-CT followed by 3D reconstruction of the saccule, DR, and initial portion of the scala media. Finally, univariate measurements of these structures were taken using the software 3D Slicer.

Results: The DR takes the form of a narrow tube which has a subtle hourglass-like shape expanding slightly on both ends contiguous with the saccule and scala media. The DR is curved concavely when viewed anteroposteriorly, adhering to the inferior bony wall of the vestibule just anterior to the posterior ampulla. 3D visualizations of the DR illustrate its small intralumenal width (<0.2mm) and its proximity to the round window with the lateralmost end of the DR situated 0.25mm (on average) superior to the posteromedial corner of the round window in humans.

Conclusions: Our results provide new visualizations of the DR, demonstrating its relationship to surrounding structures. Of particular importance is its proximity to the round window. Our observations indicate that care should be taken for surgical procedures involving the round window so as not to disturb the DR situated immediately superior to it. Our results also support the possibility of dislodged otoconia blocking the duct itself, which may impede endolymphatic flow and play a role in endolymphatic hydrops and Meniere's Disease. Plontke SK, et al. A case series shows independent vestibular labyrinthine function after major surgical trauma to the human cochlea. Communications Medicine 2021, in press.

Utility of Exome Sequencing for Genetic Diagnosis of Hearing Loss in a Large, Clinically Heterogeneous Cohort of Pediatric Patients

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Background: Hearing loss (HL) is a common sensory deficit, affecting 3/1000 newborns. Pinpointing the etiology of pediatric HL in a timely manner is critical in order to provide prognostic information, ensure access to appropriate habilitation as early as possible, and allow for time-sensitive counseling. Pediatric HL may be attributed to infectious, anatomic, or genetic causes, with over 50% of instances of sensorineural HL being genetic in etiology. Due to its genetic heterogeneity, the standard of care for identifying the cause of pediatric HL is a targeted panel that sequences a list of known deafness-causing genes. However, with recent developments in technology, exome sequencing (ES), which sequences all protein-coding regions of the genome, has become more accessible and presents an alternative strategy for obtaining a molecular diagnosis for HL. Several studies have investigated the efficacy of ES for genetic diagnosis of HL, with overall diagnostic rates from 31%-47.3%. The objective of the current study was to build upon previous literature by investigating the efficacy of ES for genetic diagnosis in a large clinically heterogeneous cohort of pediatric patients affected by HL.

Methods: Patients seen in the Boston Children's Hospital Department of Otolaryngology and Communication Enhancement with confirmed HL, without a known genetic or environmental etiology, as well as their biological relatives were eligible for enrollment. Written informed consent was obtained from all participants, and ES was performed using DNA derived from buccal swabs. Variant filtering and analysis focused on 366 known and candidate deafness-causing genes as well as ACMG59 secondary findings for interested participants. Clinically confirmed results were reported back to patients who elected to receive them, and appropriate follow-up care was coordinated for patients with syndromic and secondary findings.

Results: Exome analysis has been completed for 161 probands to date. This was a highly heterogeneous cohort consisting of patients with known anatomic abnormalities; various lateralities, degree, configuration, and age at

onset of HL; and various familial inheritance patterns. Positive findings were identified for 51 (31.7%) patients in 28 different genes, with inconclusive findings identified in an additional 13.7% of patients. The diagnostic (positive) rate was highest for patients with a positive family history, symmetric HL, and prelingual onset of HL. 19.6% of children with positive findings were diagnosed with syndromic forms of hearing loss. Secondary findings were identified in seven patients in six different genes.

Conclusions: ES offers several advantages over HL gene panel testing, including an effective diagnostic rate, identification of secondary findings, and an efficient research pipeline for discovery of novel deafness-causing genes. Furthermore, the results of this study support increased access to genetic testing for patients affected by HL in order to provide valuable prognostic information and facilitate timely access to appropriate habilitation and molecular therapies.

Examining Balance, Cognitive Load and an Auditory Balance Device in Children and Young Adults With Cochleovestibular Loss

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Background: The present study aimed to determine in children and young adults with cochleovestibular loss: 1) the interaction between balance and cognitive load, and 2) effects of an auditory balance prosthesis on balance and cognitive load. Children with sensorineural hearing loss often experience vestibular loss and balance difficulties. The BalanCI is an investigative balance prosthesis that provides auditory head-referencing cues through the user's cochlear implants so they can adjust their posture and remain upright. Maintaining balance requires cognitive resources and is typically prioritized over concurrent cognitive tasks. Balancing and performing a cognitive task simultaneously may lead to dual-task costs to either or both tasks. It was hypothesized that 1) children and young adults with cochleovestibular loss demonstrate greater dual-task costs when performing a working memory task while engaging in a static balance stance than typically-developing peers, and 2) using the BalanCI will reduce the cognitive load introduced when balancing, thereby improving performance on both the cognitive and balance tasks.

Methods: Fifteen typically-developing children aged 7-18 (mean age±SD = 13.6±2.75 years, 6 female) and 8 children and young adults with cochleovestibular loss who were bilateral cochlear implant users (mean age±SD = 19.5±5.45 years, 6 female) completed two working memory tasks; a backwards auditory digit span task, and a backwards visuospatial dot matrix task. Each working memory task was completed in three balance conditions: while seated, while standing in tandem stance on a firm stationary surface with the BalanCI off, and while standing in tandem stance with the BalanCI on. Path length of motion capture markers worn on the head, upper body, pelvis and feet was used as a measure of overall stability. Typically-developing children received cues from the BalanCI through insert earphones.

Results: Participants in both groups achieved better scores on the backwards visuospatial dot matrix task than the backwards auditory digit span task (p < 0.0001) and scores were poorer overall in CI users than typically-developing children (p = 0.038). Balance condition did not affect performance on either working memory task in either group (p = 0.56). CI users had larger path lengths than typically-developing children during both working memory tasks (p's < 0.01). In CI users path lengths were also larger during the auditory digit span task than the visuospatial dot matrix task (p = 0.0023).

Conclusions: Children with cochleovestibular loss showed impaired working memory and postural stability. Both working memory and stability were more impaired in the auditory digit span task than the visuospatial dot matrix task in CI users, potentially reflecting a stabilizing effect provided by the visual input available from the matrix, or an auditory task disadvantage for CI users. The introduction of the BalanCI use did not improve or reduce cognitive or balance task performance.

Olfaction and Smell Identification Tests: Can Olfaction Be Used to Predict Cochlear Implant Outcomes?

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Background: A cochlear implant (CI) is a prosthetic implantable device that is available for people who do not benefit from traditional, non-invasive means of amplification. There is significant variability in CI outcomes. Currently, the patient-related factors that appear to correlate with CI outcomes are limited to age and duration of deafness. Recent work has suggested cognition, specifically cognitive decline, may be an additional patient-related

factor that contributes to poor CI outcomes. This work is based on the concept that processing of sound requires intact central processing and neurocognitive function. While the relationship between cognition and hearing is evolving, it is well-established that olfactory dysfunction is an early indicator of cognitive decline. We reasoned that olfaction might provide information regarding one's ability to process auditory information, which relies heavily on central functioning and cognition. We hypothesized that olfaction and smell identification scores on the University of Pennsylvania Smell Identification Test (UPSIT) would correlate with CI outcomes. More specifically, we hypothesized that olfactory dysfunction might serve as a potential predictor of poor CI outcomes.

Methods: 48 patients were recruited to the study across two institutions of those, 32 ultimately ended up being included in final analysis. Study participants were administered the UPSIT immediately prior to CI surgery. Participants were sorted into 3 groups: normosmia, mild hyposmia, and moderate/total/severe hyposmia. CI outcomes were primarily assessed using AzBio scores, collected at 3 months and 6 months postoperatively. Univariate and multivariate analyses were performed on the data gathered. A statistical analysis program (SPSS) was used to run analysis of variance testing to evaluate whether a correlation between olfaction and CI success was present.

Results: By our analysis there was no statistically significant relationship between UPSIT score and any of the measured speech outcomes by either absolute or percentage change at either of the collected postoperative time points. Our study was limited by small sample size and the difficulty of standardizing time to CI activation in our study population.

Conclusions: Although we did not find a correlation between olfaction and cochlear implant outcomes in our small study, this does not diminish the importance of research investigating the link between cognition, hearing loss, and CI outcomes. The complexities of the current neurocognitive testing need to be simplified. In theory, a test like the UPSIT (which might be able to detect early cognitive decline) could have the potential to replace the role of neurocognitive testing that some institutions are performing for CI candidacy evaluation. While our results are not statistically significant, they do serve to deepen our understanding into this new field of research that integrates peripheral neurosensory testing, auditory processing, and central neuroscience.

Feasibility of a Totally Implantable Artificial Basilar Membrane System: A Conceptual Design
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Background: Cochlear implants (CIs) have become the standard treatment for patients who suffer from sensorineural hearing loss due to damage or loss of hair cells in the cochlea. However, conventional CIs have some challenges, such as problems caused by the use of extracorporeal devices, and have a very high power consumption for frequency analysis measurements. To overcome these challenges, a fully implantable CI (FICI) was developed. Even the newly developed FICI has some limitations, such as too much ambient noise produced from a subcutaneous microphone and a battery that requires frequent recharging. To solve these two problems, artificial basilar membranes (ABMs) made of piezoelectric materials have been studied. This study aimed to verify the conceptual idea of a totally implantable ABM system.

Methods: Using the ABM we developed in a previous study, we constructed an electronic module (EM) for the amplification of electrical output from our ABM and investigated the auditory brainstem responses of deafened guinea pigs that were stimulated by the amplified output of electricity generated by the ABM in combination with the EM in response to an actuator. Further, we implemented an optimal method for coupling ABMs to the middle ear ossicle and explored the possibility of a bioelectronic middle ear microphone.

Results: In the ABM plus EM in vivo test, the signal, which was generated from the ABM and amplified by the EM, was able to induce auditory brainstem responses in deafened guinea pigs, indicating its capacity to mimic basilar membrane functions. In the tube-type connector coupled to the umbo, we measured 120 μV of electrical output from the ABM, which was stimulated by sound (110 dB SPL, 750 Hz).

Conclusions: We developed a prototype of the totally implantable ABM system, consisting of the ABM, EM, and electrode, and assessed its feasibility. We obtained meaningful auditory brainstem responses by implanting it into guinea pigs. The power of the entire ABM system was 100 times lesser than that of conventional CIs. In the case of the ABM system with umbo connection, the electrical output was 10 times lesser than that of the ABM system without coupling. Although at the time there was insufficient electrical power to operate the entire system, we found a possibility of a self-powered ABM system, which might be one of the future options for a completely implantable device. Improving the efficiency of the ABM and developing an efficient ossicular connection (coupling) technology are challenges that need to be studied further.

Quantitative Assessment of Semicircular Canal Duct Histopathologic Dimensions in Ménière's Disease

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Category: Clinical Otolaryngology and Pathology

Background: Ménière's disease (MD) is a clinical syndrome with unknown pathophysiology; however, endolymphatic hydrops is frequently observed on histopathology in patients with clinical MD. Recently, a paradoxical relationship between video-head impulse testing (vHIT) and caloric testing, which examine the same vestibular end-organ, has emerged—vHIT responses are normal and caloric responses are often diminished. Enlargement of the membranous labyrinth within the semicircular canals has been proposed to cause this dissociation. In this study, we quantified the dimensions of the membranous and bony labyrinths within the semicircular canals to examine this hypothesis.

Methods: MD temporal bone specimens from the Johns Hopkins Human Temporal Bone Collection and the Massachusetts Eye and Ear Temporal Bone Image Library (n=21 ears, mean age 73±11) and age-matched controls (n=37 ears) were obtained. We assessed specimens for hydrops within the inner ear and measured the dimensions (long axis, short axis, cross-sectional area) of the bony and membranous labyrinths of each semicircular duct (horizontal/superior/posterior canals; HC/SC/PC). A sub-group analysis of patients with diminished caloric responses was performed as well. MD ears were compared to controls using an unpaired t test with Welch's correction or non-parametric Kolmogorov-Smirnov test.

Results: Endolymphatic hydrops was seen in the cochlea (95% of samples), saccule (80%), and utricle (70%) of MD patients and none of the controls. Within the membranous labyrinths of the semicircular canals, however, there was no difference when compared to controls in any of the dimensions (p>0.05). For the bony canals, the HC/PC long axes were significantly shorter in MD ears (HC: p=0.0049; PC: p=0.0031), while the PC short axis was also shorter (p=0.0044). The cross-sectional area of the bony HC and PC were significantly smaller (HC: p=0.016, PC: p=0.0015), resulting in a greater membranous-to-bony area ratio in MD patients compared to controls in those canals. For the SC, no significant differences were found. In the sub-group with diminished caloric responses, smaller SC bony canal area and width (in MD patients, p<0.05 for both) were the only significant differences.

Conclusions: Taken together, our results demonstrate that endolymphatic hydrops in the semicircular canals alone cannot be the answer for the caloric testing abnormalities in MD. This study also provides dimensions for the membranous and bony labyrinths within the semicircular canals. Smaller HC/PC bony labyrinths and therefore greater membranous-to-bony ratios may represent a new histopathologic correlate for MD.

Dynamic Lateral Inhibition Regulates Utricle Patterning During Inner Ear Embryonic Development

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Background: The mammalian vestibular system, sensing acceleration and gravitation, includes 5 organs. Each organ consists of alternating pattern of sensory hair cells (HCs) and non-sensory supporting cells (SCs). The embryonic development of those organs is a dynamical process that includes differentiation, cell division, delamination and morphological transitions (i.e. intercalation and shape changing). Recent studies have shown that inner ear development is affected by biochemical-signaling (e.g. Notch mediated lateral inhibition), but also by mechanical forces (e.g. mechanical pressure effects cell division rate). Elucidating the factors affecting vestibular system development and how it differs from the development of the organ of Corti can contribute to developing treatments for balance disorders, by inducing HC regeneration at post-natal stages.

Methods: We developed a live imaging assay of vestibular system explants, focusing on the horizontal acceleration sensory organ, the utricle. To track different dynamical processes, we use mice containing ZO1-EGFP (marking apical boundaries) and Atoh1-mCherry (marking differentiation of HCs). Using insights from our experimental results, we formulate a mathematical model, that includes biochemical signaling and mechanical forces. Our model results are then compared with experiments, using quantitative analysis. In addition, we use mechanical and chemical perturbations (e.g. Laser ablation, Notch inhibition) to validate and improve our model.

Results: Our preliminary imaging results of utricles at embryonic day 17.5 (E17.5) show that cell division, delamination and differentiation take place frequently at this stage, but almost exclusively for Atho1 negative cells (SCs). Results from new-born mice (P0), in contrast, only rarely exhibit cell division, delamination or

differentiation. Laser ablation experiments at both E17.5 and P0 show that ablation of HCs promotes differentiation and delamination of nearby SCs. Our preliminary modeling results, based on a 2D vertex model, show that a model that includes a feedback between Notch mediated lateral inhibition and mechanical forces (elastic forces and cell-cell adhesion) can capture the main observations.

Conclusions: The embryonic developmental of mice utricle is a dynamic process that is regulated by both Notch mediated lateral inhibition and mechanical forces. In contrast to the organ of Corti development, the developmental process is dynamic, containing both cell division and differentiation in parallel with reorganization. The interplay between mechanical forces and regulatory processes determines the dynamics at the single cell level (i.e. the "decision" of a cell to differentiate or divide). Damaged HCs may be regenerated by the differentiation of nearby SCs, even at early postnatal stages. What are the exact factors that affect single cell decisions in the utricle development remains to be tested as this project proceeds.

Cochlear Implant Positioning and Fixation Using 3D-Printed Patient Specific Surgical Guides; A Cadaveric Study

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Background: Positioning and fixation of the cochlear implant (CI) are commonly performed free hand. Applications of 3-dimensional (3D) technology now allow us to make patient specific, bone supported surgical guides, to aid CI surgeons with precise placement and drilling out the bony well which accommodates the receiver/stimulator device of the CI. We aimed to develop and validate the optimal design and evaluate accuracy of individualized 3D- printed surgical guides for cochlear implantation.

Methods: Cone beam CT (CBCT) scans were acquired from temporal bones in 9 cadaveric heads (18 ears), followed by virtual planning of the CI position. Surgical, bone-supported drilling guides were designed to conduct a minimally invasive procedure and were 3D-printed. Fixation screws were used to keep the guide in place in predetermined bone areas. Specimens were implanted with 3 different CI models. After implantation, CBCT scans of the implanted specimens were performed. Accuracy of CI placement was assessed by comparing the 3D models of the planned and implanted CI's by calculating the translational and rotational deviations.

Results: Median translational deviations of placement in the X- and Y-axis were within the predetermined clinically relevant deviation range (< 3 mm per axis); median translational deviation in the Z-axis was 3.41 mm. Median rotational deviations of placement for X-, Y- and Z-rotation were 5.50°, 4.58° and 3.71°, respectively.

Conclusions: This study resulted in the first 3D-printed, patient- and CI- model specific surgical guide for positioning during cochlear implantation. The next step for the development and evaluation of this surgical guide will be to evaluate the method in clinical practice.

Cochlear Implant Awareness: Development and Validation of a Patient Reported Outcome Measure

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Background: Surgical success of cochlear implantation is usually measured through speech perception and quality of life questionnaires. Although these questionnaires cover a broad spectrum of domains, they do not evaluate the consciousness of wearing a cochlear implant (CI) and how this impacts the daily life of patients. To evaluate this concept we aimed to develop and validate a standardized patient reported outcome measure (PROM) for use in cochlear implant users.

Methods: Development and evaluation of the COchlear iMPlant AwareneSS (COMPASS) questionnaire was realized following the COSMIN guidelines in three phases: (1) item generation, (2) qualitative pilot study to ensure relevance, comprehensiveness, comprehensibility and face validity, and (3) quantitative survey study for the assessment of reliability (test-retest) with 54 participants.

Results: Nine themes of CI awareness were identified through literature research and interviews with experts and patients. These resulted in the formulation of 18 items which were tested with a pilot study, after which 3 items were deleted. The final 15-item COMPASS questionnaire proved to have good validity and satisfactory reliability. The intraclass correlation coefficient calculated for items with continuous variables ranged from 0.66 to 0.89 with

seven out of eight items scoring above the acceptable level of 0.7. The Cohen's kappa calculated for items with nominal variables ranged from -0.4 to 0.78 with 11 (sub)items out of 15 scoring above fair to good agreement. Measurement error analysis for items with continuous variables showed a mean difference of -2.18 to 0.22. The calculated 95% limits of agreement for these items revealed no statistically significant difference between the two administered questionnaires. For items with nominal variables, the percentages of agreement calculated, ranged between 0% and 95%, and 83.3% and 96.6% for positive and negative agreement, respectively.

Conclusions: The COMPASS questionnaire is a valid and reliable PROM for evaluating the cochlear implant awareness, and it can be easily used in routine clinical practice.

Quantifying Cochlear Synaptopathy Related Perception Changes

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Background: Over the past decade, 'hidden hearing loss' – hearing difficulty in noisy environments, seemingly without accompanying audiometric threshold shifts – has inspired a wealth of research, including what and how morphological changes in the auditory periphery might give rise to this phenomenon. For example, the stochastic undersampling model (Lopez-Poveda and Barrios, 2013) suggests that auditory deafferentation can potentially introduce internal noise in the subsequent auditory processing stages. However, the parameters used in the original stochastic undersampling model don't fully capture the complexity of physiological response characteristics, thus leaving unclear the quantity of information conveyed by the auditory nerve fibers. Here, we introduce a physiologically more realistic encoding-decoding method that extends the stochastic undersampling model to study the perceptual consequences of auditory nerve fiber loss.

Methods: Stochastic undersampling is an encoding model of the auditory periphery; it models each auditory fiber as a sampler that samples the input sound at its own stochastic rate, and the loss of auditory nerve fibers is mimicked by reducing the number of samplers. In our study, half-wave rectification, refractoriness, and three auditory nerve fiber types (low, medium, and high spontaneous rate) are added to the original stochastic undersampling model to better mimic the actual information transmission in the peripheral auditory pathway. In addition, we use an artificial-neural-network-based stimulus reconstruction approach to decode the modelled auditory nerve fiber responses back into an audio signal. This reconstruction contains a feedforward neural network (Akbari et al., 2019) and a speech synthesizer (Morise et al., 2016). Our encoding-decoding method explicitly models auditory nerve fiber (type) loss within a more realistic physiological setting, which allows us to relate the level of fiber loss to auditory perceptual changes in human listeners.

Results: We tested three different levels of auditory nerve fiber loss (0, 90, 95%) in a pure tone (250, 1000, 4000 Hz) in noise detection task and a hearing in noise task via MTurk. The noise was white noise fixed at 65 dB SPL, and the speech material was HINT sentences. The results indicate that both pure tone detection threshold in noise and speech perception degrade significantly from a healthy ear to 95% auditory nerve fiber loss, at a rate that is commensurate with modeling predictions (Oxenham, 2016). We are currently acquiring additional data (e.g., speech in silence perception, sentence matrix test) to further support and broaden these results.

Conclusions: In conclusion, our encoding-decoding method enables artificial introductions of lesions in the peripheral auditory pathway (e.g. selective frequency, or fiber type loss) and thus can benefit the study of auditory pathology and hearing with cochlear implants.

Characterizing Hair Cell Regeneration in the Larval Zebrafish Inner Ear

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Background: A common cause of deafness and vestibular impairment is death of the sensory hair cells of the inner ear. Adult mammals are unable to regenerate auditory hair cells and have only a limited ability to regenerate hair cells in the vestibular organs. In contrast, robust hair cell regeneration throughout life is common in non-mammalian vertebrates such as fish and birds. Understanding the mechanisms of hair cell regeneration in these highly regenerative vertebrates will inform preventative measures and therapeutic approaches for hair cell loss in humans. The work presented here aims to define hair cell regeneration using a systematic and quantitative approach and identify support cells that act as hair cell precursors in the larval zebrafish inner ear. The zebrafish inner ear has been historically understudied in the context of hair cell regeneration, despite its high level of conservation with the mammalian inner ear and potential for in vivo imaging. This work examines hair cell

regeneration in the zebrafish inner ear during the larval stage of development, at which point the cristae and utricle become fully functional.

Methods: To study hair cell regeneration, we are employing a novel, genetically-encoded method for inner ear hair cell ablation. This method results in complete ablation of crista hair cells after just one hour of treatment, and the fish exhibit a robust regeneration response. In an effort to identify the hair cell progenitors involved in this process, we have analyzed a single-cell RNA-seq dataset of approximately 7,000 zebrafish inner ear hair and support cells.

Results: In order to distinguish hair cell addition during organ growth from addition during regeneration, we have established the baseline rate of homeostatic hair cell addition during the larval stage. Our preliminary data suggest that little hair cell turnover occurs naturally during this time. Following inner ear hair cell ablation, hair cells are rapidly regenerated during the 48 hours post-ablation. Our single-cell RNA-seq analysis reveals multiple transcriptionally distinct clusters of hair and support cells.

Conclusions: The larval zebrafish inner ear is a promising model system for studying the mechanisms of hair cell regeneration in the inner ear in vivo. Distinct types of hair and support cells exist in the zebrafish inner ear that may be analogous to cell subtypes in other vertebrate systems. Future work will include fate mapping support cell subtypes during regeneration to identify those that act as hair cell precursors and to determine the underlying mechanisms of regeneration.

Human Temporal Bones for Otopathology Research – A Pilot Study Using a Methyl Methacrylate Embedding Resin and Laser Microtomy

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Background: Histologically processed human temporal bones (hTBs) are an important resource for research on otologic and neurotologic disorders. Celloidin is the standard embedding medium for hTBs since the late 19th century due to its excellent light microscopic preservation of the complex and delicate hTB anatomy. However, years-long processing times, high costs, laborious protocols, and limited compatibility with molecular biological methods are major drawbacks of celloidin. To overcome these limitations, we here tested the methyl methacrylate resin Technovit® 9100 New (TV) for histological processing of hTBs.

Methods: Three formalin-fixed, non-decalcified hTBs were embedded in Technovit® 9100 (TV) and sectioned at 20 µm thickness using a laser microtome (TissueSurgeon®). Standard histology stainings and immunohistochemistry using antibodies against epithelial, neuronal, and bone tissue antigens were used to assess the histomorphology and tissue antigenicity, respectively. DNA isolated from a single tissue section per hTB was used for deep DNA sequencing (DNA-seq).

Results: Histological processing of hTBs using TV and laser microtomy is 12-times faster and 9-times more cost-effective as compared to celloidin and conventional microtomy. In TV-embedded hTBs the histomorphological preservation is excellent, tissue antigenicity and the applicability of immunohistochemical protocols are superior as compared to celloidin. Isolation of DNA in high quantity and quality that enables whole genome sequencing is possible with a single TV embedded hTB section but failed in our celloidin embedded hTB sections.

Conclusions: TV embedding and laser microtomy of non-decalcified hTBs has several key advantages over the standard celloidin method and in particular enhances the application of modern molecular methods in otopathology research – a prerequisite to address contemporary clinical questions on otologic and neurotologic disorders.

Speech Perception and Implicit Learning in Post-Lingual Adult Cochlear Implant Users

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Background: Speech perception outcomes in cochlear implant (CI) users are quite variable, particularly in daily listening environments. Although portions of this variability can be explained by demographic, audiological and cognitive factors, considerable unexplained variability remains. Different forms of implicit learning are though to play a role in speech perception in listeners with intact hearing and in listeners with hearing loss. The goal of this study is to explore the potential contributions of perceptual, statistical and incidental learning to the perception of challenging speech in post lingual adult CI users.

Methods: 30 post lingual adult CI users (age range 35-77, M= 55) participated so far. Participants completed a comprehensive test battery including challenging speech perception tests (sentences presented in speech shaped noise as well as four-babble talker noise - HeBio, and as natural fast speech). HeBio (the Hebrew version of the AzBio sentences). The test battery also included cognitive measures (vocabulary and memory), a perceptual learning task (time-compressed speech TCS) and two visual learning tasks (statistical and incidental). Accuracy in the speech tasks was modeled with a series of generalized mixed linear models that accounted for demographic, cognitive and speech-related factors before accounting for the contribution of the learning tasks.

Results: No association was found across the different learning tasks; however, perceptual, statistical, and incidental learning had unique contributions to the perception of HeBio sentences in 4-talker babble noise. A better performance in each of the learning tasks (one SD increase in the performance) predicted about 14% increase of the odds of correctly recognizing Hebio sentences in noise. Among the learning tasks, only the perceptual learning, assessed by TCS sentences, had a significant contribution to the perception of natural fast speech sentences.

Conclusions: CI users exhibited implicit learning, suggesting that implicit learning is either maintained or restored with the use of the implant. This learning accounts for some of the individual differences in speech recognition in noise. Similar findings were reported previously in normal-hearing young adults and in older adults with age-related hearing loss. Thus, across populations, rapid implicit learning might serve as a skill listeners can use to support speech recognition. In CI users, the ability to rapidly adjust to on-going acoustical challenges is one of the factors associated with good CI outcomes.

On the Effects of Stroke Lesions on Binaural Hearing

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Background: Many stages of the auditory system are involved in creating a spatial representation from the sound waves arriving at the two ears. Some insights into the role of specific brain regions and nuclei stem from anatomically characterized lesions and their relation to functional impairments. As stroke patients very commonly suffer an asymmetric infarction, spatial hearing is a particularly interesting functional investigation for these individuals. The mapping of sound sources to the auditory space is predominantly represented in the contralateral cortical hemisphere so that contralesional impairments are to be expected and have previously been reported. Less clear is the relationship between the location of the infarction along the auditory pathway and functional impairments or possible differences between the effects on implicit or explicit use of binaural cues.

Methods: Binaural release from masking and a lateralization task based on interaural level difference (ILD) and interaural time difference (ITD) was measured. To date, these experiments were conducted in the acute phase of stroke with 56 individuals (mean age of 63 years). Participants were not chosen based on a specific region of interest. Lesions are located in various brain regions including brainstem, basal ganglia, thalamus, cerebellum and cortical areas in either hemisphere.

Results: Binaural release from masking of at least 8 dB was present in 86% of all stroke subjects, including some with strongly impaired ITD-based lateralization. As expected, the most common lateralization impairment was found for stimuli in the contralesional hemifield in both ITD and ILD-based tasks. An ipsilesional lateralization bias was primarily observed in patients with a thalamic lesion. Side-oriented lateralization was evident in patients with brainstem and cerebellar infarcts, predominantly in tasks of ITD-based lateralization.

Conclusions: Binaural hearing is compromised in many subjects in the acute phase of stroke. The lesion location appears to have a systematic influence on the type of binaural hearing impairment. Some observed tendencies are in line with previously published reports while, to our knowledge, others are novel, such as the observed effects of basal ganglia or cerebellar lesions. The growing data set is expected to contribute to the understanding of the role different brain regions have for binaural and spatial hearing. Furthermore, it is thought to inspire the development of compensatory binaural algorithms for patients with neurologic disorders.

Lack of Slack (Slo2.2) K+ Channels Does Not Aggravate Cochlear Synaptopathy After Mild or Moderate Noise Trauma in Mice

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Category: Inner Ear: Damage and Protection
Background: The Na+-activated K+ channel Slack (Slo2.2, Kcnt1) expressed in spiral ganglion neurons (SGN) may modulate SGN firing patterns and excitability in response to acoustic stimulation. A mild noise trauma that does not increase hearing thresholds may nevertheless cause irreversible loss of synapses between inner hair cells and SGN, which is called noise-induced cochlear synaptopathy. Here we analyzed the effects of a mild and a moderate noise trauma on hearing performance and ribbon synapses in mice globally lacking Slack (Slack-/-) and in wildtype Slack+/+ (WT) mice on a C57Bl6/N background.
Methods: Eight-week-old mice were exposed to 100 or 106 dB SPL broadband noise (8-16 kHz) for 2 h. Hearing thresholds and ABR wave I amplitudes were determined with auditory brainstem response (ABR) audiometry before, directly after and up to 28 days post trauma. Finally, number and pairing of IHC ribbon synapses were analyzed by co-immunolabeling whole-mount preparations of the organ of Corti for presynaptic CtBP2 and postsynaptic HOMER-1.
Results: Before noise trauma, Slack-/- mice showed slightly elevated thresholds by 5-10 dB in the range from 2-11 kHz compared with WT. After the trauma, frequency-dependent ABR (f-ABR) thresholds were more strongly elevated in Slack-/- compared with Slack+/+ mice. In both genotypes, the 106 dB trauma caused a larger threshold elevation in the high frequency range, and this effect was extended towards lower frequencies compared with the 100 dB trauma. The effects of the traumata on genotype were inconsistent: whereas the 100 dB trauma caused a smaller transient threshold shift in the Slack-/- compared with WT, it was the opposite in the 106 dB condition. In the 106 dB trauma, Slack-/- f-ABR thresholds recovered better compared with WT after 28 days. The effects were however small (5-10 dB). Growth functions of ABR wave I amplitudes were irreversibly reduced at day 28 from 11.3-22.6 kHz indicating loss of functional auditory nerve fibers in both genotypes and trauma conditions. Surprisingly, lack of Slack appeared to partially ameliorate the trauma-induced reduction of wave I amplitude 4 weeks later.
In contrast to CBA/CaJ mice, orphan ribbons were rare while orphan HOMER-1-positive postsynapses were frequent for both traumata at mid-to-high frequency cochlear locations. An analysis of the effects of trauma and genotype on synapse survival is ongoing.
Conclusions: Our data confirm the susceptibility of ribbon synapses to a noise trauma of ≥ 100 dB SPL in C57Bl6/N mice. Unexpectedly, the lack of Slack K+ channels did not aggravate a noise-induced permanent threshold shift or reduction in ABR wave I amplitudes. Peripheral dendrites were frequently preserved despite partial trauma-induced loss of IHC synapses in both genotypes indicating that peripheral dendrites bearing IHC postsynapses are less vulnerable than presynaptic ribbons in C57Bl6/N mice.
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Simultaneous Dual Recordings From Type I Hair Cells and Their Calyx Afferents in the Mouse Vestibular Epithelium

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Background: In the reptilian vestibular epithelium, synaptic transmission between Type I hair cells and their enveloping calyces is multiplexed on three time scales. In addition to conventional unidirectional quantal transmission from hair cell to afferent operating on a millisecond time scale, there are two forms of bidirectional transmission attributable to potassium accumulation in the cleft, one that proceeds over the course of microseconds and the other that evolves over tens of milliseconds. These findings were initially obtained using two-electrode simultaneous recordings from Type I hair cell-calyx afferent pairs in the red-eared slider turtle, Trachemys scripta elegans. In the present study, we conducted similar recordings from vestibular hair cells in C57BL/6 mice of both sexes, ages P6-20, to assess the synaptic repertoire of the mammalian vestibular periphery.
Methods: Whole-cell recordings were obtained from Type I hair cells and their calyces in mouse anterior semicircular canal cristae. The recording pipettes were filled with a mixed anionic solution, KF:KCl, 118:12 mM.

One pipette was positioned at the apical neck of the hair cell and the other was placed on the basal aspect of the calyx. Both pipettes were dye–filled: Type I hair cells with Alexa Fluor 488 and calyx afferents with Alexa Fluor 568. Both pre- and postsynaptic partners were examined in voltage clamp.

Results: Hair cell depolarizations from a holding potential of –100 mV generated an initial inward current and a two component outward current. Contemporaneous inward currents associated with the major outward hair cell current were induced in the associated calyx held at –100 mV. The data using dual electrode recordings in the mouse crista show the biophysical signature of potassium accumulation in the synaptic cleft following hair cell depolarization. Based on previous findings in the turtle, these are likely to represent potassium fluxes from the hair cell into the cleft, which subsequently increases the inward current through an HCN conductance in the afferent. Therefore, this potassium accumulation generates large, slow depolarization of both the hair cell and calyx by shifting the potassium equilibrium potential for channels facing the synaptic cleft. In early postnatal development of mammalian Type I hair cells, there is a rapid inward current, and multiple outward currents that contribute to potassium elevation in the cleft during synaptic transmission.

Conclusions: The precise identities and degree to which the conductances expressed in the first postnatal weeks in mammals are retained into adulthood remain to be determined, as does the role of these conductances in shaping the dynamics of the three modes of synaptic transmission previously described in reptiles. Supported by NIH, NIDCD grants R21DC017577 and R01DC019953

OTO-825 Gene Therapy Rescues Hearing Loss and Cochlear Degeneration in a Clinically Relevant Inducible Mouse Model of GJB2 Congenital Hearing Loss
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Background: GJB2 gene mutations cause the most common form of congenital non-syndromic deafness in humans. GJB2 encodes the gap junction protein Connexin 26 (CX26), required in the inner ear for the function of non-sensory cells such as support cells and fibrocytes. In general, the onset of hearing loss is prelingual and moderate to severe, however, in some subjects, hearing loss due to loss of CX26 can be mild and progressive. Human temporal bone studies have revealed degeneration of hair and support cells in GJB2 mutant cochleae, whereas spiral ganglion neurons remain primarily unaffected. Here, we report evaluation of OTO-825, an AAV-based gene therapy candidate, in an inducible mouse model of GJB2-deficiency.

Methods: Since homozygous Cx26 knockout is embryonic lethal in mice, we utilized Cx26 conditional knockout (Cx26 cKO), generated by crossing Cx26loxp/loxp mice with a tamoxifen inducible cre (Rosa-creER) mouse line to study the effect of losing CX26 protein in the cells of the inner ear. Further, we developed an AAV based gene therapy candidate (OTO-825) after screening different capsids, promoters, and optimized GJB2 codons. We also created OTO-825-FLAG that expresses a FLAG-tagged CX26 and administered it via the intracochlear (IC) route in wildtype animals to determine the tropism of AAV derived CX26 in the inner ear by tracking FLAG expression. To study the efficacy of gene therapy, OTO-825 or vehicle were administered to Cx26 cKO mice postnatally via the IC route. Auditory Brainstem Responses were measured at postnatal day (P) 30, and the cochleae were processed for histology to determine the morphology and CX26 expression.

Results: Adjusting the timing of tamoxifen administration allowed temporal control of Cx26 knockout, resulting in varying degrees of hearing loss and cochlear defects dependent on the time of cre activation. Early postnatal cre activation caused severe to profound hearing loss in the Cx26 cKO mice at P30, whereas later cre activation caused a progressive mild to moderate type of hearing loss. Histological examination revealed little to no CX26 expression in the Cx26 cKO mice. IC administration of OTO-825-FLAG to naïve mice confirmed AAV transduction in the support cells and fibrocytes. Cx26 cKO animals injected with OTO-825 showed substantial rescue of ABR thresholds across multiple frequencies, restoration of Cx26 expression and preservation of cochlear morphology, relative to vehicle injected Cx26 cKOs.

Conclusions: A single intracochlear administration of OTO-825 successfully restored hearing, the expression of CX26 in the relevant cochlear cell types and rescued cochlear morphology in a Cx26 cKO mouse model that mimics the auditory deficits found in human GJB2 patients. Taken together, these findings support the use of OTO-825 as a clinical candidate to treat congenital hearing loss caused by GJB2 deficiency.

IEG Expression in the Mouse Auditory Cortex Shows the Importance of Natural Ordered Vocalization Sequences Over Random Ones

Amiyangshu De*[1], Swapna Agarwalla[1], Sharba Bandyopadhyay[1]
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Category: Primary Auditory Cortex

Background: Mouse ultrasonic vocalizations (USVs) are of communicative significance sharing parallels with human speech. Both have successive acoustic units occurring in non-random fashion creating a dependence structure in them. Mouse USVs are also modulated in different contexts not only in terms of various features, but also in terms of the entire sequence structure. However, it is not known whether mouse auditory neurons encode syllable sequences as a whole or not. To address the above question, we investigated the role of the cortical and subcortical regions with the help of the neuronal activity dependent expression of the immediate early gene (IEG) c-Fos.

Methods: We evaluated the expression of the c-FOS as a proxy of neural activity from adult virgin female mice in three different contexts:
1. Absence of auditory stimuli (CN);
2. Playback of natural sequences from three different contexts (SN) and
3. Playback of sequences with syllables, maintaining syllable probabilities as in 2, but randomized in order (SR). We primarily looked at the role of the auditory cortex and its sub-regions, along with other subcortical areas involved in regulating the perception of structured and unstructured acoustic stimuli.The experiment was designed as follows: initial baseline duration without auditory exposure, followed by stimulus presentation span and ended with silent latent period for stabilization of neurons for auditory encoding.

Results: We quantified the number of c-Fos+ neurons in all the three contexts, namely, CN, SN, and SR, as described above. Murine primary auditory cortex (A1) is found to be majorly modulated when exposed to SN. Interestingly, not only A1, but also in dorsal auditory cortex (AuD), and ventral auditory Cortex (AuV), c-Fos+ cell count got enhanced for SN compared to CN. However, no such modulatory effect was observed for SR. Observations similar to the ACX was made in some subcortical areas like lateral hypothalamus (LH), basolateral amygdala (BLA), ventral tegmental area (VTA).

Conclusions: A higher number of activation of c-Fos+ cells in the auditory cortex for SN compared to SR indicates the differential encoding for natural sequences with statistical regularities over random ones. Interestingly, all the subregions of ACX, i.e., A1, AuD, and AuV, were actively involved in encoding SN, but no such effect was observed in SR with respect to CN. A similar trend was observed for modulatory areas for SN but not for SR. This provides evidence that mice might give more weightage to structured stimuli compared to random order stimuli. Our study takes us a step further in establishing the mouse as a potent model to investigate perception of auditory objects formed based on temporal order.

Differential Nature of Harmonic Based Enhancement and Suppression in the Mouse Auditory Cortex

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Background: Mouse ultrasonic vocalizations (USVs), which are of communicative significance, are an attractive tool as a model to gain insight into social communication, socio-cognitive and neurodevelopmental disorders. Mouse USVs contain a variety of syllable classes with distinct spectrographic features. Two-tone harmonic complexes (TTHC) are present in mouse USVs, especially in the context of courtship and mating, and appear to be a distinct sound object rather than two individual tones presented together, especially as they are produced repetitively as a whole sequence. Voiced speech production by its mechanism produces spectrographic content that has sequential harmonic structures. Establishing connections of mouse USVs with human neurodevelopmental disorders with speech comprehension deficits or communication deficits like ASDs thus requires an understanding of coding of harmonics in the auditory pathway.

Methods: We use extracellular electrophysiology to record from single units and also employ single-cell resolution 2-photon Ca 2+ imaging in Layer 2/3 in the anaesthetized mouse primary auditory cortex (A1). Tone response maps of single neurons were obtained first. Next, responses to a set of TTHCs at appropriately matched sound levels were collected whose component frequencies (F0 and F1, fundamental and the first harmonic) were present in the tone response maps.

Results: We quantified the effect of the two tones over single tones as either suppressive, enhancing, or no effect based on the position of the TTHC relative to the best frequency (BF) of the neurons. We find that TTHC has a strong suppressive effect on A1 single-unit responses when the TTHC is "near" BF, while more enhancements in response were observed in "far" from BF cases, compared to "near" BF cases. Similar observations in the micro-network level were witnessed in Thy-1 positive excitatory pyramidal neurons (EXNs) and somatostatin positive inhibitory neurons (SOM-INNs) in A1. Both SST INNs and Thy1 EXNs show enhancement "far" from BF. However, a larger population of SOM INNs showed suppression with harmonics "near" BF compared to Thy1 EXNs. A higher degree of functional connectivity was observed through noise correlations suggests that harmonic based enhancement or suppression could depend on the property of the network.

Conclusions: We hypothesize that off-BF connections between SOM INNs and EXNs may underlie these observed differential enhancements and suppression. We find differential coding of harmonics with enhancement and suppression, both being observed, depending on the location of component tones relative to BF. SOM INNs may be instrumental in forming this differential representation of harmonics of TTHC in EXNs.

Verification - Validation of Amplification Using the Acoustic Change Complex

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Background: Verification of hearing aid fittings in infants with hearing loss utilize "real ear" measures of acoustic gain, and validation is based upon parent observations of the infant's awareness of sound and emerging milestones of oral language. Our research focuses on providing bio-neurologic indicators of hearing aid benefit, including validation of speech feature discrimination. As a step towards that goal, we studied adults with sensorineural hearing loss. The aims were to use the ACC to evaluate the effects of stimulus level and amplification on vowel contrast discrimination and to analyze the relationship between the ACC, the aided vs. unaided stimulus acoustics, and an individual's hearing loss.

Methods: Ten adults with bilaterally symmetric sensorineural hearing loss (mean PTA = 47 dB HL) participated. The participants were fit with Phonak Naida IX BTE hearing with gain settings determined for the individual's hearing loss using the NAL-NL2 fitting algorithm and verified using real-ear measures. Noise reduction, frequency compression, directionality, and the volume control were disabled. Vowel contrasts /a/-/i/ and /o/-/u/ were presented in the sound field at 0 degrees azimuth at 30 dBA and 60dBA. ACC recordings were made using the IHS Smart-EP system with vowel tokens presented in an oddball paradigm; the ACC was the averaged response to the infrequent token.

Results: In the unaided condition, 9/10 subjects had ACCs present at 60dBA, and all had ACCs in the aided condition.   None had ACCs at 30 dBA in the unaided condition, and only 3 participants had responses in the aided condition. ACC amplitudes in the aided conditions at 60 dBA were larger than those in the unaided condition. The aided /a/-/i/ contrast ACC amplitudes were larger than those for the /u/-/o/ contrast. There were no statistically significant differences in ACC component latencies owing to amplification. In the aided condition, ACC latencies were shorter for the /a/-/i/ contrast compared to the /u/-/o/ contrast.

The aided real-ear measures for the /a/-/i/ stimuli were simulated using a KEMAR system, and the hearing aid gain settings used for each listener. The change in /a/-/i/ ACC amplitudes due to amplification correlated significantly with improved audibility for the vowel contrast in the low-frequency spectral region (0-450 Hz) but not in higher spectral regions.

Conclusions: The data suggest efficacy for using the ACC to validate hearing aid fittings in listeners who cannot provide reliable perceptual responses, such as in immaturity (infants) or other neurocognitive conditions (e.g., dementia). The ACC provides evidence of the sensory and neural capacity to detect and discriminate vowel sound contrasts at the level of the cortex. This is neuro-biological validation that could be coupled with electroacoustic verification.

Serum Prestin in Aging and Noise Exposed Humans

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Background: Prestin, an inner ear motor protein, has been proposed to be a biomarker with potential to inform on hearing sensitivity and the health of the human cochlea (Parham, 2015). Prestin generates electromotility—the physical change in length of the outer hair cells (OHCs) as a function of membrane polarization and is critical for

sensitive hearing (Zheng et al. 2000). Serological measurement is a viable approach to studying prestin in humans. Our group has provided proof-of-concept studies in animals (Parham and Dyhrfjeld-Johnsen, 2016; Naples et al., 2018; Parham et al., 2019) and humans (Parker, Parham and Skoe, 2021). This work has shown serum prestin levels to have high test-retest reliability and to be related to otoacoustic emissions (OAEs). The primary goal of our current work is to evaluate the relation between prestin and two well-known culprits of OHC loss/dysfunction: noise exposure and aging.

Methods: We collected blood samples from two different groups: (1) 33 young adults, recruited for varying noise exposure histories (including university musicians) but with hearing thresholds in the clinically normal-normal range, and (2) 64 healthy adults, ranging from 18-82 years old. For both groups, venipuncture was performed and prestin levels were measured in the serum using the MBS167508 enzyme-linked immunosorbent assay (ELISA) kit. Additionally, all participants underwent audiological evaluation that included audiometry, OAEs, and speech perception in noise. For Group 1, the use of personal noise dosimetry allowed for three weeks of objective measurement of noise exposure.

Results: Group 1: Our analyses suggest a relationship between prestin levels and noise in Group 1, showing a negative correlation where individuals with higher routine noise exposure levels tended to have lower serum prestin levels. Moreover, when subgrouping participants based on their risk for a clinically significant noise-induced hearing loss (based on NIOSH standards), we found that prestin levels differed significantly between these subgroups, despite similarity in their behavioral thresholds and OAEs. Group 2: Preliminary analyses have confirmed both a relation between age and other audiometric measures, and a negative relation between age and serum prestin, as hypothesized.

Conclusions: Our work shows that those who lead louder lives were found to have lower levels of the inner-ear protein prestin circulating in their blood, even when other measures of inner-ear function could not differentiate participants with the most and least noise exposure. This reveals the potential for serum biomarkers of the cochlea to separate those at-risk for hearing loss/dysfunction from those with lower risk. These results support further study of serum prestin levels, with the potential to improve early detection of hearing loss.

The Effects of Mild Cochlear Neuropathy on Sound Encoding in the Early Auditory Pathway
A noveBrian Lam*[1], Nicholas Lesica[1]
[1]University College London

Background: The selective loss of high-threshold auditory nerve fibers (HT-ANFs) is thought to be the underlying cause of hidden hearing loss, a condition associated with poor speech recognition in the absence of an audiometric threshold shift. The effects of this neuropathy in the cochlea have been well described, but its functional consequences downstream in the central auditory pathway remain unclear. HT-ANFs are predominantly found in high-frequency preferring regions of the cochlea and, thus, the impact of their loss on the encoding of speech, which is dominated by low frequencies, is difficult to predict. If the encoding of speech in the central auditory pathway is impacted by HT-ANF loss, it is likely to be through subtle distortions of temporal activity patterns rather than changes in the overall activity related to audibility. We aimed to assess the encoding of speech and other complex sounds in populations of neurons in the early auditory pathway following mild cochlear neuropathy in Mongolian gerbils, Meriones unguiculatus.

Methods: Mild cochlear neuropathy was induced through either noise overexposure or round window infusion of ouabain in young adult gerbils. The anatomical and physiological effects of the noise or drug exposure were assessed through a combination of immunohistochemistry, measurement of otoacoustic emissions, recordings of neural activity from the round window, and auditory brainstem responses. Several weeks after noise or drug exposure, in vivo neural recordings using multi-channel electrode arrays were made in the cochlear nucleus or inferior colliculus under anesthesia in response to pure tones, modulated sounds, and speech with and without background noise.

Results: Responses to sinusoidal amplitude modulated (SAM) noise and moving ripples (MRs) at different modulation depths and intensities were analyzed to assess the fidelity of envelope encoding under different conditions. Responses to consonant-vowel syllables at different intensities with and without background noise were analyzed to identify the features of neural activity that encode different elements of speech and the degree to which these features enabled a machine learning classifier to identify consonants and vowels from neural activity patterns.

Conclusions: This project provides an explicit assessment of the impact of mild cochlear neuropathy on coding in the central auditory pathway and a comprehensive description of the changes in the neural representation of speech and other modulated sounds caused by neuropathy across a range of conditions.

POSTER BLITZ

Friday, February 4, 2022
Poster Blitz Competition
11:30 a.m. - 1:00 p.m.

Organizers: Karolina Charaziak, Ph.D. & Hari Bharadwaj, Ph.D.

Long-Term Auditory Training Prevents Age-Related Changes to Population Activity in the Primary Auditory Cortex
(Poster Blitz: Student)
Jonah Mittelstadt, *Johns Hopkins University*

Category Primary Auditory Cortex
Background: More than one in three people over the age of sixty-five suffer from age-related hearing loss, presbycusis. Presbycusis can be caused by changes in the peripheral or central auditory system. Changes in the peripheral auditory system have been well studied, but the changes in the central auditory system have been less studied. Changes in the central auditory system are thought to include changes in the primary auditory cortex (A1).
Indeed, our studies of mouse A1 have shown that aging increases the correlation of neuronal activity while decreasing the stimulus sensitivity of neuronal responses. These attributes of the aging A1 combine to decrease the encoding stimuli information compared to young A1 contributing to central hearing loss (Shilling-Scrivo, Mittelstadt, and Kanold in press).
In humans, auditory training of various durations has been shown to improve auditory performance, thereby indicating that sensory plasticity of the central auditory system might be effective in preventing some of the changes with aging. For example, life-long instrumentalists and vocalists maintain better hearing compared to non-musicians in their age group. Despite this, it is not known how long-term auditory training affects the central auditory system of older individuals. Given that one major effect of aging in A1 is the increase in correlated activity in A1, we speculated that long-term auditory training might be effective in preventing this increase in activity correlations.
Methods: To examine this hypothesis, we continuously trained mice on an auditory task for a period of at least six months until they reached old age (>17 months) utilizing automated home-cage training systems. We then compared the sound-evoked responses of large populations of single neurons (>7800 neurons) in A1 of old trained (N=16) and old naive (N=7) mice using in vivo 2-photon Ca2+ imaging.
Results: We found that training prevented many of the age-related functional changes in A1 associated with aging. Specifically, we found that long-term auditory training prevented the rise in correlation of neuronal population activity, some of which we speculate may be responsible for lower auditory performance in older individuals.
Conclusions: Our results suggest that long-term auditory training has the potential to prevent changes to neuronal population dynamics that occur during aging. While numerous therapeutic options exist to treat peripheral hearing loss, no such options exist for central hearing loss. Long-term auditory training is an accessible, simple, and quick tool that can be rapidly deployed to the aging population that may postpone or minimize functional changes to the central auditory system with aging.
This work was supported by NIH P01 AG055365 and NIH RO1DC009607 to POK.

Early Pathological Changes in Cochlear Microvasculature Precede Hair Cell and Hearing Loss in a Mouse Model of Norrie Disease
(Poster Blitz: Student)
Valda Pauzuolyte, *University College London*

Category Genetics B: General
Background: Norrie disease is a rare recessive X-linked disorder, manifesting as congenital blindness and progressive hearing loss. It is caused by mutations in the NDP gene, which encodes Norrin, a secreted Wnt-analog that induces canonical Wnt/β-catenin signaling through a Fz4/Lrp5/6/Tspan12 complex. Currently, there are no treatments for Norrie disease and the role of Norrin in the cochlea is not well understood. This study sought to identify the early sequence of pathological events leading to the onset of hearing loss in mice with a loss-of-function mutation in Ndp.

Methods: Cochlear tissue and auditory function of Ndp knockout mice (auditory function of male and female Ndp knockout mice (allele Ndptm1Wbrg; Ndp-KO) were analysed from postnatal day 10 to 2 months. Vasculature morphology and perivascular cell distribution in the cochlear lateral wall were analysed by TEM and by confocal microscopy. Integrity of the blood-cochlea barrier formation was assessed by qRT-PCR, immunohistochemistry, and vascular tracer assays. At 1 and 2 months inner and outer hair cells survival was mapped in full-length organ of Corti whole mounts, and Endocochlear Potential (EP), Distortion Product OtoAccoustic Emmission (DPOAE) and Auditory Brainstem Responses (ABR) were recorded.

Results: The cochlear microvasculature in the spiral ligament showed an abnormal morphology as early as postnatal day 10 in the Ndp-KO, prior to the onset of hearing. Vascular barrier function was defective by P20 showing leakage of the fluorescent tracer and dysregulation of Cldn5, Plvap, Cav1 gene expression in the lateral wall. Subsequent marginal cell pathology, significant reduction of EP and onset of outer hair cell degeneration in the mid-frequency range was found from 1 month onwards. DPOAE and ABR readouts of Ndp-KO differed significantly from control littermates at 2 months.

Conclusions: Cochlear microvascular pathology and reduction of EP in the Norrie disease mouse model has an early postnatal onset and precedes the degeneration of outer hair cells and detectable hearing loss indicating it is a primary site of Norrie disease pathology. Identifying this sequence of pathological events in the cochlea helps provide outcome measures for the evaluation of targeted therapeutic interventions in the in vivo model.

Neural Correlates of Perceptual Invariance in the Ferret Auditory Cortex
(Poster Blitz: Student)
Carla Griffiths, *UCL*

Category Auditory Cortex and Thalamus: Structure and Function

Background: Perceptual invariance, the act of recognising auditory objects across identity-preserving variation and in the presence of other auditory stimuli, is critical to everyday listening. In this study, we tested whether perceptual invariance for an auditory object can be established in the ferret auditory cortex (AC) and whether the perception of an auditory object is encoded within the AC.

Methods: To test perceptual invariance, we trained four ferrets in a Go/No-Go water reward task where ferrets identified a target word ("instruments") from a stream drawn from 54 other British English words (distractors). We then manipulated the mean fundamental frequency (F0) within and across trials.

We recorded neural activity from the auditory cortex using an Omnetics WARP32 chronic implant in one ferret (F1702) and considered sites with a sound-onset response for Euclidean distance decoding. We computed a decoding score for pairwise discrimination of the target word from seven high-occurrence distractors, the target word reversed, and pink noise equal in duration and spectrally matched to the target word. We also computed a decoding score for pairwise discrimination of the target word from a corresponding behavioural response of a correct Go response or an incorrect No-Go response for within-trial F0 manipulated trials and control F0 trials.

Results: The ferrets identified the target word (chance=33% hit rate) when the F0 was roved within a trial with hit rates (Female/Male speaker) of 60%/40% for F1702, 68%/38% for F2002, 43%/38% for F1803, and 48%/52% for F1815. For whole trial modified F0, the hit rate was 55%/40% (F1702), 61%/48% (F2002), 48%/47% (F1803), and 56%/39% (F1815).

An analysis of auditory cortical responses based only on correct trials, from animal F1702, revealed neural responses that discriminated target from distractor responses across variation in F0. Decoder discrimination performance was significantly higher when classifying between F0-manipulated distractor and target stimuli for both F0 manipulations across and within trials. In most cases, these responses did not carry F0 information either in target or distractor responses.

To elucidate neural correlates of perception we considered both correct Go responses (hits) and incorrect No-Go responses (misses) to the target. We then asked whether we could discriminate the neural response to the target on these trials. Several sites showed weak, but significant information about whether the trial was a hit or a miss.

Conclusions: Our preliminary results suggest that auditory objects are represented in the AC and that these responses are resistant to F0 change. Moreover, our findings suggest the AC neural representations of auditory objects align with the behavioural perception of auditory stimuli rather than the ground-truth classification of the stimuli itself. Future work will incorporate hippocampal recordings to determine whether temporal coherence between the hippocampus and AC is required for auditory object recognition.

Cochlear Implant Positioning and Fixation Using 3D-Printed Patient Specific Surgical Guides; A Cadaveric Study

(Poster Blitz: Student)

Laura Markodimitraki, *Department of Otorhinolaryngology and Head and Neck Surgery, University Medical Center Utrecht, Utrecht, the Netherlands*

Category Clinical Otolaryngology and Pathology

Background: Positioning and fixation of the cochlear implant (CI) are commonly performed free hand. Applications of 3-dimensional (3D) technology now allow us to make patient specific, bone supported surgical guides, to aid CI surgeons with precise placement and drilling out the bony well which accommodates the receiver/stimulator device of the CI. We aimed to develop and validate the optimal design and evaluate accuracy of individualized 3D- printed surgical guides for cochlear implantation.

Methods: Cone beam CT (CBCT) scans were acquired from temporal bones in 9 cadaveric heads (18 ears), followed by virtual planning of the CI position. Surgical, bone-supported drilling guides were designed to conduct a minimally invasive procedure and were 3D-printed. Fixation screws were used to keep the guide in place in predetermined bone areas. Specimens were implanted with 3 different CI models. After implantation, CBCT scans of the implanted specimens were performed. Accuracy of CI placement was assessed by comparing the 3D models of the planned and implanted CI's by calculating the translational and rotational deviations.

Results: Median translational deviations of placement in the X- and Y-axis were within the predetermined clinically relevant deviation range (< 3 mm per axis); median translational deviation in the Z-axis was 3.41 mm. Median rotational deviations of placement for X-, Y- and Z-rotation were 5.50°, 4.58° and 3.71°, respectively.

Conclusions: This study resulted in the first 3D-printed, patient- and CI- model specific surgical guide for positioning during cochlear implantation. The next step for the development and evaluation of this surgical guide will be to evaluate the method in clinical practice.

Application of Genome Editing to a Mouse Model of DFNA20

(Poster Blitz: Student)

Samantha C. Lau, *Inner Ear Gene Therapy Program, National Institute On Deafness and Other Communication Disorders (NIDCD), National Institutes of Health*

Category Inner Ear: Drug Delivery

Background: Sensorineural hearing loss is a common disorder which affects the world's population. Recent studies of inner ear gene therapy have demonstrated promising results in improving auditory and vestibular functions in mouse models of sensorineural hearing loss. While many studies of gene therapy focus on mouse models of 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 due to the later onset and slower progression of hearing loss that offers a wider window of opportunity for therapeutic interventions. In this pilot study, we applied genome editing to a mouse model of DFNA20, which carries a pathogenic amino acid substitution of the wild type proline at residue 264 with a leucine (Actg1P264L).

Methods: Neonatal (P0-P5) Actg1P264L/P264 mutant mice were used in this study. AAV2.7m8-Cas9 and AAV2.7m8-gRNA-GFP were injected simultaneously into the inner ears of Actg1P264L/P264 mutant mice through the posterior semicircular canal. Auditory brainstem response (ABR) recordings were used to assess auditory function at P30. Scanning electron microscopy (SEM) was performed to examine stereocilia morphology. Immunohistochemistry was used to evaluate cell morphology and viral transduction efficiency.

Results: Actg1P264L/P264 mutant mice develop early onset hearing loss due to outer hair cell stereocilia bundle staircase formation defects and are profoundly deaf by 6-7 weeks. SEM imaging revealed improved outer hair cell stereocilia morphology in Actg1P264L/P264 mutant mice treated with AAV2.7m8-Cas9 and AAV2.7m8-gRNA-GFP (with the gRNA designed to target the mutant P264L allele), compared to non-treated mutants. The treated Actg1P264L/P264 mutant mice also showed improved hearing at P30 compared to non-treated mutant mice.

Conclusions: Our results showed that CRISPR genome editing was able to improve the stereocilia morphology and auditory function in the Actg1P264L/P264 mutant mice.

Dexamethasone Eluting Arrays Suppress MHCII Mediated Antigen Presentation in Cochlea Following Cochlear Implantation

(Poster Blitz: Student)

Peter Eckard, *University of Iowa Carver College of Medicine*

Category Auditory Prostheses

Background: Cochlear implants (CIs) provide auditory rehabilitation to indicated patients with sensorineural hearing loss and can considerably improve their quality of life. Surgical implantation of a CI invariably induces an inflammatory and foreign body response (FBR) that can contribute to loss of residual acoustic hearing in hearing preservation post-CI and may impact CI performance and hearing outcomes. Studies from temporal bone records of implanted humans and animal models have shown that both macrophages and lymphocytes are involved in the FBR following implantation. With single-cell RNA-sequencing in a murine model, we have found evidence for involvement of lymphocytes and MHCII mediated antigen presentation in the post-CI inflammation in the cochleae. MHCII mediated antigen presentation by macrophages to lymphocytes has been shown to play a key role in implantation in other organs. The role of MHCII mediated antigen presentation in post-CI inflammation is unknown. Moreover, we and other authors have shown in animal studies that corticosteroid-eluting CIs can mitigate intracochlear macrophage infiltration and FBR. Using a murine model, this study aims to explore whether cochlear implantation activates MHCII mediated antigen presentation within the cochlea and how dexamethasone-eluting CI electrode arrays affect that presentation.

Methods: 12-week-old CX3CR1-GFP Thy1-YFP C57BL6 mice were implanted with either regular or dexamethasone-eluting CIs in the left ear with the contralateral ears acting as controls. The implants were stimulated from postoperative day 7 for up to 28 days and sacrificed at 10, 28, or 56 days postoperative. The cochleae were fixed with 4% PFA and cryosectioned at 30 micrometer thickness. Macrophages and neurons were intrinsically labeled, and mid-modiolar sections were labeled with DAPI for nuclei and immunostained with antibodies against MHC class II. Images were taken using confocal microscopy, and quantitative image analyses were performed. The cochlear regions of the scala tympani, Rosenthal's canal, lateral wall, and modiolus were manually traced, and the quantification of three parameters of MHCII+ macrophages were automated using IMARIS: density of CX3CR1+ MHCII+ macrophages, ratio of total and MHCII+ macrophages, and intensity of MHCII expression on macrophages.

Results: Cochleae implanted with regular, non-dexamethasone-eluting CI electrodes developed a robust immune response with significantly increased density of CX3CR1+ MHCII+ macrophages in the scala tympani of the basal cochlear turn and lateral wall across the whole cochlea up to 56-days postoperative. Specimens implanted with dexamethasone-eluting cochlear implant electrodes displayed significantly reduced the density of CX3CR1+ MHCII+ macrophages at all time points in scala tympani, lateral wall, modiolus, and spiral ganglion. The ratio of MHCII+ and total macrophages and the expression of MHCII on macrophages are reduced by dexamethasone-eluting implants.

Conclusions: This study suggests that activation of MHCII mediated antigen presentation is involved in the inflammatory and FBR following implantation and can be mitigated by immunosuppressive dexamethasone-eluting cochlear implants.

Dual Effect: BRAF Inhibitor Dabrafenib Protects Against Cisplatin-Induced Kidney Injury and Hearing Loss

(Poster Blitz: Student)

Darby Keirns, *Creighton University School of Medicine*

Category Other, Dual Protection for Hearing and Kidney

Background: Cisplatin is a chemotherapy agent used for cancers of the head and neck, reproductive system, lungs, and others. Its beneficial use, however, is limited by side effects including acute kidney injury (AKI) and hearing loss. There are currently no FDA-approved drugs to treat these significant side effects, which can also be dose-limiting. Recently, the oral drug dabrafenib was identified as protective against cisplatin-induced hearing loss in mice. Dabrafenib is a specific BRAF inhibitor shown to reduce activation of the MAPK pathway in inner ear cells after cisplatin treatment. BRAF is also expressed in kidney cells, and the MAPK pathway is upregulated in vivo after cisplatin treatment. Thus, a similar mechanism may contribute to damage in both tissues, making dabrafenib a potential therapeutic candidate for cisplatin-induced kidney injury.

Methods: Initial experiments in a human kidney proximal tubular cell line (HK2) were done to determine IC50 values and confirm the upregulation of the MAPK pathway in kidney cells. Then animal models were used to further assess the nephroprotective effect. Dabrafenib (12 mg/kg body weight) was administered by oral gavage for three consecutive days, twice daily, to FVB mice receiving a single dose of cisplatin (30 mg/kg body weight). Following this model, nephroprotection was evaluated through BUN levels, histology, apoptosis, and mouse survival.

Results: The initial cell viability assay of HK2 cells showed a dose-dependent increase in cisplatin-induced cell death and an IC50 of 5 μM. Treatment with dabrafenib alone showed no toxicity and improved cell viability when

given with cisplatin at an IC50 of 0.77 μM. Western blot analysis showed upregulation of the MAPK pathway in HK2 cells after cisplatin treatment and downregulation of the pathway when co-treated with dabrafenib and cisplatin.

In the mouse models, BUN, a kidney injury marker, was evaluated on day 3, which show increased levels in cisplatin-treated mice that are then reduced with administration of dabrafenib. On day 21, histological analysis by H and E and PAS staining show kidney injury in cisplatin-treated mice which is significantly reduced with dabrafenib treatment. Immunofluorescent staining demonstrates decreased expression of P-ERK, a downstream target in the MAPK pathway, in mice treated with both cisplatin and dabrafenib. Additionally, reduced levels of apoptosis were seen in the dabrafenib and cisplatin-treated mice via TUNEL assay. Importantly, there is a significant increase in survival of mice in the group treated with cisplatin and dabrafenib compared to cisplatin alone.

Conclusions: Overall, dabrafenib demonstrates reduction of cisplatin-induced kidney damage and improved survival in mice, in addition to protection against cisplatin-induced hearing loss. This provides promising results that dabrafenib could reduce both the hearing loss and AKI side effects commonly seen in those requiring cisplatin chemotherapy for treatment of various cancers, including those of the head and neck.

Can't Touch This: The Ventral Nucleus of the Trapezoid Body is Spared in an Animal Model of Autism Spectrum Disorder
(Poster Blitz: Student)
Yusra Mansour, *Henry Ford Health System and Lake Erie College of Osteopathic Medicine*

Category Brainstem: Structure and Function
Background: Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by repetitive behaviors, poor social skills, and difficulties with communication and hearing. The hearing deficits in ASD range from deafness to extreme sensitivity to routine environmental sounds. Previous research from our lab has shown drastic hypoplasia in the superior olivary complex (SOC) in both human cases of ASD and in an animal model of autism. However, in our study of the human SOC, we failed to find any changes in the total number of neurons in the ventral nucleus of the trapezoid body (VNTB) or any changes in cell body size or shape. Similarly, in animals prenatally exposed to the antiepileptic valproic acid (VPA), we failed to find any changes in the total number, size or shape of VNTB neurons. Based on these findings, we hypothesized that the neurotransmitter profiles, ascending and descending axonal projections of the VNTB are also preserved in these neurodevelopmental conditions.
Methods: VPA exposure in dams was completed via oral doses of the drug in peanut butter on embryonic days 10 and 12. Pups were weaned on postnatal day 28 and only male pups were included in this study. To investigate neurotransmitter profiles of the VNTB, we utilized immunohistochemistry for acetylcholine using choline acetyltransferase (ChAT) and for GABA using glutamate decarboxylase (GAD). Projection patterns were investigated via stereotaxic injections of retrograde tracers Fast Blue or Flourogold in the cochlea, central nucleus of the inferior colliculus (CNIC) and medial geniculate (MG).
Results: We found no difference between control and VPA-exposed animals in the number of VNTB neurons immunoreactive for ChAT or GAD. Our results indicate no significant differences in the ascending and descending projections from the VNTB between control and VPA-exposed animals despite drastic changes in these projections from surrounding nuclei.
Conclusions: These findings provide evidence that certain neuronal populations and circuits may be protected against the effects of neurodevelopmental disorders.

Anatomic, Physiologic, and Proteomic Consequences of Repeated Microneedle-Mediated Perforations of the Round Window Membrane
(Poster Blitz: Student)
Stephen Leong, *Columbia University Vagelos College of Physicians and Surgeons*

Category Inner Ear: Drug Delivery
Background: We have developed 3D-printed microneedle technology for diagnostic aspiration of perilymph and intracochlear delivery of therapeutic agents. Single microneedle-mediated round window membrane (RWM) perforation does not cause hearing loss, heals within 48-72 hours, and yields sufficient perilymph for proteomic analysis. In this study, we investigate the anatomic, physiologic, and proteomic consequences of microneedle-mediated perforations of the same RWM at multiple timepoints. By developing this technique, we establish a means of monitoring the response to inner ear interventions over an extended period of time.

Methods: 100-μm-diameter hollow microneedles were synthesized using two-photon polymerization (2PP) lithography. The tympanic bullae of Hartley guinea pigs (n=7) were opened with adequate exposure of the RWM. Distortion product otoacoustic emissions (DPOAE) and compound action potential (CAP) were recorded to assess baseline hearing. The hollow microneedle was introduced into the bulla and the RWM was perforated; 1 μL of perilymph was aspirated from the cochlea over the course of 45 seconds. Following perforation, DPOAE was obtained and the bulla was closed. 72 hours later, the above procedure was repeated with aspiration of an additional 1 μL of perilymph. 72 hours after the second perforation, final CAP and DPOAE were obtained and the animal was euthanized. RWMs were harvested immediately following euthanasia for confocal imaging. Perilymph proteomic analysis was completed using mass spectrometry-liquid chromatography. Two-tailed paired t-tests and repeated measures ANOVA tests were used to evaluate for significance; the Hochberg procedure was used to adjust for multiple comparisons.

Results: CAP, DPOAE, and confocal microscopy were completed for 6/7 animals; proteomic analysis was completed for all 7 animals. Hearing tests demonstrated mild hearing loss at 2-4 kHz most consistent with conductive hearing loss. Hearing at 0.5 kHz – 40 kHz was otherwise intact across all three timepoints. Confocal microscopy demonstrated complete healing of all perforations with full reconstitution of the RWM. Perilymph proteomic analysis identified 1855 proteins across 14 samples (2 aspirations each for 7 animals). The inner ear protein cochlin was observed in all samples, indicating successful aspiration of perilymph. Non-adjusted paired t-tests with $p < 0.01$ revealed significant changes in 13 of 1855 identified proteins (0.7%) between the first and second aspirations. The majority of these proteins were ubiquitous proteins with largely unrelated functions, and none had a role in acute inflammation. Importantly, after adjustment for multiple comparisons using the Hochberg procedure, no proteins had significant changes between the two aspirations.

Conclusions: We demonstrate that repeated microneedle perforation of the RWM is feasible, does not directly cause hearing loss, allows for complete healing of the RWM, and does not change the proteomic expression profile. Thus, microneedle-mediated repeated aspirations in a single animal can be used to monitor the response to inner ear treatments over time.

Predicting Pathology: Towards Using Sift-Ms to Facilitate Early Intervention for Age Related Hearing Loss
(Poster Blitz: Student)
Amy Worrall, *Keele University*

Category Aging
Background: Age related hearing loss (ARHL) affects the majority of those over 65 years old, with existing treatments unable to offer continued improvement as the illness progresses. However, research suggests where degradation of cochlear fibrocytes leads to downstream damage, biological interventions may be possible. Thus, an early detection strategy for fibrocyte damage is required to enable timely intervention. This research presents progress towards such a strategy, laying groundwork for a method wherein selected ion flow tube mass spectrometry (SIFT-MS) of ear wax (a biofluid known to indicate patient health) may be used to ascertain health of cochlear fibrocytes non-invasively.
Methods: This research examined levels (parts per billion) of volatile organic compounds (VOCs) in the headspace of murine cochlear fibrocyte cultures (healthy and inflammatory states) and human ear wax samples in real time using SIFT-MS. Murine cochlear fibrocytes were isolated from tissue explants, with cell type characterised via immunocytochemistry (NaKATPase, S100B, Aqp1). Inflammation was induced via the addition of IL-1β to culture media. Inflammatory state was confirmed by examination of IL-6 and IL-8 production. Fibrocytes and ear wax swabs were analysed via SIFT-MS (Transpectra Profile 3) to obtain measures of VOCs in the headspace of samples, with observations across H3O+ and NO+ precursors for compounds of m/z 1-180. Statistical analysis of multi ion mode samples was performed via Mann Whitney U test.
Results: Cochlear fibrocytes were successfully explanted to culture with type indicated as II, IV or V. SIFT-MS shows cellular samples indicate unique compounds (suggested as: pyrrole, hexyl acetate and menthone) even at low, biologically relevant, numbers (15,000 cells per sample), with significant differences between conditions identified in means of compounds: acetaldehyde (U(Nmedia=N15000=3)=2, z=-2.193, p<0.05), butyric acid (U(Nmedia=N15000=3)=2, z=-2.193, p<0.05), benzaldehyde (U(Nmedia=N15000=3)=0, z=-2.611, p<0.05) and pyruvic acid (U(Nmedia=N15000=3)=0, z=-2.611, p<0.05) for H3O+ precursor multi ion monitor data. SIFT-MS of inflamed cells demonstrates detectable differences between undosed and dosed cultures, with notable differences identified in the levels of significant compounds (noted above). SIFT-MS of ear wax similarly demonstrates unique compounds, with known wax markers identified. Results suggest distinct, detectable metabolic profiles for cochlear fibrocyte and ear wax samples.

Conclusions: The suggested novel method for SIFT-MS based measurement of cochlear fibrocyte cultures and ear wax samples appears successful thus far, with research demonstrating progress towards the establishment of cochlear fibrocyte health profiles and the use of ear wax as a biofluid for their detection. The next stage of research, therefore, will focus on the detection of poor auditory health via ear wax. Overall, though this research represents only the initial development of the technique, it may be reasonably stated that, if successful, the capacity to non-invasively detect early auditory issues in real time via ear wax may well be a breakthrough for the practice of Otolaryngology.

Interferon Gamma Expression in the Mouse Cochlea After Congenital CMV Infection
(Poster Blitz: Student)
William Marshall, *Washington University School of Medicine*

Category Inner Ear: Damage and Protection
Background: Congenital CMV (cCMV) infection is a leading cause of hearing loss in infants and children worldwide. cCMV occurs in 1/200 births in the USA, and 10% of infected newborns develop hearing loss. Despite years of study, the mechanism by which cCMV causes hearing loss is unknown. Our murine model of congenital CMV infection using peripheral viral inoculation in newborn mice produces hearing loss in approximately 60% of infected mice. This provides an excellent model for studying the effects of cCMV on the cochlea. Our previous work with this model has shown active virus in cochlear macrophages and endothelial cells between 7-14 days post infection.
Methods: In order to learn more about the immune response in the inner ear to cCMV, we examined the production of interferon gamma (IFNg) in the cochlea using IFNg-YFP reporter mice exposed to mCMV at birth. Previous studies of CMV infection in the brain have shown that IFNg plays a critical role in the immune response to infection and potentially to neurodegeneration. We believe that IFNg may play an important role in CMV induced hearing loss. Initially, we sought to determine the timing and location of IFNg expression in the inner ear after CMV infection. IFNg-YFP reporter mice were inoculated with 200 pfu of mCMV at birth. Immunofluorescence microscopy was performed to identify cells that expressed IFNg post CMV. Mice were examined at 7 and 14 days post infection and their cochleae were studied by sections and whole mount preparations. Additionally, bulk-RNA sequencing was used to evaluate differential gene expression in control and mCMV infected mice at p7 and p14.
Results: CMV infected mice at both P7 and P14 showed an increase in IFNg expression when compared to control mice. This finding coincided with the bulk-RNA sequencing data that showed a significant upregulation in IFNg expression in the mCMV infected mouse cochlea. IFNg expression frequently colocalized with CX3CR1+ cochlear macrophages. Additionally, IFNg expression was found in cells among developing cochlear hair cells and auditory nerve fibers. The identity of these cells is currently under investigation.
Conclusions: Previous work with cCMV has shown loss of spiral ganglion neurons 4-6 weeks post infection suggesting a possible interaction between neuronal loss and IFNg expression.

Auditory Cortical Plasticity Associated With Socially Reinforced Complex Sounds
(Poster Blitz: Student)
Dakshitha B Anandakumar, *Georgia Institute of Technology*

Category Primary Auditory Cortex
Background: We often encounter contexts where complex sounds reliably predict rewarding social interactions, like the distinctive footsteps of a friend approaching or the unique ringtone from a partner's phone call. Social rewards may serve as a potent driver for the sensory learning of such arbitrary, communicative sounds, but how they may be encoded in the auditory system and altered by social experience is unknown. By contrast, much research using natural vocalizations, such as in the maternal mouse model of ultrasonic vocalization (USVs) communication, has helped elucidate the auditory cortical processing of socially salient, evolved signals. Yet whether findings from that framework generalize to when novel sounds become associated with rewarding social interactions, or only applies for ethological communication, is unclear. Prior studies with USVs have suggested that motherhood increases both pup call-evoked suppression in frequency bands lateral to the USVs (Galindo-Leon etal, 2009), and the prevalence of call-excited Off-only responses (Chong etal, 2020). Do these changes also occur when an arbitrary sound is socially reinforced?
Methods: We trained mother mice on a T-maze (Dunlap etal, 2020) to use a sinusoidally and linearly frequency modulated target tone with characteristics outside the normal USV range, as a cue to localize and retrieve a pup – an innate behavior that is highly rewarding and stereotyped. We then compared neurons recorded from the

auditory cortex of awake, passively listening mothers that were either trained or exposed to the sound in the T-maze.

Results: Akin to the plasticity observed for USVs in mothers versus virgins, the target-evoked average response in frequency bands lateral the target sound was significantly suppressed in trained compared to sound-exposed animals (n=138, trained; n=101, sound-exposed; $p<0.05$ Wilcoxon ranksum). By contrast, lateral band neurons in both trained and exposed mice responded to spectrally similar but behaviorally irrelevant control sounds with overall excitation, indicating that the lateral band suppression was specific to the socially salient sound. Moreover, the prevalence, but not firing rate, of late Off-only responses to the target increased after training (n=59/309, trained; n=28/277, exposed; $p<0.05$ Fisher's Exact), as typically seen for pup USVs that gain relevance for mothers. This was most apparent for layer 5/6 neurons (n=53/104, trained; n=25/89, exposed; $p<0.05$ Fisher's Exact). Finally, for both deep and thalamorecipient cortical layers, response latencies became less concentrated at sound onset and more widely distributed throughout the target after training ($p<0.05$, kruskal-wallis), similar to a reduced Onset firing rate for pup USVs seen in mothers.

Conclusions: Together, our results suggest that lateral band suppression, Off excitation, and reduced Onset firing, are general coding features for socially salient sounds, whether they are natural or not, prompting future questions into their functional role in coding perceptual qualities and social behavioral actions. Supported by R01DC008343.

Deep Neural Network Algorithms for Noise Reduction in Cochlear Implants
(Poster Blitz: Student)
Agudemu Borjigin, *Purdue University*

Category Auditory Prostheses
Background: Despite excellent performance in quiet, cochlear implants (CIs) only partially restore normal levels of intelligibility in noisy environments. Current state-of-the-art signal processing strategies in CIs provide limited benefits in terms of noise reduction or masking release. Recent developments in the field of machine learning have resulted in deep neural network (DNN) models achieving high levels of performance in both speech enhancement and separation tasks. However, there are no commercially available CI audio processors that make use of DNN models.
Methods: We implemented two DNN models for CIs: a recurrent neural network (RNN)—a lightweight template model, and the SepFormer—the current state-of-the-art, top-performing speech-separation model in the literature. The models were trained with a custom, 30-hour dataset that included 4 conditions: speech in non-speech noise (environmental sounds), speech in 1-talker, 2-talker, and 4-talker babble backgrounds. An equal number of unique speech-noise mixtures were generated for each condition at signal-to-noise ratios (SNRs) ranging from 1 to 10 dB in 1 dB steps. The enhancement of the target speech (or the suppression of the noise) by the models was evaluated using commonly used acoustic evaluation metrics of quality and intelligibility such as (1) signal-to-distortion ratio (SDR), (2) "perceptual" evaluation of speech quality (PESQ), and (3) short-time objective intelligibility (STOI). We also tested the models behaviorally with CI users listening to the mixtures via their clinical CI processor and direct audio input.
Results: Both models introduced significant improvements in all acoustic metrics we tested (SDR, PESQ, and STOI). Preliminary data on 2 CI subjects also showed improvements in speech-in-noise intelligibility scores when the noisy speech was processed by the models, as compared to the unprocessed conditions.
Conclusions: In conclusion, the evaluated DNN models were able to significantly enhance the target speech within a noisy mixture while suppressing the background noise. This work serves as a proof of concept that DNN technology has the potential to be integrated into CIs to improve the user's listening experience in noisy environments. We hypothesize that the ongoing data collection from more CI subjects will corroborate the promising preliminary results, and guide the next steps towards developing machine-learning-based signal processing algorithms for noise reduction in CIs.

Vestibular Hair Cell Survival and Stereocilia Bundle Morphology in Usher Syndrome Type 1 Patients
(Poster Blitz: Post-doc)
Richard T. Osgood, *Massachusetts Eye and Ear, Harvard Medical School*

Category Vestibular: Basic Research and Clinical
Background: Usher syndrome is a devastating disease characterized by hearing loss, balance deficit, and progressive loss of sight (retinitis pigmentosa). It is classified into three subtypes based on severity. Usher syndrome type 1, is the most severe form. Patients typically have profound hearing loss at birth; experience

progressive visual loss before age 10, worsening over several decades; and display developmental delay in balance related behaviors, with balance deficit increasing in severity over time. Given the recent progress in the development of inner ear gene therapy for Usher syndrome, it is critical to better characterize the otopathology in these patients, in order to identify opportunities for therapeutic intervention.

Methods: Here, we utilized the human temporal bone archive at Massachusetts Eye and Ear to investigate the survival of hair cells in the vestibular organs of Usher syndrome type 1 patients. Of the seven Usher cases in the collection, three (aged 64-84) were diagnosed as type 1. Hair cell survival, and stereocilia morphology, in the vestibular organs of these three cases were assessed by differential interference contrast microscopy and confocal microscopy using the endogenous fluorescence of the eosin in the archival stained sections. Data from Usher cases were compared to age matched normal specimens.

Results: In all cases, we saw many surviving vestibular hair cells carrying stereocilia bundles. Thus, vestibular hair cells in Usher syndrome type 1 patients survive for many decades. In one case, hair cells were examined by transmission electron microscopy, enabling a high-resolution assessment of stereocilia bundle morphology. The survival of these cells to later life, even in the most severe cases of Usher syndrome, is strong evidence for the presence of vestibular hair cells in early development, in this number or greater.

Conclusions: These data, obtained in 64–84-year-old patients, provide support for an extended postnatal therapeutic window for gene therapy approaches to rescue vestibular function. This presents the exciting opportunity to rescue the sense of balance in patients with Usher syndrome, providing the potential to significantly increase quality of life in these individuals.

In Vivo Real-Time Imaging Reveals That Megalin Transports Aminoglycosides Into the Mouse Cochlea and Its Inhibition is Otoprotective

(Poster Blitz: Post-doc)
Jinkyung Kim, *Stanford University*

Category Inner Ear: Drug Delivery

Background: Aminoglycosides (AGs) are highly effective broad-spectrum antibiotics used worldwide to combat gram negative bacteria. Irreversible cochlear hair cell (HC) loss is a critical side-effect of AGs treatment, yet how AGs enter the cochlea and then target HCs remains unresolved.

Methods: We developed an in vivo imaging method to track AGs transport into adult mouse cochlea in real-time. An imaging window (IW) was created on otic capsule bone via novel chemo-mechanical cochleostomy, that enables us to observe multiple cochlear cells from a live mouse. Auditory brainstem responses (ABR) showed no effects from the surgical approach. Texas Red-labeled gentamicin (GTTR) was systemically administered to the mice to track its pathway of entry into the cochlea in vivo using two-photon imaging. A separate multidose model of ototoxicity was used and evaluated with ABR responses and immunohistochemistry.

Results: GTTR enters the cochlea via the stria vascularis, then selectively enters HCs in the organ of Corti. The GTTR uptake into HCs was completely abolished in transmembrane channel-like protein1 (TMC1) knockout mice where mechanotransducer (MET) channels are not functional, indicating the MET channel is the major pathway of AG transport into HCs. As an initial entry site of AGs into the cochlea, we targeted megalin which is an endocytic transporter found in strial marginal cells and Reissner's membrane, because it tightly binds to AGs, and is the major transporter of AGs in the kidney. Co-administration with cilastatin, a binding competitor of megalin, prevent the GTTR accumulation in HCs, suggesting megalin is a critical route of AG transport into endolymph. Lastly, cilastatin treatment markedly reduced AG-induced HC degeneration and hearing loss in vivo.

Conclusions: Together, our in vivo real-time tracking of megalin-dependent AG transport across the blood-labyrinth barrier identifies new therapeutic targets for preventing AG-induced ototoxicity.

Auditory Effects of Antiretroviral Exposure During Pregnancy and Breastfeeding

(Poster Blitz: Post-doc)
James DeBacker, *National Center for Rehabilitative Auditory Research*

Category Inner Ear: Damage and Protection

Background: The World Health Organization (WHO) recommends that pregnant and nursing women with human immunodeficiency virus (HIV) take highly active antiretroviral therapy (HAART) in order to avoid transmitting HIV to their children. To date, studies of HAART exposure in utero have indicated that exposed children are at increased risk for neurological abnormalities, including auditory effects. Previous studies have suggested that non-auditory damage resulting from exposure to HAART is caused by mitochondrial damage and endoplasmic reticulum stress, and that these toxicities vary across cells, organs, and tissues. To date, there have been conflicting

findings in clinical populations on the auditory effects of post-natal HAART exposure and no published studies of the effects of exposure during pregnancy and breastfeeding (PaB). The first objective of this project was to characterize the auditory effects of these exposures during PaB. Outside of the direct risks of HAART exposure, the use of aminoglycoside antibiotics is more common in people with HIV for the treatment of opportunistic infections. As such, this study also evaluated how exposure to HAART during PaB may increase future susceptibility to aminoglycoside-induced hearing losses.

Methods: This study utilized a well-understood mouse model of ototoxicity, the CBA/CaJ mouse, to evaluate the effects of HAART exposure during PaB on auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs). 10 female breeding mice were exposed to one of four combinations of WHO-recommended HAART for pregnant and nursing mothers or a control in order to compare the auditory effects of these drugs on their offspring. 96 offspring who were exposed to HAART during PaB underwent ABR and DPOAE testing at wean age after completing breastfeeding. A subset of the offspring were exposed to 900 mg/kg of the aminoglycoside kanamycin daily for 12 days. Auditory measures were repeated on day 7 of kanamycin exposure, 24 hours after the final exposure, and again 28 days after the final exposure. Following data collection, ABR P1-N1 amplitudes and P1 latencies were evaluated to assess the physiologic index of the afferent synaptic pathway between the IHCs and the spiral ganglion neurons.

Results: The findings can be summarized in three major themes: 1) Exposure to zidovudine (AZT)- and efavirenz (EFV)-containing HAART regimens during PaB caused ABR threshold elevation at wean with no significant impairment of DPOAEs; 2) Exposure to HAART during PaB increased the risk of kanamycin-induced mortality, especially in animals receiving EFV; 3) kanamycin-induced hearing loss is worsened by exposure to HAART during PaB, especially in animals receiving EFV and AZT.

Conclusions: These findings suggest that HAART exposure during PaB has direct effects on the developing auditory system that both causes small but significant hearing loss at wean and elevates subsequent auditory risk associated with kanamycin exposures after birth.

An in Vivo Biomarker for Evaluating the Biologic Characteristics of Ototoxic Drugs and Novel Therapeutics That Mitigate Ototoxicity
(Poster Blitz: Post-doc)
Joseph Bellairs, *University of Washington*

Category Inner Ear: Damage and Protection
Background: A biomarker is a reliable and efficient method to determine the presence or progress of a disease that can be used for either diagnostic purposes or to evaluate the effects of treatment. We previously presented proof of concept data highlighting the dose and time relationship between systemically administered aminoglycosides and neonatal mouse cochlear hair cell accumulation. Here, we present two applications of this biomarker: evaluating differential ototoxin accumulation in sensory and non-sensory cell populations and investigating the time- and dose-dependent effects of ORC-13661, a hearing protection drug.

Methods: Neonatal mice (P5) were given systemic injections of geneticin (G418), a gentamicin congener, conjugated with Texas Red (G418-TR) either alone or in conjunction with ORC-13661 pretreatment. Dosage, injection schedules, and survival times of both drugs were systematically varied. Blood samples were taken, followed by euthanasia, otic capsule dissection with fixation by local perfusion, and dissections of whole mount preparations in addition to cryostat sections. Tissue was immunolabeled with anti-myosin7a and/or phalloidin and imaged with confocal microscopy. Utilizing myosin7a as a hair cell mask, G418-TR fluorescence was quantified within hair cells.

Results: Here, we show that neonatal hair cells accumulate G418-TR over 6 hours, and then retain nearly unchanged levels of G418-TR fluorescence over 72 hours after a single injection. When evaluating other tissue types from within the cochlea, we find that the stria vascularis shows an early peak in G418-TR accumulation before returning to near baseline levels. Both supporting pillar cells and spiral ganglia neurons demonstrate minimal G418-TR uptake. Finally, we demonstrate that ORC-13661 blocks G418-TR accumulation in a time-dependent manner. ORC-13661 demonstrates robust G418-TR uptake inhibition when animals are pretreated for 2 hours and then exposed to G418-TR for 3 hours, but when the paradigm is shifted to 2 hours pretreatment followed by G418-TR for 6 hours, only the highest doses demonstrate an inhibitory effect.

Conclusions: We previously highlighted that neonatal hair cell uptake of aminoglycosides serves as a proxy for in vivo mature hair cell uptake. When evaluating uptake and retention within the neonatal cochlea, we find that aminoglycosides are retained in the sensory epithelium but not other cell populations, suggesting that both uptake and prolonged retention of aminoglycosides contribute to hair cell ototoxicity. Finally, we demonstrate that ORC-13661 pretreatment blocks accumulation of aminoglycosides in vivo in a time-dependent manner, suggesting that

either serial dosing or enteral sustained release could enhance otoprotective effects. We believe that this preparation will serve as a valuable translational tool to better understand mechanisms of ototoxicity and as a rapid model to assess biologic characteristics of therapeutic strategies designed to mitigate ototoxicity.

Function of Cortical NDNF Interneurons in Sound Frequency Discrimination
(Poster Blitz: Post-doc)
Maryse Thomas, *Massachusetts Eye and Ear/Harvard Medical School*

Category Primary Auditory Cortex
Background: Frequency tuning is a defining property of neurons in the primary auditory cortex, with highly tuned neurons responding to a more narrow range of sound frequencies. Both inhibitory and disinhibitory cortical circuits are known to shape the tuning selectivity of excitatory neurons; however, the full extent of this circuitry has yet to be described. The present study focuses on the potential contribution of a recently identified class of interneurons that express neuron-derived neurotrophic factor (NDNF). In the auditory cortex, NDNF interneurons reside primarily within layer 1 and are known to inhibit the distal dendrites of excitatory neurons while simultaneously disinhibiting their somata via projections to parvalbumin-expressing inhibitory interneurons. This circuitry suggests that subsets of NDNF interneurons are well-poised to shape excitatory neuron tuning via either inhibition or disinhibition.
Methods: We performed in vivo two-photon calcium imaging in transgenic mouse lines expressing GCaMP in NDNF interneurons (NDNF-cre x Ai148-GCaMP6f mice) or layer 2/3 excitatory neurons (Thy-1-GCaMP6s mice). Simultaneous imaging of NDNF interneurons and layer 2/3 neurons was additionally undertaken using multiplane imaging of NDNF-cre x Ai148 mice injected with a pan-neuronal AAV-GCaMP8. Pure tones of varying frequencies (4-32kHz) and intensities (0-80dB) were presented to awake, head-fixed mice to determine frequency-intensity receptive fields. We trained a subset of mice on a Go/No-Go frequency discrimination task, in which they distinguished trains of repeating pure tones from trains of two alternating tones. In some experiments, we expressed an inhibitory chemogenetic receptor (AAV-DREADD-hM4Di) in NDNF interneurons and injected mice with either saline or agonist (CNO or C21) to establish the contribution of NDNF interneuron activity on frequency tuning and discrimination performance.
Results: We first characterized the tuning properties of NDNF interneurons in response to pure tones. We found that a subset of NDNF interneurons exhibit robust frequency and intensity tuning comparable to excitatory neurons in layer 2/3. We next used our behavioral paradigm to establish reliable frequency discrimination thresholds in mice. We recorded the responses of both NDNF interneurons and layer 2/3 excitatory neurons to the training stimuli under both passive and behaving contexts. We identified individual neurons in both classes capable of discriminating between repeating or alternating tones. Finally, ongoing experiments using chemogenetic silencing of NDNF interneurons will test the necessity of their activity for task performance and tuning specificity.
Conclusions: As tuning selectivity is thought to contribute to frequency discrimination acuity, understanding the cortical circuits that shape tuning will be important for identifying the neural mechanisms underlying perceptual deficits. This work will help establish the function of NDNF interneurons in frequency tuning and further elucidate the contribution of specific inhibitory cortical circuits to sound processing.

Prdm1 Drives a Fate Switch Between Inner Ear and Lateral Line Hair Cells in Zebrafish
(Poster Blitz: Post-doc)
Jeremy Sandler, *Stowers Institute for Medical Research*

Category Regeneration
Background: The lack of regenerative ability in the cochlea after the death or damage to mechanosensory hair cells is at the core of permanent hearing loss in mammals. Driving the regeneration of hair cells is a central strategy for restoring hearing in mammals, but triggering proliferative regeneration and maturation of functional hair cells remains elusive. Recently, the co-manipulation of Atoh1 with other genes or reprogramming with a cocktail of transcription factors has produced promising results, including the detection of proliferation and hair cell regeneration after hair cell killing. However, the generation of fully mature and functional hair cells has not been achieved. The zebrafish Danio rerio has an array of mechanosensory hair cell-containing neuromasts along the trunk, called the lateral line. Both the hair cells and surrounding support cells in zebrafish share genetic, functional, and structural similarity with mammalian inner ear hair cells and support cells, but the hair cells of zebrafish readily and rapidly regenerate following death to restore full function. Through our use of scRNA-seq during homeostasis and in a fine time series during hair cell regeneration in zebrafish, we identified sets of genes

with both spatially and temporally regulated expression. One such gene is the transcription factor prdm1a, which is expressed increasingly in the maturing hair cell lineage, and in support cells shortly following hair cell killing. prdm1a is not expressed in the hair cells of the zebrafish inner ear. Previously, prdm1 has been shown to control a fate switch in various cell types, including maturing B lymphocytes and maturing photoreceptors in the retina. We mutated prdm1a in zebrafish and found a drastically reduced number of hair cells during development and during regeneration, accompanied by a reduction in the proliferation of support cells during regeneration. We performed scRNA-seq on prdm1a mutants and siblings and discovered a cell type fate switch between lateral line and inner ear hair cells, with many specific inner ear hair cell genes being ectopically expressed in lateral line hair cells of the mutants. We performed ATAC-seq and ChIP-seq to characterize the enhancers of hair cell genes and identified that Prdm1a binding sites are enriched in the promoters and enhancers of these ectopically expressed genes. These findings show that prdm1a plays a crucial role in repressing an inner ear hair cell fate in lateral line organs. Prdm genes might also be central drivers in hair cell type specification and regeneration in other vertebrates. Combined our data show that Prdm1 or other family members are important genes to consider in future regeneration attempts in the mammalian cochlea.

Methods: N/A
Results: N/A
Conclusions: N/A

Loss of PEX1 Affects Inner Hair Cell's Ribbon Synapse Maturation and Auditory Function

(Poster Blitz: Post-doc)
Stephanie Mauriac, *Boston Children's Hospital, Harvard Medical School*

Category Other, Inner ear/Hair cells: Genetic and Synapses
Background: Peroxisome Biogenesis Disorders (PBD) or Zellweger syndrome spectrum disorders (ZSD) are a group of rare genetic multisystem disorders characterized by partial or total defect in peroxisome synthesis, assembly, and/or function. PBD-ZSD is associated with neurosensory hearing loss, retinopathy, multiple organ dysfunction and psychomotor impairment. Mutations in 14 peroxin (PEX) genes have been found to cause PBD-ZSD. Mutations in PEX1 are the most common, representing 70 percent of the cases (Reuber et al. 1997). Based on genotype-phenotype correlations, PBD-ZSD has been classified into class I (less severe, survival of 2 years to above 45 years) or class II (more severe, survival of less than 12 months). Limited research has focused on the impact of peroxisomal disorders on auditory function, hampering the development of treatments for PBD-ZSD patients. As hair cells are particularly sensitive to metabolic changes, we hypothesize that mutations in PEX1 cause hearing loss by affecting hair cell functions and survival along the cochlea.
Methods: Global deletion of the Pex1 is neonatal lethal in mice impairing any postnatal studies. To overcome this limitation, we created a conditional knockout (cKO) mouse by breeding a novel floxed Pex1 mouse with VGlut3-CRE mouse to allow for selective deletion of Pex1 in inner hair cells (IHCs). We measured auditory brainstem responses (ABR) to click and pure tone stimuli from 5.6 to 32KHz in mice from 4 to 16 weeks old. Whole mount cochleae were stained with Myo7a, CtBP2 and GluR2 antibodies to assess IHC's synapse. Samples were imaged using the Zeiss LSM800 confocal microscope and analyses were done using Imaris Cell Imaging software (Oxford Instruments).
Results: Pex1 excision in IHCs leads to progressive hearing loss that is more pronounced in the low frequency range and was associated with significant decrease in ABR wave I amplitudes (P<0.0001). To determine if this change was caused by alterations in IHC-SGN synapses, cochleas were stained with CtBP2 (pre-synaptic) and GluR2 (post-synaptic) markers. We observed a decrease in ribbon synapse number and volume especially in the low frequency region of Pex1 cKO mice.
Conclusions: These results suggest a critical function of Pex1 in development and maturation of IHC-SGN synapses as well as hearing function in the low to mid-frequency range.

Dimensions of a Living Cochlear Hair Bundle

(Poster Blitz: Post-doc)
Katharine Miller, *Stanford University*

Category Hair Cells: Anatomy and Physiology
Background: The hair bundle, the mechanosensory organelle of hair cells, consists of a group of stereocilia – "hair-like" insertions in the hair cell's apex. Hair bundles from different species, sensory organs, positions within an organ, and hair-cell types respond best to different kinds of stimulation due to differences in their stereociliary heights, widths, and organization, including their insertion-point separations. While hair-bundle dimensions

dictate bundle mechanics, these measurements have only been determined to a limited degree. In particular, mammalian cochlear data are either incomplete, lack control for age or position within an organ, or have artifacts owing to fixation or dehydration. Here, we report the most complete, accurately controlled, and precise blueprint of a living mammalian cochlear hair bundle to date, a postnatal day (P) 11 mouse apical inner hair cell. We rigorously quantified artifacts owing to fixation and dehydration and determined how they affect calculations of the hair bundle's mechanical properties.

Methods: Hair bundles from P11 C57Bl6/J mouse apical inner hair cells were measured from tissue treated with a membrane-stain for live imaging, a mild PFA-fixative and phalloidin for fluorescent imaging, or a strong PFA/glutaraldehyde fixative and subsequently dehydrated for scanning electron microscopy (SEM). The left and right ears of each mouse were treated differently – either for live-cell or mildly-fixed imaging – and SEM imaging was performed on littermate cochleae, allowing us to determine the scaling factors between preparations. Each protocol permitted us to collect large numbers of stereociliary measurements from bundles located in a precise region between the 90th-160th inner hair cells from the cochlea's apex. Measurements include stereociliary heights, widths, organization, insertion separations, and numbers of stereocilia. Using these measurements, we determined how fixation and dehydration affect calculations of hair bundle mechanical properties.

Results: We found that hair bundles mildly fixed for fluorescence had the same dimensions as living hair bundles, whereas SEM-prepared hair bundles shrank uniformly (66 +/- 4% on average) in their stereociliary heights, widths, and insertion separations. By determining the shrinkage factors, we imputed live dimensions from SEM that were too small to observe optically. Accordingly, we created the first complete blueprint of a living inner-hair-cell hair bundle. SEM-prepared measurements greatly affect calculations of the bundle's mechanical properties – overestimating stereociliary deflection stiffnesses (200-300% of live) and underestimating the fluid coupling between stereocilia (55-75% of live).

Conclusions: By comparing live-cell, mildly-fixed, and SEM-prepared conditions, we have shown that mildly-fixed tissue can be used as an alternative to live-cell imaging for many bundle measurements and have created a tool to impute live hair-bundle dimensions from SEM-prepared tissue. These conversion techniques will be particularly useful for measurements of rare samples, such as human hair bundles. Finally, we have shown that accurate and precise measurements are critical for understanding hair-bundle mechanotransduction.

Development of Novel Helper Dependent Adenoviral Vectors for Inner Ear Gene Therapy
(Poster Blitz: Post-doc)
Osama Tarabichi, *University of Iowa*

Category Genetics A: Genomics and Gene Regulation

Background: Hearing loss is the most common sensory disorder worldwide and most often occurs from dysfunction within the inner ear sensory organ. Currently, no FDA approved biological therapeutics are available. Viral vector mediated gene therapy has emerged as a promising strategy to target underlying molecular mechanisms of hearing loss. Adeno-associated virus (AAV) is the most commonly studied vector for inner ear gene therapy. However, the packaging capacity of AAV is small (~4.8 kb) which limits its potential use in therapeutic applications that require expression of large or multiple transgenes. Helper-dependent adenovirus (HdAd) is a safe, non-toxic viral vector that has a large packaging capacity (~37 kb). We have found that HdAd Type 5 serotype transduces multiple cell types in the inner ear in guinea pig and mouse models but with a low efficiency. The adenovirus fiber knob protein is used for attachment of the virus to specific receptors on the cell surface. One strategy to improve efficiency and/or target subpopulations of cells is to engineer vectors with alternative fiber knob proteins which can alter cell surface receptor interactions and tropism. We sought to develop chimeric HdAd and first-generation Ad vectors and evaluate their ability to transduce cochlear tissues in adult guinea pig and mouse models.

Methods: HdAd 5, HdAd 5/35 and first-generation Ad 5/50 reporter vectors with CAG promoters were developed. Vector was injected in mouse and guinea pig models using three approaches: 1) round window 2) round window with canal fenestration and 3) direct injection into scala media. Cochleae were harvested 7 days after injection. Immunohistochemical staining and confocal microscopy were performed to study transduction patterns.

Results: Transduction in multiple cell types was achieved using HdAd5 and chimeric vectors. Transduction was most notably seen in the spiral ligament, peri-lymphatic lining, modiolar region and supporting cells.

Conclusions: Transduction of various cell types of the inner ear is feasible with HdAd-based vectors at 7-days post injection. Studies are ongoing to further characterize the transduction patterns, safety, and stability of HdAd in the inner ear.