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Wednesday, February 24, 2021

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

Podium 28: "Gene Expression, Regulation and Inner Ear Therapeutics"

Moderators: Dr. Ronna Hertzano and Dr. Zubair M. Ahmed

Category: Genetics A: Genomics and Gene Regulation, Genetics B: General

PD 28.1 Adult Cochlear Hair Cell Transcriptional Diversity Revealed With Single-Cell RNA Sequencing

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Background

The paucity of cells and difficulty associated with accessing viable mature cochlear cells embedded in dense bone, have limited molecular and biochemical studies in the mammalian cochlea. Single-cell RNA sequencing and computational data analysis has helped to alleviate some of these limitations. These technological advances are accompanied by the availability of transgenic reporter mice and cell sorting methods to isolate viable individual cells from the mature cochlea. Here, we present differential gene expression analysis of single-cell RNA-Seq data of flow cytometrically isolated mature cochlear hair cells. Our results suggest the existence of two hair cell (HC) states and provide validation of our bioinformatic predictions, via in situ hybridization.

Methods

Single-cell RNAseq was performed on post-natal day 28 (P28) hair cells isolated with fluorescently activated cell sorting (FACS). We utilized transgenic Myosin15aCre/Ai14-tdTomato mice that express red fluorescent tdTomato within hair cells. Data was aligned and annotated using STAR and RSEM algorithms and analyzed in the R programming language. RNA in situ hybridization was conducted via Hybridization chain reaction (HCR) methodology.

Results

Our bioinformatic analysis revealed distinct groups of 418 inner hair cells (IHC), 22 outer hair cells (OHC), and contaminant cells. Three subpopulations of IHCs were identified. One population is suggestive of a physiologically active state and another is indicative of a recovery hair cell state, while the third was determined to be either compromised or dying IHCs. Differential gene expression analysis allowed us to identify distinct marker genes that are associated with each group of cells. In situ hybridization validated the differentially expressed genes between subpopulations of IHC and OHC in whole-mount P28 cochlea preparations.

Conclusions

Our results confirm and extend a recent single-cell RNAseq study from hand-picked HCs (Ranum et al., 2019). Quantitative HCR is being used to support our computational result of two distinct hair cell states. Specifically, we hypothesize that the two proposed states of active and recovering HCs can be identified in situ using mRNA quantification in individual neighboring HCs. We further compared the expression profiles of mammalian cochlear hair cell genes with avian cochlear hair cell genes, and conclude that despite some overlap, there is a surprising divergence of gene expression profiles. Evolutionarily, this observation supports the notion that the mammalian organ of Corti and the avian basilar papilla are not necessarily homologous organs. The presented work increases our understanding of the transcriptomic landscape of mature auditory

hair cells; it provides new candidate genes for hearing disorders and has the potential to identify new molecular targets to be used for diagnostic and therapeutic strategies.

PD 28.2 Combined RNA-Seq and ATAC-Seq of Developing IHCs vs OHCs Reveals Differential Open Chromatin, mRNA Splicing, Transcription Start and a Novel Promoter

Chuan Zhi Foo

Northwestern University

Background

We previously found that nascent IHCs and OHCs express differential transcriptomes and that these are transient, as most of the differentially expressed genes in this early stage are no longer expressed in the mature cells (Wiwatpanit et. al., 2018). We wondered whether these transient differences in mRNA expression were due to differences at the chromatin level or whether they resulted from different transcriptional regulators acting on equally accessible promoters and enhancers. We also wondered whether, in addition to differing in the levels of mRNA expression, developing IHCs and OHCs also differed in mRNA splicing and use of alternative promoters.

Methods

We separately collected neonatal IHCs and OHCs by FACS as previously done for RNAseq but instead extracted their DNA for ATACseq. Libraries (3 per hair cell type) were sequenced using the Illumina NextSeq 500 with 40bp paired end reads at a depth of 50-100 million reads. We aligned the reads to the genome using Bowtie2 and viewed the alignments using IGV. RNA-Seq previously obtained from separate neonatal IHCs and OHCs (100bp paired end reads) was aligned to the genome using STAR, isoform level quantified with RSEM and EBSeq, and analysed for differential splicing using rMATS.

Results

ATACseq revealed numerous differences in open chromatin between IHCs and OHCs: 294 proximal elements (PEs; promoters, centered on the Transcription Start Sites) were preferentially accessible (“open”) in IHCs and 103 in OHCs, in addition to 3,889 distal elements (presumed enhancers) in IHCs and 1,777 in OHCs. Promoters and enhancers display equally open chromatin for genes equally expressed in IHCs and OHCs (such as *Atoh1* and *Pou4F3*) but are differentially accessible for genes preferentially-expressed in IHCs (*Slc18a8/Vglut3* and *Fgf8*) or OHCs (*Insm1* and *Neurod6*). Most of the differentially expressed genes examined had either a differentially accessible promoter or a nearby element/enhancer. By comparing ATACseq peaks of open chromatin with RNAseq sashimi plots we discovered putative novel promoters that drive the expression of novel isoforms of several genes that have not been described elsewhere, an example being *Brip1* in the IHCs. And by further examining RNAseq for splice differences, we find that some genes that are not expressed at different levels have differences in the relative abundance of specific isoforms, such as *Myo7A*, whose isoform 1 is more abundant in IHCs than OHCs.

Conclusions

Our data reveals that the difference in expression levels of many genes between developing IHCs and OHCs is likely due to the differential chromatin accessibility of their promoters and/or enhancers, and that alternative splicing and promoter usage further distinguished the transcriptomes of these cells during their differentiation.

PD 28.3 Characterization of Rare Spindle and Root Cell Transcriptional Profiles in the Stria Vascularis of the Adult Mouse Cochlea

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Background

The stria vascularis (SV) in the cochlea plays a pivotal role in normal hearing by generating and maintaining the endocochlear potential (EP). It is located on the lateral wall of the cochlear duct and consists of three main layers, composed predominantly by marginal, intermediate and basal cells, as well as other rare cell types, including spindle cells, macrophages, endothelial cells, and pericytes. We have previously characterized

transcriptional profiles of the major cell types in the adult mouse SV by single-cell (sc) RNA-Seq and single-nucleus (sn) RNA-Seq. Despite our previous work, transcriptional profiles of rare SV cell types, specifically SV spindle cells and root cells of the spiral prominence, remained poorly distinguished in our previous study.

Methods

In this study, we utilize two sample preservation methods, methanol fixation and RNAlater treatment, in snRNA-Seq to characterize rare cell transcriptional profiles in the adult SV. Samples were sequenced using the 10X Genomics Chromium platform, and reads were mapped to GRCm38 (mm10) mouse reference genome. Downstream data analysis was performed using Scanpy (v1.4) single cell analysis platform.

Results

We distinguish spindle cells from root cells in the lateral wall of the cochlea and characterize their transcriptional profiles. In particular, we identify novel spindle cell markers as well as potential spindle cell-specific gene regulatory networks. Datasets obtained with sample preservation methods demonstrate similar transcriptional profiles of major SV cell types compared to previously published datasets.

Conclusions

By using sample preservation methods combined with snRNA-Seq, we define distinct transcriptional profiles for rare SV spindle cells and root cells in the spiral prominence. These novel findings will provide new insights for future work in SV-related hearing loss and hearing fluctuation.

PD 28.4 Formin Protein Fhod3 Depletion Leads to Hearing Loss and Disruption of Stereocilia Structure in Mice

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Background

Formin proteins play a critical role in regulating actin cytoskeleton dynamics. A meta-analysis GWAS implicated the mammalian protein Fhod3, specifically, as a novel gene candidate in age-related hearing loss. In our previous work, mouse models demonstrated that overexpression of Fhod3 in hair cells leads to disruption of stereocilia structure and progressive, high frequency hearing loss. Here, we conversely examine Fhod3 depletion in mouse hair cells to further understand its role in regulating stereocilia structure and subsequent effects on hearing loss.

Methods

We generated a floxed Fhod3 allele in hair cells by breeding Math1-cre expressing mice with Fhod3 loxP-flanked ("floxed" or "fl") mice. We compared knockouts (KO; fl/fl,Math1-cre+), heterozygotes (Het; fl/+,Math1-cre+), and controls (+/+,Math1-cre+ or Math1-cre-) using auditory brainstem response (ABR) thresholds and scanning electron microscope (SEM) imaging of the organ of Corti.

Results

In 6-week-old mice, ABR thresholds were higher for KOs (N=9) vs. controls (N=16) at 32kHz (50.1dB vs 33.2dB, p=0.04986). At 12 weeks, both KOs (N=8) and Hets (N=5) had higher ABR thresholds than controls (N=13) at 24kHz (52.9dB, 55.0dB, vs. 33.8dB, p<0.01) and 32kHz (73.3dB, 82.4dB, vs. 39.5dB, p<0.01). Similarly at 18 weeks, KOs (N=6) and Hets (N=4) showed higher ABR thresholds than controls (N=13) at 24kHz (53.8dB, 67.3dB, vs. 35.7dB, p<0.01) and 32kHz (80.8dB, 86.0dB, vs. 50.7dB, p<0.01). SEM imaging of the organ of Corti from KOs reveals dysmorphic, fused stereocilia and loss of the shortest row of stereocilia in the basal turn of the cochlea compared to controls.

Conclusions

Here, we build on our previous data to show that not only overexpression, but also full and partial depletion (KO and Het) of Fhod3 leads to phenotypic hearing loss and hair cell stereocilia defects. Results suggest a specific balance in the protein's level of expression is critical for the normal structure and function of hair cell stereocilia.

PD 28.5 Outcomes of AAV-Mediated Gjb2 Delivery to the Mouse Cochlea

Yehoash Raphael

The University of Michigan

Background

Mutations in GJB2, which encodes connexin26 (Cx26), are the most common cause of autosomal recessive severe-to-profound hereditary deafness. In mice, adeno-associated viral (AAV) vectors can transduce hair cells and supporting cells, making them potential candidates for therapeutic interventions affecting gene expression. Previous experiments have shown that AAV-mediated Gjb2 overexpression can restore gap junction function in neonatal Gjb2 knockout mice, but phenotypic rescue of function has remained a challenge. Here, we tested the effects of AAV vector mediated Gjb2 delivery to mature ears of mice in which Gjb2 depletion was induced either early in development or upon cochlear maturation.

Methods

We used Gjb2^{flox/flox} controls or Sox10CreERT2;Gjb2^{flox/flox} (Gjb2-cKO) animals in which tamoxifen was injected to delete Gjb2 expression in supporting cells. AAV-hGJB2, with and without a Flag-tag, was administered to the inner ear at P28 via the round window with posterior semicircular canal fenestration. Auditory Brainstem Responses (ABRs) were measured 4 and 8 weeks later and then temporal bones were assessed by immunofluorescence for Cx26/Flag and Myosin VIIa.

Results

Cx26-Flag protein was detected in multiple outer sulcus supporting cells, where Gjb2 is normally expressed, and where the original Cx26 had been ablated after tamoxifen injection. Importantly, Gjb2 expression was observed primarily in the cell membrane. Ectopic Cx26 was also detected in hair cells despite their lack of gap junctions under normal conditions. Cx26 appeared to localize to the cell membrane of inner hair cells (IHC) but was dispersed in the cytoplasm of outer hair cells (OHC). No OHC loss was observed, but many IHCs were missing in ears injected with the vector. IHCs in the basal cochlear turn of contralateral (un-injected) ears also exhibited low level of Cx26; however, no IHCs were missing.

Conclusions

In Gjb2-cKO and Gjb2^{flox/flox} mice, AAV-hGJB2 transduced supporting cells and successfully expressed the human GJB2 protein. In addition, the transduction of IHCs and subsequent ectopic expression of hGJB2 resulted in partial IHC loss. To enhance the suitability of AAV gene transfer for treating Gjb2 deficiency in mature ears, it may be desirable to reduce IHC ectopic expression or inhibit its translocation to the cell membrane of IHC.

Supported by NIH-NIDCD grant R01-DC014456. We thank Leda Dimou for the Sox10CreERT2 mice.

PD 28.6 Restoration of Progressive Hearing Loss in Myosin Vi Dominant Mutation Mouse Model Using CRISPR/Cas9 in Vivo

Yilai Shu

Eye & ENT Hospital, Shanghai Medical College, Fudan University

Background

About 60% of sensorineural hearing loss in neonates is caused by defects in individual genes. For genetic deafness, the new, specific CRISPR/Cas9-based genome editing is an idea treatment to mediate targeted gene therapy. We created a humanized knock-in mouse model to mimic the p.C442Y variant found in human DFNA22 patients previously. Here we study whether adeno-associated virus (AAV) based Staphylococcus aureus Cas9 (SaCas9-KKH) system could restore progressive hearing loss that caused by Myosin VI (Myo6) p.C442Y mutant.

Methods

We screened and validated a sgRNA that specifically and efficiently edited the mutant allele in Myosin VI (Myo6) p.C442Y mutant in vitro. We then performed hearing test, immunohistochemistry, SEM and electrophysiology on the Myo6 mouse model in vivo after SaCas9-KKH allele-specific gene disruption.

Results

The hearing of Myo6^{WT/C442Y} mice was improved up to six months post injection of AAV based-SaCas9-KKH -sg2 agents. Auditory brainstem response thresholds (ABRs) was observed improved in injected ears

than in uninjected ears. Meanwhile, the potency of wave I at 8 and 16kHz in the injected ears showed lower than those in non-injected ears. In addition, higher cell survival rates, better hair bundle morphology, higher calcium current (ICa) and higher membrane capacity (Cm) were also observed in injected ears rather than in non-injected ears.

Conclusions

These findings suggest that AAV based SaCas9 allele-specific gene disruption system could represent a potential therapeutic approach for human Myosin VI p.C442Y mutation.

PD 28.7 Gene Therapy to Treat Mouse Model of Human Recessive Deafness Caused by a TMPRSS3 Mutation

Corena Loeb

Harvard Medical School

Background

Expected to afflict every one-in-ten individuals by 2050, hearing loss poses physiological, emotional, social and a growing worldwide annual economic toll. Although half of hearing loss cases are caused by genetic factors, there remains no available treatment to reverse or prevent genetic deafness. In an effort to develop AAV therapy for hearing loss, here we evaluate the efficacy of gene therapy to restore auditory function in a TMPRSS3-mutant mouse model. TMPRSS3, a type II transmembrane serine protease, is necessary for normal hearing and mutations in TMPRSS3 account for approximately 9% of autosomal recessive non-syndromic deafness. TMPRSS3 mutations affect the function and survival of hair cells and spiral ganglion neurons, the latter impacting the ability of TMPRSS3 patients to benefit from cochlear implants. As such, TMPRSS3 mutations account for an estimated 10% of cochlear implant failures.

Methods

To mimic post-lingual hearing loss in TMPRSS3 patients, we created a mouse model with the knock-in human mutation Ala306->Thr (c.916G->A). In humans, this mutation results in hearing loss that does not manifest until adulthood. Mice with the mutation develop delayed hearing loss starting at 12 months of age, making the model a valuable tool for the study of later intervention. As AAV2 is widely used in clinical applications, we constructed an AAV2-TMPRSS3 for inner ear delivery, injected via canalostomy into 12-month-old Tmprss3 (c.916G->A) mice, then monitored hearing in the following months.

Results

We found significant hearing improvement in the injected inner ears compared to the contralateral uninjected control ears as shown by reduction in ABR and DPOAE threshold shifts. Further, we detected significantly larger wave 1 amplitudes in the treated inner ear, an indication of functional rescue of spiral ganglion neurons. Analysis of the inner ear showed markedly improved hair cell survival in the injected vs. uninjected inner ear. To evaluate translational potential of TMPRSS3 gene therapy, human iPSCs carrying the Tmprss3 (c.916G->A) mutation were differentiated into a neuronal phenotype. Treatment with AAV2-TMPRSS3 was able to protect neuronal survival after repeated challenge with glutamate compared to untreated control cells.

Conclusions

This is the first time that later intervention at 12 months by AAV-mediated gene delivery rescued hearing in a mouse model of human genetic hearing loss. Significantly, AAV delivery not only prevented continuous hearing loss, but also temporarily restored hearing. AAV2-TMPRSS3 gene delivery is being developed into a clinical program as treatment for patients with TMPRSS3 mutations who otherwise lack treatment options. Evaluation of differentiated human iPSCs carrying the same mutation suggests that human cells may also be rescued by TMPRSS3 gene therapy.

PD 28.8 Identification of Novel Bio-reducible Lipid Nanoparticles for Efficient in Vivo Gene Editing in Adult Cochlea

Mingqian Huang

Massachusetts Eye and Ear/Harvard Medical School

Background

While CRISPR/Cas9 system can effectively modify DNA in vitro, efficient in vivo editing is the major bottleneck preventing its applications in translational medicine. A number of methods have been developed to deliver CRISPR/Cas9 into mouse inner ear to correct mutations causing genetic hearing loss. We have shown that liposome-mediated ribonucleoprotein (RNP) delivery targeting hair cells in neonatal mouse cochlea rescued hearing in Tmc1 mutant Beethoven mouse model. However, the same RNP approach failed to edit the target gene in adult mouse cochlea. It is essential therefore to develop novel delivery vehicles capable of targeting different cell subtypes in adult cochlea in vivo in order to apply gene editing-based therapy in human.

Methods

We have used combinatory approach to synthesize a library of bio-reducible lipid nanoparticles (LNPs) and screened them in vitro and in vivo to identify formulations capable of adult inner ear delivery. We injected LNPs mixed with Cre recombinase or GFP protein or mRNA into cochleae of adult Ai14 mice with tdTomato (tdT) reporter via canalostomy or cochleostomy. The LNPs inducing tdT-positive cells in cochlea were selected for in vivo delivery of Cas9 protein or mRNA mixed with the sgRNA targeting stop codons that prevent tdT expression.

Results

We identified several LNPs that efficiently deliver Cre recombinase protein or mRNA into the non-sensory cells on the Reissner's membrane and basilar membrane via canalostomy through posterior semicircular canal (PSCC). However, sensory cells including hair cells and supporting cells were not transfected. By switching the injection route to cochleostomy so LNP/Cre or LNP/GFP mRNA or protein complexes were directly injected into endolymph, we identified the LNPs capable of delivering mRNA and protein into major inner ear cell subtypes in the sensory region, indicating that those LNPs cannot pass through the endolymph-perilymph barrier. Surprisingly, the same LNPs, when tested with Cas9 mRNA/sgRNA, showed little editing in the sensory region despite effective delivery of Cre mRNA and protein. Further screening identified additional LNPs capable of delivering Cas9 mRNA with sgRNA leading to Cas9-mediated editing in the supporting cells.

Conclusions

These results demonstrate the utility of LNPs in delivering diverse cargos (mRNA, protein and RNP) into adult mammalian inner ear cell subtypes. It further illustrates that Cas9 mRNA/sgRNA delivery for efficient editing is different from the delivery of molecules such as Cre mRNA. Combined we identified a number of novel LNPs that target different cell types in sensory and non-sensory regions in adult cochlea in vivo. This is an important step toward CRISPR/Cas9 treatment of genetic hearing loss in human.

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

Podium 29: "Binaural and Spatial Hearing - Psychophysics, Neurophysiology and Models"

Moderators: Dr. Yi Zhou and Dr. Jonas Klug

Category: Binaural Hearing & Sound Localization

PD 29.1 Binaural Detection as a Joint Function of Masker Bandwidth, Masker Interaural Correlation, and Interaural Time Delay: Behavioral Data and Quantitative Modeling

Leslie Bernstein

University of Connecticut Health Center

Background

We recently published studies showing how a wide variety of binaural detection and discrimination data obtained over decades and across laboratories (including ours) could be explained and accounted for

quantitatively. The model employed incorporated a central stage of interaural correlation, coupled with stages of peripheral auditory processing and included both peripheral and central internal noises. Predictions were made from the outputs of the model by a decision variable implemented via a signal-detection theory analysis that utilized interaural correlations of samples of noise-alone and signal-plus-noise. Here, we report new empirical data showing how binaural detection is affected by joint variation of masker bandwidth, masker interaural correlation, and common interaural time-delay (ITD) imposed on both masker and tonal signal. The bulk of the data were obtained with stimuli centered at 500 Hz; additional data were obtained with stimuli centered at 4 kHz.

Methods

We measured detection thresholds using 100-Hz- and 900-Hz-wide Gaussian noise maskers and tonal signals. The masking configuration of primary interest was based on the standard $N\rho S\pi$ configuration where ρ indicates the interaural correlation of the masker. An ITD, τ , is imposed on the entire $N\rho S\pi$ signal-plus-masker waveform to yield the configuration $(N\rho S\pi)\tau$. Note that when $\rho = 1$ and $\tau = 0$, the configuration yields $NoS\pi$. For each bandwidth, $(N\rho S\pi)\tau$ thresholds were measured as a joint function of masker ρ and the τ imposed on the waveform. Values of ρ tested were 1.000, 0.992, 0.874, and 0.498. Values of τ tested were 0, 500, 1000, 2000, and 3000 μ s. A second configuration, $(NoS\pi)\tau$, consists of the standard $NoS\pi$ configuration but with the imposition of an ITD on the signal-plus-masker waveform. That configuration provides no binaural cues for detecting the signal. It served as a control to assess whether the imposition of an ITD, per se, affects detection.

Results

The data reflect that: 1) consistent with earlier findings, as masker ρ is decreased progressively from 1.0 to 0.498, threshold S/Ns increase; 2) as ρ is decreased, increasing the ITD (τ) from 0 to 3 ms yields progressively smaller additional increases in threshold S/N. Our theoretical approach accounted for the entire set of data while principally using values of model parameters found to predict successfully earlier data obtained in our laboratory. Importantly, the new stimulus conditions were designed to produce controlled changes in the nominal interaural correlations and sampling distributions of the stimuli as processed by the model. The latter were used to form the model's decision variable.

Conclusions

The success of the enterprise attests to the robustness of the approach and the generality of the model's ability to make accurate predictions of binaural performance. A manuscript reporting these results is, at this writing, under review in JASA.

Support: ONR Award No. ONR N00014-18-1-2437

PD 29.2 Clarifying the Mechanisms of Binaural Masking Level Difference With Deep Learning

Samuel Smith

University of Nottingham

Background

The binaural masking level difference (BMLD) quantifies the improvement in the perceptual detection of a signal in noise observed for different interaural configurations. Durlach (1963; JASA 1206-1218) derived equations that accurately predict much of the human BMLD psychophysical data. However, disparities between psychophysics-based theory and physiology findings have left the mechanisms underlying human BMLDs somewhat ambiguous. We report here results from deep neural networks (DNNs), trained on perception, bearing striking similarities to key physiology findings.

Methods

Random-phase pure tones were presented amongst randomly distributed broadband noise and simulated as emanating from two random positions in a virtual azimuth coded by interaural time difference (between far left and far right). We trained deep neural network variants (Iten et al., 2018; arXiv: 1807.10300) to predict the values of BMLDs given by Durlach's equations. Once trained, we examined the DNNs' predictive capabilities and crucially the emergent dynamics within the networks.

Results

The DNNs accurately predicted BMLDs for randomly generated stimuli whose interaural time differences were within or larger than the range plausibly produced by a human head. Despite being an extremely coarse imitation of neurobiology, accurate prediction of BMLDs was accompanied by an emergence of dynamics with broad similarities to physiology data: the DNNs learnt a sensitivity to interaural time differences with response peaks at long delays, and their nodes produced rate-versus-level responses characterized by stimulus synchronicities.

Conclusions

The simplicity of the stimuli, task paradigm, and modelling assumptions, promote the generality of the observed network dynamics. Further, the method adopted directly connects them to BMLDs. Overall, the work demonstrates how DNNs can be used to consolidate theoretical and experimental understanding in the field of binaural hearing.

PD 29.3 Dichotic Listening Deficits in Amblyaudia Are Characterized by Aberrant Neural Oscillations in Auditory Cortex

Sara Momtaz Bokharaei
The University of Memphis

Background

Children diagnosed with central auditory processing disorder (CAPD) show deficits in processing complex sounds that are associated with difficulties in higher-order language, learning, cognitive, and communicative functions. Amblyaudia (AMB) is a subcategory of CAPD identified as having binaural integration deficits and abnormally large ear asymmetries in dichotic listening tasks.

Methods

Here, we examined frequency-specific neural oscillations and functional connectivity via high-density EEG in children with and without AMB during passive listening of nonspeech stimuli.

Results

Time-frequency maps of these “brain rhythms” revealed stronger phase-locked β/γ (~35 Hz) oscillations in AMB’s within bilateral auditory cortex for sounds presented to the right ear suggesting a hypersynchronization and imbalance of auditory neural activity. Brain-behavior correlations revealed neural asymmetries in cortical responses predicted the larger than normal right-ear advantage in AMB’s dichotic listening. Additionally, we found weaker functional connectivity in the AMB group from right to left auditory cortex, despite overall stronger neural responses.

Conclusions

Overall, our results reveal abnormally large auditory sensory encoding and an imbalance in communication between cerebral hemispheres (ipsi- to -contralateral signaling). We suggest these neurophysiological changes might lead to functionally poorer behavior capacity to integrate information between the two ears in AMB and related variants of CAPD.

PD 29.4 Effects of Background Babble on Spatial-Change Tuning of Speech in Older Adults

Erol Ozmeral
University of South Florida

Background

In complex acoustic scenes, listeners take advantage of spatial cues to selectively attend to sounds, such as speech, that are deemed immediately relevant while ignoring sounds that are deemed to be irrelevant. Using electroencephalography, we have shown modulatory effects of attention on spatial-change tuning, a related neural phenomenon to the minimum audible movement angle. Younger and older listeners show comparable effects of attention, whereby spatial-change tuning to noise bursts is sharpened in directed attention tasks. However, older adults show broader spatial-change tuning than younger listeners when passively listening to moving stimuli, indicating a diminished bottom-up signal representation of spatial change. In a more recent study, younger listeners were presented with speech stimuli that moved periodically in the free field either in quiet or in background babble. As with noise bursts, moving speech in quiet evoked robust event-related

potentials and sharp spatial-change tuning, and though background babble tended have some suppressive effects, younger listeners still showed robust neural responses to the moving speech.

Methods

The present study tested the effect of background babble to spatial-change tuning of speech stimuli in an older population. Listeners were tasked with either passively listening to the speech or actively indicated whether the speech had moved to a specific location. Because older listeners are known to have greater challenges understanding speech in complex acoustic scenes, and because they have also been shown to have limited spatial-change tuning in quiet, we hypothesized that older listeners would lack the necessary signal representation to have significant effects of selective attention when background babble was present.

Results

As was the case with moving noise bursts, older adults showed clear spatial-change tuning to speech in quiet albeit broader than young adults; however, older adults were limited in their ability to meaningfully represent spatial change when background babble was present. Responses from directed attention conditions demonstrated intact top-down modulatory effects of attention when the signal could be adequately encoded (i.e., in quiet), but not when spatial-change tuning was absent altogether (i.e., in background babble).

Conclusions

The results of the present study are clear indicators that although older listeners show intact spatial selective attention, they are limited in their ability to take advantage of selective attention when the bottom-up signal is poorly encoded. Despite the normal hearing status of the present listeners, the results of this study may also provide evidence for the use of sound enhancing technology for those with clinically normal hearing thresholds.

PD 29.5 Modeling Binaural Masking Release Based on Interaural Phase Distribution

Bernhard Eurich

University of Oldenburg

Background

In complex listening environments, listeners can benefit from utilizing interaural differences to separate a signal from a masker. Differences in interaural phase configuration between a target and a masker can lead to substantial binaural masking release, even in case of large interaural masker delay. To model this unmasking behavior a neural delay compensation mechanism is widely assumed. An alternative hypothesis states that binaural unmasking behavior can be explained solely based on the interaural phase distribution. However, using this approach, it has not yet been possible to account for the decreased unmasking performance in scenarios with several noise sources (double-delayed noise, van der Heijden & Trahiotis, JASA 105-1 1999, p. 388). As a potential solution, Marquardt & McAlpine (JASA 126, EL177, 2009) suggested detrimental interaction across frequency channels. The goal of the present study is to develop this concept into a computational model in order to explain psychoacoustic data that was previously only explained by employing delay compensation.

Methods

This study proposes a model that extracts time dependent interaural phase differences (IPD) in each frequency channel. Interactions across frequency channels are introduced in order to decrease unmasking performance in case of low interaural coherence in off-frequency channels. Monaural cues are considered by assessing changes in the channels' envelope power. The model is evaluated as an artificial observer in an alternative forced choice paradigm.

Results

The model is shown to account for several relevant psychoacoustic phenomena concerning binaural masking level differences (BMLD) including the dependence on the interaural noise delay. It further accounts for the reduced BMLD in double-delayed noise. Parameter optimization is ongoing.

Conclusions

The proposed computational model was shown to account for BMLD data solely based on IPD distributions and envelope power. Thus, at this stage, across-channel interaction effects appear to provide an explanation

for the reduced binaural benefit in listening scenarios with multiple masking noise sources and large noise delays.

PD 29.6 Neural Encoding of Individual Source Locations in Multi-Speaker Listening Scenes

Kiki Heijden

Radboud University Nijmegen

Background

Everyday listening scenes typically contain concurrent, spatially separated sound sources. Humans are capable of individually localizing sound sources in such listening scenes when two or even three spatially separated sources are present (e.g. Good & Gilkey, 1996; Lorenzi, Gatehouse, & Lever, 1999; Hawley, Litovsky, & Colburn, 1999). Yet, little is understood about location encoding of individual sound sources in spatially separated, multi-source listening scenes. Here, we use invasive recordings of neuronal population responses and neuronal population modelling techniques to investigate the representation of individual sound locations in multi-source listening scenes.

Methods

We recorded neuronal population activity using stereotactic electroencephalography (sEEG) in seven participants. Participants listened to blocks of spatialized, spoken sentences. Blocks contained trials with either a single speaker (male or female), or two, spatially separated speakers (male and female) at -45° and/or $+45^\circ$. At the start of a block, participants were instructed to attend either the male or female speaker. The spatial location of the attended speaker varied pseudo-randomly over trials. In between trials, participants were asked to indicate the position of the attended speaker (left or right). We extracted the envelope of the high-gamma band (70 – 150 Hz) as a measure of neuronal population activity.

To assess spatial selectivity, we calculate a spatial selectivity index (SSI) as the normalized difference between the population response to sound on the left and right. For multi-speaker conditions, we compute SSI as a function of the locus of spatial selective attention for those time instants in which only one speaker is present. Further, we construct a model of early spatial hearing based on peripheral auditory processing (ref Patterson) to derive the expected spatial input to the neuronal populations (i.e. binaural disparity cues and interaural coherence). Next, we use this information to model multivariate temporal receptive fields (TRFs, Crosse et al., 2016) for each neuronal population for three different feature spaces: spectral profile, spatial position, and interaural coherence. We assess the validity of the mTRFs by predicting population responses to unseen listening scenes and assess the relative contribution of each feature space (De Heer et al., 2017).

Results

Neuronal populations in primary (Heschl's gyrus) and higher-order regions (planum temporale and superior temporal gyrus) exhibited spatial selectivity in single-speaker conditions. Our data furthermore showed that spatial selective attention affects spatial selectivity in multi-speaker scenes across auditory cortex. TRFs reveal mixed representations of spectral, spatial, and coherence features across auditory cortex.

Conclusions

Our study investigates the neuronal mechanisms underlying the encoding of single source locations in multi-source listening scenes. These findings have important implications for understanding speech-in-noise perception in normal hearing- and hearing-impaired listeners.

PD 29.7 Neural Modulation to Direction and Speaker in Spatial Multi-talker Speech Perception

Prachi Patel

Columbia University

Background

Humans can attend to a single conversation in crowded acoustic scenes particularly when the talkers are separated in space. How the human auditory cortex represents spatially separated multiple talkers and how attention to a talker's location or voice modulates the neural representation of attended and unattended speech is unclear.

Methods

We measured neural responses from electrodes implanted in neurosurgical patients as they performed single- and multi-talker speech perception tasks, with and without spatial separation of talkers.

Results

We found that spatial separation between talkers caused a preferential encoding of the contralateral talker's speech irrespective of the focus of attention. Moreover, talkers' speech and location were encoded in distinct component of the neural response and these components were differentially modulated by attention to either talker's voice or direction.

Conclusions

These findings shed light on interplay of bottom-up and top-down mechanisms which creates a selective and dynamic neural code that can support speech perception in crowded acoustic scenes.

PD 29.8 Validation of Online Testing for Behavioral Auditory Experiments: Speech Intelligibility in Quiet and Noise

Emily Burg

University of Wisconsin-Madison

Background

The binaural system helps listeners localize sounds and understand speech in noisy environments. Psychophysical studies that aim to measure these abilities are typically conducted in highly controlled laboratory environments with carefully calibrated stimuli. However, participant recruitment and testing can be cumbersome. An increasingly popular alternative is to move towards remote data collection outside the laboratory. Remote testing provides access to diverse patient populations and efficient collection of large sample sizes. Unlike experiments conducted in laboratory sound booths, remote testing may introduce variabilities that reduce data quality, such as the home environment and consumer-grade audio devices. Therefore, validation of remote testing protocols is essential in order to understand the impact of such variabilities as we adopt a more flexible approach to data collection.

Methods

We utilized Gorilla, an online research platform, to replicate two experiments that explored contralateral and binaural unmasking in normal hearing listeners (Goupell et al., 2016 & Bernstein et al., 2016). The current experiment measured speech reception thresholds (SRTs) at 50% correct in normal hearing adult listeners in four listening configurations: "Quiet," "Contralateral Separation," "Monaural," and "Dichotic Separation." Contralateral unmasking was calculated as the difference between SRTs in the "Monaural" and "Contralateral Separation" conditions, and binaural unmasking was the difference between the "Monaural" and "Dichotic Separation" conditions. Both target and masker stimuli were comprised of five-word sentences containing a name, verb, number, adjective, and object. Target stimuli were spoken by a female, and masker stimuli were spoken by a female or a male. Stimuli were presented over wired headphones and scaled using a subjective rating of the participants' most comfortable loudness level. A series of perceptual screenings were included to safeguard audio quality.

Results

The goal of this work is to assess whether data collected remotely produces similar findings to those obtained when data are collected in the lab. One possibility is that, due to increased ambient noise level in the home environment and more variable audio device quality across participants, remote testing will produce poorer SRTs in all conditions, but effect sizes of contralateral and/or binaural unmasking will be similar.

Conclusions

Data from this study will provide empirical evidence regarding the feasibility and reliability of remote data collection for behavioral auditory experiments, with potential applications for testing large numbers of participants with normal hearing and hearing loss.

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

Podium 30: "Recent Advances in Age-Related Hearing Loss"

Moderators: Dr. Fan-Gang Zeng and Dr. Shinichi Someya

Category: Aging

PD 30.1 Ibuprofen Treatments Improve Auditory Functions of Aging CBA/CaJ Mice

Parveen Bazard

University of South Florida

Background

Currently, there is no FDA approved drug for age-related hearing loss (ARHL), also known as presbycusis. Here we test the effects of ibuprofen – a well non-steroidal anti-inflammatory drug, on auditory function. Our rationale is that chronic inflammation with age occurs for various aging tissues of the body, including the brain and cochlea. Also, we have shown that aldosterone, a naturally occurring mineralo-corticoid hormone can help preserve auditory function during aging for CBA/CaJ mice via prevention of cell loss in spiral ganglion neurons and stria vascularis ionic changes. Hence, we have explored the combinatorial effects of aldosterone and ibuprofen here.

Methods

Aging 19-22-month-old CBA/CaJ mice were used. Treatment groups were divided into two groups: ibuprofen alone, and a combo treatment – aldosterone and ibuprofen. Ibuprofen was fed in the chow, and aldosterone was systemically administered using time-release pellets. Auditory functions; auditory brain stem responses (ABRs), distortion product otoacoustic emissions (DPOAEs), and temporal processing abilities of the mice using the ABR gap-in-noise (ABR GIN) – responses to a silent gap duration inserted in between wide band noise burst stimuli; were measured each two months. Western blot experiments were performed using two auditory cell lines: stria vascularis (SVK-1) and hair (HEI-OC1) to investigate underlying drug actions and biological mechanisms.

Results

For the initial experiments, chow-fed ibuprofen concentration was 375 ppm. Significant effects were observed on DPOAEs amplitudes and thresholds due to ibuprofen treatment. During 6 months of treatment, ibuprofen showed significantly fewer aging declines of DPOAE amplitudes and threshold elevations compared to the control group of mice of the same age. ABR-GIN responses are representative of temporal processing, i.e., the shortest gap duration at which there was a response to the second noise burst. The ABR-GIN thresholds for the aging control group was 8 ms. For second noise burst ABR, peak 1 and peak 4 amplitude shifts during the 6-month treatment period were smaller for the ibuprofen mice at each gap duration tested: 8, 12, 16, 32 and 64 ms. Also, ABR latency shifts for the 2nd noise burst during the 6-month treatment period were larger for the control group at each gap duration. Improved suprathreshold ABR levels (80 dB SPL), were observed for the combo treatment group. Preliminary molecular biology experiments indicate that ibuprofen treatment affects prestin motor protein and inflammatory biomarker expression levels.

Conclusions

Further dose optimization (e.g., decrease; 263 ppm ibuprofen) and biomarker experiments are ongoing to understand the complete effects of these treatments. In conclusion, ibuprofen-alone treatment slows the decline of outer hair cell functions as well temporal processing in aged CBA/CaJ mice. Addition of aldosterone further augments the effects, especially for improvement of suprathreshold responses.

Work supported by NIH-NIA: P01 AG009524.

PD 30.2 Gene Expression and Potential Treatment Analyses of Connexin 30 and 43 for Age-Related Hearing Loss

Jennifer Pineros

University of South Florida

Background

Connexin proteins (Cx) are essential for intercellular communication. Cx gene mutations have been linked to human syndromic and nonsyndromic deafness. Our previous work showed Cx30 downregulating with age in the stria vascularis (SV), but the role of other Cx's in age-related hearing loss (ARHL), as well as localization of Cx isoforms in the cochlea is still to be determined. We analyzed age-associated changes in Cx30 and Cx43, as well as investigating potential treatment options for ARHL.

Methods

CBA/CaJ mice were used: young adult (3 mon, n=6) and old (30 mon, n=6). Gene expression of Cx30 and Cx43 using SV and Organ of Corti (OC) samples was done with RT-qPCR. Auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE) were recorded to measure hearing changes. Western blots were used to measure Cx30 and Cx43 protein expressions in SVK-1 and HEI-OC1 cell lines for different chemicals treatments – hydrogen peroxide (H₂O₂), aldosterone (ALD), and melatonin (MEL). Similar treatments as in the cell lines were given for cochlear lateral network (LW) and OC organotypic cultures collected from rat pups (n=10). Confocal laser scanning imaging was used for Cx30 and Cx43 biomarker detection.

Results

Signal-intensity comparisons between the young adult and old cochlear samples using RT-qPCR and t-tests confirmed an upregulation for both Cx30 and Cx43 genes in the OC and downregulation for both in the SV. Dose-dependent treatment with H₂O₂ produced a decrease in protein expression for Cx30 in the SV. The treatments with ALD and MEL for both Cx proteins are underway. Preliminary data from the dose-dependent treatments of H₂O₂, ALD, and MEL showed all three chemicals impacted the expressions of both Cx proteins. These findings support further quantitative analysis for measuring the exact effect of each treatment. Moreover, confocal images confirmed the presence of both Cx proteins in the cochlea as well. Time-course experiments using SVK-1 cell samples treated with H₂O₂ have also been collected to analyze the protein expression differences at different intervals over 24 hours, which will be helpful for us to understand the molecular mechanisms of ARHL on connexin family expression levels in the peripheral auditory system.

Conclusions

Our study indicates a trend of down-regulation of both Cx genes in the SV and an upregulation in the OC with aging. The in vitro cell treatments currently appear to be good options to investigate changes among Cx expression levels and may result in possible treatments that will prevent or reverse ARHL. Western blot experiments for Cx30 and Cx43 using protective treatments, ALD and MEL, are ongoing. The long-term goal is to determine the mechanisms affecting the connexin family gap junction proteins during ARHL and the possible feasibility of potential treatments.

Support: NIH grant P01 AG009524.

PD 30.3 Age Related Apoptosis in CBA/CaJ Mouse Cochlea Can Be Emulated in Vitro Using Cochlear Cell Lines Treated With Hydrogen Peroxide

Robert Frisina

University of South Florida

Background

Cell loss is a biological hallmark of aging, including the aged cochlea. How cell loss contributes to cochlear age-related deterioration is partially understood, but greater insights into its mechanisms may lead to new strategic therapies to prevent the progression of presbycusis, a predominant age-related sensory disorder worldwide. Other than classical pathway guided apoptosis, alternative cell death programs such as apoptosis-inducing factor (AIF) can be mediated by non-caspase proteases as their regulators. We are interested in understanding whether exogenous hydrogen peroxide (H₂O₂) stimulation, simulating age-related oxidative stress, will lead to cell death induced by AIF activation.

Methods

CBA/CaJ mice were divided into three groups: Young adult, middle-aged and old, with equal amounts of males/females per group (N=6/group). Auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs) were recorded to measure hearing changes with aging. In vitro cell

treatments using SV-K1 and HEI-OC1 cell lines were performed using H₂O₂ with different experimental paradigms: dose-dependent and time-course. RT-qPCR was used to measure gene expression. Western blots and confocal laser scanning microscopy were used with immunohistochemistry for the analysis of biomarker changes including AIF pathway markers.

Results

We found that AIF translocation to the nucleus is increased in the cochlea with parallel hearing threshold elevations as aging occurs. Using Ligation-Mediated PCR (LM-PCR), an increase in DNA fragmentation was found in aged cochlear samples, suggesting that there is a possible link between AIF nuclear translocation and cell loss in the aged cochlea. Additionally, we examined if the presence of hydrogen peroxide (H₂O₂) concurrently induces cell death in cochlear cell lines (HEI-OC1 and SV-K1). Subjecting the cells to lower doses of H₂O₂ (10nM-100µM) induced increasing AIF nuclear translocation and DNA fragmentation (apoptosis) without caspase-dependent pathway activation. Further analysis of siAIF-transfected (AIF depletion) cells clearly demonstrated that nuclear translocation and condensation are the most important factors for cellular apoptosis as a result of H₂O₂ exposure.

Conclusions

Our results indicate that oxidative stress and the activation of the AIF caspase-independent pathway, resulting in AIF nuclear translocation and condensation, are associated with aging in the cochlea and therefore, is a probable biological mechanism for age-related hearing loss. The mechanisms by which cells undergo age-related and H₂O₂ stimulated (oxidative stress) AIF-mediated cell death is not completely clear at present, however efforts are being undertaken to increase our understanding so as to achieve the clinical goal of preventing the progression of presbycusis. Overall, our findings support the idea that oxidative stress and subsequent AIF nucleus distribution enhancement with aging are mechanisms underlying age-related hearing loss.

Supported by NIH-NIA grant P01 AG009524.

PD 30.4 Autophagic Concepts and Mechanisms of Age-Related Hearing Loss May Be Helpful to Remedy the Novel Coronavirus

Freyda Mannering
University of South Florida

Background

The coronavirus 2019 (COVID-19) pandemic has taken the world by surprise as it spread through the continents with rapidly increasing mortality rates. It is of the utmost urgency to study the effects of antiviral drugs such as hydroxychloroquine and remdesivir, since the FDA has approved these drugs for clinical trials. The autophagic pathway is a highly conserved cellular degradation and recycling process. Our previous study showed that aging processes promotes autophagy inhibition and auditory dysfunction. It is an essential factor for cell survival and hearing maintenance in mouse models. Likewise, we hypothesize that autophagy may play a role for survival and invasion of COVID-19.

Methods

Young adult (3 mon), middle age (12 mon) and aged (30 mon) CBA/CaJ mice served as auditory animal models. Cochlear cell lines SV-K1, HEI-OC1, and human lung epithelia cells (HBEpC) were utilized in different experimental paradigms: dose-dependent, time-course, and transduced by lentivirus mCherry-GFP-LC3 vectors pseudo-typed with spike proteins. Western blots and laser scanning confocal microscopy were used to identify signal proteins in the mTOR (mechanistic target of rapamycin) pathway including LC3, p62 and 4E-BP. An OriGene Lentivirus kit was used for the measuring infectivity of the virus. Ligation mediated PCR (LA-PCR) was used to detect DNA laddering (cell death biomarker assay).

Results

Apoptosis (programmed cell death) was detected in the aged cochlea and HEI-OC1 cells treated with chloroquine (CQ). Autophagy biomarkers, LC3II and P62, were increased in the old cochlea compared to young adult cochlea, similar to the results of our in vitro experiment with the autophagy inhibitor CQ in SV-K1 cells. Similarly, we found that remdesivir (Re) and hydroxychloroquine (HCQ) induced LC3II and 4E-BP

protein expressions increases in HBEPc cells compared with nontreated cells, indicating autophagy flux was blocked by interfering with the fusion between AP and AL, and we also observed that the mTOR pathway was activated. We infected the HBEPc cells with lenti-mCherry-GFP-LC3. Comparing the controls to the highest doses of Re and HCQ, both mCherry and GFP signals were decreased. mCherry signals are less than GFP signals in treated cells, suggesting Re and HCQ inhibit virus proliferation at the point of autolysosome formation. We collected the supernatants three days post-infection of lenti-mCherry-GFP-LC3, to assess viral production by the HIV p24 ELISA method. In both Re and HCQ treatment groups, viral concentrations had significantly decreased compared to the untreated controls.

Conclusions

The incidence of age-related cochlea autophagy dysfunction and Re/HCQ-induced viral autophagy inhibition suggests shared mechanisms for cell survival maintenance for normal hearing and COVID-19 virus spreading. Further characterization of the molecular mechanisms and biomarkers involved in regulating selective autophagy is valuable for drug development for new therapies.

Funding: Univ. of South Florida, NIH-NIDCD R21 DC017039, and NIH-NIA P01 AG009524.

PD 30.5 Age-Related Changes to the Size and Pigment Content of the Stria Vascularis in Mouse and Human Temporal Bones

Nicholas Andresen

Johns Hopkins Hospital

Background

The stria vascularis (SV) is a pigmented structure located in the lateral wall of the cochlea that generates the endocochlear potential (EP). Decreased EP has been linked to age-related hearing loss. Melanocytes are thought to be involved in the generation of the EP and are thought to convey antioxidant protection in the cochlea. Converging evidence shows that African Americans have a 40-60% lower risk of age-related hearing loss, and stria vascularis from older African American adults shows greater pigmentation compared to Caucasians (Sun et al. 2014). However, it remains unclear if increased cochlear pigmentation confers lifelong protection against hearing loss or if this finding indicates differential age-related changes in cochlear pigmentation. We studied changes to the size and pigment content of the stria vascularis in a mouse model of age-related hearing loss and compared those changes to pediatric and adult temporal bones.

Methods

Mouse temporal bone specimens were collected from CBA/CaJ strain juvenile (3-4 week) and adult (30-32 months) laboratory mice housed in a quiet environment. Pediatric and adult temporal bones of individuals who identified as Caucasian or African American were examined from the Johns Hopkins Temporal Bone Collection. Information regarding Fitzpatrick skin type were unavailable and self-identified race/ethnicity was used as a proxy. Images were taken using light microscopy at 20x magnification. Image J software (v1.53) was used to measure stria vascularis area and pigment content, calculated as a percent area of the stria vascularis.

Results

Among mouse specimens, stria vascularis area remained unchanged overall, but pigment content increased with age. Among human specimens, stria vascularis area decreased and pigment content increased with age. African American individuals had higher amounts of pigment within the stria vascularis, among both pediatric and adult populations.

Conclusions

Pigmentation in the stria vascularis increases with age in mouse and human temporal bone specimens. Individuals who identified as African American had higher pigment content within the stria vascularis both as children and as adults. These results suggest that pigment within the stria vascularis may have a protective role against age-related hearing loss. Our findings also underscore the need for representation of participants who reflect the racial/ethnic diversity of the aging population both within temporal bone studies and hearing-related studies more broadly.

PD 30.6 Cochlear Inflammation in CBA/CaJ Mice Increases During Age-Related Hearing Loss

Ben Seicol

The Ohio State University

Background

Age-related hearing loss (ARHL) is a major hearing impairment in patients that is widely studied in research animals. Hair cells in the cochlea transduce mechanical sound waves into electrical signals, and specialized ribbon synapses reliably transmit auditory information to the peripheral terminals (cochlear synapses) of spiral ganglion neurons. Loss of hair cells and cochlear synapses lead to sensorineural hearing loss in ARHL. CBA/CaJ mice exhibit late-onset, human-like progression of hearing loss during normal aging making them a fitting model for studying ARHL in mice. Cochlear macrophages and circulating monocytes drive cochlear inflammation following physical and chemical insults to cochlea, and in humans, cochlear inflammation has recently been confirmed in post-mortem tissue samples of aged patients. However, well-controlled animal studies of cochlear inflammation in late-onset ARHL are needed to advance our mechanistic understanding of the contribution of inflammation to sensorineural hearing loss in aging.

Methods

To address this, we have conducted a series of immunohistochemical studies examining cochlear inflammation in young (~2-3 months) and aged (~30 months) CBA/CaJ mice.

Results

Iba1-labeled resident macrophages in the cochlea show increased area and elevated levels of CD68 with age, indicating increased activation.

Conclusions

This study confirms that CBA/CaJ mice are a suitable model for studying inflammation in ARHL, and our results suggest that increased cochlear inflammation during aging may contribute to the pathophysiology of ARHL.

PD 30.7 Hair-Cell Neurotransmission Modulates Neomycin Susceptibility

Daria Lukasz

NIH

Background

The aminoglycoside antibiotic neomycin is effective at eliminating life-threatening infections. Neomycin is also a potent ototoxin that has been shown to 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 ototoxins. 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. Upon activation, hair cells rely on mechanotransduction and neurotransmission to convert stimuli into a chemical signal. Although previous work has implicated the metabolic demands of mechanotransduction in ototoxin susceptibility, the contribution of neurotransmission to this phenomenon is unknown.

Methods

For our studies we used larval zebrafish, which offer several advantages over rodent models. Larvae are transparent and possess externally located lateral line hair cells. These features allow for easy in vivo and in toto imaging of individual cells following bath pharmacological or fluorescent dye treatment. We used these approaches to examine *cav1.3*^{-/-} and *otoferlin*^{-/-} zebrafish mutants. *CaV1.3* is required for presynaptic calcium influx and calcium-dependent vesicle fusion at the hair cell synapse. The protein *otoferlin* is a calcium sensor that couples presynaptic calcium influx with vesicle fusion. These mutants enable us to investigate the contribution of hair cell neurotransmission to neomycin susceptibility.

Results

We found that both cav1.3^{-/-} and otof^{-/-} mutants exhibit significantly augmented hair cell survival relative to wildtype sibling controls when challenged with neomycin across multiple concentrations. Transient block of Cav1.3 channels with the antagonist isradipine during neomycin exposure has no effect, which indicates that prolonged loss of neurotransmission is required for protection. Furthermore, we utilized the cytosolic ROS indicator cellROX Orange and the mitochondrially-localized ROS indicator mitoTimer to reveal a significant reduction in oxidative stress in both mutants relative to controls. The mitochondrial potential indicator TMRE also revealed reduced mitochondrial activity in both cav1.3^{-/-} and otof^{-/-} mutant hair cells likely resulting from reduced ATP demands.

Conclusions

These results suggest that neurotransmission contributes to hair-cell neomycin susceptibility. In neurotransmission-deficient mutants, a reduction in neomycin susceptibility correlates with reduced mitochondrial activity levels and reduced mitochondrial ROS byproduct accumulation. The finding that neurotransmission at the level of vesicle fusion is highly energy demanding has only recently been demonstrated in neurons and now appears to hold true in hair cells. This work brings us closer to creating targeted therapies to prevent hearing loss, particularly in the context of slow long-term damage associated with aging or presbycusis.

12:30 p.m. - 2:30 p.m.

Symposium 31

SYMP 31. Integrating Biology to Innovate Hearing Restoration: How Young Investigators Can Shape the Future

Chair: Jennifer Harre
Hannover Medical School

SYMP 31.1 Understanding the Pathophysiology of Hearing Loss to Identify Novel Therapeutic Targets

Ellen Reisinger
University of Tuebingen Medical Center

New therapeutic strategies are emerging to ameliorate hearing disorders. Gene editing, cell replacement and gene replacement strategies are being developed, all of which provide causal therapies for hearing impairment. Which strategy is most suitable for a given form of deafness? Basic research, unravelling the pathophysiology of each hearing disorder, is detrimental to develop a targeted strategy against hearing loss. Furthermore, for any form of deafness, a detailed knowledge of the sites of lesion in the auditory system helps to validate the efficiency of a therapeutic approach.

SYMP 31.2 Mammalian Inner Ear Development and Stem Cell Biology at Single Cell Resolution

Taha Jan
UCSF

State of the art single cell transcriptomics technology has revolutionized the fundamental understanding of biological processes, including stem cell biology and developmental dynamics. Recently, these techniques have been applied to the mammalian inner ear in both mice and humans to gain an in depth understanding of the diversity of cells and their fates during development. The next phase of discovery involves diseased and damaged states of mammalian tissues using single cell transcriptomics to gain new molecular insights into responses after damage and identification of targets for potential therapeutics.

SYMP 31.3 Towards Personalized Medicine Through Advancing Molecular Genetic Diagnostic Approaches

Barbara Vona

Tübingen Hearing Research Centre, Eberhard Karls University Tübingen

The post-genomic era has delivered a remarkable flood of knowledge about the genes and mutational signatures involved in hereditary hearing loss. This breakthrough has enabled remarkable navigation through genetically heterogeneous hearing loss and has come with its own set of new challenges. We present our experience with routine molecular genetic diagnostics for hearing loss using a series of approaches that range from targeted gene panels to genome sequencing and the integration of RNA-seq as a companion diagnostic approach for achieving unparalleled information about the transcriptome.

SYMP 31.4 Lentiviral Vector-Based Gene Therapy to Treat Hereditary Forms of Hearing Loss

Juliane Schott

Hannover Medical School

Genetic causes can make up to 50% of sensorineural hearing loss (SNHL) cases. Thus, application of gene therapy to provide an intact gene copy is an attractive option to treat SNHL. We have established a lentiviral vector production and administration protocol that allows efficient in-vivo transduction of cochlear cells, including hair cells and spiral ganglion neurons. Following a systematic approach, we currently identify the most suitable pseudotype and promoter variants and develop strategies for specific cell targeting. First results demonstrate the success of lentiviral gene therapy by improved hearing and balance scores in a relevant murine knock-out disease model.

SYMP 31.5 A Novel Composite Coating for Neuronal Electrodes: Nanoporous Platinum Containing Nanoporous Silica Nanoparticles

Mosaieb Habib

Leibniz Universität Hannover

Platinum is currently used as electrode material within the cochlear implant (CI). By utilizing a composite material, composed of nanoporous silica nanoparticles (NPSNPs) enclosed within the pores of nanoporous platinum (NPpT) as a drug delivery system on the surface of the CI electrode, residual spiral ganglion neurons and cochlear hair cells could be preserved. Silica-core-polystyrene-shell-nanoparticles as hard template particles were deposited into which interspaces porous platinum is deposited via electrodeposition. The novel material combines the favorable properties of NPpT and NPSNPs, specifically the excellent electrochemical behavior simultaneously with the large pore volume and a variety of possible surface modifications.

SYMP 31.6 Does CI Stimulation With Uninformative Interaural Time Differences (ITDs) Prevent the Development of ITD Sensitivity?

Nicole Rosskoth-Kuhl

University Medical Center Freiburg

Early deafened patients with cochlear implants (CI) show poor sensitivity for interaural time differences (ITDs), an important cue for sound localization and speech comprehension. Current CI processors are unable to synchronize their internal clock binaurally resulting in inconsistent delivery of ITD cues when electrically stimulating the auditory system and thus preventing the “normal” development of ITD sensitivity through reliable exposure. We investigate to what extent varying, uninformative presentation of ITDs interferes with the development of ITD sensitivity in hearing inexperienced CI users and whether this is reversible by delivering bilaterally synchronized, informative ITDs.

12:30 p.m. - 2:30 p.m.

Symposium 32

SYMP 32. From Bench to Bedside – Translational Oto-Therapeutic Approaches

Chair: Monica Serban
University of Montana
Co-Chair: Rebecca Lewis
Whisper

SYMP 32.1 Validating Candidate Otoprotective Therapeutics in Medically-Relevant Preclinical Models

Peter Steyger
Creighton University

Until recently most ototoxicity studies were conducted in healthy preclinical models that did not reflect the clinical settings in which these medications were used. For example, systemic bacterial infections treated with aminoglycoside antibiotics also induce severe inflammation. Recent preclinical studies have shown that inflammation potentiates aminoglycoside-, and cisplatin-induced cochleotoxicity. These data indicate the importance of validating the otoprotective efficacy of candidate pharmacotherapeutics in medically-relevant preclinical settings. This will increase the prospect of candidate otoprotective therapeutics advancing to clinical trials while also de-risking the investment in such clinical trials. Other challenges in translating candidate othotherapeutics to market will be discussed.

SYMP 32.2 Translational Approaches for the Management of Cytomegalovirus Mediated Sensorineural Hearing Loss

Albert Park
University of Utah

Cytomegalovirus (CMV) is an underappreciated but important cause of pediatric hearing loss. Longitudinal studies have reported CMV infected children becoming deaf. An unmet need is an effective treatment that will prevent this loss. We have developed a murine model for CMV and reported that the antiviral drug, ganciclovir mitigates hearing loss during active infection. We are currently embarking on an NIH funded international multi-institutional placebo controlled clinical trial to determine the efficacy and safety of an oral form of ganciclovir in CMV infected children with isolated hearing loss. We will discuss preliminary findings and future directions for treatment.

SYMP 32.3 Othotherapeutics for Childhood Hearing Loss: Needs and Challenges

Dylan Chan
University of California, San Francisco

Childhood hearing loss can be present at birth or develop postnatally and differs significantly from adult-onset hearing loss with respect to etiology, feasible strategies for drug delivery, and functional impact. Thus, othotherapeutic development targeted toward adult indications and delivery methods may be ineffective or impractical for translation to pediatric populations. In this presentation, I will use two common examples of hearing loss in children - sensorineural hearing loss due to cisplatin, and to enlarged vestibular aqueduct - to illustrate the challenges and unmet needs for othotherapeutics in pediatric hearing loss.

SYMP 32.4 Bridging the Gap Between Researchers and Patients in an Academic Setting

Lukas Landegger

Vienna General Hospital, Medical University of Vienna

As a practicing otolaryngologist who took a break from clinical work for six years to pursue a PhD and a postdoctoral fellowship, I was fortunate enough to interact with some of the best role models in the field of hearing research in seven countries. After carrying out in vitro and ex vivo work, animal studies with diverse species like mice, gerbils, guinea pigs, sheep, and non-human primates in addition to retro- and prospective clinical studies, I proceeded to combine my clinical work with establishing a research group. In this session, I hope to elaborate on academic research at several levels.

SYMP 32.5 Lost in Translation: Overcoming Obstacles to Advance the Field of Inner Ear Therapeutics

Katharine Fernandez

NIDCD

Millions of cancer survivors struggle with the social and emotional toll of cisplatin-induced hearing loss. We recently showed that the cholesterol-lowering drug atorvastatin is associated with reduced hearing loss in patients undergoing cisplatin therapy to treat head and neck cancer. I will discuss the challenges we encountered in this drug-repurposing project and some strategies that helped us address those challenges. I will then discuss the obstacles we still face as we work to bring this potential therapy to the clinic and suggest some resources that might facilitate translation of basic discoveries into clinical therapies for ototoxic drug-induced hearing loss.

SYMP 32.6 From Research to Preclinical Development: Translating In Vitro/Ex Vivo Research Into Clinically Relevant In Vivo Studies for Hearing Loss

Bonnie Jacques

Otonomy Inc.

One of the hurdles in drug development is understanding the ability of in vitro or ex vivo models to predict in vivo or clinical efficacy. This includes how severe a particular damage model may be, what level of efficacy is meaningful as well as the best method for safely delivering a drug or obtaining sufficient drug levels in desired tissues. Some of these preclinical aspects will be discussed as well their implications for regulatory approvals and future clinical studies based on Otonomy's experience in otic drug development from research to commercialization, using specific examples from current hearing loss programs.

SYMP 32.7 Translation of Genetic Medicines for the Inner Ear

Michelle Valero

Akouos Inc.

Individuals with genetic hearing loss have limited treatment options. While cochlear implants and hearing aids can improve hearing and quality of life, the field has yet to advance a therapeutic alternative to restore physiologic hearing. Several gene therapy approaches show striking efficacy in animal models of genetic hearing loss, but translation to the clinic requires the development of a new route of administration. Here we will review Akouos's precision genetic medicines platform, using a novel delivery approach for direct intracochlear administration of therapeutic nucleic acids to the ear using the Anc80 variant of adeno-associated viral vector.

12:30 p.m. - 2:30 p.m.

Symposium 33

SYMP 33. Age-Related Plasticity Changes in the Central Auditory System

Chair: Robert Frisina

University of South Florida

Co-Chair: Josef Syka

Institute of Experimental Medicine, ASCR

SYMP 33.1 An Alternative Aging Model Produces Central Auditory Deficits That Precede Peripheral Deficits

Edward Bartlett

Purdue University

Oxidative stress likely contributes to presbycusis, and oxidative stress from hyperglycemia is known to cause hearing deficits. We investigated D-galactose induced hyperglycemia in young adult rats using auditory evoked potentials that are sensitive to aging. Animals were evaluated at baseline, nine weeks afterwards, and nine months afterwards. Tone thresholds were unaffected by D-galactose at nine weeks but declined significantly more than in normal aging after 9 months, whereas later ABR waves and AM following responses declined after 9 weeks. This model produces a window of central auditory decline without peripheral decline, decoupling electrophysiological markers of cochlear damage from central dysfunction.

SYMP 33.2 Age-Related Reductions in Temporal Salience Engage Top-Down Predictions in Auditory Thalamus

Donald Caspary

Southern Illinois University School of Medicine

To understand speech in challenging environments, older adults increasingly use top-down cognitive and contextual resources. MGB integrates ascending signals and descending predictions, gating auditory representations based on salience and context. MGB units show age-related increases in preference for predictable modulated (SAM) stimuli. We tested if degrading the temporal reliability of SAM stimuli in young rats would increase top-down prediction coding and if this was reversed by blocking corticothalamic projections. Reducing SAM salience increased prediction coding which was reversed by blockade of top-down projections. These findings support the hypothesis that less salient acoustic signals engage top-down resources to disambiguate acoustic signals.

SYMP 33.3 Age-Related Changes in the Processing of Temporal Parameters of Acoustical Signals in the Auditory Cortex

Josef Syka

Institute of Experimental Medicine, ASCR

Age-related changes in neural temporal processing were found in the rat auditory cortex. When stimulated with wide-band sounds, such as broad-band noise or clicks, the aged auditory system of experimental animals exhibited a decline in the precision and reliability of both the rate code and temporal code. However, in response to sinusoidal or nearly sinusoidal auditory stimuli, the aged animals appeared to have the same or even an improved processing ability as young adult animals. In addition to this, differences were observed between the reactions of neurons in the left and right auditory cortices, both in young adult and aged animals

SYMP 33.4 Neural Plasticity in the Aged Auditory System Induced by Exposure to Spectral and Temporal Augmented Acoustic Environments

Joseph Walton
University of South Florida

There is growing evidence that targeted auditory training results in neural plasticity and can aid in the treatment of central auditory-processing disorders in children and young adults. These studies use auditory training or passive listening to modulate neural plasticity. However, there is a paucity of reports which have examined the effects of augmented acoustic environments on hearing function for age-related hearing loss (ARHL). Here, we use augmented acoustic environments having various acoustic characteristics that span spectral and temporal domains in an attempt to improve neural correlates of central auditory processing in animal models of ARHL.

Supported by NIH (Grant #P01-AG009524)

SYMP 33.5 Of Mice and (Wo)Men: Alterations in Central Auditory Nervous System Function Across Aging Animal Species

Sandra Gordon-Salant
University of Maryland

The Neuroplasticity and Auditory Aging project conducts corresponding investigations with mouse, ferret, and humans of the impact of auditory/cognitive training paradigms on alterations in neurophysiology in central auditory pathways and behavior with aging. This presentation will share initial results that quantify these age-related alterations prior to intervention. The findings range from changes in cortical connectivity in aging mice to alterations in reconstructions of degraded speech in auditory cortex in aging ferrets and humans. Similarly, psychophysical discrimination of and steady-state responses to pulse rates are altered by the aging process in humans. Implications will be discussed. Supported by NIH (Grant #P01-AG055365)

SYMP 33.6 Effects of Age on Binaural Processing: The Relative Contributions of Peripheral and Central Processing

Brian Moore
University of Cambridge

Several aspects of binaural processing worsen with increasing age, including the detection of differences in the temporal fine structure and the envelopes of sounds at the two ears (more so for the former) and binaural unmasking. However, the relative contribution of peripheral and central processes to the effects of age is unclear. The evidence pertaining to this issue will be reviewed. It is concluded that both peripheral and central processes play a role. In addition, the ability to use binaural information for auditory scene analysis depends on cognitive abilities, which tend to decline with increasing age.

SYMP 33.7 Age-Related Auditory Declines and Perceptual Decision-Making for Speech Recognition in Noise

Kenneth Vaden
Medical University of South Carolina

Speech recognition in noise is hypothesized to benefit from perceptual decision-making processes, which gather sensory evidence until reaching a decision criterion for recognition. We have observed that lower speech intelligibility can result in slower evidence accumulation, and lower criteria for faster recognition. Our new neuroimaging findings suggest that cingulo-opercular activity can benefit speech recognition by adjusting decision criteria for expected difficult listening conditions. We will discuss how these findings relate to hearing loss and central auditory declines that can slow evidence accumulation, and compensatory criteria

adjustments that could produce more extensive frontal activity for older adults during challenging auditory tasks.

3:00 p.m. - 5:00 p.m.

Podium 34: "Ototoxicity, Noise Damage and Protection"

Moderators: Dr. Lavinia Sheets and Dr. Sharon Kujawa

Category: Inner Ear: Damage & Protection

PD 34.1 Cdkn2a Deletion and Ototoxic Insults Independently and Synergistically Promote Glial Cell Proliferation in the Inner Ear

Kendra Stansak

University of Mississippi Medical Center

Background

Glial cells play critical roles throughout the nervous system but are generally less studied than neurons. In addition to their more generalized neurotrophic functions, some glial cells act as progenitors which can give rise to neurons or glia, and many glial cells retain the ability to proliferate well into adulthood. Thus, glial cells represent a cell pool that could be used to promote the survival and possibly regeneration of non-regenerative neurons. However, when glial cell proliferation is unchecked, it can lead to tumorigenesis. Due to the fact that non-regenerative eighth cranial nerve neurons can be lost to various insults including atrophy subsequent to hair cell loss, and due to the fact that schwannomas can arise in or around the eighth cranial nerve, there is an imperative need to better understand the genetic and environmental factors that influence glial cell proliferation in the inner ear. The Cdkn2a locus gives rise to two different tumor suppressor proteins. Accordingly, loss of function mutations or deletion of Cdkn2a exons results in increased cellular proliferation in a number of tissues. However, the effect of Cdkn2a deletion on peripheral glial cells, or on any cells of the inner ear, is not well elucidated.

Methods

In the current study, young adult (P30) male and female Cdkn2a knockout mice and their wild type counterparts were given saline vehicle or subjected to ototoxic damage by furosemide and kanamycin treatment. Mice were provided with the thymidine analog BrdU in their drinking water for 2 weeks.

Results Vehicle-treated Cdkn2a knockout mice demonstrated a modest, but significant, increase in cellular proliferation throughout the modiolus compared to wild type littermates. Mice injected with furosemide and kanamycin exhibited greater levels of cell proliferation compared to vehicle injected mice; and within this ototoxic condition, proliferation was significantly greater in Cdkn2a-null mice. Cells were labeled with antibodies against proliferating cell nuclear antigen (PCNA) and markers for glial cells (S-100), macrophages (CD68) and neurons (Hu-D and Tuj1). While some PCNA positive cells appeared to be CD68 positive macrophages, the vast majority of proliferating cells were S100 positive suggesting that they are glial. In only one of the experimental groups, the kanamycin treated Cdkn2a-null cohort, were any proliferative cells co-labeled by the neuron-specific antibodies Tuj1 and Hu-D., but these were rare.

Conclusions

The data therefore suggest that, while Cdkn2a deletion or kanamycin ototoxicity can each cause a moderate increase in glial cell proliferation in the inner ear, the combined effect of these two manipulations is a robust, synergistic increase in proliferation. These results and future directions will help us to better understand molecular and environmental causes of glial cell proliferation in the inner ear with potential implications for vestibular schwannomas and possibly neuronal regeneration.

PD 34.2 Cisplatin Induces Aberrant Stress Granules: Implications for Ototoxicity

Jack Martin

UCL Ear Institute

Background

Cisplatin is a highly effective platinum-based chemotherapeutic used to treat a variety of solid tumours. There are however several serious dose limiting side effects such as, neurotoxicity, nephrotoxicity and ototoxicity. Hearing loss developed by patients undergoing chemotherapy with cisplatin is a bilateral, progressive, and irreversible sensorineural hearing loss. The mechanisms underlying cisplatin ototoxicity are not fully understood and there is no effective treatment for the prevention of cisplatin induced hearing loss. Cisplatin kills rapidly dividing cells, such as those in tumours by forming DNA adducts that are recognized by the cell, leading to apoptosis. However, the cochlear cells damaged by cisplatin treatment undergo little or no proliferation, which suggests a cisplatin-protein interaction rather than a cisplatin-DNA interaction as a possible cause of the toxicity of cisplatin to these cells. There are multiple proteins that have been shown to interact with cisplatin, several of which are involved in the stress granule (SG) response. We previously identified the role of SGs in protecting against aminoglycoside antibiotic induced ototoxicity (Goncalves et al. 2019). Therefore, here we investigated the assembly and clearance of SGs in response to cisplatin to assess its possible impairment by sequestering of SG proteins by cisplatin.

Methods

Two cell lines, mouse cochlea-derived UB-OC2s and human retinal pigment epithelial RPE-1s were treated with 100µM cisplatin for 24hrs before fixation and immunostained with the SG markers Caprin1 and HuR. To assess SG clearance, cells were allowed to recover in fresh medium for up to 24hrs before fixation. Cisplatin induced SGs and their rate of clearance were compared to SGs induced by sodium arsenite, an inducer of oxidative stress.

Results

Cisplatin induced SGs were significantly ($p < 0.001$) fewer and smaller than arsenite induced SGs in both cell lines. Additionally, cisplatin induced SGs were persistent 24hrs after removal of cisplatin, in stark contrast to arsenite induced SGs that were cleared within 2hrs after removal of arsenite. Cisplatin treated cells were also unable to mount a stress granule response to subsequent arsenite or heat shock exposure.

Conclusions

The reduced size and quantity of SGs induced by cisplatin and their persistence compared to arsenite induced SGs, implicates an impaired SG response to cisplatin possibly due to impaired activity of one of the previously identified cisplatin binding proteins involved in SG assembly/clearance. Furthermore, the inability of cisplatin treated cells to mount a SG response to subsequent stress stimuli suggests cisplatin could sensitize cochlear cells to future insults by compromising the protective function of stress granules. Manipulation of the SG pathway may provide a novel target for therapeutics preventing cisplatin ototoxicity.

PD 34.3 Circadian Rhythms Effect of Gentamicin Ototoxicity: A Zebrafish Study

Alejandro Garcia

Massachusetts Eye and Ear

Background

Aminoglycosides are broad-spectrum antibiotics commonly used in humans due to its high bactericidal effectiveness, large availability and low cost. However, one of the main side effects is the damage to sensory hair cells of the inner ear which induces sensorineural hearing loss (SNHL). The zebrafish lateral line contains hair cell-bearing organs called neuromasts that are homologous to hair cells of the mammalian inner ear. Hence, zebrafish is a well-known model to study ototoxicity. In rodent model's ototoxicity severity varies with circadian time, being higher during the day when the animal is resting. Therefore, extrapolating these results to humans is difficult given the rodent's active nocturnal nature. The present study seeks to determine if there are variations in the toxicity of hair cells produced by the administration of gentamicin at different times of the day in the diurnal zebrafish model.

Methods

A total of 448 Zebrafish larvae (TABS type) 5 dpf were treated with gentamicin for 30 minutes at different dosages (100, 250, 400 uM) in 7 different circadian times (ZT1, ZT5, ZT9, ZT13, ZT17, ZT21, ZT1', ZT0 = Lights On). Structural integrity of 10 neuromasts (SO1, SO2, IO1-IO4, M2, MI1, MI2, O2) were evaluated with DASPEI dye under fluorescence microscope according a semiquantitative damage scale (0 to 4 points

per neuromast). Functional neuromasts integrity were analyzed using a vibratory startle response test (distance moved one second after stimulus) using a DanioVision equipped with a tapping device. For the statistical analysis, a multivariate ANOVA was used to evaluate cellular damage among the three different gentamicin doses and the control. A t-test was used to evaluate the differences in the distance traveled by each fish after a startle response.

Results

The zebrafish presented higher cellular structural damage when exposed to a gradual increase dosage of gentamicin ($p < 0.001$). The 400uM dose had significant effects compared to the two lower dosages ($p < 0.001$). The zebrafish vibratory startle response vary according to circadian times, showing a peak at ZT13 and a valley at ZT9. The startle response curves were similar for the animals treated with gentamicin showing a circadian pattern. There were shorter distances traveled at night for the 400 uM larvae compared to the lower dosage-treated larvae (ZT17 vs. ZT9, $p < 0.05$). Animals treated with gentamicin 400uM at night did not have a peak reaction compared to those treated during the day, indicating severe tissue damage.

Conclusions

Aminoglycosides cause damage to zebrafish neuromasts in a dose dependent manner. The animals startle response follows a circadian rhythm. Animals treated with high dose of gentamicin do not show the typical peak reaction at night. These results suggest that ototoxicity could vary with the time of administration in diurnal animals, which could be extrapolated to humans.

PD 34.4 Characterization of Quinoxaline Derivatives for Protection Against Latrogenic-Induced Hearing Loss

Marisa Zallocchi

Creighton University School of Medicine

Background

Hair cell death is the leading cause of hearing and balance disorders in humans. It can be caused by many factors including noise, aging, and therapeutic agents. As society becomes more technologically advanced and the availability of novel therapeutic compounds with ototoxic side-effects increases, so does the threat to the human hearing health. Previous studies have shown the therapeutic potential of quinoxaline against drug-induced ototoxicity.

Methods

We synthesized and screened a library of 68 quinoxaline derivatives for protection against aminoglycoside-induced damage of hair cells from the zebrafish lateral line.

Results

We identified Qx28 as the best quinoxaline derivative that provides robust protection, against both aminoglycosides and cisplatin in zebrafish and mouse cochlear explants. FM1-43 uptake and antibiotic efficacy studies reveal that Qx28 is neither blocking the mechanotransduction channels nor interfering with aminoglycoside antibacterial activity, suggesting that it may be protecting the hair cells by directly counteracting the ototoxin's mechanism of action. Finally, we assessed the regulation of NF- κ B pathway in vitro in mouse embryonic fibroblasts and in vivo in zebrafish larvae. Those studies showed that Qx28 protects hair cells by blocking NF- κ B canonical pathway activation.

Conclusions

Qx28 is a promising and versatile otoprotectant that can act across different species and toxins.

Funding: NIH-R01DC015444, NIH-R01DC015010, USAMRMC-RH170030, ONR-N00014-18-1-2507, LB692/Creighton, Bellucci Foundation Award. University of Nebraska Medical Center, Advanced Microscopy Core Facility (Fred and Pamela Buffett Cancer Center Support Grant -P30CA036727, Institutional Development Award (IDeA) from the NIGMS of the NIH -P30GM106397, the Nebraska Research Initiative Grant and Nebraska Center for Cellular Signaling CoBRE -NIH P30GM106397.

PD 34.5 Effects of Acoustic Trauma of Varying Strength on Threshold Shifts, Synaptic Ribbons and Glutamatergic Postsynapses in Inner Hair Cells of C57Bl6/N Mice

Inner Ear: Damage & Protection

Philipp Schätzle¹, Philip Derleder¹, Pauline Schepsky¹, Fahmi Nasri¹, Philipp Fischer¹, Kerstin Blum¹, Simone Kurt¹, Jutta Engel^{*1}

¹Saarland University

Background

In noise-induced cochlear synaptopathy, synapses between inner hair cells (IHC) and spiral ganglion neurons are irreversibly lost after noise trauma despite normal hearing thresholds. Broadband noise (8-16 kHz) at 100 dB SPL for 2 h damage part of the synaptic connections in the high-frequency region of the cochlea without causing a permanent threshold shift (PTS) in CBA/CaJ mice (Kujawa and Liberman, 2009). Here we analyzed how noise traumata of varying strength affected the synaptic connections of IHCs, the number of OHCs, and hearing performance along the entire frequency range in C57Bl6/N mice.

Methods

Eight-week-old mice of both sexes were exposed to 100, 106, or 112 dB SPL broadband noise (8-16 kHz) for 2 h. The shift in hearing thresholds determined with auditory brainstem response (ABR) audiometry was assessed directly after and up to 28 days post trauma. Thereafter, the number of IHC synapses, specifically the pairing of presynaptic ribbons (CtBP2) and postsynaptic AMPA receptor scaffold (HOMER-1) clusters, was analyzed along the cochlear length in whole-mount preparations of the organ of Corti. Further, the number of OHCs was determined.

Results

Four weeks after a trauma of 106 dB SPL, a PTS was observed in the high-frequency region that extended towards the middle-to-low-frequencies for the 112-dB trauma. Growth functions of ABR wave I were strongly reduced in the high frequency region for both the 106- and 112-dB SPL trauma. OHC loss was negligible compared with unexposed control mice. Synaptic pairs were reduced in the midbasal and basal cochlear region in all trauma conditions. In contrast to CBA/CaJ mice, orphan ribbons were rare while orphan HOMER-1-positive postsynapses were frequent in all trauma conditions.

Conclusions

These data confirm the susceptibility of ribbon synapses and ABR wave I amplitudes to a noise trauma of ≥ 100 dB SPL. Surprisingly, peripheral dendrites were frequently preserved despite trauma-induced loss of part of the IHC synapses. This indicates that peripheral dendrites bearing IHC postsynapses are less vulnerable than presynaptic ribbons in C57Bl6/N mice.

Supported by DFG SFB 894 (A8 to J.E.) and IRTG 1830/2 (P1 to J.E.)

PD 34.6 IGF-1 via PI3K/AKT Activation Promotes Survival and Constructive Metabolism in HEI-OC1 Auditory Cells

Ángela García-Mato

Instituto de Investigaciones Biomédicas "Alberto Sols"

Background

Insulin-like growth factor 1 (IGF-1) is an anabolic hormone that promotes survival in otic progenitors and auditory cells. Mutations in the IGF1 gene cause human sensorineural hearing loss (1). Null Igf1 mice are deaf and show early apoptosis in the neurosensory cochlear cells (2). Actions of IGF-1 are mediated by binding to its high affinity transmembrane receptor, IGF1R. This interaction typically leads to the activation of the PI3K-AKT pathway and of the MAPK-ERK cascade (3).

Methods

To gain insight into the molecular mechanisms involved in IGF-1 downstream signaling in the sensory hair cells, we have used the HEI-OC1 cell line derived from the ImmortomouseTM hearing receptor (4). We studied the actions and main downstream signaling pathways of IGF-1. Apoptosis and cell viability were studied by

flow cytometry and XTT assay, respectively, whilst activation of target proteins was measured by Western blotting. To further characterize the molecular actions of IGF-1, autophagy flux and metabolic pathways were analyzed by Western blotting and by live microscopy. The consequences of blockage of IGF-1 actions were also investigated by using specific IGF1R inhibitors and XTT assay.

Results

The study of the relative expression of genes of the IGF system by RT-qPCR showed that IGF system factors and receptors are expressed in both progenitors and differentiated auditory cells. In auditory progenitor cells, IGF-1 engagement with its receptor, IGF1R, activated AKT/mTORC1 signaling to increase survival, proliferation and constructive metabolism, such as glycogen storage and protein synthesis, and reduce autophagic flux and apoptosis. In striking contrast to the proliferative state, differentiating HEI-OC1 auditory cells maintained active anabolism and enhanced autophagic flux, even in the presence of IGF-1, which triggered anabolic signaling through mTORC1 but did not affect autophagy. Finally, the response to ototoxic agents was explored to establish the otoprotective potential of IGF-1.

Conclusions

Our data indicate that HEI-OC1 cells can be used as a model to understand the actions of IGF-1 in hair cells, to identify novel targets and to unravel the molecular mechanisms involved in IGF-1 deficiency-associated otic damage.

Funding

This work was supported by Spanish FEDER/SAF2017-86107-R-HEARCODE to IVN. AGM holds an FPU fellowship (FPU16/03308; MECD), BC held a Marie Curie contract and LR-dR has a contract supported by CIBERER (Institute of Health Carlos III).

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PD 34.7 Transcriptomic Drug Screening Identifies Niclosamide, an FDA-Approved Drug Against Cisplatin-and Noise-Induced Hearing Loss

Sarath Vijayakumar

Creighton University School of Medicine

Background

Acquired hearing loss due to chemotherapy and noise exposure is a major health problem affecting millions of people worldwide. Cisplatin is one of the most effective platinum-based chemotherapeutic agents for the treatment of various types of malignancies, including head and neck, ovarian, and lung cancer. Despite its efficacy, cisplatin treatment often causes permanent drug-induced hearing loss from damage to the inner ear sensory cells and neurons. Noise-induced hearing loss is the second most common form of sensorineural hearing impairment, after presbycusis. However, there are no FDA-approved drugs for the treatment of cisplatin- or noise- induced hearing loss creating an imperative need for new treatments to diminish the ototoxicity of these insults.

Methods

We derived a cisplatin-resistant transcriptome profile from 15 cancer cell studies and conducted a high-throughput in silico screen for over 4,000 compounds using the L100CDS2 small-molecule database. Of 500 hits that we identified to be associated to cisplatin resistance, we further tested 42 top compounds in HEI-OC1 and zebrafish models to determine dose-response curves. Our top-hit compound niclosamide had a low IC50 and high efficacy in both models. Niclosamide

was then tested in an FVB mouse model exposed to 30 mg/kg of cisplatin, and ABR and DPOAE was recorded. Additionally, we assessed the otoprotective effect of niclosamide against 2-hour exposure to 100 dB SPL 8-16 kHz octave band noise in FVB mouse model of noise-trauma.

Results

Our in silico screen revealed over 500 small-molecule hits, with 42 of those compounds having an overlap score of 10% with the cisplatin-resistant phenotype. Dose-response curves and IC50s of each compound were then measured in an HEI-OC1 cell model. Our top-hit candidate niclosamide demonstrated both full protection and low IC50 (171 nM). Additionally, our zebrafish screen of these 42 compounds revealed niclosamide is efficacious against cisplatin-induced neuromast damage. After treatment with 30 mg/kg of cisplatin, niclosamide when injected IP at 10 mg/kg, demonstrated significant protection against cisplatin-induced hearing loss in adult FVB mice (~20 dB protection at 8, 16, and 32 kHz frequencies). Additionally, when the same dose was administered prophylactically niclosamide significantly reduced the permanent threshold shifts after noise-trauma by ~20 dB at octave-step frequencies tested in 8-64 kHz range in the drug treated group when compared to the vehicle treated group. Preliminary results based on cochlear immunoblots suggest that niclosamide modulates multiple signaling pathways. Studies are ongoing to delineate the possible mechanism of action for niclosamide's otoprotection.

Conclusions

Niclosamide has demonstrated protection against both noise and cisplatin ototoxicity in multiple models. As a repurposed FDA-approved drug, we see potential for expedition through the FDA-approval process and entry into clinical trials for both cisplatin- and noise-induced ototoxicity. This work was supported in part by NIH-R01DC015444, NIH-R01DC015010, 1R43DC018762-01, USAMRMC-RH170030, ONR-N00014-18-1-2507, and LB692/Creighton.

PD 34.8 Influence of Mitochondrial Homeostasis on Mechanically Induced Hair-Cell Damage and Synapse Loss in the Zebrafish Lateral Line

Melanie Holmgren

Washington University in St. Louis

Background

Sensory hair cells rely on mitochondria to produce enough energy to meet the high metabolic demands of mechanotransduction and synaptic transmission, as well as contribute to the homeostatic control of intracellular Ca²⁺. Overstimulation of hair cells, such as by noise, is particularly stressful to hair-cell mitochondria. Mitochondrial Inner Membrane Protein 17 (Mpv17) functions as a non-selective channel and plays a role in maintaining mitochondrial homeostasis. We have previously demonstrated a model with which we can mechanically overstimulate zebrafish lateral-line hair cells and found that mpv17 (-/-) mutant hair cells were more vulnerable to morphological disruption than their wild-type siblings. We also observed mpv17 (-/-) mutant hair cells displayed significantly elevated levels of ROS, indicating impaired mitochondrial function.

Methods

To determine the role of mitochondrial homeostasis on hair-cell synapse integrity, we surveyed synapse number in mpv17 (-/-) mutants and wild-type siblings as well as the sizes of presynaptic ribbons and postsynaptic densities following mechanical overstimulation.

Results

We observed overstimulated mpv17 (-/-) lateral-line organs lost more hair cells, yet lost fewer synapses per hair cell relative to wild-type. We also quantified the sizes of hair cell pre- and postsynaptic structures, and observed significantly enlarged wild-type postsynaptic densities, yet relatively little change in the size of mpv17 (-/-) postsynaptic densities following stimulation. Additionally, mpv17 (-/-) mutant hair cells at rest showed significantly reduced uptake of the dye FM1-43, indicating smaller resting mechanotransduction currents.

Conclusions

These results suggest hair-cell mitochondrial activity influences susceptibility to mechanically induced damage to lateral line organs and may provide insight on mechanisms of noise-induced hair-cell damage.

3:00 p.m. - 5:00 p.m.

Podium 35: "Auditory Cortex and Auditory Perception"

Moderators: Dr. Ariel Hight and Dr. Kath Wood

Category: Auditory Cortex: Anatomy, Physiology & Function

PD 35.1 Auditory Cortex Responses Are Enhanced During Social Learning

Nihaad Paraouty

New York University - Center for Neural Science

Background

The acquisition of new skills can be facilitated by social experience, typically studied by exposing a naïve animal to a conspecific that is performing a well-defined behavior. In a previous study (Paraouty et al., 2020), we showed that naïve gerbils acquired a sound discrimination task significantly faster when exposed for five days to a demonstrator gerbil that was performing the task. In fact, facilitation of learning occurred even when observer and demonstrator were separated by an opaque divider, suggesting that observers were learning auditory cues. Here, we asked whether such social learning is associated with changes to auditory cortex (AC) processing.

Methods

To test whether AC is necessary for social learning, naïve gerbils received bilateral infusions of an agent that blocks activity (muscimol) in AC during exposure to the demonstrator gerbil. Demonstrators performed an amplitude modulation (AM) discrimination task, using an appetitive Go-Nogo procedure. Following 5 days of exposure, naïve observer gerbils practiced the AM task without any drug infusion. To test whether AC neuron coding properties were modified during social learning, 64-channel silicon probes were chronically implanted in AC, and wireless recordings were obtained from freely moving naïve gerbils during the exposure sessions, as well as during subsequent practice sessions.

Results

Naïve gerbils that received bilateral AC infusions of muscimol during exposure to the demonstrator required a significantly greater number of days to learn the Go-Nogo discrimination task, as compared to saline-infused controls. In separate experiments, electrophysiological recordings from single AC neurons revealed significant changes in firing rate responses to the Go and Nogo stimuli across exposure days. Furthermore, using a spike pattern classifier, we computed a neural d' value for each AC neuron during the exposure sessions. To assess whether learning-induced changes occurred during the exposure sessions, we compared neural d' values during early exposure sessions to those in late exposure sessions. For the majority of AC neurons, the neural d' values improved across exposure sessions.

Conclusions

Together, these results suggest that exposure to a demonstrator performing a sound discrimination task is causally linked to social learning and is associated with AC neuron plasticity. It is therefore plausible that such changes in coding properties of AC neurons may support the social facilitation of learning.

PD 35.2 Contextual Sensitivity of Auditory Cortical Neurons Remains Stable Across Days of Recording in Awake Mice

Marios Akritas

UCL

Background

Perceptual salience of sounds depends on the acoustic context in which they appear. Neural correlates of this contextual sensitivity can be investigated using nonlinear-linear “context models” of neuronal responses to complex sounds (Williamson et al. 2016 *Neuron*). Context models capture the spectrotemporal sensitivity of a neuron in a “principal receptive field” (PRF) and its sensitivity to local acoustic context in a “contextual gain field” (CGF). The CGF provides a compact description of how the responsiveness of the neuron depends

on sound combinations, such as pairs of tones occurring at different frequencies and/or times. Previous studies of CGF and PRF structure have revealed strong neuron-specific patterns of nonlinear contextual sensitivity modulating linear spectrotemporal tuning in auditory cortical neurons of anaesthetized mice.

Methods

To investigate the stability of CGF and PRF structure in awake animals, we obtained 514 spike-sorted recordings (315 single-unit, 199 multi-unit) from core auditory cortex in 4 awake male mice, during repeated presentations of dynamic random chord stimuli over multiple days. Adapting a previously developed method (Tolias et al. 2007 J Neurophysiol), we matched spike waveforms across recording sessions to identify 76 unique units recorded over at least 2 days.

Results

We quantified stability of CGFs or PRFs within and between days by calculating the alignment of their estimates from different recording sessions using the normalized dot product. Stability was only slightly lower between days than within a recording session and declined only modestly across additional days. For example, median CGF stabilities within day 1, between days 1&2, and between days 1&5 were 0.75, 0.75 and 0.57; corresponding PRF values were 0.91, 0.85 and 0.85. Histograms of both average change and average absolute change in alignment per day were strongly peaked near zero for CGFs as well as for PRFs.

Conclusions

We conclude that contextual sensitivity is an essential and stable feature of the neural code in auditory cortex of awake animals. In ongoing work, we are examining how contextual sensitivity of auditory cortical neurons is affected by the animal's behavioural state.

PD 35.3 Effects of Intermodal Attention in Primary and Secondary Auditory Cortex

Jennifer Mohn

University of California, Davis

Background

Our sensory systems are constantly receiving an immense amount of information that must be quickly evaluated in order to interpret and interact with our environment. This can necessitate shifting attention between sensory modalities in order to focus on the most relevant stimuli to the current behavioral goals. How the encoding of sensory information may vary under particular modality demands, remains unclear.

Methods

To investigate the neural mechanisms underlying this attentional shift, we recorded single-unit activity in primary (A1) and secondary (ML) auditory cortex from two monkeys as they performed an intermodal attention task. The animals were presented with audio-visual stimuli and were required to switch between performing an auditory task and a visual task. The auditory task was amplitude modulation (AM) frequency discrimination, where AM frequency was easily differentiated and fixed (within a session) but AM depth was varied across the animals' perceptual range. The visual task was random-dot motion direction discrimination, difficulty matched to the auditory task for each animal. Additionally, unimodal blocks of trials were presented to control for the possible effects of a distracting stimulus on encoding and discriminability.

Results

We used signal detection theory metrics (ROC area) to evaluate the neural discriminability of AM frequency by both rate- and temporal-based measures. We found neurons in both areas with activity that was significantly modulated by attentional context. Additionally, we found a population of neurons in ML, but not A1 with significantly higher firing rates during the visual-only stimulus blocks.

Conclusions

This suggests that behavioral context may have a larger effect on neural responses in ML than A1.

PD 35.4 Emergence of Stream Segregation in Neural Responses in Ferret Auditory and Frontal Cortices

Neha Joshi

University of Maryland, College Park

Background

This study concerns the neural mechanisms involved in the perceptual segregation of sound sources, a process that appears effortless in humans and animals despite its complexity. Since the ferret frequency range of hearing encompasses the human range, and their auditory cortical responses have demonstrated complex spectrotemporal representation of sound, ferrets are an excellent animal model for phoneme discrimination. They can be behaviorally trained to differentiate “target” words (trisyllabic sequences) from various alternative “reference” words.

Methods

In this study, ferrets learned to attend to a unique target speaker’s voice and to respond to a specific trisyllabic sequence target (speech-like “word”) only when uttered by a specific target speaker, using a conditioned avoidance Go/No-Go behavioral paradigm. Ferrets successfully performed this word recognition task (in the presence of other non-target trisyllabic words) in a 1-speaker context, and also demonstrated speech segregation by learning to reliably detect the same target word uttered by speaker 1, in a complex 2-speaker scene with overlapping words (including the target trisyllabic sequence) uttered by the second speaker. We recorded neuronal activity of quietly listening and task-engaged ferrets in the 1-speaker and 2-speaker contexts in the primary and secondary auditory cortices (A1, n = 127, PEG, n = 78 neurons), and also in the dorsal frontal cortex (dFC, n = 54 neurons).

Results

Neurophysiological evidence from our recordings in the auditory cortices suggests that there is differential processing in the frequency domain of the target speaker compared to the unattended speaker during active task engagement, even in A1. We observed complete suppression of neural responses in the speaker-specific frequency regions of the interfering speaker in the behaving animal, but not in the passively listening animal. This suppressive effect built up as the trial progressed. Responses in dFC also showed attention-modulated differential processing, where responses to the target word spoken by the attended speaker became considerably enhanced relative to responses to the reference words during attention in a single talker and multi-stream scenario. In dFC, suppression of responses to the interfering speaker occurred at the level of average peri-stimulus time histograms within the temporal domain upon deviation from the target syllable sequence.

Conclusions

These results demonstrate that neurons in the auditory and frontal cortices encode words selectively depending on learning and attentional context, indicating that in this task the animal suppresses target syllables based not only on their syllabic sequence identity but also on their vocal source. The findings from this behavioral neurophysiological study help us better understand the role of attention and underlying neural mechanisms in the emergence of stream segregation in multi-speaker environments and provide valuable insight into pre-processing of ignored and attended speech during auditory scene analysis.

PD 35.5 Evidence for Multiple Mechanisms of Top-Down Suppression in Auditory Cortex During Marmoset Vocalization

Steven Eliades

University of Pennsylvania School of Medicine

Background

Vocal communication requires auditory self-monitoring of our vocal output to ensure accurate production. During both human speech and animal vocal production, there is a prominent vocalization-induced suppression of activity in the auditory cortex, thought to reflect the comparison of sensory feedback with expected vocal acoustics. However recent evidence has also suggested a non-specific suppression of auditory responses during more general motor tasks, like locomotion. This raises a question about the existence of multiple sources and mechanisms of auditory cortical suppression during vocalization.

Methods

Here we investigated the possibility of multiple, separable top-down motor inputs by examining temporal response patterns in the auditory cortex of vocalizing marmoset monkeys, focusing on marmoset ‘twitter’ calls, vocalizations with multiple individual phrases. We recorded multiple neurons in the auditory cortex for freely moving marmosets while the animals’ produced self-initiated vocalizations. We analyzed responses during twitter vocalizations, separating out activity during the phrases and the inter-phrase intervals.

Results

We found the presence of two components of vocalization-induced suppression. The first was a tonic suppression that began prior to vocal onset and persisted across the duration of vocal production. The second was a phasic component that occurred only during individual vocal phrases, was more selective in the neurons affected, and was sensitive to perturbations in vocal feedback.

Conclusions

The separability of these two components suggests the possible presence of two distinct mechanisms of vocal suppression: a more tonic inhibition consistent with a non-specific vocal corollary discharge, and a phasic inhibition consistent with an efference copy that relays specific predictions about expected sensory feedback. These results have important implications about the mechanisms of top-down sensory prediction in the auditory system.

PD 35.6 Visually Influenced Neurons in Primary Auditory Cortex of Awake Cats

Xiaohan Bao

McGill University

Background

Deaf subjects are identified to have enhanced visual abilities compared to hearing subjects. It is hypothesized that such behavioral compensation is provided by functional reorganization, in which auditory cortex, deprived of normal acoustic input, is cross-modally reorganized to process visual information. To evaluate changes in auditory cortex following hearing loss, it is necessary to examine visual influences in auditory cortex prior to hearing loss.

Methods

Therefore, adult hearing cats were trained to make 5-sec visual fixations while multi-unit spikes from A1 neurons in response to auditory and/or visual stimuli were recorded from a 32-channel chronically implanted electrode array.

Results

The data show that, while responses driven solely by visual stimuli were not observed, visual modulation of auditory responses occurs frequently. More recording sites displayed visual suppression than facilitation. This modulation depended on both frequency of the auditory stimulus and audiovisual temporal disparity. The temporal pattern of audiovisual interaction demonstrated periodicity at a selective range of cycle rates (e.g. 10-Hz oscillation), which has been previously reported in auditory-somatosensory and audiovisual interactions in macaque A1.

Conclusions

Furthermore, our findings on visually modulated neurons in hearing A1 are consistent with reports of recordings in anesthetized ferrets or awake macaques. Next, we plan to examine if visual responses and visual modulation of auditory inputs occur in A1 of awake deaf cats.

PD 35.7 Simple Temporal Traits Are Represented Orthogonal to the Tonotopic Axis in Guinea Pig A1

Peter Baumhoff

Hannover Medical School

Background

The mammalian brain accesses the spectro-temporal traits of complex sounds. It combines those into behaviorally meaningful information. The primary auditory cortex is characterized by a tonotopic gradient in

all species investigated so far, but it remains unclear which feature is represented orthogonally to the tonotopic axis. Here we studied this representation in guinea pigs.

Methods

We used surface electrode arrays (ECoGs, 16 channels) to record local field potentials (LFPs) from the primary auditory cortex of Hartley guinea pigs (N=4). The stimuli were clicks (50 μ s, 0-90 dB SPL pe) pure tones (1-32 kHz, 4st./oct., 0-80 dB SPL), and click trains (thr.+30 dB, 10-320 Hz, 4st./oct.). All signals were stored with a sampling frequency of 25 kHz. The tonotopic gradient of the primary auditory cortex (A1) was reconstructed from the LFP signals. The LFP responses to click trains were analyzed by calculating vector strength of response maxima, cross correlations with the stimulus shape, LFPs per stimulus (period sums), and periodograms of the responses. The data were used to reconstruct surface maps for the representations of both spectral and temporal information in A1.

Results

The tonotopic gradient within A1 could be reconstructed from LFP data in all cases. A rostro-caudal progression from low to high frequencies is apparent from the recordings. The axis is tilted dorso-ventrally. An inversion of the frequency gradient signified the border between A1 and the dorso caudal field (DC). All LFP responses to click trains had a low-pass characteristic, i.e. the synchronization with the stimulus dropped sharply for high repetition rates. Measures of LFP synchronization revealed gradients in dorso-ventral direction. They were angled relative to the tonotopic axis by 60° to 90°. The highest repetition cut-off frequencies for vector strength, cross correlations, and periodograms ranged from approx. 100 Hz to above 200 Hz for period sums. High repetition cut-offs were represented at the dorso-caudal border of A1, with the exception of the period-sum analysis that found the highest values in a ventro-rostral area.

Conclusions

The results of this study suggest the presence of a temporal processing gradient in the guinea pig A1. It traverses the tonotopic gradient. The antidromic gradients for mainly synchronization based (periodograms, vector strength, cross correlation) and mainly response strength based (period sums) measures potentially indicate a switch from synchronization code to rate code along the temporal processing axis. The findings presented here enable us to study the processing of degraded temporal structure in the guinea pig model in a next step.

WK received funding from a MHH HiLF scholarship and AK was supported by the Cluster of Excellence Hearing4All (DFG Exc. 2177).

PD 35.8 Morphometric Analysis of Feline Auditory Cortex – an Effect of Experience

Peter Hubka

Hannover Medical School

Background

Development of the structure and function of cerebral cortex is initially driven by genetic factors and subsequently refined by spontaneous and experience-related neuronal activations. Recently, a considerable progress was achieved in the understanding of genetic substrates which governs development of the cortical morphology. An effect of experience on the morphologic characteristics of the cortical structure is, however, not well understood yet. Here we propose a complex morphometric approach to quantify characteristic structural properties of the feline temporal cortex (auditory and surrounding areas). We assess an effect of experience on the cerebral cortex structure by comparing morphometric parameters of normally hearing and congenitally deaf cats.

Methods

The morphology of the cerebral cortex was evaluated by 7T magnetic resonance imaging (MRI; Bruker, USA) of feline brains that were extracted after transcatheterial whole-body perfusion and PFA fixation of the animals. The cerebral cortex was subsequently segmented from MRI scans using 3D Slicer software (<http://www.slicer.org>). After segmentation, a 3D model of the cortical ribbon was constructed. Morphometric parameters such as folding index, surface curvature, cortical area and volume were computed from this model. Additionally, cortical thickness was computed through diffeomorphic registration of the

gray-white surface with the pial surface. To assess general morphology of the feline temporal cortex, distributions and dependences of the morphometric parameters were evaluated. The described procedure was tested in the MRI images of the cats with different degree of auditory experience during development (normally hearing, congenitally deafness and single-sided deafness).

Results

We have developed and tested a framework for morphometric evaluation of the complex characterization of the cerebral cortex morphology in 3 dimensions. The extracted parameters enable a statistical comparison between the cortices in the animals with different degree of auditory experience and are thus suitable for a comparison of effects of developmental auditory experience on the architecture of the auditory cortex.

Conclusions

In the present study, we have proposed a quantitative comprehensive cortical mapping framework for analyzing structural changes of auditory cortex in developmental disorders. It can result in identification of novel structural biomarkers, which will enhance interpretation and informative value of noninvasive imaging techniques used in clinics.

3:00 p.m. - 5:00 p.m.

Podium 36: "Cochlear Synaptopathy & Auditory Nerve Function, New Insights"

Moderators: Dr. Sarah Verhulst and Dr. Kenneth Henry

Category: Auditory Nerve

PD 36.1 Age-Related Reduction in Frequency-Following Responses and Its Connection to Peripheral Neural Degeneration

Jonatan Märcher-Rørsted

Technical University of Denmark

Background

Previous studies have reported an age-related reduction in frequency-following responses (FFRs) in listeners with clinically normal audiometric thresholds. This has been argued to reflect an age-dependent decline in neural synchrony in the central auditory system. However, age-dependent degeneration of auditory nerve (AN) fibers may have little effect on audiometric sensitivity and may yet affect the suprathreshold coding of temporal information. This peripheral loss of temporal information may not be recovered centrally and may thus also contribute to reduced phase-locking accuracy in the auditory midbrain. Here, we investigated whether age-related reductions in the FFR could, at least in part, reflect age-dependent peripheral neural degeneration.

Methods

Ten young and ten older normal-hearing listeners participated in the study. FFRs to cosine frequency sweeps in the range from 0.2 to 1.2 kHz as well as to static pure-tone stimuli at mid (706 Hz) and lower (326 Hz) frequencies were measured to explore the frequency-dependent neural coding at brainstem level in these two age groups. Additional measures potentially related to AN degeneration, such as the strength of the middle-ear muscle reflex (MEMR) and extended high-frequency audiometry, were also evaluated. The experimental electrophysiological data were compared to the output of a phenomenological model of the AN presented with the same stimuli. To explore the possibility of a reduction of synchronous activity with AN degeneration, model outputs with different degrees of AN loss were investigated. To investigate potential effects on the FFR due to of high-frequency outer hair cell (OHC) loss typically seen in aging listeners, we impaired the AN model to match age-normal sensitivity loss profiles.

Results

A reduction in the FFR response in the older listeners was found across stimulation frequencies for both sweep and static pure-tone stimulation. Older listeners also showed significantly shallower MEMR growth level

functions compared to the younger listeners, which could indicate a loss of low spontaneous rate fibers in the AN. Despite having clinically normal audiometric thresholds, the older listeners had significantly reduced sensitivity at frequencies above 8 kHz compared to the young group. The computational simulations suggested that such experimental results can be accounted for by neural degeneration already at the stage of the AN whereas a loss of sensitivity due to OHC dysfunction at higher frequencies could not explain the observed reduced FFR in the older listeners.

Conclusions

Age-related reductions in the FFR were observed across frequency. These results could, at least in part, reflect an age-dependent neural degeneration in the cochlea. The model results support the idea that reduced neural synchronization at the level of the brainstem could be influenced by peripheral neural degeneration even without central desynchronization. Based on age-normal sensitivity loss profiles, the modeling suggests that OHC loss cannot account for the observed experimental data.

PD 36.2 An Encoding-Decoding Method for Studying Cochlear Synaptopathy

Jiayue Liu

Duke University

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 & 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 capture the complexity of physiological response characteristics, thus leaving unclear the quantity of information conveyed by the auditory nerve fibers. Here, we propose 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, which samples the input sound at its own stochastic rate, and the loss of auditory nerve fibers is mimicked by specifying 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, a stimulus reconstruction is used to decode the modelled auditory nerve fiber responses back to an audio signal. This reconstruction contains a spectral energy map (Mesgarani et al., 2009) and a phase extraction process. 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 conducted 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 sounds used in these two tasks are degraded using the encoding-decoding model, implementing three different levels of auditory nerve fiber loss (0, 90, 99%). Preliminary results indicate that the pure tone detection threshold in noise increases with a decrease in the number of fibers. However, the overall threshold shift is larger from 90% to 99% fiber loss than from 0% to 90% fiber loss. Analysis of the speech reception task is still ongoing.

Conclusions

In conclusion, our model combines detailed physiological responses with the stochastic undersampling model and thereby creates a more realistic computational model which can be tested in human listeners. 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 cochlear implants.

PD 36.3 Can Spectro-Temporally Modulated Signals be Used to Assess the Contribution of Synaptopathy to Speech-in-Noise Intelligibility Deficits?

Emmanuel Ponsot
Ghent University

Background

Difficulties in understanding speech-in-noise (SPiN) cannot only be explained by the loss of cochlear outer-hair cells (OHC) monitored with audiometric measures, since individuals with normal audiograms also suffer from these problems. Among other potential deficits arising in subsequent stages of the auditory system, synaptopathy, i.e. damage to the synapses connecting cochlear inner hair cells to the auditory nerve (AN), is thought to play an important role. However, current attempts to assess the role of synaptopathy for SPiN intelligibility differences among listeners with similar audiograms have led to mixed results. One explanation for these discrepancies relates to the different types of stimuli that were used to quantify the two measures in the relationship: synaptopathy diagnosis is made on the basis of amplitude-modulated tones whereas SPiN intelligibility is assessed using speech material. To align both measures more optimally, it is critical to evaluate synaptopathy with supra-threshold stimuli (i) that resemble speech, i.e. that contain variations along both spectral and temporal dimensions in similar frequency regions, and (ii) that can quantify the respective contributions of OHC damage and AN deficits.

Methods

Here, we investigate whether spectro-temporal modulation (STM) signals – noise carriers whose envelope is jointly modulated in time and frequency as a tractable and parameterized model of speech formants – can overcome this challenge and be used for synaptopathy diagnosis and SPiN deficit assessment. We tested this idea by investigating how STM stimuli are encoded at the level of the AN and how this neural code is affected by different hearing deficits. We simulated population AN responses to a variety of STM signals (spectral and temporal modulations in the range crucial for speech intelligibility; noise carriers in low [250Hz-1kHz] or high [1-4kHz] frequency regions) using a biophysical model of the auditory periphery. We simulated different hearing profiles with different amounts and combinations of OHC loss / AN deficits.

Results

The simulations show that STM stimuli with carriers in different frequency regions produce distinguishable AN response patterns depending on the deficit type. By contrasting responses to different STM signals, it is theoretically possible to tease apart the respective contributions of OHC deficits / synaptopathy.

Conclusions

Overall, this modelling work sheds further light on the complex neural code of STM signals and shows how the different types of information carried by these signals (envelope, temporal fine structure) are affected by different hearing deficits. Together, these results suggest novel behavioural and electrophysiological proxies of synaptopathy to optimally assess its contribution to SPiN intelligibility deficits, which remains to be tested experimentally.

Work supported by Fondation pour l'Audition (FPA-Ponsot) and European Research Council (ERC-StG-678120, RobSpear).

PD 36.4 Histopathological Correlates of Auditory-Nerve Damage From Kainic Acid in the Budgerigar

John L. Wilson
University of Rochester School of Medicine & Dentistry

Background

Sensorineural hearing loss involving damage to hair cells and auditory-nerve afferent neurons affects millions of individuals worldwide, with significant consequences for interpersonal communication and quality of life. While hair-cell damage is readily detectable with the audiogram, detection of auditory-nerve damage (or cochlear synaptopathy) has remained a major clinical challenge. Auditory-nerve damage progresses steadily with increasing age and sound overexposure and has been proposed to cause 'hidden hearing loss' associated with perceptual difficulties in noise. Prior work in our laboratory has used intracochlear kainic-acid infusions in budgerigars (*Melopsittacus undulatus*) to produce an animal model of auditory-nerve loss, as part of an

ongoing effort to characterize the impact of this prevalent pathology on complex-sound perception. Kainic-acid infusions in budgerigars caused permanent, 40-70% reduction of compound auditory-nerve responses and wave I of the auditory brainstem response, consistent with substantial auditory-nerve loss. Envelope following responses are also reduced across a wide range of stimulus modulation frequencies. However, the histopathological changes associated with these electrophysiological changes remain unknown.

Methods

Histological analyses of both cochleae were performed in control animals and those with unilateral or bilateral damage from kainic acid to assess histopathological changes caused by the infusion. Cochleae were processed using standard immunohistochemical techniques as frozen transverse sections to quantify the impact of kainic-acid exposure on hair cells and auditory-nerve afferent neurons.

Results

Several commercially available antibodies were identified to label hair cells and neural elements in the budgerigar cochlea. Antibodies were against Myosin VIIa for labelling hair cells, beta-III tubulin for labeling neuron cell bodies in the auditory-nerve ganglion, myelin basic protein for labeling afferent nerve fibers, and CtBP2 for labeling hair-cell synaptic ribbons (a component of the afferent AN synapse).

Conclusions

Comparison of results between cochlea from control and kainic acid-exposed cochleae using confocal microscopy will verify the specific anatomical changes underlying diminished auditory-nerve responses in the budgerigar model of auditory-nerve loss. Further, behavioral studies in this animal model, which shows human-like behavioral sensitivity to many simple and complex sounds, hold promise to identify the aspects of auditory perception impacted by this common pathology. This research was supported by grant R01-DC017519 from NIDCD.

PD 36.5 Predicting Synapse Counts in Living Humans by Combining Computational Models With Auditory Physiology

Brad Buran

Oregon Health & Science University

Background

Approximately 40% of human cochlear synapses are lost by 50 years of age. Cochlear synaptopathy is predicted to result in perceptual deficits including tinnitus, hyperacusis, and difficulty understanding speech-in-noise. However, there is currently no method for diagnosing synaptopathy in living humans. Without a means of diagnosing synaptopathy, we cannot conclusively determine if noise-induced synaptopathy occurs in humans, identify the perceptual consequences of synaptopathy, or test potential drug treatments.

The auditory brainstem response (ABR) wave I amplitude is a non-invasive physiological measure shown to be sensitive to synaptopathy in animal models. However, wave I amplitude alone is insufficient to detect synaptopathy in individual human subjects. This is, in part, due to the likelihood of outer hair cell (OHC) damage co-existing with synaptopathy in humans with sensorineural hearing loss. Thus, a diagnostic assay based on noninvasive auditory evoked potentials must be able to account for OHC dysfunction. An existing computational model of the auditory periphery (CMAP; Verhulst et al. 2018) can predict distortion product otoacoustic emissions (DPOAEs) and ABRs for any combination of OHC and synaptic loss.

Methods

Using Bayesian regression analysis to fit the Verhulst CMAP, synapse counts were predicted from measured ABR and DPOAE data for individual subjects in a sample of 74 young military Veterans and non-Veterans with normal audiograms. The model was fit in two stages. First, a machine learning approach was used to adjust cochlear tuning given measured DPOAEs, thereby creating a DPOAE-adjusted CMAP for each subject. Simulated DPOAEs generated using the DPOAE-adjusted CMAPs closely matched measured DPOAEs. Second, the DPOAE-adjusted CMAP for each subject was used to generate simulated ABR responses to 3, 4, and 6 kHz toneburst stimuli for low, medium and high spontaneous rate auditory nerve fibers (ANFs) distributed uniformly along the tonotopic axis of the human cochlea. The average number of ANFs per IHC along the length of the cochlea (i.e., a synaptogram) was modeled as a parabola with priors for the coefficients derived from human temporal bone synapse counts (Wu et al. 2019).

Results

Predicted synapse counts show the expected relationships with synaptopathy risk factors and predicted perceptual consequences. Groups with higher noise exposure histories have lower predicted synapse counts than controls, consistent with noise-induced synaptopathy. Report of tinnitus, a predicted consequence of synaptopathy, is also associated with lower synapse counts. In addition, although not statistically significant, lower predicted synapse counts are weakly correlated with poorer speech-in-noise perception, another expected impact of synaptopathy.

Conclusions

These results illustrate the feasibility of this modeling approach and demonstrate the utility of a model that reduces multiple sources of information, including DP-grams and ABRs measured at multiple levels and frequencies, into a single synaptogram.

This work was supported by VA RR&D C1484-M/C2104-W/C9230-C, NIDCD R21 DC01696, and ERC 678120.

PD 36.6 Representation of Voice Pitch in Discharge Patterns of Auditory-Nerve Fibers Following Noise-Induced Hearing Loss

Satyabrata Parida
Purdue University

Background

Pitch plays an important role in everyday communication, including speech and music perception. Broadly speaking, pitch is encoded in two neural mechanisms: (1) temporal and (2) temporal-place. The temporal mechanism is mediated by unresolved harmonics of the fundamental frequency (F0) in a stimulus. The temporal-place mechanism is primarily mediated by resolved F0 harmonics. Psychoacoustic studies have suggested that people with hearing impairment rely more on the temporal mechanism and less on the temporal-place mechanism to perceive pitch; however, these hypotheses have not been simultaneously tested neurophysiologically.

Methods

Here we recorded spike trains from single auditory-nerve fibers of anesthetized male chinchillas with either normal hearing or mild-moderate hearing loss due to acoustic trauma in response to a natural speech sentence. Temporal pitch coding was evaluated using the spectrum of the alternative-polarity peristimulus time histograms. Temporal-place pitch coding was evaluated using average-localized synchronized response and cepstral analysis.

Results

Results showed an enhanced temporal representation and a degraded temporal-place representation of pitch for the mild-moderate hearing-loss group.

Conclusions

Overall, these findings help to explain the neural basis for pitch perception in natural speech by people with hearing impairment.

PD 36.7 Simulating the Human Compound Action Potential Elicited by Clicks and Amplitude Modulated Carriers

Yousef Alamri
University of Utah

Background

The compound action potential (CAP) represents the summed electrical activity generated by synchronous firing of auditory nerve (AN) fibers in response to sound. The CAP is an essential component of the electrical response of the cochlea and is used in clinical and research settings to assess the sensitivity and neural encoding of the auditory system to sound. Computational modeling of the CAP provides a vehicle for evaluating possible neural coding mechanisms and relating such mechanisms to auditory perception. In this

study, we simulated the human CAP measured from an electrode placed on the tympanic membrane. We focused on simulating the morphology and level-dependence of click-evoked CAPs. In addition, we obtained preliminary simulations for human CAPs evoked by amplitude-modulated (AM) sinusoids.

Methods

Model CAPs were obtained by convolving the peri-stimulus time histograms of an array of characteristic frequencies (CFs) with the unitary response, which accounts for the transfer function of the system of tissues and fluids between the AN and the recording electrode. Peri-stimulus time histograms were obtained from a computational model of the auditory periphery that simulates middle ear filtering, cochlear nonlinearity (i.e., compression, suppression, level-dependent tuning), and neural adaptation. We incorporated the relative contributions of high, medium, and low spontaneous rate AN fibers as well as the CF-dependent neural innervation density along the cochlear duct. We predicted the effect of varying stimulus parameters (e.g., intensity, polarity) on the morphological and temporal aspects of the CAP observed in human subjects.

Results

For simulated CAPs evoked by 100-microsecond clicks, we found that the peak-to-peak amplitude of the CAP increases while the latency of the CAP decreases with increasing click intensity. In addition, the first negative peak narrows, and the following positive peak splits into two parts with increasing click level. These morphological and temporal characteristics of the simulated CAPs are consistent with observations in human data. Further, preliminary simulations of CAPs evoked by AM stimuli are overall consistent with data collected from human subjects.

Conclusions

This modeling approach provides a first step to evaluating potential neural coding mechanisms revealed by the human CAP. The evaluation of such mechanisms is essential to understanding cues that mediate auditory perception. Our future work will expand the current simulations to evaluate the extent to which gain control mechanisms, such as the medial olivocochlear reflex, classic firing rate adaptation, and dynamic range adaptation contribute to the human CAP and the perception of AM sounds.

PD 36.8 The Envelope Following Response in Young Military Veterans Suggests Noise-Induced Cochlear Synaptopathy

Naomi Bramhall
VA RR&D NCRAR

Background

Although several non-invasive physiological measures appear to be sensitive to cochlear synaptopathy in animal models, including auditory brainstem response (ABR) wave I amplitude, the middle ear muscle reflex (MEMR), and the envelope following response (EFR), human studies of these measures in samples that are expected to vary in terms of the degree of noise-induced synaptopathy have resulted in mixed findings. One explanation for the differing results may be differences in the type of noise exposure across studies. For example, the synaptopathy risk for recreational noise exposures such as concerts, night clubs, and bars may be much lower than the synaptopathy risk for military noise exposures such as firearms, artillery, and explosions. We previously demonstrated that young Veterans reporting high noise exposure during their military service show a reduction in ABR wave I amplitude and MEMR strength compared to non-Veterans who have never used a firearm, consistent with animal models of noise-induced synaptopathy. If young Veterans with a significant history of military noise exposure suffer from synaptopathy, animal studies suggest that a reduction in EFR magnitude should also be observed among these individuals.

Methods

EFRs in response to a 4 kHz carrier tone modulated at 110 Hz were measured in young military Veterans and non-Veterans with normal audiograms and robust DPOAEs. ABRs were measured in response to a 100 dB peSPL 4 kHz toneburst. Wideband MEMRs were measured for a contralateral broadband noise elicitor. Self-reported noise exposure was assessed using the Lifetime Exposure to Noise and Solvents Questionnaire (LENS-Q). Participants also completed a questionnaire that asked if they experienced frequent or constant tinnitus.

Results

Mean EFR magnitude was lower for Veterans compared with non-Veterans with minimal noise exposure and no history of firearm use. Lower EFR magnitudes were associated with lower ABR wave I amplitudes and weaker MEMR growth functions. The perception of tinnitus was also associated with lower EFR magnitudes.

Conclusions

The relationship between Veteran status and EFR magnitude in the context of a normal audiogram is consistent with animal models of noise-induced cochlear synaptopathy. This suggests that EFR measurements may be informative in human studies of synaptopathy. Positive relationships between EFR magnitude and both ABR wave I amplitude and MEMR strength in this sample suggest that these three measurements are providing similar information about synaptic/neuronal function in humans. In addition, the observed reduction in EFR magnitude among Veterans reporting tinnitus confirms previous findings and adds support to the hypothesis that some forms of tinnitus may be a perceptual consequence of cochlear synaptopathy.

3:00 p.m. - 5:00 p.m.

Poster Session 5

W1. Towards Universal Automated Classification of the Acoustic Startle Reflex With Machine Learning

Aging

Ryan Longenecker¹, Timothy Fawcett², Joel Berger³, Alexander Galazyuk⁴, Merri Rosen⁵, Richard Salvi⁶, Joseph Walton^{*2}

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Background

The acoustic startle reflex (ASR), a robust, motor response to an intense auditory stimulus has been used as a tool to assess psychophysiology in humans and animals for almost century in diverse fields such as schizophrenia, bipolar disorder, hearing loss, and tinnitus. ASRs have been collected and assessed using different metrics to quantify or detect the response resulting in considerable variability in ASR results from different studies, laboratories and species.

Methods

To address this issue, we recently developed a machine learning model to automatically classify ASR waveforms in mice (Fawcett et al., 2020). Startle waveforms from standard input/output function, and various stimulus prepulse trials were manually classified as startles or non-startle for mice, rats, guinea pigs, and gerbils. We also assessed several different modalities of startle including whole body startle response collected by both piezoelectric transducers and load cell platforms. In addition, Pryor reflex startle responses commonly observed in guinea pigs were also part of the data set. Machine learning algorithms were used to combine startle-related features into a robust predictive model for each animal/modality and testing platform. Features were extracted from each waveform, as well as, their respective power spectral densities and continuous wavelet transforms. A universal set of features were obtained by generalizing the specific details (time and frequency) allowing for “common features” across all animals/modalities. Features were then partitioned into two sets (training and test) with repeated k-fold cross validation employed to help reduce overtraining to specific subsets of data. Machine learning methods from different families of algorithms were trained using the generalized features extracted from ASR waveforms from each individual animal/modality to predict an ASR waveform to be a startle or non-startle. Individual models were then stacked to produce extremely robust ensemble models for each animal/modality. Finally, the generalized features from each animal/modality were combined into a universal machine learning model.

Results

Startle waveforms between animals and modalities can differ dramatically which can lead to wide variety of methods used to classify startles waveforms. Using the most critical extracted features of all of startle waveforms across species and acquisition methods based on our previous machine learning study, we found that our machine learning random forest algorithm could accurately classify ASR waveforms for each species with greater than 97% accuracy. This machine learning classification procedure validated startle waveform verification and dramatically improved consistency in detecting gap-induced and prepulse-induced inhibition of the acoustic startle reflex in mice by lowering the variance of the raw startle data.

Conclusions

We have developed a universal startle waveform analysis that can be used by any laboratory utilizing the ASR methodology. This machine learning based method will improve data reliability and translatability between labs using startle reflex to assess auditory perception.

W2. Human Otopathology of Age-Related Ossicular Changes in Presbycusis

Aging

Prithwjit Roychowdhury^{*1}, Melissa Castillo Bustamante¹, Marc Polanik¹, Elliott Kozin¹, Aaron Remenschneider¹

¹*Massachusetts Eye and Ear Infirmary*

Background

Age-related hearing loss (presbycusis) is a prevalent condition. The cause of presbycusis is attributed to inner ear dysfunction; however, little is known about age-related changes in the ossicular joints. Altered compliance of ossicular joints may lead to high frequency conductive hearing loss that is not captured on standard audiometry. Furthermore, age-related changes to the ossicular chain are poorly described. Herein, we performed a length and volumetric analysis of the incudomalleolar joint (IMJ) and incudostapedial joint (ISJ) in specimens from the National Temporal Bone Registry with audiometrically documented presbycusis to describe age-related ossicular joint findings in presbycotic patients.

Methods

Specimens from the National Temporal Bone Registry with audiometrically documented presbycusis without clear evidence of sensory, neural, strial, or mixed features were analyzed by light microscopy and included in the analysis. Non pathologic specimens from individuals <45 years of age with normal hearing were included as controls. Length measurements of the IMJ and ISJ were completed and volumetric measurements of the IMJ synovial space were obtained after 3-dimensional reconstruction. All measurements were completed by two researchers in ImageJ with good agreement.

Results

Twelve patients (22 ears) and ten controls (15 ears) were examined. Mean age for the presbycusis group was 76.3 ± 10.9 (Range 59-93) and 33.5 ± 9.38 (Range 19-45) for controls ($p < 0.0001$). The IMJ length analysis revealed an increase in the total ($9.97 \mu\text{m}$) and calcified ($9.58 \mu\text{m}$) cartilage along the surface of the incus in presbycotic patients when compared to controls ($p < 0.05$). The ISJ analysis revealed smaller cartilage length along the surface of the incus ($31.4 \mu\text{m}$) between the presbycotic patients and the controls, but this was not statistically significant ($p = 0.0729$). In the volumetric analysis of the IMJ, there were no significant differences in either the total IM volume or the synovial space volume alone.

Conclusions

Otopathological analysis revealed significant differences in the IMJ when comparing presbycotic patients to the controls. The IMJ was narrower and revealed a greater degree of calcification in the presbycotic patients. Taken together, these findings suggest that age-related changes occur within the ossicular joints. Further work should explore whether functional effects occur as a result of observed otopathologic changes. Measurements of middle ear impedance and high frequency conductive hearing as a function of age are necessary.

W3. Use of RNAseq to Identify Early Biomarkers of ARHL

Aging

Dominic Cosgrove*¹, Jacob Madison¹, Daniel T. Meehan¹, Grady Phillips², Lauren Jacobs², Diana Jarocki², Michael Anne Gratton²

¹Boys Town National Research Hospital, ²Washington University

Background

Presbycusis affects 33% of Americans over the age of 65 and 50% over 75. Metabolic presbycusis, based upon strial pathology accounts for 25% of presbycusis, while a mixture of metabolic and sensory dysfunction forms the basis for mixed presbycusis. To identify the early pathobiology of metabolic dysfunction in mixed presbycusis, we performed RNA-seq using lateral wall RNA from 3, 12, and 24-month-old CBA/CaJ mice.

Methods

RNA was extracted from microdissected lateral wall mouse cochleae (3 mice per time-point) at 3, 12, and 24 months of age. RNA samples were processed by the Illumina NextSeq 550 platform. The resulting fastq files were sent through a proprietary pipeline utilizing the Trimmomatic suite, and a 2-Pass STAR protocol equipping the GRCm38 genome (mm10) from the National Center for Biotechnological Information (NCBI) and a curated genomic regions file.

The aligned .bam files were processed with the featureCounts program of the Rsubread package from BioConductor. Counts per million (CPM) values were derived from a list of curated genomic regions that were synonymous with the Mouse Genome Index (MGI) and the NCBI, the same genomic regions file used for alignment. The resulting 13731 genomic features were used to score the alignments in featureCounts.

CPM were then analyzed with the Gene Set Enrichment Analysis (GSEA) Software Package v4.0.3 from the Broad Institute using the continuous mode with a Pearson Correlation Coefficient metric to discover linearly expressed genes and gene sets from those which are provided in the software package. We chose the Pearson model to visualize the genetic changes in a linear pathology of hearing loss over time.

Results

The results show: 1/ progressively reduced expression of transcripts encoding proteins involved in reduced G-protein receptor signaling, reduced ligand-gated ion channel activity, 2/ reduced endothelial cell proliferation, 3/ elevated sodium phosphate symporter activity, 4/ positive regulation of gamma-interferon production, 5/ reduced angiogenesis, 6/ decreased expression of fibrillar collagens, and 7/ a progressive increase in expression of genes regulating cell death. Additionally, many other pathways were progressively influenced with aging but were related to aging in general vs. ARHL specifically.

Conclusions

This work shows molecular changes in gene expression in the lateral wall that predict structural and functional deficits as early as 12 months of age-well before notable sensory cell loss. Minimally elevated ABR thresholds and reduced EPs at 15 months suggest functional deficit in the lateral wall at 15-months. A multitude of pathways are progressively up- or down-regulated as a function of aging in the CBA/CaJ mouse lateral wall. We expect that many of these will overlap with models of sensorineural presbycusis indicating pathways related to aging, but not metabolically based ARHL. These early RNA changes may reflect points of intervention that might prevent or attenuate pathologic changes.

W4. Rate Discrimination Training May Partially Restore Age-Related Temporal Processing Deficits

Aging

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¹University of Maryland

Background

The ability to understand speech in complex environments depends on the brain's ability to preserve the precise timing characteristics of the speech signal. Temporal processing is known to decline with age, and this decline is a factor in the older adult's experience of communication difficulty in noisy backgrounds. This study's purpose is to evaluate the potential for improving temporal processing through the use of rate discrimination training. A second purpose is to evaluate the extent to which perceptual learning on specific rate discrimination tasks can be generalized to other temporal processing tasks and measures of speech understanding.

Methods

A double-blind, randomized control design was used to assign young normal-hearing (YNH), older normal-hearing (ONH), and older hearing impaired (OHI) listeners to one of three treatment groups: 1) experimental (rate discrimination for 100-Hz and 300-Hz pulse trains, nine sessions), active control (tone detection in noise, nine sessions), and passive control (no training). All listeners are seen for a battery of perceptual tests including rate discrimination for 100-, 200-, 300-, and 400-Hz pulse trains, gap detection thresholds, gap duration discrimination, and temporal rate discrimination. Recognition of sentences presented in quiet, babble noise, reverberation, and in time-compressed form is measured. Auditory steady-state responses (ASSRs) are recorded to the pulse trains used in the rate discrimination task. All listener groups are evaluated at four time points relative to training: pre, post, one month, and six months.

Results

Data collected to date show significant improvement in rate discrimination thresholds for the trained frequencies (100 and 300 Hz) across training groups, but there was a session $\times$ training group interaction driven by a greater threshold improvement in the training group than in the active and passive control groups. There was also a significant improvement in rate discrimination thresholds for the untrained frequencies (200 and 400 Hz), and there were no differences in the extent of improvement between training groups. ASSR spectral energy significantly increased for 100 Hz and 200 Hz, but only in the YNH and OHI experimental groups. Spectral energy decreased in the active control groups and remained stable in the passive control groups. The effects of training did not generalize to the other temporal processing or speech recognition measures.

Conclusions

Rate discrimination training appears to improve temporal processing, as reflected in decreased behavioral thresholds and increased spectral energy, but the effects appear to be specific to the trained rates and do not generalize to other rates or performance on other temporal processing tasks. More definitive conclusions will be made when the target number of subjects is reached for listener and training groups.

Funding: This research was supported by the National Institute on Aging of the National Institutes of Health under Award Number P01AG051603 (Gordon-Salant).

W5. Blast-Overpressure Induced Morphological Changes in Central Auditory Neuraxis of Chinchilla

Auditory Cortex: Anatomy, Physiology & Function

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¹SUNY Buffalo

Background

By the very nature of their duties, military service members are at increased risk of exposure to dangerously high levels of sound, both from prolonged and/or multiple transient acoustic insults. The positive pressure phase of brief duration blast-overpressure (BOP) (> than ten kPa) produces mechanical damage to cochlear structures up to auditory cortex which differs from that observed from longer duration, lower level continuous-noise induced cochlear damage. In the present study, we aimed to quantify the morphological changes in the central auditory neuraxis (cochlear nucleus (CN), inferior colliculus (IC) and auditory cortex (AC)) of chinchilla in 1-month post-BOP.

Methods

A two-group (Control and BOP exposed) of chinchillas each containing three animals were used for this study. BOP exposed group were unilaterally (left side) exposed to 172 dB SPL using a compressed air-driven acoustic shock tube. At 1 month post-BOP, the 4% paraformaldehyde-fixed chinchilla brains were processed for routine paraffin sectioning and CN, IC and AC coronal sections (10-micron thickness) of both control and BOP group were subjected for morphometric quantification using cresyl-fast violet (CFV) and modified (crossmon's) trichrome (MT) method. Viable/dead cells and apoptotic cells were quantified using CFV and MT method respectively. Quantitative assessments were performed using ImageJ cell counter plugins.

Results

When compared to control, viable cells were decreased along with increased dead/apoptotic cells on both sides of AC in BOP exposed animals. Interestingly, there is a significant increase in dead cell count on contralateral IC when compared to controls. However, there is no significant change in the cell counts of the ipsilateral IC when compared to control. In CN, dead cell count was found to be increased on both sides of BOP exposed animal when compared to controls. Between the hemibrain of BOP exposed animals, more dead cells/apoptotic cells were evident in ipsilateral CN when compared to contralateral CN.

Conclusions

Our preliminary results indicate that BOP compromised the cytoarchitecture of central auditory neuraxis of chinchilla especially at AC and CN regions at 1 month. In future, quantification using robust morphometric features will provide more insights on region-specific BOP induced pathology in Chinchillas.

W6. Decoding the Perceptual Consequences of Stimulus Specific Adaptation

Auditory Cortex: Anatomy, Physiology & Function

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Background

The environment contains a large set of regularities that can be exploited by the brain to optimize an organism's behavior. In the auditory system, commonly occurring stimuli (standards) have been shown to elicit a local adaptation in response. Unexpected, deviant stimuli on the other hand elicit larger responses, whose size increases along the processing hierarchy. This phenomenon - commonly termed Stimulus Specific Adaptation (SSA) - has been linked to predictive coding, as it can be interpreted as signaling both the predictable and unpredictable parts of the sensory environment through decreased or increased responses respectively.

Methods

While the presence of SSA has been demonstrated in multiple areas and species, previous studies have not addressed the consequence of SSA for the perception of stimuli. Here, we specifically probed the effect of SSA on cortical populations using a modified oddball paradigm in urethane-anesthetized rats (82 cells) and awake mice (102 cells) using extracellular recordings. The resulting representation was estimated using population analysis and neural decoding to estimate the perceptual consequences. Data decoding was realized by two types of biologically plausible decoders - the classical (vector) decoder and a more general likelihood decoder. The data are then related to different models that could explain the observed effects.

Results

We find that adaptation to the standard ('adaptor') leads to a significant, repulsive shift around this tone's frequency in a subset of the data. This leads to an increase in the local steepness of the relation between actual and decoded tones, which could be interpreted as an increase in local sensitivity. This could have interesting behavioral consequences in tasks such as change detection or discrimination of similar stimuli.

Conclusions

The underlying population response adapts in a semi-local way to the standard. We account for this in a multistage adaptation model, with thalamic neurons integrating biases and their response being integrated in the cortex. While this provides a first look at the perceptual consequences of SSA, further investigation is required to understand its influence on perception.

W7. Neural Correlates of Modulation Detection Interference

Auditory Cortex: Anatomy, Physiology & Function

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Background

The auditory detection threshold of a sinusoidal amplitude modulation (AM) applied to a tone carrier is increased by the presence of an amplitude-modulated tone in a remote audio-frequency region, a phenomenon known as modulation detection interference (MDI). Psychoacoustic studies have revealed that MDI increases with the proximity in AM rates between the target and masker AMs, leading to an interference of up to 15 dB. Recent work suggests that MDI is due to difficulty in associating the near-threshold target AM with its appropriate carrier when other carriers are present, and therefore represents a form of informational masking. The aim of this study was twofold: (i) investigate the neural correlates of MDI (ii) as well as their relationship with behavioural performance. The AM following response (AMFR), a steady-state auditory response was used as an index of AM neural encoding.

Methods

Normal-hearing adults (n = 26) aged 18 to 35 years were tested using a 2-interval, 2-alternative forced choice with constant-stimuli procedure. Target and comparison intervals consisted of three sinusoids (750, 1500 and 4500 Hz). In each trial, listeners were asked to identify which of the two intervals contained a 29.7-Hz target AM applied to the 1500-Hz sinusoid. In the “baseline” condition, the 750 and 4500-Hz sinusoids were unmodulated; in the “MDI” condition, the 750 and 4500-Hz sinusoids were modulated sinusoidally in amplitude at 17.3 Hz (masking AMs). Target and masking AMs were presented well above individual AM detection thresholds measured in a preliminary session. AMFR and behavioural performance were simultaneously recorded in response to the two intervals.

Results

Performance was near ceiling in the baseline condition, and above chance in the MDI condition. In the baseline condition, an AMFR was significantly evoked at 29.7 Hz. In the MDI condition, two AMFR peaks emerged at both target and masking modulation rates. The amplitude of the target AMFR was significantly smaller in the MDI than in the baseline condition. Yet, in the MDI condition, the AMFR evoked by the maskers was smaller when the target was modulated than unmodulated. Notably, it was negatively correlated with behavioural detection performance.

Conclusions

Together, these results indicate that the AMFR can be used as an index of MDI. In particular, the amplitude of the AMFR evoked by the interfering modulations appears to be related to the behavioural performance in MDI. This raises the possibility that MDI is related to the neural suppression of the masker.

W8. Capturing Audiovisual Integration Effects With Steady-State EEG

Auditory Cortex: Human Studies

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Background

Learning to read requires linking familiar speech sounds with visual letters. fMRI studies have revealed enhanced neural responses to congruent-letter sound pairs in adults and children, known as a congruence effect. Additionally, ERP studies have shown topographic differences when processing congruent and incongruent familiar words, and neural and behavioral markers of audiovisual (AV) integration have been found to predict future reading fluency.

To further this line of research, we investigated evoked responses to the integration of letters and speech sounds in adults using a steady-state EEG paradigm and a data-driven component analysis. Our paradigm drove both low-level effects reflecting stimulus modality and higher-order psychological effects (i.e., letter-sound congruence mapping). To the best of our knowledge, our findings present the first evidence of a congruence effect using English letter-sound pairs.

Methods

Stimuli comprised six visual letters and natural speech sounds corresponding to single letters (p, n, k, g, d). We presented alternating stimuli at a rate of 2 Hz in audio-only and audiovisual conditions. In the audio-only condition, stimuli alternated between intact and reversed speech sounds. In the audiovisual condition, visual and auditory stimuli alternated between congruent (e.g., seeing p and hearing /p/) and incongruent (e.g., seeing p and hearing /t/) letter-sound pairs. Twelve adult native English speakers were asked to indicate when identical stimuli appeared twice in a row while 128-channel EEG was recorded. We used Reliable Components Analysis (RCA) to compute spatial components by maximizing trial-to-trial covariance. RCA was trained twice for each condition to investigate (1) low-level effects from the stimulus oscillation at 2 Hz, and (2) higher-order effects from the psychological oscillation at 1 Hz. We assessed the significance of complex-valued component-space data using Hotelling's t^2 test.

Results

Both conditions produced low-level stimulus effects related to general modality processing. For both the audio-only and AV paradigms, the maximally reliable component had a fronto-central topography consistent with auditory processing. The AV condition also produced a second RC with an occipito-parietal topography, consistent with visual processing. Critically, our steady-state paradigm drove psychological effects related to stimulus manipulations and produced different component topographies for speech reversal and AV congruence effects.

Conclusions

Using a steady-state paradigm in conjunction with a data-driven spatial filtering technique, we recovered modality-relevant topographies from audio-only and AV conditions. Additionally, the significant activations in component spaces reflected psychological effects - a reversal effect in the audio-only condition, and a congruency effect in the AV condition. This congruence mapping significantly impacted signal amplitudes in component space, providing evidence of multisensory integration driven by steady-state stimuli. Toward our larger goal of studying neural correlates of reading development, we next plan to repeat the investigation with children; this paradigm has potential to study processing differences in dyslexia as well.

W9. Intensity Just-Noticeable Differences and Event-Related Potentials of a Masked Tone in Masking Release Conditions

Auditory Cortex: Human Studies

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Background

The human auditory system enables communication in noisy environments by making use of beneficial signal properties. One example of such perceptual phenomenon is masking release. Behaviorally, masking release can be observed as the enhanced audibility induced by beneficial cues. One can quantify masking release with a difference in detection threshold of a masked pure tone between the conditions with and without beneficial cues. For example, a decrease in detection threshold induced by a comodulation cue is comodulation masking release (CMR) and the one by interaural phase differences (IPD) cue is called binaural masking level difference (BMLD). Many studies have shown that these measures can easily amount to 10-20 dB in thresholds. Few have investigated the audibility at supra-threshold levels when beneficial cues are present. Previous studies used scaling methods to measure the audibility at supra-thresholds and results were dependent on the listeners' subjective criteria for the scaling. Here, we applied a just-noticeable-difference (JND) measure that can overcome such subjective scaling pitfalls. Furthermore, we investigated the neuronal representation of masked signals in masking release conditions with electrophysiological event-related

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potentials. We hypothesized that the amplitudes of late auditory evoked potentials (LAEPs) are correlated with the intensity-JND measures in masking release conditions.

Methods

For intensity-JND measures, we used a three-alternative forced-choice paradigm (3-AFC) and with the task to select the loudest interval. JNDs were measured at +0dB, +5dB, +10dB, +15dB, +20 dB and +25dB above the individually measured masked threshold. The LAEPs were measured with the same masking release conditions and at +15dB, +20 dB and +25dB above the individually measured masked threshold. Eight different masking release conditions with different combinations of three cues were investigated: comodulation, IPD and onset asynchrony.

Results

All conditions show a decrease in JNDs as the level of the tone increases. Conditions with lower detection thresholds show higher JNDs compared to the ones with higher detection thresholds, or small amounts of masking release. In other words, JNDs are inversely proportional to the sound intensity. This indicates that the auditory system uses separate mechanisms for detection and discrimination tasks. In addition, the growth in amplitudes of LAEPs show only little increase when JNDs are high in contrast to conditions with low JNDs.

Conclusions

This study will contribute to a better understanding of neuronal encodings of the tones in masking release conditions and suggests a separate processing of the detection and discrimination task.

W10. The Role of Auditory Nerve Phase-Locking in Human Hearing: Evidence From Deep Neural Networks

Auditory Nerve

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Background

The peripheral auditory system converts sounds to neural representations with exquisitely precise spike timing. The role of phase-locked fine temporal structure remains controversial because no physiological mechanism for extracting this information monaurally has been identified. Nonetheless, fine temporal structure in the periphery is widely believed to support hearing in noise. One prevailing hypothesis is that speech recognition difficulties of hearing-impaired listeners in noisy environments reflect an inability to use fine temporal structure. Here, we investigate the role of auditory nerve phase-locking with deep neural networks optimized to perform everyday recognition tasks from simulated auditory nerve input whose phase-locking limit was manipulated.

Methods

We trained deep artificial neural networks to recognize spoken words, talker identity, and environmental sounds from simulated auditory nerve representations of natural stimuli. The training stimuli consisted of speech excerpts superimposed on recorded auditory scenes. We manipulated the upper frequency limit of auditory nerve phase-locking in the peripheral model 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. They were able to recognize voices and words, even at low signal-to-noise ratios, and error patterns resembled those of human listeners. Notably, reducing the upper frequency limit of auditory nerve phase-locking to just 50 Hz (eliminating virtually all fine temporal structure in the peripheral representation) had very little effect on network speech recognition performance, but substantially impaired voice recognition at lower signal-to-noise ratios.

Conclusions

The results suggest that the fine temporal structure in auditory nerve responses is critical for accurate voice recognition -- but not speech recognition -- in noisy environments. Networks optimized for peripheral

representations devoid of phase-locked spike timing exhibit speech-in-noise recognition comparable to that of networks with full access to fine temporal structure, and to that of humans.

W11. Use of Sound Time Reversal to Measure Ongoing Delay

Auditory Nerve

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Background

Auto- and crosscorrelograms provide a powerful way to analyze temporal aspects of neural responses, essentially by tallying intervals between spikes in different sets of spike trains. Such analysis allows, for example, the measurement of microsecond changes in temporal patterns with changes in sound pressure. A disadvantage of these methods is that they do not provide absolute measures of latency, i.e. measures of delay between features in the stimulus and the subsequent spikes triggered by these features. While various methods exist to obtain such measures, e.g. based on onsets of post-stimulus-time histograms, phase-frequency plots, or delay of reverse correlation (revcor) functions, it is desirable to have a measure which uses the same framework of counting inter-spike intervals.

Methods

Responses to wideband noise were obtained from auditory nerve fibers of anesthetized chinchillas. The noise token was played in two directions: forward and reversed, and each of these versions was also presented in two polarities, which were each other's inverse. The spike trains in response to the forward noise were then compared with the reversed spike trains in response to the reverse noise, by means of crosscorrelograms. Assuming that the same trigger features are present in the forward and reversed noise, these features should trigger spikes with the same delay for both noises. Correlograms between the spike trains to the forward noise and the reversed spike trains to the reversed noise should then show a maximum at twice the value of this delay. As in our previous work, we also correlated responses to forward and reversed noise with opposite polarity and examined the difference and sum of the correlograms to identical and opposite polarity (difcor and sumcor). We refer to these as rocfid and rocsum.

Results

We find that indeed rocfid (for low-frequency fibers) and rocsum (for high-frequency fibers) show maxima at delays of several ms, yielding delay estimates that fit values reported in the literature. The delays show the expected inverse dependence on characteristic frequency. However, both rocfid and rocsum frequently show asymmetries around their maximum, along the delay axis. This is to be expected because spikes are not directly triggered by sound features: there is filtering interposed between sound and spike output. For low-frequency fibers, a linear estimate of this filter can be obtained with the revcor function, calculated from the same spike trains. We found that the revcor of spike trains to forward and reverse noise was identical, as expected. To predict the rocfid, we crosscorrelated the revcor obtained in response to forward noise with the reversed revcor (the rocver) to reversed noise. We found an excellent match between this crosscorrelation and the rocfid.

Conclusions

Use of time-reversed noise provides a quick and easy method to measure ongoing delay based on inter-spike intervals.

W12. Chromatin Remodeler CHD7 Regulates Spiral Ganglion Growth and Schwann Cell Myelination in the Mouse Cochlea

Auditory Nerve

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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 the development of neurons and myelinating Schwann cells in the cochlear spiral ganglion.

Methods

Auditory Brainstem Responses (ABRs) were recorded in adult male 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 and cell counting. Immunohistochemical staining for hair cell synaptic markers CtBP2 and GluA2 was conducted on 4-week old wild-type and Chd7Gt/+ cochleae, which were imaged via confocal microscopy.

Results

Analysis of ABR recordings in Chd7Gt/+ adult animals showed prolonged latency of the Wave I peak at 4 kHz, suggesting delayed propagation of auditory input through the cochlear nerve at lower frequency stimulation. Wave I peak latencies at 16 and 32 kHz were similar in wild-type and Chd7Gt/+ animals. Histological analysis of adult Chd7Gt/+ cochleae by TEM revealed hypermyelinated Type I spiral ganglion axons, as well as significantly variable axon diameter. Proportions of neurons and Schwann cells in the apical spiral ganglia were reduced in Chd7Gt/+ mutant mice. Staining for CtBP2 and GluA2 showed no differences in hair cell synapse formation in Chd7Gt/+ mutant cochleae.

Conclusions

Collectively these results suggest a critical role for CHD7 in the development of neurons and glia in the cochlear spiral ganglion and the importance of appropriate myelination for normal sensorineural hearing. Interestingly, Chd7 haploinsufficiency disproportionately affects the apex of the cochlear spiral ganglion. In contrast, CHD7 appears dispensable for hair cell synapse formation in the mouse cochlea. This study offers critical insight into the pathophysiology of CHARGE syndrome and may lead to clinical advancements in treating sensorineural hearing loss in patients with CHARGE and related disorders. This work was supported by NIH R01 DC014456 (D.M., Y.R.), NIH R01 DC018404 (D.M.), and NIH T32 DC000011 (E.R.).

W13. Short Term Plasticity of Inhibitory Synapses Onto Medial Olivocochlear Neurons

Auditory Pathways: Brainstem

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Background

The synaptic plasticity of synapses onto medial olivocochlear (MOC) efferent neurons in the brainstem is poorly understood. Inhibitory synaptic 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 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 (e)PSCs from MOC somata while electrically stimulating presynaptic MNTB axons to release neurotransmitter. AMPA type glutamate receptors were blocked with CNQX to isolate inhibitory neurotransmission.

Results

Different extracellular calcium concentrations from 0.5 to 2.0 mM were used to test the dependence of synaptic plasticity on 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 action potential rates, followed by

faster stimulation rates to mimic sound-evoked activity. 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. When low rates of stimulation were applied to mimic spontaneous MNTB activity, subsequent faster synaptic activity was reduced in amplitude relative to control conditions.

Conclusions

These results suggest that MNTB suppression of MOC activity is reduced over the course of a sustained stimulus, and that MNTB synapses onto MOC neurons may be in a depressed state even in the absence of sound.

W14. Altered Spine Morphology in the LSO of Fragile X Syndrome Mice

Auditory Pathways: Brainstem

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Background

Cellular and circuit hyperexcitability are well known hallmarks of Fragile X Syndrome (FXS), the most common monogenetic form of autism. Sensory hypersensitivity, and in particular auditory hypersensitivity, is a common symptom and complaint of patients with FXS potentially due to these cellular and circuit hyperexcitability phenotypes in many brain areas. Recent work has shown that there are alterations to areas of the brain responsible for the earliest sound processing steps. These have been described at the cellular, circuit and behavioral levels. The exact mechanisms for this auditory hypersensitivity and underlying alterations at the brainstem level are unknown.

Methods

Studies from other brain areas have shown alterations to dendritic spine morphology/excitability in FXS and are thought to contribute to overall excitability phenotypes. To test whether dendritic spine morphology is altered in the auditory brainstem of FXS mice compared to wildtype, animals were injected with delta-G rabies anterograde tracer into the lateral superior olive (LSO). Spines were then measured with both SIM and STED super resolution techniques to obtain 2D and 3D renderings of labeled LSO spines, followed by software analysis and semi-automated quantification of these super resolution images. Spine number, neck length, spine length, aspect ratio, head area, and width of the spine neck were quantified and compared between FXS and wild type.

Results

While spine numbers and spine heads appeared to be unaltered, we found longer and more immature spines in FXS mutant mice compared to controls.

Conclusions

This study adds a possible mechanism underlying overall auditory hypersensitivity, as immature and elongated spines has been shown to be associated with alterations in synaptic strength in other neural systems. The results also add to the growing literature on auditory deficits, including auditory brain stem and sound localization pathway deficits, in FXS. Additionally, it is the first study to use super resolution techniques to examine and characterize LSO spine morphology independent of FXS phenotype.

W15. Inhibition and Gap Junction Effects on Synchronization Enhancement in Bushy Cells of Ventral Cochlear Nucleus

Auditory Pathways: Brainstem

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Background

Auditory nerve fibers (ANFs) show synchronized firing behavior to low-frequency input stimuli. This kind of behavior is even more prominent in bushy cells of ventral cochlear nucleus (VCN; Joris et al., 1994; Joris and Smith, 2008). Synchrony enhancement in globular bushy cells, which receive synaptic inputs from many ANFs, can be explained by a coincidence detection mechanism performed by these cells. However, the possible mechanisms behind spherical bushy cell synchrony enhancement are still unclear, since they receive very few excitatory inputs from ANFs.

Methods

In this study, bio-physically detailed neural network models of globular and spherical bushy cell microcircuits of VCN are created based on the approach of Manis and Campagnola (2018). Temperature scaling and alternative sodium channel models are also included in the model implementation. The model takes its excitatory inputs from phenomenological ANF model of Bruce et al. (2018). The effects of broadband inhibition (coming from D-stellate cells) and narrowly tuned inhibition (coming from tuberculoventral cells of the dorsal cochlear nucleus) are inspected. Since the models used in this study are computationally expensive, the simulations are performed in the high-performance computing environment Shared Hierarchical Academic Research Computing Network (SHARCNET).

Results

Preliminary results suggest that, while inhibition can affect the synchronization of model spherical bushy cells to low-frequency tones, it cannot fully explain the degree of synchronization and enhancement exhibited by the published data.

Conclusions

Inclusion of gap junctions between bushy cells may be required for the synchronization enhancement behavior seen in physiological recordings.

W16. Enhanced Brainstem Phase-Locking in Low-Level Noise Reveals Stochastic Resonance in the Frequency-Following Response (FFR)

Auditory Pathways: Brainstem

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Background

In nonlinear systems, the inclusion of low-level noise can paradoxically improve signal detection, a phenomenon known as stochastic resonance (SR). SR has been observed in human hearing whereby sensory thresholds (e.g., signal detection and discrimination) are enhanced in the presence of noise. Here, we asked whether subcortical auditory processing (neural phase locking) shows evidence of SR.

Methods

We recorded brainstem frequency-following-responses (FFRs) in young, normal-hearing listeners to near-threshold (40 dB SPL) complex tones (150 Hz) presented concurrent with low-level noise (+20 to -20 dB SNRs).

Results

Though variable across ears, listeners showed improved auditory detection thresholds with subthreshold noise confirming SR psychophysically. At the neural level, low-level FFRs were initially eradicated by noise (an expected masking effect) but were surprisingly reinvigorated at select masker levels (local maximum near ~35 dB SPL). These data suggest brainstem phase-locking to near-threshold periodic stimuli is enhanced in optimal levels of noise, the hallmark of SR

Conclusions

Our findings provide novel evidence for stochastic resonance in the human auditory brainstem and suggest that under some circumstances, noise can actually benefit both the behavioral and the neural encoding of complex sounds.

W17. Relations of Two Metrics for Quantifying the Degree of Phase-Locking: Correlation Index and Vector Strength

Auditory Pathways: Brainstem

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Background

Auditory neurons generate time-locked action potentials (or spikes) in response to temporally changing acoustic stimuli. A spike response pattern is called phase-locked, when its neuronal discharges occur preferentially at a certain phase of a periodic stimulus. In the auditory system, various degrees of phase-locking can be seen from auditory nerves to cortical neurons. The classical metric for quantifying phase-locking is the vector strength (VS), first introduced by Goldberg and Brown (1969, J. Neurophysiol.). Mathematically, VS is the absolute value of the Fourier component of a phase-locked spike train normalized by the total number of spikes. To quantify the temporal response characteristics of a neuron, another metric, called the correlation index (CI), was proposed by Joris et al. (2006, Hear Res.). The CI is defined as the peak value of the shuffled autocorrelogram (SAC), which captures the temporal reproducibility of a neuronal spiking response. Both VS and CI have been used in the field of auditory neuroscience to investigate how temporal information is processed and transferred along the auditory pathways. The analysis of physiological data by Joris and colleagues (2006, Hear Res.) from trapezoid body fibers in cats suggested that these two metrics should covary. However, it remains still unknown how they are generally related to each other.

Methods

In the present study, we derive a rigorous relationship between VS and CI, under certain mathematical assumptions for phase-locking. More specifically, we assume Poissonian spike trains with a temporally changing intensity function modeled with a von Mises distribution, and demonstrate that VS and CI are mutually related via the concentration parameter that determines the degree of phase-locking.

Results

These theoretical results are confirmed to be largely consistent with physiological data recorded in the auditory brainstem of various animals (Ashida and Carr, 2010, Front. Neurosci.). Furthermore, we generate artificial phase-locked spike sequences, for which recording and analysis parameters can be systematically manipulated. Our analysis results suggest that the deviation of the empirical data from the theoretical prediction is often due to multimodal or skewed distribution in the period histogram that generally lead to a reduction of VS. Additionally, we find that the analysis bin width is a critical parameter for computing a CI. A large bin width generally results in an underestimation of SAC peaks, while a small bin width can lead to an imprecise estimation of the SAC due to a larger variation of spike counts.

Conclusions

Overall, our results provide general relations between the two measures of phase-locking, VS and CI, and will aid comparisons of physiological data that are characterized with these metrics.

W18. The Role of Contralateral Spike Timing in Determining Sound Localization Precision in a Spiking Neural Network Model of the Auditory Brainstem

Auditory Pathways: Midbrain

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Background

In mammalian hearing, the sound localization circuit enables the brain to capture differences in the arrival time of binaural sounds at the two ears on a microsecond level. The resulting neural activity propagates initially in separate monaural neural circuits and monaural nuclei, and eventually converges onto binaural neurons. How the micro-second precise integration of the two sets of signals that have propagated across several fiber bundles, synapses, and neuronal types is accomplished, is not entirely understood. Models of sound localization circuits commonly symbolize the propagation of action potentials as constant time delays based on the physical distance between nuclei. Considering the auditory brainstem contains axons with different diameters and myelination patterns resulting in different conduction velocities, the differences in axonal properties and their resulting effects on temporal precision and spatial hearing are not well represented in most existing models.

Methods

In this study, a spiking network model of the mammalian auditory brainstem with multi-compartment axons was developed to examine its temporal dynamics during interaural time difference (ITD) discrimination. Additionally, a decoding model was used to estimate the effects of spike timing on sound localization.

Results

In the model simulation, the axonal conduction velocity of the contralaterally originating inhibitory pathway -the globular bushy cell (GBC) to the medial nucleus of the trapezoid body (MNTB) pathway- had an impact on ITD tuning curves. Additional decoding analysis demonstrated that contralateral spike timing influences the amount of information represented in population responses, and the accuracy of sound localization.

Conclusions

These findings indicate that the contralateral spike timing is a key attribute to the sound localization precision and suggest the linkage between axon myelination and spatial hearing perception.

W19. Transient Complement Expression and Distinct Microglial Subpopulations in the Multisensory Midbrain During an Early Critical Period

Auditory Pathways: Midbrain

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Background

The lateral cortex of the inferior colliculus (LCIC) is a multimodal shell nucleus of the midbrain which receives segregated somatosensory and auditory afferents. Its characteristic modular-extramodular organization develops during an early postnatal critical period (postnatal day, P0-P12), with somatosensory inputs targeting glutamic acid decarboxylase (GAD)-specific modules and auditory inputs surrounding calretinin (CR)-positive extramodular zones. Microglial cells (MGCs) have been implicated in the development and refinement of analogous discretely organized maps in a variety of unimodal systems. Two pathways involved in neuronal-glial interactions whereby exuberant or underutilized synapses are selectively tagged for elimination are the classical complement cascade and the fractalkine signaling pathway. The present study examines the potential involvement of complement signaling in refining early LCIC networks. We also address whether distinct microglial subsets exist within the nascent LCIC, each with unique molecular signatures, and thereby perhaps serving different developmental roles.

Methods

Immunostaining was performed in GAD67-GFP and CX3CR1-GFP mice at designated ages during the defined LCIC critical period (P0, P4, P8, and P12), as well as later timepoints to determine any transience in early expression patterns. GAD67+/GFP mice facilitate easy visualization of emerging LCIC modular fields, while CX3CR1+/GFP mice highlight MGCs expressing the fractalkine receptor. CD11b (a subunit necessary for forming complement receptor 3, CR3), C3 (an upstream complement protein), TMEM119 (a highly specific microglial marker), and CR (an LCIC extramodular marker) antibodies were used in various combinations for immunostaining. Z-stacks of coronal sections throughout the LCIC were acquired and analyzed using a Nikon Eclipse imaging system and associated Nikon Elements software.

Results

CD11b expression is homogeneous throughout the IC at birth, prior to localizing to the LCIC by P4. Coincident with LCIC compartmental sharpening and segregation of interfacing multimodal afferent streams from P8-P12, CD11b expression becomes highly concentrated to layer 2 modular fields. As CD11b is a key protein in the recruitment of MGCs in other systems, such patterns hint towards complement signaling involvement in refining early LCIC multimodal connectivity. C3 is also present in the LCIC during this critical period. Its expression is similarly diffuse at birth, however, unlike CD11b's cellular appearance, C3 staining is more punctate and largely restricted to the neuropil. Interestingly, in CX3CR1+/GFP mice, CD11b labeling does not colocalize with GFP-labeled MGCs. Furthermore, in P12 CX3CR1+/GFP mice stained for TMEM119, distinct MGC subpopulations were again observed.

Conclusions

The lack of colocalization between fractalkine receptor expressing MGCs and both CD11b and TMEM119 markers suggests that unique subsets of microglia exist in the developing LCIC with different molecular signatures and perhaps different responsibilities. Ongoing triple-labeling experiments aim to determine if there is any observed overlap amongst these (CX3CR1, CD11b, TMEM119) and other widely utilized MGC markers (e.g. Iba-1).

W20. Living Electrodes for Auditory Rehabilitation

Auditory Prostheses

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Background

Stimulation and recording of the peripheral and central auditory system currently rely on metallic electrodes that suffer from biocompatibility and field stimulation issues. A biologically-based interface could overcome these limitations, allow for chronic monitoring of the auditory pathways, and form the foundation for a new generation auditory implants. Here we describe the fabrication and implantation of a 'living electrode' derived from cultured cortical neurons and a custom scaffold designed for implantation to provide axonal inputs into the inferior colliculus of a rat.

Methods

Cortical neurons were harvested from whole-brain explants of embryonic rats and cultured in supportive media. Adherent cells were dissociated and induced into aggregates via gentle centrifugation in inverted pyramid micro-wells. Aggregate cultures were virally transduced to express the fluorescent calcium reporter GCaMP6 to enable optical imaging of neuronal activity. A custom scaffold was fabricated with 3% agarose molded with glass capillary tubes and acupuncture needles to form a cylinder with outer diameter of 389 um and inner diameter of 160 um. The inner channel was filled with ECM (rat tail collagen and mouse laminin). Aggregates were then positioned at the ends of the micro-column under stereomicroscopy before returning to culture allowing for neurite extension along the ECM filled column. The construct was then implanted into the inferior colliculus of a rat under stereotactic guidance with the aggregate of cell bodies remaining at the surface and the terminal end of the micro-column within the inferior colliculus. Intravital multiphoton microscopy was used to evaluate for viable cells in the implanted aggregate and spontaneous firing monitored through high speed fluorescent microscopy of the spontaneous activity of the GCaMP expressing neurons.

Results

Cortical neurons were successfully harvested, cultured, formed into aggregates and transfected with GCaMP. These aggregates were formed into living electrodes by culturing at the ends of a custom, ECM filled construct that allowed for neurite extension along its length. This was then successfully implanted into the inferior colliculus of a rat with the implanted neuronal cell bodies accessible for monitoring and stimulation at the brain surface. Neuronal survival and spontaneous neuronal firing from the implanted neuronal cell bodies was directly measured at one-month post-implant.

Conclusions

Significant advancement in auditory implants has been limited by the biocompatibility and field effects of traditional electrode technology. Biologically based stimulation techniques promise to alleviate these drawbacks by allowing for synapse-specific stimulation and the avoidance of non-biologic material for chronic implantation. Here we show the first steps of such an implant utilizing neurons cultured in a biocompatible scaffold to create a 'living electrode'. In vivo implantation is demonstrated with viability at one month. These findings form the foundation for further studies to evaluate the properties of such implants for chronic central auditory monitoring and stimulation.

W21. Development of a New Surgical Approach for Implantation of a High-Density Penetrating Electrode Array in the Feline Auditory Nerve

Auditory Prostheses

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Background

Direct intraneural stimulation of the auditory nerve offers benefits for auditory rehabilitation over contemporary intrascalar cochlear implantation. Direct contact between the stimulating electrode and its neural targets has potential for greater frequency specificity, lower stimulation thresholds, and a broader range of frequency representations. The auditory nerve implant (ANI) being developed is composed of a SYNCHRONY 2 cochlear stimulator (MED-EL) connected by a helical lead to a Utah Slanted Electrode Array (USEA, Blackrock Microsystems). Prior to a human clinical investigation, preclinical animal studies are required to establish device safety, durability, and efficacy. These studies require a surgical approach to the auditory nerve in large animal models. The cat model has ample temporal bone and cochlear nerve anatomical data and suitability for electrophysiologic testing of auditory implants based. Previous attempts to access the auditory nerve in cats have yielded suboptimal nerve exposure and severe iatrogenic injury. This study aimed to develop a safe and feasible surgical approach to the internal auditory canal (IAC) and auditory nerve of the cat that promotes durable positioning of an implantable auditory nerve prosthesis.

Methods

The optimal surgical approach for implantation of the feline auditory nerve was developed in 2 stages. First, systematic microdissections of feline cadavers were performed to evaluate several individual and combined candidate approaches including transbullar, retrolabyrinthine, translabyrinthine, retrosigmoid, and transcochlear approaches, each based on modifications of established human surgical skull base approaches to the IAC. Access corridors and nerve diameters were measured and feasibility of neural implantation of the prosthesis was estimated. In the second stage, a series of acute experiments (i.e., non-recovery surgeries with anesthetized cat) with full neural implantation of USEAs were performed to confirm the feasibility and efficacy of the candidate approach for durable neural implantation.

Results

The retrosigmoid approach required significant cerebellar compression and aspiration, resulting in an unacceptable degree of morbidity, and offered suboptimal visualization of the auditory nerve. Similarly, the retrolabyrinthine and transcochlear approaches afforded limited exposure of the auditory nerve. By contrast, the modiolar, intracanalicular, and cisternal segments of the auditory nerve were safely accessed via the

transbullar translabyrinthine approach. This exposure provided adequate visualization of all auditory nerve segments, thus facilitating implantation of a penetrating nerve array without appreciable contact with the vestibular nerves or facial nerve.

Conclusions

The transbullar translabyrinthine approach to the IAC facilitates precise placement of a penetrating USEA into the feline auditory nerve. Neural function is preserved following exposure and electrode array placement. This will permit anatomic investigations to refine ANI design and facilitate live animal 6-month pre-clinical testing. These tests will establish safety with respect to trauma and stimulation as well as electrophysiologic performance outcomes towards initiation of a pilot clinical investigation in humans.

W22. Frequency-Specific Activation of the Peripheral Hearing System: Controlled Modulation of Intensity

Auditory Prostheses

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Background

For the development of auditory prostheses, the controlled activation of the hearing system is necessary. One colored laser can be modulated to predictably activate different frequency channels of the auditory system through the optoacoustic effect when applied on vibratory structures of the peripheral hearing organ (Stahn et al. 2019). The next important step towards the development of an auditory prosthesis is the controlled modulation of the activation intensity.

Methods

We inserted a 365 µm diameter optic fiber into the outer ear canal of a guinea pig ex vivo and focused the laser pulses at the umbo as the central point of the tympanic membrane (TM). 10 ns laser pulses were applied modulated as sinusoids and the vibration velocity of the TM was recorded with a scanning laser Doppler vibrometer (LDV, PSV-500, Polytec GmbH, Waldbronn, Germany). We calculated the vibration displacement of the TM from the recorded velocity data. The applied laser modulation rates (LMR) ranged between 1 and 10 kHz, the laser pulse rates (LPR) were 32 kHz or 50 kHz and the maximal power was between 20 and 320 mW. We present herein the ex vivo results in comparison with the frequency specific recordings in vivo within the central nucleus of the inferior colliculus (ICC) from our in vivo studies (Stahn et al. 2019).

Results

Considering the single side displacement spectrum, the signal to noise ratio (SNR) of the main peak at the fundamental frequency f₀ increased with increasing power values for the applied LMR/LPR combinations. The SNR of additional peaks at the 2nd harmonics and the LPR increased in a similar manner but were below the main peak SNR for 50 kHz LPR. The maximal displacement amplitude at the umbo was comparable to the displacement amplitude after acoustic stimulation with pure tones up to at least 60 dB SPL. This was in line with the results at ICC level.

Conclusions

The results demonstrate ex vivo and in vivo that intensity can be modulated through variation of the incoming optical power values. Displacement amplitudes comparable to at least 60 dB SPL could be reached in guinea pigs. Studies are in progress to further optimize the stimulation parameters improving the energy consumption and increasing the induced sound amplification through the optoacoustic stimulation of the peripheral hearing organ.

Reference:

*Stahn, P., Lim, H. H., Hinsberger, M. P., Sorg, K., Pillong, L., Kannengießer, M., ... & Wenzel, G. I. (2019). Frequency-specific activation of the peripheral auditory system using optoacoustic laser stimulation. *Scientific reports*, 9(1), 1-13.

W23. Simulation Study of Novel Auditory Nerve Implant: Model and Expected Percepts

Auditory Prostheses

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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 Microsystems). The overall goal of the ANI is to potentially improve hearing performance over current hearing treatment options. The ANI will be directly implanted into the auditory nerve with the aim of achieving more focused activation and greater transmission of auditory information to the brain compared to the cochlear implant. The ANI consists of a MED-EL stimulator attached to a Utah Slanted Electrode Array (USEA) with 15 electrodes in a 3x5 configuration. A computational model of ANI stimulation was created to simulate the 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 to determine the potential performance of the device.

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, and tonotopic frequency organization across the nerve. This 2D model of auditory nerve fibers was translated into 3D by extending each fiber in a third dimension. 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 along the nerve was employed by applying an electrical current at the electrode tips to simulate excitation profiles. From these excitation profiles, vocoded stimuli were created to be presented to normal-hearing subjects to simulate the expected percepts from the ANI. 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. Threshold, comfortable loudness level, and dynamic range were determined for each of the 15 simulated electrodes for each of 10 normal-hearing subjects. Loudness balancing was also performed. The loudness-balanced stimuli were used in the pitch-ordering study, which 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 due to the complex organization of frequency fibers within the auditory nerve.

Conclusions

A computational model of the ANI was created to simulate the expected effects of directly stimulating 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.

Funded by NIH UG3NS107688 and NSF UtB DGE 1734815

W24. Towards Decoding Selective Attention in Single Sided Deafened Subjects Using Cochlear Implant Electrodes

Auditory Prostheses

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Background

Decoding the attention of a listener to a given speaker in a situation with multiple concurrent speakers, background noise and reverberation is a promising research line in cochlear implant (CI) research. It has been

shown that the envelope of an attended speech stream can be decoded from single-trial electroencephalography (EEG). Applications of this technique can be found in future closed-loop CIs that can be fitted objectively or include brain-steered speech enhancement algorithms. State-of-the-art high-density EEG systems can be used for this purpose but are unsuitable to be used in the clinical routine or in daily life. The current trend to develop small and portable EEG systems points out the need for EEG systems that are easily applicable and less visible than common EEG caps. CI backward telemetry systems provide the unique possibility to record electrophysiological signals invasively without the need for an extra surgery. This study aimed at recording cortical signals with a novel CI backward telemetry system that allows us to record a single-channel signal continuously.

Methods

Six single-sided deafened subjects implanted with an Advanced Bionics CI participated in the study. In a first step, we recorded cortical auditory evoked potentials (CAEPs). In a second step, we recorded the cortical response to continuous speech stimuli in a selective attention paradigm where the subject had to attend to one speech stream while ignoring a second one. All stimuli were presented acoustically through an inserted earphone to the normal hearing ear. EEG was recorded with the CI electrodes in the contralateral ear and additionally with a high-density scalp EEG system. The EEG recordings were used to reconstruct the envelope of the attended speech stream using a linear model.

Results

CAEP morphologies were successfully detected in all subjects with high-density scalp EEG and in some subjects with CI-based recordings. It was possible to reconstruct the envelope of the attended speech streams, whereby the decoding performance was best with high-density scalp EEG. CI-based recordings varied largely among patients, probably due to different CI electrode locations and orientations with respect to the auditory cortex. We found decoding accuracies above chance level in three out of six subjects using CI-based recordings. The results indicate that it is possible to record cortical responses with CI electrodes, at least in some subjects.

Conclusions

To conclude, this study demonstrates the feasibility to reconstruct the envelope of an attended speech stream from single-channel scalp EEG recordings. Moreover, in some subjects it is possible to record cortical signals with CI electrodes. These findings pave the way towards making EEG recordings more applicable in the clinic, e.g. for an objective closed-loop CI fitting, and for future brain-steered CIs. However, the study also points to the need to further improve the CI backward telemetry systems.

W25. The Effect of Age on the Functional Status of the Cochlear Nerve in Cochlear Implant Patients

Auditory Prostheses

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Background

Older cochlear implant (CI) patients generally have worse speech perception capabilities compared to younger CI patients (e.g. Lin et al., 2012; Roberts et al., 2013; Sladen & Zappler, 2005). While declining cognitive function may contribute to these speech perception deficits (Budenz et al., 2011; Lin et al., 2012), a recent human temporal-bone study has shown degeneration of the cochlear nerve (CN) with advancing age (Wu et al., 2019). However, the effect of age on the functional status (i.e. number and responsiveness of nerve fibers) of the CN has not been well established. We recently developed an analytical model for quantifying the overall functional status of the CN based on results of electrophysiological measures of the electrically evoked compound action potential (eCAP, Skidmore et al., 2020). Building on this previous work, we created a new analytical model for quantifying local CN function and assessed the effects of advanced age on local CN function in CI users.

Methods

The input (predictive) variables of this new analytical model included eCAP threshold, slope of the eCAP amplitude growth function, negative-peak (i.e. N1) latency, and absolute refractory recovery time estimated

based on the refractory recovery function. The output variable of this model was local CN index. To create this model, eCAP results measured in children with cochlear nerve deficiency and children with normal-sized CNs were used as the training dataset. Compared with the original model (Skidmore et al., 2020), the eCAP variables measured at different electrode locations were not combined based on the known neural survival patterns of those patient populations. Rather, the new model provides an optimized combination of eCAP parameters that represent the CN responsiveness to electrical stimulation delivered by individual electrodes along the electrode array. To date, we have calculated the local CN index for 38 adults ranging in age from 48.8 to 80.8 years (mean: 66.6, SD 8.9) at three electrode locations across the array. Pearson correlation analyses were performed to compare local CN indices with age.

Results

Our preliminary results showed that age was significantly correlated with the local CN index at the basal ($r = -0.41$, $p=0.005$) and middle ($r = -0.43$, $p = 0.003$) regions of the cochlea, but not at the apical region ($p>0.05$).

Conclusions

Based on our preliminary results, we conclude that the functional status of the CN is significantly affected by the age of CI patients. This effect of age is stronger in the basal and middle regions of the cochlear than in the apical region.

W26. Relationship of Immune and Non-Immune Cells to the Foreign Body Response in the Mouse Cochlea to Electrode Implantation

Auditory Prostheses

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Background

Loss of hair cells results in sensorineural hearing loss. The function of the hair cells can be bypassed by a cochlear implant to restore hearing. The foreign body response (FBR) in the cochlea to the cochlear implant electrode array is believed to play a role in the loss of efficiency of the implant seen in a subset of recipients. We have characterized the cells involved in the FBR in a mouse model of cochlear implantation with and without chronic electrical stimulation.

Methods

10-12-week-old CX3CR1-GFP, Thy1-YFP reporter mice of C57Bl6 background with normal hearing (verified by ABR and DPOAE), were implanted with 3 channel cochlear implant in the left ear via a round window insertion. The right ear was the non-operated control. 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 5 hrs/day, 5 days/week for either 1, 2 or 3 weeks with low (30 CL below NRT threshold) or high stimulation (at the level of behavioral response). Cochleae were harvested for immunohistochemistry 14, 21 or 28 days post-operatively, fixed with 4% PFA, cryopreserved with serial concentrations of sucrose solutions, and embedded in OCT. For each cochlea, 3 consecutive, 30 μ m thick sections, parallel to the mid-modiolar plane were labeled with antibody against CD45 (common leukocyte antigen) to quantify immune infiltration. The outlines of scala tympani, Rosenthal canal, and lateral wall for each turn were traced manually to measure the volume of each and to quantify spiral ganglion neurons (SGNs) and immune cell density.

Results

Irrespective of duration of implantation and level of stimulation, cochlear implantation resulted in significant infiltration of CD45+, immune cells and CD45-negative, non-immune cells into the cochlea. The infiltration is predominantly localized to scala tympani in the base of the cochlea adjacent to the electrode array; scala media and Rosenthal canal are largely devoid of any cellular infiltration. CD45+ immune cells are either CX3CR1+ or CX3CR1-, indicating macrophage and non-macrophage immune cells, respectively. There was no significant degeneration of Thy1+ SGNs as a result of implantation.

Conclusions

These data suggest role of immune (macrophage and non-macrophage) and non-immune cellular infiltrates as part of the post-implantation FBR in mouse model. Further molecular characterization of these individual cell types is underway.

W27. Effect of Advanced Age on Neural Adaptation and Recovery of the Electrically Stimulated Auditory Nerve in Adult Cochlear Implant Users

Auditory Prostheses

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Background

Cochlear implants (CIs) have become a common treatment for severe-profound sensorineural hearing loss in patients 65 years and older (Lin et al., 2011). Speech perception capabilities in this population often are poorer than younger CI users. Temporal encoding properties of the auditory nerve (AN), such as neural adaptation and recovery from adaptation caused by prior stimulation, produce peaks in the discharge rate of the AN which serve to enhance acoustic onsets in the speech waveform (Delgutte, 1997). Alteration in AN encoding such as excessive neural adaptation and/or slow adaptation recovery could potentially lead to poor AN representation of temporal envelope cues (Jeng et al., 2009), ultimately affecting speech perception (Nelson & Donaldson, 2001). Aging is known to be associated with AN degeneration (Wu et al., 2019), which could affect adaptation and recovery processes. This study aimed to evaluate the effects of age on neural adaptation and adaptation recovery at the AN in CI users.

Methods

To date, 30 postlingual deaf adult CI users have participated. Participants had a Cochlear™ Nucleus® device in the test ear and were split in to two groups, <65 and ≥65 years (average age: 52.1 and 72.6, respectively). For both adaptation and adaptation recovery paradigms, 100-ms pulse trains were presented at 2 Hz in a monopolar-coupled stimulation mode at four carrier pulse rates: 500, 900, 1800 and 2400 pulses per second (pps). Stimuli were delivered to individual CI electrodes at the maximum comfortable level (i.e., C level) measured at 2400 pps at four electrode locations (e.g., 3, 9 15, 21). The amount of neural adaptation at each rate was quantified using the adaptation index (AI; He et al., 2016) defined as the averaged normalized electrically evoked compound action potential (eCAP) amplitude measured within three-time windows over the course of the pulse train. Adaptation recovery was evaluated by measuring eCAP amplitude in response to a probe pulse at different time points after a masker-pulse-train ceases (i.e., the masker-probe interval, MPI) using a modified-forward masking paradigm. MPIs tested were 359, 500, 1054, 2000, 4000, 8000, 16000, 32000, 64000, 128000, and 256000 μs. Mathematical modeling was used to estimate the time constants of the adaptation recovery function.

Results

Preliminary results suggest AIs increased with carrier rate of the pulse train, with the smallest AI measured at 2400 pps. Aging had a significant negative effect on the amount of AI only at 1800 and 2400 pps. Two distinct patterns of adaptation recovery function were observed. Different techniques were investigated to identify optimized mathematical model(s) to estimate adaptation recovery time constants.

Conclusions

Preliminary results suggest the electrically-stimulated AN exhibited greater neural adaptation with advanced age at high carrier rates. Additionally, neural adaptation recovery function varied among subjects, and across electrode locations.

W28. Spectral Weighting of Sound Localization Cues: Impacts of Simulated Hearing Loss and Noise Masking

Binaural Hearing & Sound Localization

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Background

Listeners' ability to localize a complex sound relies upon spatial cues (interaural-time-differences [ITDs] and interaural-level-differences [ILDs]) distributed across the frequency spectrum. For listeners with normal hearing (NH), the duplex theory of sound suggests that ITDs dominate the localization of broadband and low-frequency stimuli while ILDs play a greater role in the localization of high-frequency stimuli [e.g. Macpherson & Middlebrooks 2002, J Acoust Soc Am 111:2219-36]. Folkerts and Stecker [2018, ARO 42:505] employed observer weighting methods to quantify spectral weighting functions (SWFs) for various complex sounds. Frequency components with the highest weight fell within the spectral "dominance region", around 400-800 Hz [Bilsen & Raatgever 1973, Acustica 28:131-132]. Folkerts and Stecker [2020, ARO 43:865] extended this work to measure the effect of component intensity on SWFs. Frequency components with the highest weight followed "level dominance" [Berg, 1990, J Acoust Soc Am 88:149-158] with increased weights for the loudest components. The current study further extends this work by measuring the effect on SWFs when complex sounds are filtered through a high-frequency sloping hearing-loss simulation (HLS) and when complex sounds are presented with competing noise.

Methods

In one condition, target stimuli were processed by HLS that reduces audibility, broadens auditory filters, and introduces temporal jitter to simulate hearing loss [Cuevas-Rodríguez et al. 2018, IEEE (SIVE)]. In a second condition, targets were presented in a spatially complex noise background at various signal-to-noise ratios (SNR); in that condition, target components were presented at equal intensity (no HLS). In both conditions, NH listeners were asked to localize 100-ms complex tones containing seven noise-band components. Each trial presented individual components with random azimuth variation of 0°, ±5, 6.25°, or ±11.25° around a "base" azimuth selected for that trial. Base azimuths varied within a ±56.25° range across trials. Multiple linear regression of the rank-transformed azimuthal response estimated the SWFs weights.

Results

In the HLS condition, weighting patterns were evaluated relative to audibility, frequency resolution, and ITD ambiguities, all of which predict weighting function maxima to shift toward lower-frequency components. Similarly, disruption of low-frequency binaural information in the competing noise condition at less favorable SNRs, was predicted to increase weighting of high-frequency components [Brungart & Simpson 2009, J Acoust Soc Am 126:3199–3208].

Conclusions

Spatial hearing may be impacted by shifts in the frequency dominance region due to reduced audibility resulting from hearing loss or complex/noisy backgrounds with competing distractors. [Supported by NIH R01-DC016643]

W29. Effects of Reduced Device Delay Mismatch on Precision and Trueness of Sound Localization Judgements in Bimodal Listeners

Binaural Hearing & Sound Localization

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Background

Asymmetric hearing loss often leads to asymmetric provision with hearing devices in both ears. In bimodal provision i.e. one ear provided with a cochlear implant (CI) and one ear provided with a hearing aid (HA), the different devices are typically not aligned in terms of signal processing delays. This temporal difference is called device delay mismatch. In earlier work Zirn et al. (2015) could show that this device delay mismatch between an ear provided with a current MED-EL CI and a contralateral ear provided with a digital HA is between 3 and 10 ms.

Methods

The presented work applied sound localization tests in an A-B-B-A test design to determine if a reduction of the device delay mismatch has beneficial effects on sound localization accuracy in bimodal listeners. For this purpose, a battery-powered, portable and programmable delay line was designed to be able to reduce the device delay mismatch. With this device the CI stimulation was delayed according to the measured HA processing delay in nine bimodal listeners.

Results

The applied reduction of device delay mismatch had significant effects on the precision (expressed by the rms error) and the trueness (expressed by the signed bias) of the listeners sound localization judgements. The rms error could be reduced from 52.6° to 37.9° and the signed bias was improved from 25.2° to 10.5° after device delay mismatch reduction. Every test subject benefited.

Conclusions

Sound source localization accuracy in the frontal horizontal plane can be improved in bimodal CI/HA users by reducing the device delay mismatch. Therefore, temporal alignment between CI and HA could be a viable step to further improve bimodal provision.

W30. Contextual Plasticity in Sound Localization Induced by Passive Exposure to Transient Sounds

Binaural Hearing & Sound Localization

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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 distractor-target trials in which the distractor was at a fixed location while the target location varied. The observed bias is always away from the contextual distractor location, even though the distractor is not present on the experimental trials. Here, two experiments were performed. Exp. 1 examined whether this phenomenon is dependent on engagement of the subject in an active localization task on the contextual trials, as used in previous studies. Here, instead, contextual trials only contained the distractor without any targets, and the listener's task was to passively listen to the context. It was hypothesized that if CP is mainly caused by adaptation to the distractors, then it would be observed also in this condition. Exp. 2 examined whether CP is also observed in virtual environments, both reverberant and anechoic. It used a setup similar to Exp. 1 and it was hypothesized that the observed CP might be stronger than in Exp. 1, in particular in anechoic virtual space, as no real-world anchoring to stimuli in real world is available. In both experiments, distractor locations were varying from block to block while the target range was fixed across blocks, to examine how CP depends on the distractor location.

Methods

In both experiments, the target stimulus was a 2-ms noise burst (click), while the distractor was a click train consisting of 12 such clicks. Six target locations were used, $\pm 33^\circ$, $\pm 22^\circ$, $\pm 11^\circ$ in Exp 1 and $\pm 30^\circ$, $\pm 20^\circ$, $\pm 10^\circ$ in Exp. 2. Distractor locations were fixed across block at 0° , $\pm 45^\circ$, or $\pm 90^\circ$ in Exp. 1 and 0° or $\pm 50^\circ$ in Exp. 2. In addition, baseline blocks contained no distractors. 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.

Results

In Exp. 1, repulsive contextual biases of up to 5° were induced by the lateral distractors, while a bias of up to 3° was induced by the central distractor. In Exp. 2, the patterns were similar, but the effect was much stronger, reaching up to 19° in the virtual anechoic condition and approximately 17° in the virtual reverberant condition. The effects were strongest near the distractor and decreased with target separation from distractor.

Conclusions

These results confirm that contextual plasticity is a general localization aftereffect phenomenon that does not require active engagement in a localization task.

[Work supported by APVV DS-FR-19-0025].

W31. Cortical Mechanisms of Binaural Integration Investigated Using Functional Near-Infrared Spectroscopy (fNIRS)

Binaural Hearing & Sound Localization

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Background

Binaural hearing depends on integration of inputs from both ears by the auditory system and facilitates perception of multiple sound sources in our auditory environment. Cochlear implants (CIs) have been used to rehabilitate binaural hearing in listeners with single-sided deafness, who have intact hearing in one ear and are deaf in the other (SSD-CI), and in listeners with bilateral deafness (BiCI). Behavioral data suggest that both groups recover some binaural hearing, but they perform poorer than do normal-hearing (NH) listeners in binaural hearing tasks. To date the binaural deficits of SSD-CI and BiCI listeners have not been studied on a neural level. We aim to assess neural mechanisms of binaural integration by measuring cortical neural activity using functional near-infrared spectroscopy (fNIRS).

Methods

To examine binaural integration, we utilize a paradigm devised by Cherry & Taylor (1954) in which sequential speech segments alternate between ears. From low to high switching rates, this stimulus produces a canonical V-shaped speech intelligibility function. At low switching rates, listeners extract speech information by switching attention between ears. At high switching rates, listeners bridge the short and frequent silent gaps in either ear to understand speech. However, at intermediate switching rates (around 4-6 Hz), neither strategy is highly effective, therefore more effort is exerted to integrate the inputs from both ears and speech intelligibility decreases. Using switching speech and fNIRS, this study assesses cortical activity in the bilateral auditory cortices (AC), which are sensitive to speech intelligibility, and in the bilateral inferior frontal gyri (IFG), which have been shown to encode effortful listening. We simulate SSD-CI and BiCI conditions for NH listeners by presenting vocoded speech in the right ear or both ears, respectively. In a third condition, non-degraded (clear) speech is presented. For all stimulus conditions, speech is presented at four switching rates: 2, 4, 8, and 32 Hz. We also record intelligibility scores for all subjects.

Results

We hypothesize that speech intelligibility directly correlates with auditory neural responses, hence fNIRS measures will yield greater cortical activity in the AC in conditions with more intelligible speech. Since our selected switching rates are expected to reproduce the V-shaped intelligibility function in the SSD-CI and BiCI conditions but not in the clear speech condition, then for the SSD-CI and BiCI conditions we expect to observe V-shaped trends in peak amplitudes of block-averaged fNIRS responses in the AC. We also hypothesize that increased effort is associated with greater cortical activity in the IFG; if the four switching rates differ in task demands based on the posited listening strategies, then we will observe greater fNIRS responses in the bilateral IFG in the more demanding conditions.

Conclusions

Research funded by the American Otological Society Fellowship Grant, and NIH DC003083.

W32. Optogenetic Activation of Medial Olivocochlear Efferent Fibers

Cellular/Systems Development

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Background

During development, inner hair cells (IHCs) in the mammalian cochlea are unresponsive to acoustic stimuli but instead present intrinsic electrical activity, crucial for the normal development of the auditory pathway. During this same period, neurons originating from the medial olivocochlear complex (MOC) transiently innervate the soma of IHCs. This synapse is mediated by acetylcholine (ACh), which activates nicotinic receptors assembled by $\alpha 9$ and $\alpha 10$ subunits and ultimately controls IHC excitability during this period. Even though this is a cholinergic synapse, previous evidence indicates the presence of abundant GABA and presynaptic GABAB receptors. Moreover, the application of GABAB receptors agonists can reduce ACh release.

Methods

To specifically drive gene expression in MOC efferent fibers, transgenic *Chatm2(crc)Lowl/J-Cre* (ChAT-Cre) and *Gad2tm1(cre/ERT2)Zjh/J-Cre* (GAD2-Cre) mice were mated with a floxed ChR2 line, Ai32. Whole cell patch clamp at the IHC were performed in P9-11 mice while optogenetically stimulated either cholinergic (ChAT-Cre/ChR2) or GABAergic (GAD2-Cre/ChR2) fibers. The expression of Cre in GAD2-Cre mice was induced by tamoxifen, injected during P1 to P5 (I.P 50 μ g/g).

Results

We showed, for the first time, that cholinergic MOC fibers can be optogenetically activated in ChAT-Cre/ChR2 mice. We found that in ChR2 homozygous mice (n=10), ACh release is triggered more robustly than in ChR2 heterozygous mice (n=7). In addition, blocking GABAB receptors with CGP36216 while stimulating ChR2-expressing cholinergic fibers increased the amplitude of the response and decreased synaptic failures, suggesting the presence of GABA together with ACh in the synaptic cleft. Optogenetic experiments were also performed to activate GABAergic fibers in GAD2-Cre/ChR2 mice. Preliminary results indicated that activation of ChR2 by light is not sufficient to elicit a measurable cholinergic synaptic response in IHC per se.

Additionally, immunohistochemistry techniques were used to characterize cholinergic and GABAergic expression in B6.Cg-Gt(ROSA)26Sortm14(CAG-tdTomato)Hze/J (tdTomato) crossed with ChAT-Cre or GAD2-Cre respectively.

Conclusions

Although our functional data in ChAT-Cre/ChR2 mice suggest that, during development, GABA is co-released with ACh from MOC terminals, a more detailed study of the GAD2-Cre /ChR2 mice would be needed to back this conclusion.

W33. Caspase-3 Activity is Specialized for Non-Apoptotic Neurodevelopmental Functions in the Auditory Brainstem

Cellular/Systems Development

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Background

The auditory brainstem contains specialized circuitry responsible for determining sound source location based on interaural differences in sound arrival time. This computation involves extremely precise neural connectivity, the development of which is a major focus of our research. We previously showed that the apoptotic protease caspase-3 is necessary for normal development of the chick auditory brainstem projection

from nucleus magnocellularis (NM) to nucleus laminaris (NL). Inhibition of caspase-3 prior to the period of developmental cell death in auditory brainstem nuclei resulted in NM axon mistargeting. However, the mechanisms by which non-apoptotic caspase-3 activity facilitates the formation of the NM-NL synapse are not known. Here we investigated processes downstream of caspase-3 by characterizing the proteins cleaved by caspase-3 in the chick auditory brainstem.

Methods

Chick embryos were treated by intraventricular injection of the caspase-3 inhibitor z-DEVD-fmk or vehicle solution on embryonic days 9 and 10. Brainstems were harvested and subjected to proteomic mass spectrometry. Peptidomes were filtered for peptides likely generated by in vivo caspase-3 activity based on two criteria: the peptide terminated in a caspase-like cleavage site (i.e. with a C-terminal aspartate or glutamate residue), and the peptide was present in vehicle-injected brainstems but absent in caspase-3-inhibited brainstems. Proteins with at least one such peptide were considered likely auditory brainstem caspase-3 (ABC3) substrates.

Results

ABC3 substrates included novel caspase-3 substrates as well as proteins cleaved by caspase-3 in other contexts. Of the latter, cleavage targets previously identified in non-apoptotic contexts were more heavily enriched among ABC3 substrates than cleavage targets previously identified in apoptotic contexts. We generated an IceLogo of the ABC3 consensus cleavage site and found that the resulting tetrapeptide sequence N-terminal of the scissile bond (DHRD) bore some similarity to that of the canonical caspase-3 consensus site (DEVD). However, the substitution of “EV” with “HR” suggested a systematic change in the usual substrate preference of caspase-3. We used functional annotation enrichment analysis to determine whether ABC3 substrates with “HR” cleavage sites were associated with a specific protein category disproportionately targeted by caspase-3 activity. Compared to the background of all ABC3 substrates, the GO term “structural constituent of cytoskeleton” was enriched among ABC3 substrates with an H or R at the same position as the consensus sequence.

Conclusions

These findings suggest that the observed ABC3 consensus sequence reflects a shift toward cleavage of cytoskeletal proteins, which are essential for axon guidance and synapse formation. This substrate preference represents a specialized, non-apoptotic function for caspase-3 in auditory brainstem circuit formation.

W34. Three-Dimensional Human Dental Pulp Stem Cell Culture for Generating Otic Neuronal Progenitor Spheroids

Cellular/Systems Development

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Background

The development of a stem cell-based therapy for auditory neuron (AN) replacement requires overcoming several challenges. One being to successfully differentiate otic neural progenitors (ONPs) from unlimited and easy access source of stem cells for autologous cell therapy. Mesenchymal stem cell (MSC) therapy is an emerging treatment modality for a number of human diseases. Human dental pulp stem cells (hDPSCs) could be an alternative stem cell type as it possesses both mesenchymal and neural characteristic features due to its ectodermal origin, ease of isolation and higher proliferation. In this study, we used neural differentiation protocol to differentiate hDPSCs towards neural progenitors and subsequently to ONPs.

Methods

We used human DPSCs isolated from dental pulp tissue by immunoselection of a cell surface marker (Stro-1) by flow cytometry to generate a homogenous cell population. MSC dental pulp Stro-1+ cells were cultured in ultra-low attachment plates to generate proliferative neurospheres in a defined medium (DMEM/F12, N2) supplemented with EGF and bFGF growth factors during 7 days in vitro. Initially, quantitative analysis (i.e., diameter,...) of spheroids was collected daily to follow their spatio-temporal dynamics over the time course of in vitro three-dimensional (3D) cultures. Cell proliferation and apoptosis within the spheroids, were evaluated

by the expression of Ki67 and Caspase3/7 assay respectively. Finally, we characterized the expression of mesenchymal, neural crest and ONP gene markers in differentiated spheroids by qPCR and immunocytochemistry analyses.

Results

Our in vitro data show that the neuro-induction of DPSCs by cell aggregation and growth factors generated homogenous regular well shaped neurospheres of about 250 to 300 μm diameter. This diameter range of spheroids has been deemed as an appropriate size to ensure factors and nutriment diffusion throughout the spheroids. In parallel, apoptotic profile assessed by CASP3/7 expression was confined to a small area within the spheroid core. Our preliminary characterization of differentiated spheroids by qPCR and immunohistochemistry indicated expression of Pax6, an earliest marker for neural fate along with other markers specific to neural crest (Nestin, Sox10,..) and ONP (Gata3, Sox2,..) lineage.

Conclusions

We generate hDPSC-derived ONP spheroids that mimic the gene expression profiles characteristic of ONPs, which are upstream in the AN lineage. We are currently testing the potential of ONPs differentiation to AN lineage in a 3D-spheroid model under different induction paradigms. This study represents a first step towards future cell-based regeneration strategies for hearing loss.

W35. Ephrin-A3 is Required for Tonotopic Map Precision and Auditory Functions in the Mouse Auditory Brainstem

Cellular/Systems Development

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Background

Sound information is transmitted to the central nervous system via spiral ganglion neurons (SGNs) in the cochlea. Auditory nerve fibers of SGNs innervate the cochlear nucleus in the brainstem in a tonotopic fashion, meaning connectivity of auditory neurons is organized by neuron's responses to different sound frequencies. Ephs and ephrins signaling molecules are known to be involved in axon guidance and have been shown to play a role in development of topographic gradients in other sensory systems. In this study, we explored whether Eph/ephrin signaling serves as a molecular guidance mechanism in tonotopic map formation.

Methods

We used in situ hybridization to screen the expression of ephrin molecules and found that ephrin-A3 molecules are expressed in a gradient along the tonotopic axis of the cochlear nucleus during development. We then conducted ephrin-A3 stripe assays to test whether ephrin-A3 forward signaling guides auditory nerve fibers to find their targets in the cochlear nucleus and thus plays a role in tonotopic map formation. We also recorded auditory brainstem responses and evaluated frequency discrimination using an acoustic startle-based approach in ephrin-A3 mutant mice. We then exposed ephrin-A3 mutants to pure tones and analyzed tonotopic bands of neuronal c-fos activation in the anteroventral cochlear nucleus.

Results

We found that auditory nerve fibers were repelled by stripes containing ephrin-A3 in a time-dependent fashion during development. In the absence of ephrin-A3, mutant mice showed delayed auditory brainstem responses and impaired sound-frequency discrimination in acoustic startle-based assays. Tonotopic bands of neuronal activation upon pure-tone stimulation also became wider in the anteroventral cochlear nucleus of ephrin-A3 mutants.

Conclusions

These findings indicate an essential role of ephrin-A3 in tonotopic map formation and auditory functions.

W36. POU3F4-Expressing Otic Mesenchyme Cells Promote Spiral Ganglion Neuron Survival

Cellular/Systems Development

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Background

Current therapies to improve hearing, including hearing aids and cochlear implants, require intact and functional auditory neurons. Within the inner ear, the primary auditory neurons, spiral ganglion neurons (SGNs), are surrounded by mesenchyme cells, which express the transcription factor Pou3f4 (also known as Brn4). POU3F4 mutations have been associated with DFNB2, the most common form of X-linked deafness. Loss of Pou3f4 includes developmental malformations of the middle and inner ear, and loss of endocochlear potential. We showed previously that interactions between Pou3f4-expressing mesenchyme cells and SGNs control SGN axon bundling during development. Pou3f4 continues to be expressed by mesenchyme cells well into adulthood, but how Pou3f4 continues to affect SGN development or maintenance following birth is not well understood.

Methods

To address the impact of Pou3f4 on SGN development and maintenance, we documented Pou3f4 protein expression in early postnatal mouse cochleae, then examined SGN survival and differentiation in Pou3f4 knockout mice (Pou3f4y/-). We also examined macrophage morphology and SGN axon outgrowth.

Results

In Pou3f4y/- mice, SGN density begins to decline by the end of the first postnatal week, with approximately 25% of SGNs ultimately lost. Interestingly, this period of neuronal loss coincides with elevations in apoptosis and the appearance of a transient population of Pou3f4-expressing cells amongst spiral ganglion cell bodies. We also found that macrophages in Pou3f4y/- cochleae appeared amoeboid, suggesting active phagocytosis and/or that loss of Pou3f4 enhances oxidative stress in the cochlea. Finally, we used a sparse SGN labeling model to examine axon outgrowth patterns in Pou3f4 nulls and found no differences in the percentages of neurons making contact with the sensory domain. This suggests that SGN loss in Pou3f4y/- cochleae does not result from loss of synaptic or trophic input from the sensory epithelium.

Conclusions

These data suggest that Pou3f4-expressing mesenchyme cells maintain SGN populations during the early postnatal period. Currently, we are determining how otic mesenchyme cells signal to SGNs to promote their survival.

W37. Programmed Cell Death Promotes Macrophage Recruitment Into the Developing Cochlea

Cellular/Systems Development

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Background

Programmed cell death plays a critical role in the development and maturation of the cochlea. In particular, cellular remodeling occurs within the greater epithelial ridge (GER) of Kolliker's organ, leading to the formation of the inner sulcus. In mice, this period of cell death normally occurs between P5-13 (Peeters et al., Mol Cell Endocrinol 2015) and is essential for normal cochlear function. Similar forms of cell death are common in many embryonic tissues, and the resulting cellular debris is often cleared by macrophages. The developing cochlea contains numerous macrophages, but the role of macrophages in otic development is unclear. This study examined the relationship between programmed cell death and macrophage numbers in the cochleae of neonatal mice.

Methods

Experiments used CX3CR1-GFP/+ mice, which express GFP in all macrophages. In initial studies, mice were euthanized at P3-60 and their cochleae were removed, fixed and processed for immunolabeling. In other studies, mice received either thyroid hormone (T3) or saline (controls) at P0 and P1, and their cochleae were fixed and examined at P3-13. Specimens were labeled with combinations of anti-GFP (GFP+ macrophages), anti-cleaved caspase 3 (apoptosis marker) and anti-SOX2 (supporting cell marker) antibodies, and cell nuclei were labeled with DAPI. All cochleae were imaged as whole mounts using confocal microscopy.

Results

Dying cells in the GER were first observed in the basal region of the developing cochlea, beginning at P5. A wave of apoptotic cells then progressed along the tonotopic axis of the cochlea, reaching the apex by P7 and terminating by ~P13. We also observed changes in the numbers of macrophages in GER during this same period. At a given spatial location, the increase in macrophage numbers lagged behind the initiation of cell death by ~2 days. High numbers of macrophages were present throughout the GER between P7-10, and we observed numerous macrophages engaged in the phagocytosis of dying cells. Prior studies have shown that the onset of cell death in the GER is regulated by thyroid hormone and that treatment of the developing cochlea with thyroid hormone (T3) can evoke early cell death (e.g., Peeters et al., 2015). In agreement with those observations, we found that injection of T3 at P0/1 evoked cell death in the GER, beginning at P3. This early induction of cell death was accompanied by early recruitment of macrophages into the GER, which began at P5.

Conclusions

Our results suggest that programmed cell death is one factor that recruits macrophages into the developing cochlea and that these macrophages engage in the clearance of dying cells.

W38. COVID-19 is Not Associated With Previous Otitis Media

Clinical Otolaryngology & Pathology

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Background

Chronic inflammatory conditions are known risk factors for infection with SARS-CoV-2, the virus that causes COVID-19. Understanding risk factors for infection is important in identifying patients more likely to contract the disease. We investigated whether a previous diagnosis of otitis media is associated with an increased frequency of COVID-19 diagnosis.

Methods

We reviewed the records of 362,015 adult and pediatric patients who sought consultation for suspected COVID-19 anytime until August 10, 2020. The main inclusion criteria were: (1) a previous diagnosis of any form of otitis media prior to March 15, 2020, whether suppurative, acute, recurrent, with effusion, or chronic otitis media with or without cholesteatoma; and (2) a laboratory or clinical diagnosis of presence or absence of COVID-19. Age (range 1-90 years) and sex were included as covariates. Standard statistical analyses using t-test, chi-square test or logistic regression were performed.

Results

A total of 650 (0.18%) otitis media patients were clinically diagnosed with COVID-19, while 381 (0.11%) otitis media patients had a laboratory diagnosis of SARS-CoV-2 infection. Only 275 (0.08%) individuals were COVID-19-positive by both laboratory and clinical diagnoses. These rates are much lower than COVID-19 or SARS-CoV-2-positive rates within the state. The mean age for COVID-19-positive patients was 46 years, while COVID-19-negative patients were younger at 26 years on average or a median of 16 years ($p < 2.2 \times 10^{-16}$). Sex was not a significant risk factor for infection. Initial chi-square analysis identified an association between otitis media type (namely suppurative, recurrent, and chronic with or without cholesteatoma) and clinical COVID-19, but these associations were not significant when corrected for age and sex.

Conclusions

Unlike other inflammatory conditions, previous otitis media is not a predisposing factor for SARS-CoV-2 infection. Additional subset analyses are being performed to further determine the role of chronic inflammatory conditions of the ear and other organ systems in predisposition to SARS-CoV-2 infection.

W39. A Retrospective Analysis of Diagnosed and Subclinical Executive Function Deficits in Children With Hearing Loss

Clinical Otolaryngology & Pathology

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Background

A growing body of evidence suggests that disrupted access to auditory input early in life alters the development of neurocognitive processes, such as executive function. When compared to their normal-hearing peers, children with hearing loss have been shown to perform more poorly on measures of executive function (e.g., attention and working memory). These group differences are noteworthy in part because executive function has been associated with language development and speech recognition in noise. However, the prevalence of executive function deficits in a clinical population of children with hearing loss remains unknown. Executive function is not routinely assessed as part of the pediatric audiologic test battery yet concerns for children's ability to "listen" and "pay attention" are often reported by parents, caregivers, and other professionals. These reports may reflect an increased prevalence of executive function deficits in children with hearing loss, which would critically inform the clinical management of these children.

The primary objective of this study is to quantify the prevalence of subclinical and diagnosed executive function deficits in children with hearing loss. A secondary objective is to determine whether the prevalence of executive function deficits differs based on the type and degree of hearing loss as well as the management and treatment options utilized.

Methods

A retrospective analysis was performed on medical records of children seen at Lucile Packard Children's Hospital Stanford. The sample was narrowed to children between 0 and 21 years of age who were evaluated for or diagnosed with hearing loss. Type of hearing loss was classified as conductive, sensorineural, or mixed. Degree of hearing loss was stratified according to four-frequency pure tone average (i.e., air conduction thresholds at 500, 1000, 2000, and 4000 Hz). Executive function deficits were characterized as diagnosed (e.g., attention-deficit/hyperactivity disorder) or subclinical based on documented concerns without explicit diagnosis (e.g., inattention, listening difficulties). Management and treatment options included: 1) fitting of devices such as hearing aids or cochlear implants; 2) placement of pressure equalization tubes; 3) surgery for treatment of hearing loss; and 4) watchful waiting.

Results

A preliminary review suggests that hearing loss in children is associated with a greater prevalence of either diagnoses associated with executive function deficits, or with subclinical concerns raised on issues of inattention or listening difficulties.

Conclusions

While preliminary, these results suggest that both diagnosed and subclinical executive function deficits are widely observed in children with hearing loss. However, the extent to which concerns about executive function reflect purely auditory phenomena or true difficulties in executive function remains unclear. This motivates the development of clinical tools that can be included in the pediatric audiologic test battery to help distinguish between auditory and executive function deficits, with the ultimate goal of better guiding patient care.

W40. Single-Cell Vs Bulk ATAC-Seq: Methodological Insights From Whole Cochlear Sequencing

Genetics A: Genomics and Gene Regulation

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Background

A cell's regulome consists of all cis-regulatory elements in its genome and encompasses information for gene regulatory networks and transcriptional activities. Many bulk sequencing technologies have been utilized over the years to study the regulome, including DNase-seq, ChIP-seq and ATAC-seq. However, these techniques do not allow for a global understanding of each individual cell population in the tissue of interest. Recent advancements in single-cell technologies have made it possible to study the regulomes of individual cells in complex, heterogeneous tissues such as the cochlea. Single-cell ATAC-seq (scATAC-seq) is the most widely used of these single-cell regulome mapping technologies because of its simple methodology, robust protocols, and scalability to large numbers of cells. Typically, the number of expressed genes or open chromatin sites detected from single cells is lower compared to bulk population-level measurements. The cause for this is multifactorial, including sparse data due to a high frequency of dropout events, transcriptional bursting (temporal fluctuation of transcript levels in individual cells), and higher levels of technical noise. Thus, in choosing techniques for mapping chromatin accessibility in experimental design we are often faced with a dilemma of resolution versus robustness of the chosen methodology.

Methods

In this study, we compare whole cochlear scATAC-seq to cochlear epithelial (CD326+) and non-epithelial (CD326-) bulk ATAC-seq data for sensitivity and complexity. We also compare three different bioinformatic methods to analyze the scATAC-seq data, including Signac, SnapATAC and scATAC-pro. In addition to global analysis of chromatin accessibility we compare the epigenetic results obtained for several of the canonical inner ear cell type-specific genes.

Results

We show the advantages of using either bulk ATAC-seq and scATAC-seq. The methodology to be used depends on the bioinformatic analyses needed to address the underlying hypothesis, overall sensitivity and complexity needed, and cell-types being studied. We also show the significant differences between multiple scATAC-seq pipelines allowing for further investigation of transcription factor binding, gene expression and developmental dynamics.

Conclusions

In conclusion, the presented analyses should help investigators in our field choose which methodology and bioinformatic pipeline best suits their experimental design.

W41. Genomic Analysis of Profound Childhood Hearing Loss in the Yoruba Population of Nigeria

Genetics A: Genomics and Gene Regulation

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Background

There are hundreds of genes in which variant alleles cause sensorineural hearing loss in children. However, the causative genes and alleles of hearing loss in Sub-Saharan African populations are still largely unknown. It is widely assumed that there is great heterogeneity of mutations causing hearing loss in Sub-Saharan Africa because of the very old age and ancient admixture of the Sub-Saharan African population. The back-migration

of non-Africans into Africa in recent history has also added to this heterogeneity. We aimed to further elucidate the variants causing hearing loss in indigenous Sub-Saharan Africans.

Methods

We ascertained 57 small families of Yoruba ethno-lingual ancestry in Ibadan, Nigeria, with at least one individual with non-syndromic, severe to profound, prelingual-onset, bilateral hearing loss that could not be attributed to nongenetic factors associated with hearing loss. We performed a combination of whole-exome and Sanger sequencing analyses of their nuclear and mitochondrial genomes.

Results

No bi-allelic pathogenic variants were identified in *GJB2*, encoding connexin 26, a common cause of deafness in many other populations. Variants predicted to be pathogenic or likely damaging were identified in genes associated with non-syndromic deafness (*ILDR1*, *MYO15A*, *TMPRSS3*, *TNC* and *WFS1*) or Usher syndrome (*CDH23*, *CIB2*, *MYO7A*, *PCDH15*, *USH2A*), and in three genes associated with other syndromic forms of hearing loss (*CHD7*, *OPA1* and *TRRAP*). Several rare mitochondrial variants, including m.1555A>G, were detected in the mitochondrial gene *MT-RNR1* but not in control Yoruba samples.

Conclusions

Pathogenic variants of *GBJ2* play a limited role in the etiology of hearing loss in Sub Saharan African population. Eighteen (29%) of 61 independent cases of hearing loss in this cohort of Yoruba families were likely caused by variant alleles of genes reported to underlie deafness in other populations. All identified pathogenic or likely damaging variants in individuals with hearing loss were private, most were detected in compound heterozygosity, and 60% were novel. These results indicate an extremely high level of genetic heterogeneity and underscore the need for more large-scale studies to identify the genetic causes of hearing loss in Africa.

W42. Expression of Hearing Loss Related Genes in iPSC Derived Cells of the Human Inner Ear

Genetics B: General

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Background

Hearing loss (HL) is the most common sensory deficit. Induced pluripotent stem cells (iPSCs) derived from patients with a known genetic etiology of hearing loss are a powerful tool to model and study the molecular and cellular phenotype of specific HL-associated variants with the potential to act as a platform for the development of novel therapeutic strategies. For this potential to be fully realized, it is essential to understand the patterns of expression of different HL-associated genes across different cell types that constitute the inner ear. The aims of this project were to derive inner ear hair cell-like cells and spiral ganglion neuron-like cells from control iPSCs and determine the expression of important hearing loss-related genes in these iPSC models.

Methods

Control iPSCs derived from an individual with no clinical hearing loss or mutations in known hearing loss related genes were induced to differentiate into inner ear hair cell-like cells and spiral ganglion neuron-like cells through previously established differentiation protocols. During the hair cell-like cell differentiation, cells were harvested for RNA extraction at the otic epithelial progenitor stage (days 6, 12, 26, and 33) and hair cell-like cell stage (day 40). During the spiral ganglion neuron-like cell differentiation, cells were harvested at the otic neural progenitor stage (days 20 and 25), and at the spiral ganglion neuron-like cell stage (day 42). qPCR was performed on cDNA derived from the RNA of each time point to determine the relative expression levels of eight hearing loss related genes, namely *P2RX2*, *GJB2*, *USH2A*, *TMPRSS3*, *SMPX*, *TMEM43*, *ATP2B2*, and *KCNQ4*.

Results

During the hair cell-like cell differentiation, *P2RX2*, *TMPRSS3*, *TMEM43* and *GJB2* increased in expression, peaking at 90x, 13x, 2.5x and 36x fold change, respectively. During the spiral ganglion neuron-like cell

differentiation, GJB2 and ATP2B2 were found to have an increase of expression (up to day 25, further results pending). These expression patterns mimic what is seen in embryonic development.

Conclusions

Hearing loss genes are expressed in iPSC derived models of the inner ear. These models can be used to study the molecular and cellular function of these genes. iPSC derived inner ear hair cell-like cells will be used to study P2RX2, TMPRSS3, TMEM43 and GJB2 and spiral ganglion neuron-like cells will be used to study GJB2 and ATP2B2. These models will also be used to develop novel therapeutics for mutations in these genes.

W43. Characterization of Novel AAV Capsid Variants for Delivery of GJB2 Gene Therapy for Congenital Hearing Loss

Genetics B: General

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Background

Congenital hearing loss is the most common inherited sensory disorder. Of the over 100 genes associated with hereditary deafness, most are expressed by either mechanosensitive hair cells or the neighboring support cells which provide structural and homeostatic support. The GJB2 gene encodes the protein Connexin26 which forms gap junctions in multiple cell types of the cochlea and mutations in this gene represent the most common cause of genetic hearing loss. Target cells that normally express Connexin26 include the lateral wall and spiral limbus fibrocytes as well as organ of Corti support cells. To develop a therapy for GJB2 deficiency, a gene delivery tool which exhibits broad tropism is preferred. AAV-mediated gene therapy is an emerging platform to safely deliver intact, functional genes to tissues exhibiting mutated alleles. The AAV capsid represents a critical component that largely dictates tropism. We conducted a head-to-head evaluation of novel and previously described AAV capsids in both an optimized ex vivo model as well as multiple in vivo models, including a non-human primate (NHP) study, to assess cochlear tropism.

Methods

P2-P4 rat or mouse pups were used for all ex vivo experiments. Whole organ cochlear explants were treated with AAV either continuously for 120 hours or for 48 hours followed by washout and 72 hours in media without AAV. Several previously characterized or novel AAV variants were tested; all capsids utilized the CBA promoter to drive the expression of reporter constructs to allow for rapid visualization of cellular transduction. For evaluation of tropism, cochlear explants were imaged on a confocal microscope and the tissue coverage of the AAV reporter was quantified. Phalloidin staining was used to assess potential cell toxicity resulting from AAV treatment. Adult NHPs were dosed intracochlearly with an AAV variant via the round window membrane then euthanized 12 weeks later. Cochleae were sectioned and evaluated for AAV reporter expression by immunohistochemistry.

Results

Our comparative study in cochlear explants identified capsids with significantly high transduction efficiency, including several with greater transduction efficiency than previously described AAV variants. Top performing capsids exhibited transduction in all target cells that natively express Connexin26. Importantly, none of the AAV capsid variants tested in this study exhibited signs of toxicity within the organ of Corti. Evaluation of tropism in NHPs showed similar patterns as in cochlear explants, demonstrating the suitability of these capsids for clinical development.

Conclusions

We identified several AAV capsids with more favorable tropism profiles than previously reported top-performing variants in the cochlea and confirmed that these capsids are capable of transducing relevant cell types in primate in vivo studies. These results support the further development of this specific AAV-based gene therapy for GJB2-related hearing loss.

W44. Rare Variants in OTOG and GRHL2 Contribute to Meniere's Disease in Adults

Genetics B: General

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Background

Most cases of Meniere's Disease (MD) are considered sporadic, however, familial aggregation, variable expressivity, and incomplete penetrance have been described. Recently, aggregation of rare variants in known sensorineural hearing loss (SNHL) genes in sporadic cases of Meniere's disease has been reported at a small scale.¹

Methods

To explore the role of rare variants in MD, using a genome-first approach, carriers of predicted pathogenic variants in known SNHL and suggestive Meniere's genes were identified in the Penn Medicine Biobank (PMBB) and tested for association between carrier status and phenotype. We also prospectively genotyped patients presenting with MD in a neurotology clinic.

Results

Adjusting for age, gender, and ancestry, rare predicted loss-of-function variants in OTOG were independently associated with an increased odds of Meniere's Disease at $p = 0.01635$ (beta = 2.4909). Additive gene burden analysis against MD was significant above Bonferroni-correct threshold for GRHL2 ($p = 0.0002916461$, beta = 2.8626435). Twelve genes showed nominal significance above $p = 0.05$. These results were replicated in United Kingdom Biobank for OTOG ($p = 0.00790267$). In a neurotology clinic, 19 patients with a variety of hearing loss and MD presentations were prospectively genotyped using a hearing loss gene panel (GeneDx, Gaithersburg, MD). Three patients carried rare OTOG variants (MAF < 0.001 in gnomAD); two of the three rare variant carriers were clinically diagnosed with Meniere's Disease by a neuro-otologist prior to genotyping.

Conclusions

Our approach of a retrospective genomic association study for MD was complemented by findings from prospective clinical genotyping for an association between OTOG rare variants and MD and suggests a possible association with GRHL2.

W45. ATOH1, a Novel Human Deafness Gene, Detected in the Jewish Population Among a Spectrum of Genes for Inherited Hearing Loss

Genetics B: General

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Background

Variants in more than 150 genes are responsible for inherited hearing loss. The Jewish population in Israel is made up of communities that differ with respect to geographic origin, spoken language, and traditions. Endogamy has led to high frequencies of recessive genetic traits, many due to founder mutations. The variety of alleles reveal a wide range of deafness-associated variants with relationships between genotype, phenotype, and ethnicity, setting guidelines for clinical genetic counseling for the deaf.

Methods

DNA from both hearing impaired and hearing members of 88 multiplex families, with non-syndromic or syndromic hearing loss, were sequenced using the HEar-Seq gene panel of known and candidate deafness genes. One of these was a five-generation family, HL263, with early onset, progressive non-syndromic hearing loss of Iraqi Jewish origin.

Results

The genetic causes of hearing loss were revealed for 60% of the families, including novel variants in 25 genes. One gene was encountered for the first time in human hearing loss: ATOH1 (Atonal), a basic helix-loop-helix transcription factor. ATOH1 was included on the panel because several mutations in the mouse ortholog lead to hearing loss. The ATOH1 c.1030delC variant co-segregated with hearing loss in family HL263. Whole exome sequencing, confirmed there were no other potentially damaging variants in any genes that co-segregated with hearing loss in this family. The ATOH1 c.1030delC causes a frameshift; western analysis revealed a significantly slower rate of degradation for mutant ATOH1 protein compared to wild-type ATOH1 protein. Cochlear explants from *Atoh1*^{-/-} mice were transfected with wild-type or mutant *Atoh1*, leading to the induction of hair cells.

Conclusions

Our results led to the identification of an ATOH1 pathogenic variant for the first time. Given the critical role of this transcription factor in inner ear development, it is not surprising that only a mutation in this region of the protein would be viable. We hypothesize that untimely expression of ATOH1 may generate immature ectopic hair cells, which interfere with development of normal hair cells and ultimately leading to hearing loss. Our results also demonstrate that genomic sequencing with a gene panel dedicated to hearing loss is effective for genetic diagnoses in a diverse population. Comprehensive sequencing enables well-informed genetic counseling and clinical management by medical geneticists, otolaryngologists, audiologists and speech therapists and can be integrated into newborn screening for deafness.

W46. The Molecular Background of Hearing Impairment Due to Severe Cochleovestibular Malformations

Genetics B: General

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Background

Rare disorders pose challenges in patient diagnosis, management and treatment. In a subset of children with profound congenital sensorineural hearing impairment, this can be due to an abnormal cochleovestibular (CV) anatomy such as cochlear aplasia. These children require a fast and suitable intervention to maximize social, mental, speech and language development. Unfortunately, the etiology of these conditions is unclear. Many genetic studies lack imaging data and several of these malformations are rare, especially those that are bilateral and non-syndromic. An exception of this is the enlargement of the vestibular aqueduct, which is a more common malformation often associated with SLC26A4 variants.

Methods

By combining detailed temporal bone imaging data with a family-based exome sequencing approach, we investigated the genetic etiology of CV abnormalities in affected children. So far, we have completed sequencing in 16 CV families and are actively completing sequencing in additional families and enrolling novel families.

Results

We have successfully identified genetic factors implicated in these disorders and have demonstrated phenotypic similarities in Danio Rerio mutant models for candidate genes. In 50% of the families, we identified a pathogenic or likely pathogenic variant, of which 50% were de novo. De novo variants are often implicated in neurodevelopmental disorders and often more harmful than inherited variants as they are not subjected to strong natural selection. In addition, our data demonstrates that children with malformations of the 8th cranial nerve share pathogenic mechanisms such as defects in neural crest regulatory molecules (e.g. GREB1L, MASP1, SIX1). Therefore, they can be classified as (cranial) neurocristopathies.

Conclusions

Although severe bilateral CV malformations are challenging to study due to their rarity, they can provide us with unique insights into the developing human ear. We aim to establish a molecular database of children with

CV malformations and encourage researchers at the conference to combine efforts to elucidate the etiology of rare disorders affecting hearing.

W47. BAIAP2L2 Localization in Hair Bundles Relies on Mechanotransduction and Ca²⁺

Hair Cells: Anatomy & Physiology

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Background

The hair bundle develops in a highly stereotyped manner to generate the consistent dimensions and features necessary for functional mechanotransduction (MET) and sensitive hearing. MET itself regulates bundle morphogenesis, and the correct targeting of several bundle proteins depend on MET. BAIAP2L2, the product of the novel mouse deafness gene *Baiap2l2*, appears to be one such protein. EPS8 and the motor protein MYO15A form a complex that is responsible for correctly targeting several bundle proteins, and we have recently shown that BAIAP2L2 targeting to stereocilia requires both of these proteins (Carlton et al., under review). We also showed by pulldown assay with recombinant proteins that BAIAP2L2 interacts with EPS8 (Carlton et al., under review). As the localization of MYO15A and EPS8 within the bundle are regulated by MET, we hypothesized that BAIAP2L2 is delivered to stereocilia tips as a cargo of the MYO15A-EPS8 complex, and that Ca²⁺ entering through open transduction channels may cause BAIAP2L2 to dissociate from this complex and persist at tips of transducing stereocilia.

Methods

We localized BAIAP2L2 in mutant mice (*Tmc1*;*Tmc2* DKO, *Tmie* KO, *Pcdh15* KO, *Myo15a* KO, *Eps8* KO) by whole-mount immuno-staining. Pull-down assays were used to examine identify where BAIAP2L2 binds EPS8. Cochlear explants were prepared using tissue from wild-type mice, cultured either in the presence of the mechanotransduction blocker tubocurarine or the cell-permeant Ca²⁺ chelator BAPTA-AM, and then immuno-stained for BAIAP2L2 and EPS8 to test the effect on BAIAP2L2 localization. The effect of Ca²⁺ on BAIAP2L2-lipid interactions was investigated by protein-lipid overlay assay and co-flotation.

Results

In *Tmc1*;*Tmc2* double knockout or *Tmie* knockout transduction mutants, BAIAP2L2 no longer enriches in row 2 stereocilia, but instead localized weakly to all tips. In *Eps8* and *Myo15a* knockout mice, BAIAP2L2 was absent from stereocilia tips. Similarly, BAIAP2L2 no longer enriched at stereocilia tips after culture with either tubocurarine or BAPTA-AM. EPS8 localization shifted from predominantly labelling row 1 tips to labelling all stereocilia tips following tubocurarine treatment, whereas its localization was unchanged after culturing with BAPTA-AM. BAIAP2L2 binds within residues 1-441 of EPS8, suggesting that BAIAP2L2 binding of EPS8 may not compete with formation of the MYO15A-EPS8 complex, as MYO15A binds within residues 535-821 of EPS8 (Manor et al., 2011). Using a protein-lipid overlay assay, we found that the BAIAP2L2 I-BAR domain bound PI3P, PI4P, and PI5P. Binding was abolished in the absence of Ca²⁺. Similarly, Ca²⁺ facilitated BAIAP2L2 I-BAR binding to phosphoinositide-containing liposomes.

Conclusions

BAIAP2L2 requires MET for correct targeting, and preliminary data suggest that the interaction of BAIAP2L2 with phospholipids depends on Ca²⁺. We suggest that BAIAP2L2 targeting to tips of transducing stereocilia may occur because the protein's interaction with the membrane environment is elevated by Ca²⁺ that enters through open transduction channels.

W48. Investigating Ultrastructural Consequences of Aging On Murine Inner Hair Cell Ribbon Synapses

Hair Cells: Anatomy & Physiology

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Background

The ribbon synapses between inner hair cells (IHCs) and auditory nerve fibers (ANFs) mediate coordinated and indefatigable neurotransmission allowing for continuous sound encoding with exceptional temporal precision (Moser and Beutner, 2000; Matthews and Fuchs, 2010). A hallmark of these synapses is an electron-dense structure, called the synaptic ribbon. These ribbons tether a halo of glutamate-filled synaptic vesicles (SVs) via proteinaceous filaments (Glowatzki et al., 2008) to support rapid SV replenishment over long periods of time (Moser and Beutner, 2000; Pangšrič et al., 2010). SVs appear to be organized in morphologically distinct SV pools such as the membrane-proximal and the ribbon-associated pool (Frank et al., 2010; Chakrabarti et al., 2018).

Recent research in mice revealed the vulnerability of these specific synapses upon age-related hearing loss. IHC ribbon synapses are among the first structures to get affected by aging, leading to disrupted auditory transmission, known as age-related cochlear synaptopathy (Stamatakis et al., 2006; Sergeyenko et al., 2013). We hypothesize that on the degeneration timeline, structural alterations such as disturbed ribbon organization or synaptic vesicle pool arrangements precede any major morphological changes like the loss of ribbons or postsynaptic ANFs. We use electron microscopy to assess the ultrastructure of presynaptic IHC active zones (AZs) of different mice models and at different age groups.

Methods

We compared the ultrastructural alterations in C57BL/6J mice, a broadly used wild-type strain with an early-onset of progressive hearing loss (Henry, 1983; Johnson et al., 2000), with the otoferlin knock-out (KO) mice, which are profoundly deaf (Roux et al., 2006). We employed high-pressure freezing (HPF) and freeze substitution (FS) to achieve a rapid immobilization and near-to-native state preservation of the tissue. Individual ribbon synapses were analyzed using electron tomography (ET), which ensures 3D visualization in high z-resolution. Parameters like ribbons' structure, size and number of SVs as well as their tethering state of the different morphological vesicle pools were determined and compared between 6 weeks-old and 10-12 months old mice.

Results

We observed a significant increase in the ribbon size upon aging in both C57BL/6J and otoferlin KO mice. Moreover, the majority of the ribbons in the older age-group harbored a hollow core, what is also known as 'fenestrated ribbons' (Sobkowicz et al., 1982; Michanski et al., 2019). As described previously, we detected large clusters of heterogeneous, irregularly-shaped vesicles (Stamatakis et al., 2006), which we termed 'pleomorphic vesicle clusters' in aging C57BL/6J. These were also present in both young & aging otoferlin-KO IHCs. These clusters were located close to the nucleus but also in direct ribbon synapse vicinity.

Conclusions

These preliminary results shed light on different ultrastructural features that occur upon aging at IHC ribbon synapses, which might correlate to synapse dysfunction and age-related hearing loss.

W49. Loss of Baiap2l2 Destabilizes the Transducing Stereocilia of Cochlear Hair Cells and Leads to Deafness

Hair Cells: Anatomy & Physiology

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Background

BAIAP2L2 is a member of the I-BAR protein family, which are able to dimerise via their I-BAR domain to detect and induce negative membrane curvature. Thus, by localising to the curvature at the tips of actin protrusions, and recruiting actin effectors such as EPS8, I-BAR proteins can regulate their growth. In the cochlea, the actin stereocilia rows of hair cells are precisely arranged to form a staircase, which is essential for mechano-electrical transduction (MET). This morphology is established and maintained by the differential

localisation of actin effectors. For example, EPS8 is localised to the tips of the tallest row and EPS8L2 is at the tips of the shorter two rows. Given the role of I-BAR proteins in recruiting EPS8 to growing actin protrusions, we hypothesised that BAIAP2L2 is essential for stereocilia development by localising EPS8.

Methods

The hearing thresholds of BAIAP2L2 null animals were investigated using auditory brainstem responses at different postnatal ages in order to determine the progression of hearing loss. Scanning electron microscopy as used to investigate the morphology of the hair bundles. Patch clamp experiments were used to record the MET current while displacing the stereociliary bundle of hair cells using a piezo-driven fluid jet. Whole mount immunolabelling of the cochlea sensory epithelium was also used to determine the localisation of BAIAP2L2 and the interaction relationship with EPS8. Pull-down assays were used to investigate protein-protein interaction.

Results

BAIAP2L2 null animals already showed a significantly elevated hearing thresholds in post-natal day 14 (P14) mice, and this hearing loss progressed to full deafness by around 8 months of age. Immunolabelling showed that BAIAP2L2 is selectively localised to the shorter rows of stereocilia. BAIAP2L2 knockout mice showed a progressive degeneration of the shorter rows of stereocilia starting from about P10 in outer hair cells (OHCs) and a few months later in inner hair cells (IHCs). This defect in bundle morphology was associated with a reduced MET current. We also showed that BAIAP2L2 interacts with key proteins known to be essential for the normal morphogenesis and maintenance of the stereociliary (CDC42, RAC1, EPS8, and ESPNL). Finally, the localization of BAIAP2L2 to the stereocilia tip requires both EPS8 and MYO15A.

Conclusions

We have demonstrated that BAIAP2L2 is essential for maintaining the shorter rows of stereocilia in both OHCs and IHCs. We propose that MYO15A, EPS8 and BAIAP2L2 are likely to be part of a stereociliary protein complex involved in the maintenance of adult cochlear hair cells transducing stereocilia.

W50. Kv1.8 Potassium Channels in Mouse Vestibular Hair Cells

Hair Cells: Anatomy & Physiology

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Background

Potassium (K⁺) currents in vestibular hair cells play dynamic roles in shaping receptor potentials. Voltage-gated K⁺ (Kv) channels are strikingly different in the type I and type II hair cells (HCI and HCII) of amniote vestibular organs. HCI have a non-inactivating low-voltage-activated K⁺ conductance (gK,L) that strongly affects the gain and time course of receptor potentials and participates in non-quantal transmission with the post-synaptic calyx. HCII lack gK,L; as in most hair cells, their KV channels activate positive to resting potential, including an inactivating A-type K⁺ conductance (gA). Here we report evidence that Kv1.8 channel expression plays a role in the expression of both gK,L and gA.

Kv1.8 (gene name: Kcna10) is highly expressed in mouse vestibular hair cells (Scheffer et al. J Neurosci. 35:6366, 2015) and, in a constitutive knockout of Kv1.8 (B6-Kcna10TM45 mice; Lee et al. Hearing Res. 300:1, 2013), the vestibular evoked potential, VsEP, in response to head accelerations was strongly reduced.

Methods

To investigate possible contributions of Kv1.8 channels to hair cell voltage-gated currents, we recorded from hair cells in semi-intact preparations of the utricles of B6-Kcna10TM45 mice (postnatal days 10-300). Sectioned utricular epithelia were stained with anti-Kv1.8 (Alomone). The level of Kcna10 transcripts in subtypes of mouse utricular hair cells was assessed using an existing single-cell RNA-Seq database that spans embryonic (E14.5) to adult ages.

Results

HCI in null mutants lacked gK,L: relative to littermate controls, steady-state maximal conductance density was reduced 95% and voltage of half-maximal activation (V_{half}) was shifted +40 mV. HCII in null mutants lacked gA: relative to littermate controls, peak maximal conductance density was reduced 66-84% and inactivation time course was greatly slowed. The HCI and HCII conductances that depend on Kv1.8 expression (gK,L and gA) have very different voltage dependence: gK,L $V_{half} -82 \pm 1$ mV (mean $\pm$ SE, $n=44$); gA $V_{half} -22 \pm 1$ mV (28). Antibody labeling of null and wildtype utricles shows specific expression in HCI and HCII basolateral membranes, and single-cell RNAseq shows Kcna10 expression in both HCI and HCII, with higher levels in HCI.

Conclusions

If Kv1.8 is a pore-forming subunit in both gK,L and gA, then the voltage dependence and inactivation rate of these conductances depend on additional factors that are differentially expressed in HCI and HCII. Single-cell RNA data suggest candidates: β subunits Kv1 β 1 and Kv1 β 2 are enriched in HCI and HCII, respectively, and the A-type channel subunit Kv1.4 is enriched in HCII. Pharmacological experiments on HCII suggest that gA may be a heteromer of Kv1.4 and another Kv1 α subunit, for which Kv1.8 is a possible candidate.

Supported by NSF Graduate Research Fellowship to HRM and NIH grant R01 DC012347. We thank T.B. Friedman and S.M. Jones for the generous gift of B6-Kcna10-TM45 mice.

W51. Confocal Laser Scanning Microscopy of Inner Ear Ribbon Synapses with a Cochlear Implant in Situ

Hair Cells: Anatomy & Physiology

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Background

Sensorineural hearing loss affects not only and not always at first the sensory cells of the inner ear, but often their synapses and subsequent neurites are initially damaged. These auditory synaptopathies are examined at the research level to identify the cascades of hearing pathologies and have also moved into the focus of therapy development. Auditory synaptopathies also play an important role in the framework of cochlear implant (CI) care, since they can lead to a loss of physiological hearing in patients with residual hearing. Accordingly, CI-dependent traumatization, which leads to synaptopathy, and corresponding therapy options are being under investigation.

We aimed to develop a method to examine implanted inner ears of guinea pigs, an animal model commonly used in hearing research, at the synapse level.

Methods

Fresh (bolt shot) and transcardially perfused and paraformaldehyde fixed cochleae from pigmented and albino guinea pigs were used. A cochlear implant was inserted into the scala tympani and secured at the round window niche using a drop of dental cement. The specimens were decalcified and subsequently immunostained for ribbon synapses and hair cells using antibodies against CtBP2, PDS95 and myosin VIIa. The cochleae were either processed for histology as intact samples or cut into three pieces (basal, medial, apical) or the bony capsule was removed. To visualize the synapse level without separating the neuroepithelium they were made transparent (methyl salicylate benzyl benzoate (MSBB)) and 3D-imaged using confocal laser scanning microscopy (CLSM).

Results

We detected a difference in fluorescence intensity with albinos giving a better response than pigmented guinea pigs. There was no difference in immunostaining intensity between fresh or perfused samples. With all three preparation methods used the complex helical structure of the cochlea remains intact and an overview image of the cochlea for assessing the CI position is possible. Synapse imaging was possible in cochleae with removed bony capsule and in those cut into three pieces. Dissection of the bony capsule can cause damage of the lateral wall whereas cutting into pieces can damage the basilar membrane.

Conclusions

We developed a technique to visualize the ribbon synapses of cleared guinea pig cochleae with a CI in situ. Removal of the bony wall and dissection into three pieces allow evaluation of the cochlear implant position as well as visualization of the sensory cells including the synapses. In our hands the removal of the bony capsule of the cochlea is superior over three-pieces dissection due to the fact that lateral wall damage is more acceptable when being focused on basilar membrane evaluation. This novel technique can be applied in a wide field of hearing research focusing on synaptopathy.

W52. Open Board

W53. Influence of Geometry on the Summating Potential From Outer and Inner Hair Cells

Inner Ear: Anatomy & Physiology

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Background

In electrocochleography (ECoChG), the summating potential (SP) is the dc offset that can be measured during tone bursts. Recently, Pappa et al., (J Neurophysiol. 121:2163-2180, 2019) showed by using ototoxins and neurotoxins that SP contributions from outer and inner hair cell populations (OHCs and IHCs) recorded at the round window (RW) have opposite polarities. A possible source of this difference is for the operating point of OHCs to be set such that acoustic stimulation reduces the standing current (more channels open than closed at rest), while the operating point is the opposite for IHCs (more closed than open channels). Another is different spatial locations with differing geometric orientations relative to the recording electrode for the two groups. Different source locations for OHCs and IHCs would also arise from different operating points, but the OHC bias could be towards more channels closed than open at rest. We report SP observations recorded after changing the placement of recording electrodes to locations other than the RW, as a means to examine changes in the composition of the SP in relation to the geometry of recording sites and the frequency organization of the cochlea.

Methods

Normal hearing (NH) or animals treated with ototoxic combinations of furosemide/kanamycin (FK, 100 mg/kg ip and 200 mg/kg sq) to remove OHCs and leave IHCs intact were used. Under urethane anesthesia (1.5 g/kg, ip), ECoChG's were recorded after application of the neurotoxin kainic acid (KA, 60 mM) to the round window. Responses in FK animals originate from IHCs alone, while the OHC contribution is obtained by subtraction of FK cases (IHCs only) from normal hearing cases (OHCs and IHCs). Stimuli, delivered through sealed tubes, were tone bursts (40ms duration from 0.25 to 32kHz at intensities from 0-90 dB SPL). Recording electrodes were placed at the RW or into holes (100 microns) drilled over the 16kHz region of scala tympani (ST), or 16kHz or 2kHz region of scala media (SM). Inverting electrodes were in the neck muscle or RW and a ground electrode was at the base of the tail. Hair cell cochleograms and the location of each recording site were reconstructed relative to a 3-D frequency map of each cochlea after processing for light sheet microscopy (see Hutson et al., J. Comp. Neurol. Epub ahead of print, 2020).

Results

For the RW, the OHCs and IHCs had opposite polarities for all frequencies as previously reported, and this was also true for the 16kHz ST and 2kHz SM recordings. However, for the 16kHz SM recordings the OHCs and IHCs sometimes had the same polarities.

Conclusions

Since the OHC and IHC polarities did not always have opposite polarities when stimulated with the same frequencies, a geometric explanation is supported.

W54. Relationship Among Selective Inner Hair Cell Loss and Auditory Brainstem Response Amplitudes in Carboplatin-Treated Chinchillas

Inner Ear: Anatomy & Physiology

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Background

Pure tone audiometry is the most common and universally accepted metric for hearing health. However, compelling evidence from animal models demonstrates that significant cochlear damage, such as loss of afferent auditory synapses or severe loss of inner hair cells (IHC), can go undetected by traditional threshold measures as long as OHCs have minimal damage. Furthermore, the relationship among threshold and suprathreshold outcomes is complex and not well correlated even in the absence of retrocochlear pathology. Pre-clinical physiological studies measuring auditory brainstem response (ABR) outcomes have shown little to no change in thresholds following selective IHC loss or synaptopathy. These cochlear lesions have been shown to reduce the amplitude of the compound action potential (CAP), synonymous with wave-I of the ABR, suggesting a reduction in cochlear output despite the absence of elevated thresholds. In contrast, wave-IV amplitudes have been found to remain normal or even enhanced in the presence of auditory fiber loss. Whereas the ABR wave-I amplitude reduction at suprathreshold level is the established hallmark of afferent synapse loss, the relationship among IHC loss and other suprathreshold metrics of the ABR are limited. For the current investigation, we sought to evaluate the relationship among ABR wave-I and wave-IV amplitudes in chinchillas before and after carboplatin, a treatment that reliably and selectively destroys IHCs in this species.

Methods

Free feeding, young adult chinchillas, were used as part of a larger study aimed at assessing psychophysical and physiological changes in cochlear status associated with selective IHC loss. ABR thresholds and distortion product otoacoustic emissions (DPOAEs) were evaluated to assay the status of normal cochlear nonlinearity and as a measure of overall hearing sensitivity. ABR wave-I and wave-IV suprathreshold amplitudes were measured bilaterally at 90, 80, and 70 dB SPL with 1, 2, 4, 8, 12, and 16 kHz tone pips. DPOAEs, Pre-, and post-carboplatin ABR measures 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 no OHC loss. Post-carboplatin assessments were performed three weeks following treatment to allow time for recovery. Animals were then sacrificed, and cochlear sensory cells and neural synaptic connections were assessed.

Results

As expected, carboplatin treatment produced significant IHC loss, with OHCs largely intact and no change on DPOAEs. ABRs were characterized by relatively low thresholds and poorer morphology following treatment. ABR amplitude data demonstrated substantial suprathreshold reductions at wave-I, even in the absence of elevated thresholds. However, wave-IV amplitudes remained unchanged.

Conclusions

These findings support previous studies suggesting the use of ABR wave-I amplitudes as an assay of selective IHC loss.

W55. Audiogram Shapes of Mutant Mouse Lines With Known Cochlear Defects

Inner Ear: Anatomy & Physiology

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Background

Finding a way to non-invasively diagnose specific types of presbycusis has long been a goal in the hearing research field. Schuknecht and Gacek classified presbycusis into three main types: sensory, neural, and strial/metabolic(1). Dubno et al(2) created a classification scheme to predict types of presbycusis by

audiogram patterns. These audiometric phenotypes include strial/metabolic (flat loss at low frequencies, with gradual, shallow sloping loss at high frequencies), sensory (little loss at low frequencies, with a steep sloping loss at high frequencies), and metabolic+sensory. We have acquired auditory brainstem responses (ABR) from multiple mutant mouse lines in conjunction with determining their primary cochlear defect. We want to determine if the audiogram shapes from mouse mutants with known cochlear defects support the use of human audiogram shapes to predict cochlear pathology; for example, will the audiogram of a mutant with a primary strial defect have a similar shape to audiograms of humans classified with strial/metabolic presbycusis?

Methods

We used ABR thresholds from mice with a primary defect of the 1) stria (S1pr2<stdf> and Sgms<tm1b(EUCOMM)Wtsi>), 2) outer hair cells (Slc26a5<tm1(EGFP/Cre/ERT2)Wtsi> and Ocm<tm1e(EUCOMM)Wtsi>), 3) synapses (Wbp2<tm1a(EUCOMM)Wtsi>), or 4) middle ear (Mcph<tm1a(EUCOMM)Wtsi> and Ywhae<tm1e(EUCOMM)Wtsi>). All of these mutants were generated and maintained on a pure C57BL/6 genetic background. Characterization of cochlear pathology of these mice have been published (3-6) or are in preparation. We created a normative baseline using the mean thresholds of ABRs from 1078 wildtype C57BL/6 mice aged 27-65 days. We converted the thresholds from dB SPL to dB HL by subtracting the baseline from the original threshold values of each individual mouse and plotted the data to be comparable to human audiograms.

Results

Our results show that the strial defect mice exhibit a flat loss across frequencies, similar to humans classified under strial/metabolic presbycusis by Dubno et al. Mutants with middle ear defects also had a flat audiogram pattern, but conductive defects are readily identified in humans so would be unlikely to be confused with strial defects. The Ocm and Wbp2 mutants showed little to no loss at low frequencies and steep sloping loss towards the higher frequencies, similar to humans classified under sensory presbycusis. The Slc26a5 mutants' audiogram was flat across frequencies but this may be due to the severity of hearing loss this mutation causes.

Conclusions

In summary, these mouse mutants with known cochlear defects do show similar audiogram patterns to the human audiometric phenotypes, supporting the use of audiogram shape as an initial step in diagnosis.

W56. Phosphoinositide Effector Proteins in the Mouse Organ of Corti

Inner Ear: Anatomy & Physiology

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Background

Phosphoinositides are phospholipid components of all cell membranes that regulate several different key cellular functions like membrane trafficking, ion channel and transporter activity, cytoskeletal dynamics, and signal transduction. Even though these components are essential for hearing, to date the role of phosphoinositides in cells of the organ of Corti is currently understudied. The purpose of this study was to establish possible roles phosphoinositides play in cellular functions that underlie hearing. Therefore, we examined several known phosphoinositide effector proteins: Tubby-like protein 3 (TULP3), a phosphoinositide- dependent regulator of G-protein coupled receptors; tubby, a putative transcription factor affected by hydrolysis of the phosphoinositide PI(4,5)P₂, and SWAP70, a cytoskeletal regulator, dependent on the production of PI(3,4)P₂.

Methods

Using immunohistochemistry on whole-mount preparations of the mouse organ of Corti, we were able to detect protein expression and cellular localization of these effectors.

Results

Here we report that TULP3 is mainly expressed in the apical parts of the inner and outer pillar cells, as well as in the phalangeal processes of the Deiters cells. Both structures are known to be filled with microtubules, which have also been shown to be regulated by phosphoinositides. We further detected TULP3 in the cell

bodies of the inner and outer hair cells. While we did not find expression of SWAP70, however tubby immune-reactivity was localized to the distal segments of the stereocilia of outer hair cells, where previously prominent PI(4,5)P2 labelling was described, and also in the nuclei of the Deiters cells.

Conclusions

Together our observations show that these effectors distribute throughout the organ of Corti, suggesting that phosphoinositides are involved in controlling a variety of important functions in hearing.

W57. Neurophysiological Basis of Enhanced Lateral Line Sensitivity in Blind Cavefish

Inner Ear: Anatomy & Physiology

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Background

Populations of Mexican tetra (*Astyanax mexicanus*) have evolved eye loss and heightened sensitivity of the lateral line system after invading subterranean cave systems. Work on blind cavefish has revealed behavioral differences from sighted surface fish attributed to variations in lateral line neuromast morphology. It remains unknown whether differences in the underlying neural circuitry of the lateral line could contribute to increased flow sensitivity.

Methods

For the first time, we apply an electrophysiological approach to understanding lateral line sensitivity in blind cavefish. Loose patch recordings of posterior lateral line were made in fictive, 4-6 days post fertilization cavefish and surface fish. Spontaneous activity, the intrinsic firing of afferent fibers in the absence of neuromast stimulation, is greater in blind versus sighted cavefish. This suggests heightened sensitivity of the cavefish lateral line. We next recorded afferent fiber activity during swimming and periods of inactivity by simultaneously measuring the ventral root activity. Here, we were interested in evaluating the role of the efferent lateral line system (i.e. corollary discharge), which is responsible for canceling out self-generated flow stimuli during locomotion to enhance receptivity to biologically relevant stimuli.

Results

Our results show that cavefish afferent fibers do not experience the inhibitory effects of locomotion caused by the efferent system as compared to surface fish. Confocal imaging data suggests that cavefish possess the same complement of efferent neurons in the hindbrain as surface fish. We observed that this lack of afferent inhibition by efferent neurons has convergently evolved across three genetically distinct cavefish populations. Furthermore, targeted ablation of efferent neurons in surface fish results in lateral line signaling similar to cavefish.

Conclusions

This leads us to propose that the cavefish efferent system is either less active or has evolved post-synaptic differences that modifies lateral line functionality compared to surface fish. We reason that cavefish rely on self-generated flow to navigate their environment (i.e. active flow sensing), such that inhibitory effects of the efferent system may be expected to be diminished. The cavefish model system is uniquely poised to investigate the evolution of sensory circuits.

W58. LHFPL5 Contains Two Omega-Type ER Retention Motifs That Preclude Plasma Membrane Localization in Heterologous Cells

Inner Ear: Cochlear Mechanics

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Background

Hair cell mechanotransduction in mammals requires a complex interplay of multiple proteins that form the mechanotransduction channel. One such component is the transmembrane protein LHFPL5, which is required for mechanotransduction and hearing. Despite containing four transmembrane domains (TMDs), as well as localizing to the plasma membrane (PM) of hair cell stereocilia, recent studies demonstrated that LHFPL5 traffics to the PM in a heterologous cell line only when co-expressed with PCDH15, a tip-link component of the mechanotransduction complex. Intriguingly, when expressed alone, LHFPL5 is invariably retained in the endoplasmic reticulum (ER) in heterologous cell lines. We sought to shed light on the protein regions that might preclude proper PM localization of LHFPL5, when expressed using the HEK293 cell line.

Methods

Plasmid constructs were designed in silico using Lasergene II software (DNASTAR, Madison, WI). The full cDNA containing AQP3-GFP was then synthesized by Genscript (Piscataway, NJ). Each mouse LHFPL5 construct was also based on custom synthesis by Genscript with its known ER retention motifs KK or RRR substituted for alanines, and codon optimized for mammalian expression in HEK293 cells. Transfections were performed using Lipofectamine 2000 (Life Technologies, Carlsbad, CA). To label the cell nucleus we used Hoechst (Life Technologies, CA). All recorded experiments were performed within two days after transfection. HEK293 cells were examined with an SP8 confocal fluorescence microscope using a 63 × 1.4 NA objective, Leica (Wetzlar, Germany).

Results

Employing the AQP3-GFP Reporter (AGR) system, we scanned the entire LHFPL5 protein and identified two omega regions, i.e. sequences that preclude PM localization. Typically, each omega signal by itself is sufficient to preclude protein trafficking to the PM. The first omega –omega 1– is located within the first exterior loop of LHFPL5 and the second one –omega 2– is contained within the last transmembrane domain. Using alanine/serine mutagenesis we narrowed down the region containing omega-1 to the sequence of amino acids 59 through 74 of LHFPL5. Interestingly, this region spans four out of five amino acid residues known to interact with PCDH15, namely Y61, N71, V72, and L73.

Conclusions

Our results further suggest that the presence of cell-specific protein partners may mask omega-type regions and thus facilitate trafficking to the PM in stereocilia. Alanine/serine mutagenesis of omega 1 is not sufficient to enable trafficking of LHFPL5 to the PM in HEK293 cells, confirming that intracellular retention activity of both omegas must be counteracted. Future studies will demonstrate whether additionally eliminating the omega 2 may enable PM localization of LHFPL5.

W59. Endolymphatic Hydrops Can Produce Shifts in the Cochlear Frequency-Place Map That Can Account for Diplacusis in Ménière's Disease

Inner Ear: Cochlear Mechanics

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Background

A common symptom of Ménière's disease is diplacusis, a perceptual anomaly in which a tone has a different pitch in the left and right ears. A hallmark feature of Ménière's disease is endolymphatic hydrops, an increase in endolymphatic fluid that causes Reisner's membrane to bulge into scala vestibuli. It has been hypothesized that the increased fluid pushes on the organ of Corti and the basilar membrane (BM), thereby stiffening the BM. A stiffer BM would increase the local characteristic frequency (CF) and shift the cochlear frequency-place map apically. If the map is different in the two ears, diplacusis would be expected to result. However, the hypothesized shift of the map has never been observed.

Methods

We created unilateral endolymphatic hydrops by surgically ablating the right-side endolymphatic sac in five guinea pigs. At 30 post-operative days when endolymphatic hydrops can routinely be seen in histology, we

used our apex-to-base perfusion technique to determine the cochlear frequency-place map. An ototoxic solution (2.16mM kainic acid in artificial perilymph) was perfused through a pipette sealed into the guinea-pig cochlear apex and was driven toward the cochlear aqueduct in the base, with the injection rate adjusted so that the solution front advanced at a constant rate of 0.5 mm/min (~5 minutes per CF octave). Auditory-nerve compound action potentials (CAPs) were measured with a round-window electrode in response to tone bursts at several frequencies and sound levels. When the solution reached a cochlear region that contributed to the CAP, the CAP amplitude was reduced and revealed the spatial origin of the CAP.

Results

In four of the five hydropic animals, CAP responses to low-frequency, near-threshold tone bursts were reduced by the apex-to-base perfusion before corresponding responses from control animals in which the endolymphatic sac was not ablated. For tones 10 dB above the CAP threshold, on average, the 50% reduction times of the five operated animals occurred before the 50% reduction times of the eight control animals by 5.8, 3.8, 4.3, 2.4 and 3.4 minutes, at 2, 4, 6, 8, and 12 kHz. These reduction time differences were statistically significant for the lowest three frequencies by a bootstrap statistical test ($p = 0.019, 0.033, 0.016, 0.144, 0.135$, respectively), thus showing a map shift in ears with endolymphatic hydrops.

Conclusions

Endolymphatic hydrops can cause apical shifts in cochlear frequency-place maps. Such shifts can account for the diplacusis in Ménière's disease. Diplacusis is easy to measure in humans, and our identification of an underlying mechanism for diplacusis may encourage the use of diplacusis for early diagnosing of Ménière's disease and treatment monitoring. With early disease identification, classical therapeutics should have a better chance of working before permanent damage is done.

W60. Formulation of Nonlinear Force of the Outer Hair Cells Based on Measurement and Numerical Analysis of Distortion Product Otoacoustic Emissions

Inner Ear: Cochlear Mechanics

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Background

Distortion product otoacoustic emissions (DPOAEs) are considered to be generated by nonlinear force of the outer hair cells (OHC), and those frequencies and levels depend on the shape of the curve of the force (Lukashkin et al., 2002). In this study, we aimed to formulate the nonlinear force of OHC based on the distortion components detected in measurements of DPOAEs.

Methods

Each order of distortion component is generally generated by different degree term of a nonlinear function which is approximated by a power series, i.e. the N-order (here, N is an integer number) of distortion components are generated by N-degree term of the function. A force of OHC, P(OHC), was formulated based on the input/output (I/O) functions of DPOAEs measured in normal-hearing human ears. The I/O functions of DPOAEs obtained from numerical analysis using the finite-element model of the human cochlea including P(OHC) were compared with the measurements of DPOAEs and the validity of the P(OHC) was evaluated. The I/O function was simulated by applying complex tone to the stapes head of the model.

Results

The I/O functions of 2f₁-f₂ component measured in all the ears were included in the normal range (Dorn et al., 2001). Third order (2f₁-f₂, 2f₂-f₁) and fifth order of distortion components (3f₂-2f₁) were generated in all the ears. By contrast, even-order of distortion components (i.e. f₂-f₁, 3f₁-f₂) were not detected. Although curve of the force of OHC is assumed to be a function with saturation, its symmetry around operating point is not clarified. A nonlinear function including both of saturability and symmetry around operating point, e.g. arctangent function, is approximated with only odd-degree terms by Maclaurin expansion around operating point. Thus, P(OHC) was formulated as a function of velocity of the basilar membrane (BM) using arctangent function, because even-order of distortion components were not detected. If the anterograde and retrograde pressure gains of the middle ear are assumed to be constant at a certain frequency regardless of sound pressure,

features of the I/O function (e.g., ease of saturation) obtained at the stapes footplate of the model are considered to be comparable to that measured in the human ear. In addition, the I/O function of P(OHC) obtained at the BM of the model where P(OHC) showed maximum value was similar to that obtained from the stapes footplate.

Conclusions

A rigorous formulation of P(OHC) by comparing the I/O function of each distortion component between the measurement and numerical analysis is possible. Those transmission gains should be considered for comparison between the I/O function obtained from measurements and the stapes-footplate of the model. When the I/O function obtained from the model is translated, the I/O function could approximately match with the I/O function of the measurement.

W61. Methodology for the Evaluation of Non-Osseous Bone Conduction Pathways Between the Cochlea and the Skull Vault

Inner Ear: Cochlear Mechanics

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Background

The role of the non-osseous pathways in bone conduction hearing is still not clearly understood. The aim of this work is the development and evaluation of a methodology for the experimental investigation of the interaction between the intracranial (i.e., cerebrospinal fluid (CSF), brain) and intracochlear content as well as the skull bone, under non-osseous stimulation.

Methods

Experiments are being conducted on a set of five Thiel embalmed whole head cadaver specimens. In order to reduce potential intersample variability, due to mechanical and geometrical differences in the skull content, the skull vault has been emptied and filled with saline water. In addition, a static pressure of 15cm water column was applied to mimic physiological reassurance in the skull. A commercial hydrophone was used as a source to activate the intracranial content using a dedicated amplifier (Type 2713, Brüel & Kjær, Denmark) to drive the hydrophone as a transmitter (source of hydrodynamic pressure). The resulting intracranial pressure was monitored via another commercial hydrophone. In parallel to that, the corresponding intracochlear pressure difference was monitored via our custom-made pressure sensor. For comparison purposes, osseous pathways were sequentially activated in the range of 0.1-10 kHz, via the electromagnetic actuator from a commercial BC hearing aid (BCHA) (Baha® 5 SuperPower). The response of the skull bone surface was monitored as motions of the ipsi-, top and contra-lateral skull surface, along with the ipsi- and contra-lateral promontories. The surface motion was quantified by sequentially measuring ~200 points on the skull surface (~ 15-20mm pitch) via a three-dimensional laser Doppler vibrometer (3D LDV) system.

Results

Preliminary data indicates up to 20 dB in intracochlear pressure measurements measurement data due to improper sealing of the cochleostomies, relative motion between the intracochlear pressure sensor and the TB, as well as parasitic mechanical effects from the mechanical support system. The promontory and skull surface underwent spatially complex motion with similar contributions from all motion components, regardless of stimulation mode.

Conclusions

A comprehensive set of experimental measurements and stimulations conditions could quantify the contribution of the non-osseous pathways between the intracranial and cochlea contents. Preliminary data has shown that the mechanical connection between the intra-cochlear pressure sensors and the temporal bone, plays a crucial role in the reliability of the measurements.

W62. Active Amplification by Low Pass Filtered OHC

Inner Ear: Cochlear Mechanics

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Background

In a recent 1-d transmission line 2DOF cochlear model (Sisto et al., 2019), the micromechanics is described in terms of two oscillators that can be identified with the basilar membrane (BM) and the reticular lamina (RL), coupled by the internal active nonlinear OHC force. The hair bundle shear is simply schematized by the relative displacement of RL and BM, giving the input to the somato-elasticity of the OHC system, represented by a nonlinear function of the relative displacement and velocity of the RL and BM. A double feedback loop was proposed by Lu et al. (2006) to schematize the OHC piezoelectric function (direct and reverse).

Methods

In this study, a third dynamical equation was added representing the low-pass filtering effect due to the slow build-up of the OHC transmembrane potential, and the active terms phases were set such that the effective anti-damping action takes place only above the local low-pass frequency. The low-pass frequency of the filter monotonically changes along the BM, as suggested by experimental data, but slower than the CF.

Results

Low frequencies in their peak region are below the local low-pass frequency of the filter, whereas high frequencies are above it. The micromechanical amplification is abruptly reduced below some critical frequency because the active force goes out-of-phase with the BM velocity; in the second, it decreases slowly with increasing frequency due to low high-frequency response of the filter. As a consequence, the maximum BM gain is limited to 15-20 dB in the mid-to-high-frequency range, and vanishes below the critical frequency, providing a natural interpretation of the apical-basal transition. Fluid volume effects (pressure focusing) could help recovering the high BM gain levels (40 dB) that are experimentally observed in the basal cochlea. A formal identity between the equations of Lu et al. (2006) and the form of the active force proposed here is discussed in different frequency ranges.

Conclusions

A system of differential equations, involving two coupled oscillators and a third dynamic equation, low-filtering the relative displacement and velocity of the two masses, BM and RL, seems to be sufficient to capture important features of the cochlear mechanics, including the so-called apical-basal transition. The pressure focusing effect, neglected in the model, could provide the additional gain necessary to get sufficient BM gain with physiologically plausible parameters.

W63. Noise Exposure and Risky Behavior in the General Population

Inner Ear: Damage & Protection

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Background

Risk taking propensity has been shown to be a reliable determinant of risk and non-risk seeking health behavior (e.g. smoking). Hearing loss is the 3rd most common health problem in the US. Exposure to hazardous noise is known to increase risk for hearing loss, with one-third of all cases of hearing loss being attributed to noise exposure. Despite this, noise exposure and noise-induced hearing loss is on the rise. Factors that moderate risky behavior leading to hazardous noise exposure are not entirely clear. The current study examined risk propensity in relation to perceptions of hearing loss and noise risk behavior in the general population. It is known that hearing loss is correlated with certain demographics such as income, education, race, gender, age, occupation, but it is not known whether people in these demographics actually vary in how they perceive risk. This study investigated if there were systematic demographic or situation factors that correlate to misperception of risk from noise exposure.

Methods

1281 adult participants from Amazon Mechanical Turk online pool completed an online survey to investigate the risk perceptions of noise exposure in the general population. Risk propensity was measured using an eight-item scale measuring general risk-taking tendencies. Perceptions of noise exposure were examined through 1) knowledge and perceptions of hearing loss, and 2) self-reported behaviors in commonly reported high-noise situations. Participants indicated their subjective perceptions of the probability of each risk on a likelihood scale ranging from “not at all likely” (1) to “very likely” (10).

Results

There were 797 male and 484 female respondents. Age was the commonly reported perceived cause of hearing loss (63%) by respondents. Less than half (43%) of respondents attributed hearing loss to hazardous noise exposure. Significant relationships were observed for gender, race, occupation, income, and education. Depression was the highest reported perceived consequence of hearing loss (61% of respondents), while dementia was only a perceived consequence in 18% of respondents. Self-perceived risk for hearing loss varied significantly by gender ($p < 0.001$), and men engaged in more noise-risk behaviors than women ($p < 0.001$). Age was not a significant factor in self-reported noise-risk behavior.

Conclusions

In a large diverse sample of the general population risk propensity and noise seeking behaviors were not found to be driven by age but varied by gender. Race, age and occupation were self-identified risk contributing factors for hearing loss. Results demonstrate limited knowledge of the common causes of hearing loss and resulting health consequences. Noise was correctly identified as a relevant risk factor in less than half the study population, independent of commonly associated demographic factors.

W64. Surgical Intervention Triggered by Real-Time Intra-Cochlear Electrocochleography During Implantation Saves Residual Hearing: Outcomes of a Randomised Controlled Trial

Inner Ear: Damage & Protection

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Background

Preservation of natural hearing during cochlear implantation is associated with improved speech outcomes, however more than half of implant recipients lose this hearing. Real-time electrophysiological monitoring of cochlear output during implantation, made possible by recording electrocochleography using the electrodes on the cochlear implant, has shown promise in predicting hearing preservation.

Sudden drops in the amplitude of the cochlear microphonic (CM) have been shown to predict more severe hearing losses. Here, we report on a trial investigating whether immediate surgical intervention triggered by these drops can save residual hearing.

Methods

A single-blinded placebo-controlled trial of surgical intervention triggered when CM amplitude dropped by at least 30% of a prior maximum amplitude during cochlear implantation. Intraoperative electrocochleography was recorded in 60 adults implanted with Cochlear Ltd's Thin Straight Electrode, half randomly assigned to a control group and half to an interventional group. The surgical intervention was to withdraw the electrode in ½-mm steps to recover CM amplitude. The primary outcome was hearing preservation 3 months following implantation, with secondary outcomes of speech-in-noise reception thresholds by group or CM outcome, and depth of implantation.

Results

Sixty patients were recruited; neither pre-operative audiometry nor speech reception thresholds were significantly different between groups. Post-operatively, hearing preservation was significantly better in the interventional group. This was the case in absolute difference (median of 30 dB for control, 20 dB for interventional, $\chi^2 = 6.2$, $p = .013$), as well as for relative difference (medians of 66% for the control, 31% for the interventional, $\chi^2 = 5.9$, $p = .015$). Speech-in-noise reception thresholds were significantly better in patients

with no CM drop at any point during insertion compared with patients with a CM drop; however, those with successfully recovered CMs after an initial drop were not significantly different (median gain required for speech reception score of 50% above noise of 6.9 dB for no drop, 8.6 for recovered CM, and 9.8 for CM drop, $\chi^2 = 6.8$, $p = .032$). Angular insertion depth was not significantly different between control and interventional groups.

Conclusions

This is the first demonstration that surgical intervention in response to intraoperative hearing monitoring can save residual hearing during cochlear implantation. There is evidence that this intervention does not have negative effects on cochlear implantation depth and may provide benefits to speech in noise reception when successful.

W65. Kainate- And Noise-Induced Cochlear Synaptopathy in Gerbils

Inner Ear: Damage & Protection

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Background

Noise overexposure can produce a cochlear deafferentation arising from loss of inner hair cell (IHC) synapses with auditory nerve fibers. The condition is called cochlear synaptopathy and may be instigated by excess sound-evoked glutamate release by the IHCs. To probe this hypothesis, we compared a pharmacological model of cochlear synaptopathy induced by a glutamate agonist (kainic acid - KA) with a model of noise-induced synaptopathy.

Methods

Experiments were performed in young adult gerbils. Animals received local KA or octave band noise exposure and were then held for varying post-exposure times. Cochlear function was assessed using outer hair cell (OHC)-based distortion-product otoacoustic emissions (DPOAEs) and compound action potentials (CAPs) of the auditory nerve. In the same animals, the cochleae were removed and immunostained to quantify the synapses and characterize their 3D distribution along the IHCs.

Results

KA infusions did not affect OHC function as assayed by DPOAEs, whereas noise produced acute DPOAE declines which recovered by 2 weeks post exposure. In contrast, both KA and noise acutely elevated CAP thresholds and reduced CAP amplitudes. Thresholds recovered to baseline after 2 weeks but amplitude declines of ~50% remained for both KA and noise. Consistent with the CAP amplitude data, the number of synapses/IHC in KA-infused cochleae was reduced by ~50% throughout the tonotopic axis, with no pillar versus modiolar difference observed. Noise-induced synaptic loss was maximum in tonotopically-appropriate cochlear regions and comparable to that of KA in the basal cochlea.

Conclusions

Ongoing experiments will provide additional pharmacologic characterization of the cochlear synaptopathy. Improved understanding of the role of excitotoxicity in noise-induced cochlear deafferentation in an animal with a hearing range similar to human may open new clinical avenues for protection and treatment. Research supported by the Office of Naval Research and the NIH/NIDCD.

W66. AAV-Mediated Islet1 Overexpression to Protect Hearing From Noise-Induced and Age-Related Hearing Loss

Inner Ear: Damage & Protection

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Background

Permanent damage to and the loss of inner ear hair cells are the major factors underlying noise-induced hearing loss (NIHL) and age-related hearing loss (ARHL). We have previously shown that hair cell overexpression of Islet1, a LIM-homeodomain transcription factor, protects ISL1 transgenic mice from NIHL and ARHL.

Methods

In this study, we used AAV-mediated local delivery of ISL1 gene to assess if the gene therapy approach could be effective in protection from NIHL and ARHL in wild type adult and aged mice. CBA/CaJ mice were injected with AAV/Anc80-ISL1-GFP at six-week-old age using canalostomy and injected mice were then exposed to noise at 1-20kHz for two hours at 110dB SPL that caused permanent threshold shifts (PTS). Subsequently, injected mice were subject to multiple noise exposures at 12 months and 15 months of age, respectively. To study AAV-ISL1 in hearing protection in ARHL, C57BL/6J mice were injected with AAV/Anc80-ISL1 at six months of age.

Results

One month after injection, mean threshold shifts of the injected ears were significantly lower compared with the uninjected contralateral control ears at low to middle frequencies, which correlated with the transduction pattern in hair cells in middle and apical turns by AAV/Anc80. Hearing test after each exposure showed sustained protection against NIHL. Analysis of the injected inner ears detected improved hair cell survival and significant preservation of synapses as the result of ISL1 overexpression. Half year after injection, mean threshold shifts of the injected ears were significantly lower compared with the uninjected control ears. We found that p-S6, a mTORC1 signaling effector, was greatly reduced in the outer hair cells of injected inner ear, indicating that ISL1 expression suppresses mTOR activity that leads to attenuation of ARHL.

Conclusions

We have thus developed a gene therapy approach by which one-time local delivery of ISL1 in adult mice protects from multiple noise exposures in long term. Our future work aims to reproduce the findings in a large animal model to lay the foundation for the development into clinic in certain patient populations.

W67. Time Course of Cochlear Synapse Counts Measured With Triple Immunohistologic Labeling of PSD95-GluA2-CtBP2 on Acute Noise-Induced Synaptopathy in Mice

Inner Ear: Damage & Protection

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Background

Noise-induced cochlear synaptopathy (NICS) can occur even at noise levels that don't cause hair cell loss. In animal model, NICS can be noninvasively detected as reduced wave-I amplitudes of the auditory brainstem response (ABR) and, histologically, by counting synapses. Here we ask, first, whether synapse loss occurs only during noise exposure or continuously deteriorates with time after noise, second, whether synapses spontaneously regenerate, and, third, how presynaptic or postsynaptic components change with time after noise. To answer these, we have longitudinally measured ABR amplitudes and threshold in a within-subject design and showed both measures recovered to the maximum level on postnoise day 3 (Hu et al, ARO 2020). Here, we examined the synapses at different time points after noise exposure in group-comparison to histologically illustrate the postnoise time course of acute NICS.

Methods

12 to 14 week-old CBA/CaJ male mice were divided into normal control, immediately after noise (PND0), postnoise day one and 14 (PND1, PND14) groups. NICS was generated with 2 hours of 100 dB SPL 8-16 kHz octave band noise. ABRs were measured at 8, 16 and 32 kHz prior to noise exposure to obtain the baseline for all mice, and also measured on PND1 and PND14 as indicated. Consistent with our previous studies, by PND14, ABR thresholds recovered to the prenoise baseline but ABR wave-I amplitudes did not. Mice were euthanized after final ABR measure or immediately after noise. Presynaptic ribbons, postsynaptic densities (PSDs) and GluA2 AMPAR subunits were labeled with, respectively, anti-CtBP2 (C), anti-PSD95 (P) and

anti-GluA2 (G) antibodies to quantify synapses/IHC in organ of Corti wholemounts at 8, 16 and 32 kHz locations.

Results

Synapses defined either as colocalization of CtBP2 & PSD95 (C&P) or as CtBP2 & GluA2 (C&G) are both significantly decreased from PND0. C&P or C&G counts are similar from PND0 to PND1, suggesting no further synaptic degeneration after noise. Ribbons significantly decreased by PND1 but showed no further decrease by PND14. PSDs or GluA2 puncta tend to slightly increase from PND0 to PND1. C&P counts were slightly higher than C&G counts by ~1-2 counts/IHC, with the greatest difference on PND0, the difference remaining similar at the no-noise control, PND1 and PND14. Orphan PSDs increased after noise with the greatest number on PND1. Orphan presynaptic ribbons are not apparent except on PND1. This implies some repair process present from PND0 to PND1.

Conclusions

We confirmed the previous reports that synapse loss occurs during noise exposure with no evident synaptic deterioration with time post-noise. Synapses defined as C&P always exceed in number those defined as C&G either in no-noise or postnoise conditions. This is consistent with a heterogeneous distribution of GluA2-containing AMPAR subunits among synapses. (Support from NIH DC02961, DoD W81XWH-14-1-0494, and an AHRF grant.)

W68. Inferior Colliculus Recordings to Assess Focal Spiral Ganglion Neuron Lesions

Inner Ear: Damage & Protection

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Background

Speech perception in CI users is hampered by heterogeneous degeneration of spiral ganglion neurons (SGN) along an individual cochlea. The interpretation of human psychophysical and electrophysiological results is difficult, as reliable markers of locally restricted SGN degeneration/ survival are still missing. Recently, we have proposed that the difference in responsiveness to cathodic versus anodic-leading stimulation (i.e. polarity effect, PE) corresponds to the presence of micro-lesions at the SGN soma and may therefore be a marker for SGN loss (Konerding et al. 2020, Hearing Research). The rationale of the PE (e.g. Miller et al. 1999, Hearing Research) is a more central spike initiation sites for anodic than cathodic stimulation, which is proposed to change after lesioning. One limitation of our previous results is the use of biphasic pulses: the second phase of opposite polarity might have reduced or even changed the polarity effect.

Methods

Thus, we used charged-balanced pseudo-monophasic stimuli with an 8 times longer initial phase, prior to the 50µs-long dominant phase eliciting the response. We performed midbrain recordings along the tonotopical axis of the inferior colliculus (IC). The recordings were made prior and after a micro-lesion (<450µm diameter) was introduced in the basal part of the cochlea. To assess local changes in the polarity effect, we compared apical and basal monopolar stimulation, using a 6-contact guinea pig-CI. We analyzed the thresholds and first spike latencies (FSL) of IC multiunit activity. The IC recording positions were chosen prior to neomycin-deafening, based on pure-tone stimulation.

Results

The response profile in the IC showed the typical broad activation to monopolar stimulation. The FSL was shorter for anodic than cathodic stimulation. Apical 'off lesion' stimulation lead to lower anodic than cathodic thresholds, both pre and post lesion. Basal 'on lesion' stimulation did, however, result in a significant change in the polarity effect after the lesion. After lesioning, the cathodic stimulation gained higher efficiency (lower thresholds) relative to the anodic stimulation.

Conclusions

Thus, we conclude that the main findings of our previous study, using electrically-evoked compound action potentials, have been validated: a) the FSL data confirmed a more central spike initiation sites for anodic than cathodic stimulation and b) the threshold PE is a marker for local changes in SGN health.

Supported by Hearing 4 All (DFG Exc. 2177), MHH Foundation and MedEl Company (Innsbruck).

W69. Sensorineural Hearing Loss Occurs in Chronic Suppurative Otitis Media Mouse Model

Inner Ear: Damage & Protection

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Background

CSOM is a neglected tropical disease affecting 330 million people worldwide and is the most common cause of permanent hearing loss among children in developing countries. *Pseudomonas aeruginosa* (PA) is the leading cause, initially generating acute otitis media and later converting from a planktonic to biofilm state. Persister cells within the biofilm can evade antibiotic attack due to low metabolic activity and robust repair mechanisms. While conductive hearing loss is an issue, it is known that permanent high frequency sensorineural hearing loss (SNHL) also occurs in CSOM patients. CSOM donors' temporal bone specimens show significant hair cells (HC) loss in the basal turn of the cochlea. However, the mechanism behind SNHL remains unknown. Our lab has created and validated a novel PA CSOM mouse model that mimics the human condition. We hypothesize that SNHL correlates highly with phenotypes of PA and number of infiltrating macrophages.

Results

PA was inoculated into the middle ear of mice either as stationary or exponential phase at different concentrations, confocal images at 3 days after inoculation (3dai) revealed near-complete outer hair cell (OHC) loss in the base and middle of the ipsilateral cochlea (IC), whereas OHC survival rate was 82% in the apex. The inoculation of 109 CFU/ml stationary phase PA treated with ofloxacin resulted in no OHCs loss at 3dai. OHC loss occurred at 7dai in both the IC and contralateral cochlea (CC). In the IC, OHC survival in the base, middle and apex was 2.7%, 55.5% and 98%, respectively, compared to the control group (PBS inoculation). In contrast, the CC demonstrated 9.5%, 82%, and 97% OHC survival in the base, middle and apex, respectively. No inner hair cell (IHC) loss occurred in either cochlea. Lastly, stationary phase PA inoculation at a low dose (107 CFU/mL) resulted in no OHC loss at 3dai and 7dai. OHC loss exclusively occurred in the IC at 14dai. OHC survival was 24%, 92% and 100% in the base, middle and apex, respectively. We further investigated the infiltrating cells in the cochleae. At 7dai, when no OHC loss occurred, Macrophages were significantly increased in IC compared to CC. Few to no neutrophils were present in IC.

Conclusions

We concluded that SNHL severity depends on the exposure to specific phenotypes of PA, and macrophages likely play a role in the pathogenesis of SNHL in CSOM.

W70. Attenuation of Hearing Loss and Inner Ear Pathologies After Cochlear Implantation Following Pre-Treatment by Near-Infrared-Light

Inner Ear: Damage & Protection

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Background

The protection of residual hearing is becoming increasingly important in cochlear implant (CI) surgery. Various approaches have been investigated for the prevention of cochlear functional damage. One effective option without side effects could be the application of near-infrared light (NIR) perioperatively. Specific wavelengths within the NIR-spectrum are known to influence cytochrome-c-oxidase activity which leads in turn to a decrease of apoptotic mechanisms. It has been shown previously that NIR can decrease the cochlear

hair cell loss significantly if applied daily after noise exposure. Recently, studies by our group indicated that even a single NIR-pre-treatment has the ability to reduce auditory threshold shifts and rescue cochlear sensory tissue when applied immediately before loud sound exposure. The present study investigated the effect of a single NIR pre-treatment before cochlear implantation on inner ear structural and functional properties in an animal model.

Methods

Normal hearing adult guinea pigs undergo a unilateral pre-treatment of the cochlea with NIR-light for 15 minutes. Immediately after light exposure, a guinea pig CI electrode array was inserted through a cochleostomy into the scala tympani of the first cochlear turn. The contralateral ear received the similar implantation but was not NIR pre-treated and served as an intra-individual control.

Results

Four weeks after implantation, the amount of residual hearing, measured by ABR recordings, was significantly higher on the NIR pre-treated side. Hair cell loss and inner hair cell synapse counts were determined immunohistochemically in histological cochlear samples and data were compared between the two ears (with or without NIR pre-treatment) in each animal.

The data demonstrate that outer hair cell loss was significantly reduced in NIR-pre-treated ears compared to the contralateral side (implanted only). No differences have been shown for inner hair cell counts as well as for the number of neuronal synaptic connections between inner hair cells and spiral ganglion neurons.

Conclusions

Our results suggest that a very effective protection of residual hearing is possible during cochlear implantation by a single NIR pre-treatment. However, the observed attenuation of cochlear pathologies alone does not sufficiently explain the reduction of auditory threshold shift. Additional sources need to be considered and discussed.

This study was supported by Advanced Bionics GmbH, Hannover, Germany.

W71. Fate Mapping Reveals Slow Turnover of Adult Cochlear Macrophages in Steady State and Distinguishes Resident From Recruited Monocyte-Derived Macrophages Following Acoustic Trauma

Inner Ear: Damage & Protection

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Background

The mammalian cochlea contains resident macrophages. 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 has not been studied extensively. We showed that macrophages play beneficial role and may promote the survival of spiral ganglion neurons (SGNs) via fractalkine (CX3CL1-CX3CR1) signaling in the injured cochlea (Kaur et al., 2015, 2018, 2019). However, it remains elusive whether CX3CR1-expressing resident or recruited macrophages are functionally distinct and differentially promote SGN survival. To determine the functional diversity of cochlear macrophages and to define the isolated roles of CX3CR1-expressing resident and recruited macrophages in SGN survival in the injured cochlea, it is critical to distinguish them phenotypically. We used fate mapping technique wherein CX3CR1-expressing resident and recruited macrophages are endogenously labeled with different fluorescent reporters.

Methods

Tamoxifen inducible Cx3cr1CreER mouse line was crossed with red fluorescent protein (RFP) reporter mouse line (R26RFP). The progeny CX3CR1YFP-CreER/wt:R26RFP were injected with tamoxifen or corn oil and euthanized at various time points post injection to determine Cre recombination efficiency in CX3CR1 lineage and their rate of turnover in both 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 or sham exposure to track and distinguish CX3CR1-expressing resident and recruited macrophages

in the injured cochlea. Tissue were analyzed by flow cytometry, fluorescent immunohistochemistry and confocal imaging.

Results

In the absence of tamoxifen, 28.5%±3.7% of CX3CR1-YFP+ CD45+ resident macrophages in the cochlea and 0.1%±0.07% of CD45+ CD11b+ Ly6G- monocytes in the blood were found to co-express RFP. After 2 days of tamoxifen treatment, 98.5%±1.0% of resident macrophages in the cochlea and 60%±8.6% of monocytes in the blood were found to co-express RFP. By 2 months, the recombination efficiency remained same in the cochlea but only rare RFP+ monocytes could be detected in the blood. Examination of recombined CX3CR1-expressing cochlear resident macrophages over a period of 120 days indicate that their turnover rate is slower than circulating monocytes/macrophages (1-3 days) and similar to long-lived microglia (brain resident macrophages). Acoustic trauma in “washed out” mice show that injured cochlea contains both YFP+ RFP+ population consistent with resident macrophages and a YFP+ RFP- population consistent with recruited monocyte-derived macrophages. There was no significant increase in YFP+ RFP+ resident macrophages, indicating that the overall increase in macrophages was entirely due to newly recruited cells. In contrast, unexposed mice only possessed YFP+ RFP+ resident macrophages.

Conclusions

These data establish the use of fate mapping to definitively distinguish CX3CR1-expressing resident macrophages from recruited monocyte-derived macrophages in both naive and injured cochlea.

W72. Discovery of Active Transport of Particles Across the Round Window Membrane

Inner Ear: Drug Delivery

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Background

Systemic pharmaceuticals have limitations for otic delivery due to the blood-labyrinthine barrier and the potential for side effects. Local delivery can increase effective dose and reduce off-target effects. Currently, injection into the middle ear places drugs in contact with the round window membrane (RWM), allowing passive diffusion into inner ear perilymph. This is effective for relatively small molecules but not as efficient for large molecules or gene therapy vectors. Opening the RWM or direct injection into the perilymph potentially expose the delicate inner ear structures to damage or infection. A mechanism for efficiently delivering large cargo across the intact RWM would be valuable.

Methods

To search for the existence of a RWM transport mechanism, we screened a library of viral vectors, each expressing one of 109 random peptides, for the ability to move large (1 µm) particles across the intact RWM of rats.

Results

We identified rare peptides that mediate efficient transport which was absent post-mortem, indicating an active transport mechanism. The peptides, which can transport viruses and DNA into the inner ear, are not similar to known cell- or tissue-penetrating peptides.

Conclusions

We have discovered an unexpected, active mechanisms of trans-RWM transport that can move large particles across the intact membrane, from the middle to the inner ear. This offers the opportunity to develop an entirely novel strategy for the noninvasive delivery of drugs, drug-containing particles and gene therapy vectors to treat inner ear disorders.

W73. Comparison of Inner Ear Gene Therapy Delivery Routes in Small Animal Models Using Helper-Dependent Adenovirus

Inner Ear: Drug Delivery

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Background

Viral vector-mediated gene therapy shows promise for treatment of inner ear diseases. Most inner ear gene therapy research has focused on the use of adeno-associated viral (AAVs) vectors for gene delivery, however, the small carrying capacity of these vectors (4.8 kb) limits their efficacy. To restore sensory function in many types of hearing loss, targeting of larger or multiple transgenes in various cell types is needed. Helper-dependent adenovirus (HdAd) has great potential for the development of such strategies due to its large capacity for genetic cargo (37 kb). The complex structure and relatively inaccessible location of inner ear tissues poses a unique challenge for delivery of viral vector-mediated gene therapy biologics. Viral transduction is critically determined by access to host cell receptors used for viral entry. Cell receptor access is critically determined by the route of viral vector delivery to the inner ear. We have generated HdAd reporter vectors for the characterization of HdAd as a platform technology for in-vivo application. Here we sought to establish HdAd transduction patterns and route-dependent differences in transduction through delivery of HdAd in the rodent inner ear using multiple surgical approaches.

Methods

HdAd vectors carrying fluorescent reporters were delivered to perilymphatic and endolymphatic compartments of guinea pig and mouse species inner ear using different surgical approaches. A comparison of transduction via different delivery routes was carried out at a 7-day time point using immunohistochemical staining and confocal microscopy.

Results

Imaging analysis revealed fluorescent reporter expression with all delivery approaches at a 7-day time point. The extent of transduction along the cochlear axis depended on the route of delivery. Differences in transduction of specific inner ear tissues depending on delivery route were also noted.

Conclusions

Inner ear transduction using HdAd varies depending on the delivery route used to access the inner ear in rodent species. Additional experiments looking at safety, stability of expression and volume-dependent transduction differences are ongoing.

W74. Generating Kcnj10 Fluorescent Reporter Mice for Visualization of Intermediate Cells in the Stria Vascularis

Inner Ear: Membranes & Fluids

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Background

The stria vascularis (SV) is a stratified epithelial tissue in the lateral wall of the cochlear duct and is responsible for potassium ion transport into endolymphatic fluid-containing scala media. The processes that result in elevated potassium concentration in the endolymph are necessary for an endocochlear potential (EP) between 80 to 100 mV, which is critical for normal hearing. The SV contains three layers of main cell types: (1) basal cells, which are adjacent to fibrocytes of lateral wall, (2) marginal cells, which directly border the scala media, and intermediate cells between these two cell types. While the basal cell layer is easily distinguishable from

others, the intermediate and marginal cells form an interdigitated network of cell projections within the SV. Kcnj10 (Potassium Inwardly Rectifying Channel Subfamily J Member 10), the gene that encodes KIR4.1, an inwardly rectifying potassium channel critical to EP generation, is highly expressed in SV intermediate cells. With the goal of enabling visual distinction of intermediate cells, we generated a transgenic mouse strain expressing fluorescent reporter gene specific for this cell type.

Methods

A ZsGreen expression cassette was used to replace the Kcnj10 coding sequence in BAC RP23-157J4. Two founder lines were generated. Transgenic mice produced with the BAC express ZsGreen in a cell-specific fashion. The expression of ZsGreen in the inner ears of transgenic mice bred to congenicity on a C57BL/6J background were examined utilizing cross-section and whole mount immunohistochemistry. Auditory brainstem responses (ABR), distortion products of otoacoustic emissions (DPOAE) and endocochlear potential (EP) were also tested in these mice.

Results

We demonstrate that transgene expression does not affect auditory function as measured by ABR, DPOAE, and EP testing when compared to wild-type C57BL/6J mice. Strong transgene expression is observed in tissue types and cells known to express Kcnj10, including intermediate cells of the SV. Signal is expressed in cytoplasm as well as cell nuclei. Co-labeling with antibodies and RNAscope probes confirmed specificity of ZsGreen signal location.

Conclusions

Transgenic Kcnj10-ZsGreen mice express fluorescent signal in SV intermediate cells and allow the delineation of SV intermediate cells from marginal cells, which may be a useful in investigating the role of cell type-specific function in the SV, allowing cellular studies to determine the mechanisms by which SV intermediate cells contribute to EP generation.

W75. Non-Invasive Measures of the Efficiency of Middle-Ear Prosthesis Placement and Length

Middle & External Ear

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Background

To replace damaged or pathological ossicles, ear surgeons use strut-like prostheses to restore the mechanical continuity between the tympanic membrane (TM) and the stapes footplate. It is known that different lengths and placement and thus tension and angle in the prosthesis can impact its sound transmission capabilities. Our goal is to determine if transmission differences resulting from differences in tension and placement can be measured non-invasively from ear canal measurements.

Methods

We developed a finite element model of the normal middle ear (ME) segmented from a stack of micro-CT images of a human temporal bone. The results were compared with physiological measurements of stapes velocity and non-invasive ear-canal input absorbance, performed on the same specimen. In order to simulate a total ossicular replacement (TORP), the incus and stapes crus were removed in the model and a prosthesis padded with cartilage was interposed between the TM and footplate. Two alternative TORP configurations were considered in which the plate of the prosthesis was placed either near the umbo (Assembly 1) or on the posterior quadrant of the flat region of the TM (Assembly 2). We calculated the stapes velocity to determine transmission efficacy and the ME admittance and absorbance near the TM to determine if efficacy can be measured non-invasively. The absorbance and admittance calculations made from 0.2-8 kHz in the presence of static pressures that ranged from -200 to +200 daPa.

Results

With a progressive increase in prosthesis length, both configurations show a significant reduction in stapes velocity below 2 kHz. The ME absorbance shows a substantial difference between the assemblies in the frequency range between 500 Hz and 7000 Hz at ambient pressure. However, Assembly 2 appears less

sensitive to a variation of the prosthesis length than Assembly 1. The admittance across input pressure at 226 Hz (similar to clinical audiometry) and 1000 Hz was calculated. Assembly 1 with a nominal length of the shaft, produced the expected symmetric curves with a peak at 0 daPa. When the length of the shaft increases from its nominal value, the peak of the tympanometry curve shifts to different pressure locations. In contrast, results with Assembly 2 are less sensitive to the shaft's length.

Conclusions

Our preliminary calculations provide early evidence that ear canal measurements of admittance and absorbance, as a function of pressure and frequency, are sensitive to prosthesis placement and length. This paves the path towards using wideband tympanometry measurements as a diagnostic tool to determine if a prosthesis was chosen with the optimal length and position. [Work supported by the Amelia Peabody Charitable Fund.]

W76. Effect of Sutures in Bone Conduction of Acoustic Waves

Middle & External Ear

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Background

The effect of the skull structure, and the sutures in particular, on bone conduction hearing is still not sufficiently understood. This work provides an experimental investigation of bone conduction (BC) sound propagation of skull bone surface waves over sutures and fractures in entire cadaver heads as well as in extracted bone samples containing sutures.

Methods

All experiments were conducted on six fresh whole head cadaver specimens. Electromagnetic actuator from a commercial BC hearing aid (BCHA) (Baha® 5 SuperPower) was utilized to provide stepped sine stimulus in the range of 0.1-10 kHz, via an implanted screw. The response of the intact skull surface was monitored as motions of the top, back, base, ipsi- and contra-lateral skull surface as well as of seven sutures per head and two fractures in high resolution. The surface motion was quantified by sequentially measuring approximately 900 different points on the intact skull surface (5-7mm pitch at the sutures and 15-20mm elsewhere) with a three-dimensional laser Doppler vibrometer (3D LDV) system and a robot arm. Before extracting bone samples, containing sutures, each location of each sample, as well as several anatomical locations, were registered via fiducial markers (0.5mm wires). Additionally, a computer tomography (CT) scan was conducted for each intact head (including fiducial markers). After extraction of the bone samples, the motion of the lateral and medial surface of each sample was measured, along it the position of the fiducial markers. Based on the fiducial markers data, the velocity and CT data were coalesced together into a single coordinate system, allowing for comparison between structural and motion data.

Results

In general, the skull surface underwent spatially complex motion regardless of the skull state and vicinity to sutures. Medial and later skull surfaces were moving without any significant modification at the sutures. The mode shapes of vibration of the extracted bone samples, containing sutures, resembles that of simple homogeneous plates, with limited effects of the sutures or the sample support. There was limited relative motion between skull surface at the two sides of a suture.

Conclusions

Merging of comprehensive experimental data from multiple domains allows for the detailed evaluation of the effects of the sutures on the motion of the skull bone.

W77. Single-Cell RNA Sequencing Uncovers Rapid Shift in the Cellular Architecture of the Murine Tympanic Membrane After Injury

Middle & External Ear

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Background

Tympanic membrane (TM) perforations are a common cause of conductive hearing loss. Although in most cases they close spontaneously, they can become chronic and cause ongoing morbidity. Despite having a high prevalence, the underlying cellular interactions that govern TM perforation healing are mostly unknown. Previous work demonstrated that upon injury, the murine TM displays a robust increase in proliferation, but which cells and what signals drive this remain to be understood. This study aimed to transcriptionally define the cell populations activated in response to injury of the murine TM to explore the coordinated healing process and identify what may be dysregulated in chronic perforations.

Methods

TM perforations were made in both ears of adult mice. Animals were sacrificed 1, 3, 7, or 14 days following injury (n = 20 ears per timepoint). Single cells were enzymatically dissociated and submitted for single-cell RNA sequencing (scRNA-seq) using the 10x Genomics platform. Two clustering algorithms, Seurat and CellFindR, were employed to identify clusters of cells in an unbiased fashion, and the resulting gene expression matrices were queried to transcriptionally characterize each population. Top differentially expressed genes were computationally identified for each cluster, allowing for pathway analysis of each identified cluster. Protein detection via immunohistochemistry and RNA detection via in situ hybridization were used to biologically validate and define the anatomic location and spatial relationships of distinct timepoints throughout the perforation healing process.

Results

Over 10,000 cells were captured per time-point (range: 10,292- 22,366). Data was merged from all conditions, and unsupervised clustering of the cumulative data revealed 97 total sub-populations of cells within 15 distinct top-level populations. The major cell types in the uninjured and wounded TM were keratinocytes, endothelial cells, immune cells, mucosal cells, mesenchymal cells, adipocytes, and red blood cells. Further analysis demonstrated the emergence of cellular subtypes unique to the injured and healing state as early as 1 day after perforation. There are waves of transcriptional shifts, first the keratinocytes and later in the mesenchymal and mucosal populations. The transcriptional data shows induction of new sub-populations of cells present from 1 day to 7 days post-injury, which then disappear almost entirely by 14 days post-injury. Among the distinct cell states identified were a gross transcriptional shift among the stem/progenitor keratinocyte population after injury and the emergence of a distinct “wounded” keratinocyte progenitor population. At later timepoints, large populations of more differentiated keratinocytes emerge.

Conclusions

Overall, this study defined the transcriptional and cellular architecture of the TM in homeostasis and wound healing. Key markers of these cell populations were identified and can be used for future investigation of the mechanism of wound resolution of the TM. This, in turn, could help inform how healing is perturbed to induce chronic perforations.

W78. Inner Ear Tropism of Natural and Engineered AAV Serotypes in Non-Human Primate Enables Therapeutic Targeting of a Diverse Set of Cochlear Cell Types

Other, Gene Therapy

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Background

Often, the AAV capsid is a primary determinant of cellular transduction for AAV gene therapy vectors, and different capsids can lead to different efficiency or specificity (tropism) of transduction. Because in some therapeutic contexts, capsid tropism can limit the desired transduction and thus enable the therapeutic approach, much research over the past decade has gone into engineering or evolving capsids with novel tropisms. Another proposed advantage of some engineered capsids is that because of their novel amino acid sequence, they may be less likely to be neutralized by pre-existing antibodies (neutralizing antibodies) present in human populations.

In the ear, several novel capsids have been proposed to provide improved transduction of therapeutically relevant cell types, including, for example, Anc80L65, AAVie, and Php.b. Most of the data supporting the value of these, however, have been generated in mice.

We sought here to examine the ability of natural and engineered capsids to transduce cells, particularly inner hair cells, in the non-human primate (NHP).

Methods

NHP were injected with various AAV serotypes via the Round Window Membrane and inner ear tropism was visualized using immunohistochemistry or RNAscope.

To assess the importance of neutralizing antibody evasion, we tested mice and NHP for serum levels of pre-existing antibodies before and after treatment with AAV.

Results

We found that essentially all capsids tested were able to transduce primate inner hair cells efficiently. Furthermore, several capsids were able to transduce outer hair cells, supporting cells, cells of the spiral ligament, and other cell types of interest.

We show, in both mice and in primates, that elevated systemic levels of neutralizing antibodies do not appear to impact cellular transduction in the ear.

Conclusions

Our data suggests that many capsids are viable options for therapeutic development in the inner ear, even those with high prevalence of seropositivity in the human population.

W79. Hearing Impairment Beyond the Ear: Brainstem and Thalamocortical Contributions to Gap-Detection Deficits

Other, Temporal Processing; Systems-level Analysis of Auditory Function

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Background

Hearing difficulty is often assumed to originate within the ear; however, certain impairments might arise entirely within the brain. For example, some people perform normally on tests of peripheral auditory function but nevertheless have difficulty detecting rapid sound changes, such as brief silent gaps in noise. These "gap-detection deficits" might originate beyond the ear, from abnormalities in the auditory brainstem, thalamus or cortex.

Methods

We used a mouse model of naturally occurring gap-detection deficits to examine the contributions of brainstem and thalamocortical structures to hearing impairment of likely central auditory origin. Ectopic BXSb/MpJ-Yaa mice have normal peripheral auditory function but exhibit behavioural deficits in their ability to detect very brief gaps in noise, compared to control littermates. We previously found a neural correlate of this gap-detection deficit in the auditory thalamus and identified a specific reduction in thalamic sensitivity to sound offsets as its likely cause (Anderson and Linden 2016 J. Neurosci). Here, we traced the origins of these thalamic deficits in both thalamocortical and brainstem structures of the "sound-offset pathway" (Kopp-Scheinflug, Sinclair and Linden 2018 TINS), by performing in vivo recordings in the auditory cortex, medial geniculate body and inferior colliculus, and in vitro recordings in the superior paraolivary nucleus.

Results

Our results reveal that gap-detection deficits in ectopic mice likely arise from both brainstem abnormalities in sound-offset response generation and thalamocortical abnormalities in noise adaptation.

Conclusions

These findings elucidate brain mechanisms of gap detection and demonstrate that naturally occurring gap-detection deficits can originate beyond the ear.

W80. Pupil Dilation, as a Continuous Measure of Listening Effort, During Sustained Attention to Competing Talkers

Other, Pupillometry, Naturalistic speech processing, Aging

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Background

Communicating in realistic listening environments often requires people to continuously track different speakers. When acoustic demands increase, listeners exhibit greater effort even when speech intelligibility remains unchanged. Researchers have attempted to quantify listening effort for word or sentence stimuli, but it is not clear how these results generalize to longer-duration naturalistic speech. Listening effort may furthermore be exacerbated in populations that experience auditory and cognitive deficits, such as older adults.

Methods

We investigated the effects of age and acoustic demands on listening effort by analyzing the time course of pupil dilation during naturalistic speech processing. Pupillometry from 19 younger and 15 older normal-hearing adults was measured while listening to 1-min speech segments. Speech stimuli were presented as a single talker and in the presence of a competing talker. Generalized additive mixed models (GAMMs) were used to analyze the pupil dilation dynamics.

Results

Listeners showed a smaller pupil dilation in the single-talker versus competing-talker conditions. No consistent differences were found between young and older participants. Furthermore, pupil dilation increased initially following speech onset and then decreased over the 1-min time course. It was also largest on the first versus second and third presentation, suggesting that familiarity with a speech stimulus affects pupil dilation.

Conclusions

In sum, we have shown that pupillometry for long speech segments is feasible in both younger and older adults, and that this measure contains valuable information about how effort unfolds in a non-linear way across time and trials.

W81. Dynamics of the Auditory Continuity Illusion

Other, Auditory scene analysis

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Background

Auditory perception happens over time, as a dynamic process. As we listen to the world around us, we continuously link related sounds together (words in a sentence, for instance) and separate unrelated sounds (voices in a crowded room). We study the auditory continuity illusion to illuminate principles underlying the dynamic processing of sound. In this illusion a tone is played with a short interruption during which the tone is replaced with a noise. If the noise is sufficiently loud, listeners perceive (incorrectly) that the tone continues through the interruption. Past studies of this phenomenon have led to conceptual understanding of this illusion (in Bregman's work on auditory scene analysis, notably), but the dynamical principles and neural circuits that generate this illusion are still being investigated.

Methods

We develop a mathematical model to represent the activity (firing rates) of populations of neurons that signal perception of tone and noise. Firing rate models of this kind are frequently applied to visual illusions and, recently, the auditory streaming paradigm. We associate equilibrium solutions (steady states) of the model with perception of tones and noise. By analyzing the existence and stability of these states, we identify stimulus conditions and neural parameters under which the continuity illusion is present in the model. We also model masking of tones by noise. This provides a necessary constraint on the model: the effect of noise in the model is context dependent. Noise suppresses activity of the tone population in masking conditions but facilitates activity of the tone population in continuity illusion conditions.

Results

We identified two distinct dynamical features that can give rise to neural dynamics consistent with the auditory continuity illusion: hysteresis and bistability. To create hysteresis dynamics, we include mutual inhibition between the tone population and a noise-driven inhibitory pool. Disinhibition in this circuit structure enables noise to preserve firing activity in the tone population during interruptions to the tone. Under bistable dynamics, neural activity in the model is driven by transient responses to sound onsets and offsets. Noise can degrade these “acoustic edges” and cause neural activity to persist when the tone is replaced by noise.

Conclusions

Our mathematical theory describes principles of neural dynamics that can create the auditory continuity illusion. Importantly, we connect our model to well-developed theories of auditory scene analysis articulated by Bregman and others. In particular, the case of hysteresis dynamics aligns with Bregman’s “Sufficiency of evidence rule” and the bistable case corresponds to his “No discontinuity rule.” Our work, therefore, provides a unifying framework that can inform experimental and model-based investigations of this illusion.

W82. The Origin Along the Cochlea of Tone Pip-Evoked Otoacoustic Emissions

Otoacoustic Emissions

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Background

Otoacoustic emissions (OAEs) are widely used for clinical and scientific investigations, as they are non-invasive and quick to record. Widely used OAEs are those evoked by transient stimuli, particularly clicks (CEOAEs). Greater frequency specificity may be obtained by using tone pip-evoked OAEs (PEOAEs). PEOAEs are hypothesized to arise from reflections of energy near the characteristic-frequency (CF) place of the tone pip, but actual PEOAEs have components with a wide range of latencies, some much shorter than expected, suggestive of a cochlear origin that is far basal relative to CF. To understand and interpret PEOAEs, we must know the origin along the cochlea of their various latency components.

Methods

We studied the spatial origin of PEOAEs by perfusing guinea-pig cochleae from apex to base with artificial perilymph containing 20 mM salicylate, which blocks cochlear amplification, reduces PEOAE and auditory-nerve compound-action-potential (CAP) responses, and reveals the cochlear location that produced each response. The perfusion flow rate was adjusted to achieve a constant rate of 0.5 mm/min (~5 minutes per CF octave). Ear-canal sound pressure and CAPs were simultaneously recorded in 16 animals in response to tone pips (6 cycles, 3-cycle rise/fall, no plateau) ranging from 1.4 to 4 kHz at 35- and 59-dB SPL. PEOAEs at 35- and 59-dB SPL were obtained by removing the tone-pip energy by scaling and subtracting the ear-canal sound pressure from 71 dB SPL tone pips. PEOAE components were identified as the peaks in the absolute values of the PEOAE waveforms. The CF place was identified by the perfusion time at which the CAP amplitude was reduced by 50%. The perfusion times for 20%, 50%, and 80% PEOAE-component reductions were also measured. To align latencies from different pip frequencies, latency was expressed relative to the expected cochlear delay (ECD) at each pip frequency, determined from published averages of guinea-pig stimulus-frequency OAE delays (Shera and Guinan 2003, JASA113:2762).

Results

PEOAE-component latencies varied from ~0.3 to 2 ECDs with a central cluster from ~0.5 to ~1.5 ECDs and the densest cluster at ~0.8 ECD. The pattern of PEOAE-component delay (in ECDs) versus PEOAE-reduction-time cochlear place was similar for all frequencies. From the 20% to 80% reduction times, cochlear amplification regions were spread over a ~3 octave range that moved basally for shorter delays. A regression line to the PEOAE 50% reduction locations, showed an amplification origin at 0.7 octaves basal of CF for ECD=1, and for every octave more basal, the latency became shorter by 0.8 ECD.

Conclusions

Most PEOAE components received cochlear amplification within 1 octave of the CF region. PEOAEs provide information about the location near the tone-pip CF region, not about regions far-basal of CF.

Supported by NIH/NIDCD R01 DC014997 (JTL)

W83. Distortion Product Generation by Outer Hair Cells: Influence of Primary Frequency Ratio on Fluid Pressure and Extracellular Voltage

Otoacoustic Emissions

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Background

Distortion product otoacoustic emissions (DPOAEs) are weak sounds measured in the ear canal in response to a two-tone stimulus. DPOAEs have been intensively studied and widely applied for the clinical assessment of hearing function for decades. Their intracochlear counterparts (distortion products, DPs) are commonly assumed to arise from the nonlinearity of the electrical response of outer hair cells (OHCs), but the DPs in the electrical output of OHCs have not been examined. This limits both our understanding of how and where the DPs are generated and the clinical interpretation of DPOAEs.

Methods

In this work, we combine a three-dimensional nonlinear computational model with mechanical-electrical-acoustic coupling with in vivo measurements of the pressure in scala tympani and the extracellular electrical OHC response to study DP generation by OHCs in the gerbil cochlea. Before studying DP generation, the model is first calibrated by comparison to measurements of pure-tone responses. Next, we used the calibrated model to simulate two-tone responses (with frequencies f_1 and f_2) to study DPs in both the pressure and the electrical response. These simulations were again compared to in vivo DP measurements.

Results

After calibration, there is excellent qualitative and quantitative agreement between the experimental data and the model predictions to single tones. Both show tuned mechanical and electrical responses around the best frequency (BF) at low stimulus levels. At high stimulus levels, the mechanical tuning broadens while the electrical response becomes low-pass.

At low stimulus intensities (50 dB SPL) and moderate to high frequency ratios ($f_2/f_1=1.25$ and 1.35), the DP potential shows two distinct peaks: when either f_2 or $f_{dp}=2f_1-f_2$ is close to BF. Analysis of the DP phase reveals that the 2nd peak results from a forward propagating wave at f_{dp} . The DP in the potential thus have two origins: generated by the nonlinearity local to the recording location and a linear response due to vibration of the hair bundle at DP frequency. In response to high level stimuli (80 dB SPL), the DP pressure drops to low values when f_2 is below the BF while the DP voltage is low-pass for all primary frequency ratios. The low pass nature of the DP voltage response implies that OHCs generate DP even at locations significantly basal to the f_2 peak location, which is consistent with recent measurements of DP on the reticular lamina. However, analysis of model simulations demonstrates that these basal DP generators have only limited influence on the fluid pressure or BM vibrations and contribute little to DPOAEs measured in the ear canal.

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.

W84. Cochlear Nonlinearity Contributes to the Initiation of Early ABR Waveforms

Otoacoustic Emissions

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Background

Human auditory brainstem response (ABR) computational models have relied on processing stages emphasizing cochlear compressive nonlinearities in order to improve the accuracy of model performance in predicting actual ABR waveform latencies from human data. The purpose of the current study was to formally examine the relationship between compression estimated from distortion-product otoacoustic emissions (DPOAE) and the absolute latencies of Wave I and Wave V in humans.

Methods

The subjects were 12 adult normal-hearing human females aged 22-28 years. The experimental procedures used in the study were approved by the University of Memphis Institutional Review Board and all subjects provided written informed consent. DPOAE input-output functions (L2=25-75 dB SPL) were measured across a two-octave frequency range and were fitted with third-order polynomials to derive compression thresholds (CT). Single-channel click-evoked ABRs were recorded differentially with disk electrodes placed at the high forehead location (~Fpz) referenced to an earlobe electrode ipsilateral to the stimulus ear with the ground electrode placed at the mid forehead. Each ABR waveform was constituted from 2000 averages. ABR waveforms were replicated to assist in accurate identification and labeling.

Results

DPOAE CT varied from 42-47 dB SPL on average and were relatively constant at 1000, 2000, and 4000 Hz. Individuals with low CT at 4000 Hz exhibited longer ABR Wave I latencies while individuals with high CT at 4000 Hz exhibited shorter ABR Wave I latencies, and this relationship was statistically significant ($r = -0.68$, $p=0.01$). No statistically significant relationships between DPOAE CT and ABR Wave V absolute latencies were detected.

Conclusions

The parallel hallmarks of cochlear amplification when the amplifier is in good condition are narrowly-tuned cochlear filters and compressive growth functions. According to filter theory, the build-up and processing times in narrowly tuned cochlear filters result in longer output delays and briefer output delays would be expected in more broadly tuned cochlear filters. The linkage between CT at 4000 Hz and ABR Wave I latency in the present study provides indirect evidence of enhanced cochlear tuning in individuals with low CT compared with individuals with high CT. These findings are consistent with known cochlear amplifier characteristics and complement previous results indicating that individuals with low CT exhibit better hearing thresholds than individuals with high CT.

W85. Neuromodulation Enhances Plasticity in a Rat Model of Cochlear Implant Use

Plasticity, Learning, & Adaptation to Hearing Impairment

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Background

Rates of auditory perceptual learning with cochlear implants are highly variable across patients. Adaptation to cochlear implants is believed to require neuroplasticity within the central auditory system. However, mechanisms by which behavioral training enables plasticity and improves outcomes are poorly understood. Here we hypothesize that neural mechanisms promoting plasticity in the rat auditory system are key to optimizing cochlear implant usage.

Methods

Specifically, we examined the effect of pairing locus coeruleus stimulation with an auditory stimulus on auditory learning when the animal has to relearn a tone identification task (self-initiated, go/no-go) using a

cochlear implant. Initial training was done using acoustic stimuli in normal hearing animals. Animals were then bilaterally deafened, unilaterally cochlear-implanted, and retrained with the new target delivered by intracochlear electrical stimulation. Prior to daily behavioral training sessions, rats underwent 5-10 min pairing sessions either with optogenetic locus coeruleus or sham stimulation.

Results

Pairing accelerated learning with cochlear implants. We then conducted multi-unit recordings in the auditory cortex to assess responses to the cochlear implant. Animals that had been trained with the cochlear implant had increased activation of the cortex, and those that underwent pairing had a sharper representation of the target cochlear implant channel. We used fiber photometry to monitor activity of noradrenergic locus coeruleus neurons. During auditory learning, cochlear implanted animals display dynamic locus coeruleus activity, specifically during the acquisition of the meaning of reward relevant tones.

Conclusions

These studies indicate that neuromodulation can play a powerful role in shaping outcomes with cochlear implant use and training.

W86. Speech Listening and Cortical Plasticity in Children Using Hearing Aids

Plasticity, Learning, & Adaptation to Hearing Impairment

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Background

Difficulty understanding speech in noise is a common complaint of hearing impairment. Improving this ability is especially important in children as they develop their language skills.

Methods

We fit experienced hearing aid users aged 6-12 years, with mild to moderately severe hearing loss, with bilateral OpenSound Navigator (OSN) or omnidirectional enabled hearing aids. To assess behavioural and cortical plasticity in response to their new hearing aids, outcome measures were completed within a week of initial fit (visit 1; V1) and after up to 13 months of hearing aid use (visit 2; V2).

Results

In a pilot study (n = 15), speech in noise, but not word learning or working memory ability improved after two months of OSN use (V2 = 2m; Pinkl et al., medRxiv). When completing a speech recognition task within an MRI scanner at V1, without hearing aids, more activation was found in visual and motor areas and less activation in the auditory cortex, compared to typically developing (TD) children. After using OSN for 2 months, our pilot participants showed increased activation re V1 in the visual areas and less use of auditory and speech processing areas. This strengthening of alternative cortical activation may indicate that, after using OSN enabled hearing aids, less attention or effort is required to complete the auditory MRI task.

We also explored cortical temporal connectivity during resting state fMRI. We used Neurosynth and GingerAle to synthesise regions of interest (ROIs) and create data-led auditory speech and sound networks (Stewart, GitHub). Connection strength within these networks at V1 differed from TD children and correlated with cognitive and language learning behavioural outcomes. However, cortical network strength changes did not correlate with behavioural outcomes at V2. Overall our pilot MRI demonstrated that children with OSN-aided mild/moderate hearing loss have dynamic alternative cortical speech and sound networks that acclimatize within two months.

Conclusions

Powered by the pilot, we recruited a further 40 children for a double-blind registered clinical trial (NCT03771287). Age and hearing loss matched pairs were randomly assigned to OSN or omni programming on physically identical hearing aids. Behavioural measures showed no differences between groups at V1 or V2 (9-13 months of hearing aid use) on speech in noise, word learning or memory ability, or on parent, teacher or self-assessments. Cortical activity and resting state connectivity results are being analysed using pipelines established in our pilot.

Disclosure: This research was funded by the Oticon Research Foundation. Design, execution and reporting of the study were completed independent of the Foundation and Oticon Inc.

W87. Early-Life Stress Alters Vocalization Preference in Gerbils

Plasticity, Learning, & Adaptation to Hearing Impairment

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Background

Early-life stress (ELS) is known to affect cognition, learning, and emotional regulation. While the mechanisms that induce ELS-derived dysfunction are broad, they include alterations in critical period regulatory elements¹. Yet despite increased plasticity of the auditory system during critical periods, it is unknown whether ELS affects simple sensory perception. This may be the case, as children from low socio-economic status environments (a proxy for ELS) have increased risk for impaired speech perception, as well as altered auditory brainstem responses to speech envelopes². Recent studies from our laboratory have indicated that ELS impairs gerbil auditory perception of temporal features, such as those expressed in gerbil vocalizations³. Here we tested whether ELS affects gerbil approach and avoidance responses to conspecific vocalizations.

Methods

To induce ELS, Mongolian gerbil pups were intermittently maternally separated and restrained from P9-P24, a time window encompassing the critical period for auditory cortex maturation. These animals and an age-matched Control group were then tested as juveniles (P30-P36) in a Y-shaped maze with speakers at the ends of two arms to allow approach behavior, and a dummy speaker at the end of the third arm to allow avoidance behavior. This design provides an opportunity for animals to approach or withdraw from a sound source at the ends of particular arms. The sound stimuli were exemplars from either greeting or alarm calls. Time spent in each arm and in a central monitoring position was scored offline.

Results

Relative to Control animals, ELS animals showed greater approach behavior, regardless of stimulus type. Notably, ELS animals decreased the amount of time spent farthest away from either sound, suggesting that ELS animals were not actively avoiding the sound. ELS animals tended to increase monitoring behavior during aversive sounds, unlike Control animals.

Conclusions

Our results indicate that ELS influences behavioral responses to ethologically relevant sounds. These behavioral changes may arise from differences in auditory perception, cognition, anxiety, or a combination of these factors. To help determine whether cognition and anxiety are affected by this treatment, we are collecting performance data on additional behavioral tasks. These data are among the first to demonstrate auditory dysfunction emerging from early life stress and will contribute to understanding how ELS in children impacts scholastic performance.

W88. Open Board

W89. Effects Hearing Protection on Spatial Awareness

Psychoacoustics

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Background

Firefighters are at increased risk for noise-induced hearing loss (NIHL) due to frequent exposure to hazardous levels of noise. Despite increased awareness of NIHL as a major occupational health problem, implementation

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of effective hearing protection interventions remains challenging in this population. Reduction of situational awareness as a result of hearing personal protective equipment (PPE) has been reported as a primary barrier to PPE use in the fire service. Accurate sound localization contributes to situational awareness, and influences rapid decision making. Hearing PPE both reduces overall access to sound and disrupts access to cues responsible for key spatial orientation tasks such as localization accuracy and front-back distinction. NIHL also reduces access to spectral cues. This study examines the effects of PPE and NIHL on spatial awareness.

Methods

The experiment was conducted with the Coordinate Response Measure (CRM) speech corpus. In each trial of the experiment, four simultaneous spatially separated phrases from the CRM corpus were presented at random from 4 equidistant speakers in 360 degree horizontal array. Listeners were instructed to listen for the target phrase, which was identified by the presence of the call sign “Baron,” amidst three masking phrases. The experimental task required the listener to locate the source of the target phrase and report the associated color-number combination of the phrase. Localization accuracy was collected using a custom head-orientation tracker. Listeners were tested under two hearing PPE conditions and two simulated NIHL conditions using a repeated measures design.

Results

Listeners were highly accurate at both localization and speech monitoring under the normal hearing condition. Localization performance and speech monitoring were negatively affected by PPE. Reaction times revealed participants required increased time to locate the target in the experimental conditions compared to normal hearing conditions. Dual task performance indicates a tradeoff between search time and accuracy under the experimental conditions.

Conclusions

Results reveal use of hearing PPE significantly disrupts spatial hearing, specifically localization performance. Compromised speech monitoring and increased reaction times revealed decreased ability to manage dual tasks when compared to the normal hearing condition. These results are consistent with the self-reported barrier to PPE use of reduced situation awareness, evidenced by impaired decision making and task performance in a controlled dual-task localization experiment.

W90. Deep Neural Networks Reveal Interplay of Peripheral Coding and Stimulus Statistics in Human Pitch Perception

Psychoacoustics

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Background

Computations on receptor responses enable behavior in the environment. Behavior is plausibly shaped by both the sensory receptors and the environments for which organisms are optimized, but their roles are often opaque. One classic example is pitch perception; whose properties are commonly linked to peripheral neural coding limits rather than environmental acoustic constraints.

Methods

We developed a model of pitch perception by optimizing artificial neural networks to estimate the fundamental frequency (F0) of their acoustic input. The networks were trained on simulated auditory nerve representations of speech and music embedded in background noise. To investigate which aspects of the auditory periphery and acoustical environment contribute to human-like pitch behavior, we optimized networks with altered cochleae and sound statistics.

Results

Human-like behavior emerged only when cochleae had high temporal fidelity and when models were optimized for natural sounds. Specifically, lowering the upper-limit of phase-locking in the auditory nerve yielded models with behavior qualitatively different from that of humans, with substantially worse F0 discrimination and a distinct dependence on stimulus characteristics. Model behavior was substantially less sensitive to changes in cochlear frequency tuning. However, the results were also strongly dependent on the

sound statistics the model was optimized for. Optimizing for stimuli with unnatural spectra, or without concurrent background noise yielded behavior qualitatively unlike that of humans.

Conclusions

The results suggest pitch perception is critically shaped by the constraints of natural environments in addition to those of the cochlea. The work provides an example of how supervised machine learning can enable normative analysis in domains where traditional ideal observers are intractable, an approach that is broadly applicable outside of pitch and audition.

W91. The Relationship Between Behavioral Gap Detection Thresholds and Electrophysiological Measurements of Neural Adaptation in Adult Cochlear Implant Users

Psychoacoustics

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Background

Poor speech perception in cochlear implant (CI) users has been attributed to deteriorations in the auditory system (Friedland et al., 2010; Roberts et al., 2013). Temporal cues are particularly important for speech perception in CI users, and the severity of temporal processing deficits has been shown to correlate to speech perception abilities (Grose et al., 2006; Fitzgibbons and Gordon-Salant, 1996). The ability of the auditory nerve (AN) to faithfully encode electrical stimulation has been proposed to be important for the perception and acuity of temporal cues. In this study, we assessed the association of behavioral gap detection thresholds (GDTs) with neural adaptation and recovery from adaptation, two properties of temporal responsiveness of the AN, at two electrode locations for each study participant. This study tested the hypothesis that GDTs were affected by the amount neural adaptation and the speed of recovery from adaptation due to prior stimulation.

Methods

Study participants included seven post-lingually deafened adult CI users ranging in age between 38 to 71 years (mean: 60 years; SD: 11 years). For one participant, both ears were tested. As a result, data were collected in eight ears. All subjects were implanted with a Cochlear™ Nucleus® device in the test ear(s) with full electrode insertions. For each subject, electrically evoked compound action potentials (eCAPs) and behavioral gap detection thresholds (GDTs) were measured at one basal and one apical electrode location, using pulse train stimuli at two carrier rates: 900 and 1800 pulses per second (pps). Behavioral GDTs were measured using a three-alternative, forced-choice, three-down one-up adaptive protocol estimating 79.4% correct detection (Levitt, 1971). One-tailed, Spearman correlational analyses between behavioral and electrophysiological measurements were performed separately for the two carrier rates.

Results

Our preliminary results showed that there was no significant correlation ($p > .05$) between behavioral GDTs and the amount of AN neural adaptation at either 900 pps or 1800 pps. The association between GDTs and recovery from adaptation is currently under investigation.

Conclusions

Based on our preliminary results, the degree of peripheral neural adaptation seems to not have a strong effect on behavioral GDTs. Neural adaptation recovery will be evaluated as the next step to this analysis.

W92. Pitch Perception Improves With Increasing Modulation Depth in Normal Hearing and Cochlear Implant Users

Psychoacoustics

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Background

Pitch has been aptly defined as the perceptual correlate of the periodicity of sounds. The fundamental frequency of voiced speech and of musical notes produces a complex pattern of spatial and temporal variations across the auditory nerve. Cochlear implants (CIs) attempt to mirror this process by delivering temporally modulated pulse trains to electrodes along the length of the auditory nerve. Cochlear implants allow for sub-microsecond control over the temporal properties of stimulation. Despite this exceptional control, pitch perception provided by CI stimulation is significantly worse than that of normal-hearing listeners even when normal-hearing listeners are limited to temporal cues associated with spatially unresolved harmonics.

Methods

This study examines the extent that resolution on a pitch-ranking task depends on modulation depth of the temporal cues. Two experiments will be described. In the first experiment, adults with normal hearing and adults who use cochlear implants were tested on pitch ranking of modulated tones with modulation depth systematically controlled. The carrier tone was a 6 kHz sinusoid with sinusoidal amplitude modulation applied for modulation frequency conditions of 110, 220, and 440 Hz. Modulation depth was systematically varied with conditions including 25, 50, 100, 200, and 400% modulation depth with 200% depth corresponding to half-wave rectification (i.e., “transposed”) and the 400% condition further temporally sharpened.

Results

Results indicate that pitch ranking is better when provided by stimuli with deeper temporal modulations for both normal hearing and CI users. The second experiment examines similar stimuli but bypassing clinical sound processing to directly test the extent modulation depth affects pitch ranking in CI users when presented amplitude-modulated pulse trains on a single electrode. Preliminary results from the second experiment further supports that pitch ranking is better provided by stimulation with deep modulations.

Conclusions

We interpret these results as evidence that modulation enhancement can improve pitch perception for cochlear implant users.

W93. The Role of Beating in Mistuning Perception

Psychoacoustics

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Background

In Western music all tones are separated by a given number of semitones. Small changes to these step sizes can make them sound more dissonant and “out-of-tune”. Earlier studies have shown that the preference for consonant over dissonant musical intervals presented in isolation was related to preference for harmonicity over inharmonicity but not with preference for sounds without beating. However, it remains unclear whether the same sensitivity to harmonicity underlies our ability to detect mistuning between two instruments in ongoing musical segments. The present study addresses this question by determining the degree to which the presence or absence of beats affects mistuning judgments.

Methods

Participants were asked to judge whether short excerpts from J.S. Bach Two-Part Inventions for keyboard were in tune, using four possible responses: “sure in-tune”, “maybe in-tune”, “maybe out-of-tune”, or “sure out-of-tune”. The stimuli consisted of complex tones that contained the first 15 harmonics, presented with equal amplitudes, and were gated with onset and offset ramps of 20 ms, superimposed on an exponential decay with a time constant of 400 ms. Mistuning was achieved by increasing or decreasing the F0s of all tones in the high voice by 0.5 semitones. The first experiment tested an unprocessed condition (including both inharmonicity and beating cues), conditions where beating was reduced or eliminated by either presenting the low and high voices to opposite ears (dichotic condition) or by lowpass and highpass filtering the low and high voices, respectively, at 1150 Hz, and a condition where the two voices were perceived in opposite ears by introducing interaural time differences (ITDs), but retained the presence of beats. The second experiment

varied the spectral slopes of the tone complexes to vary the amount of beating and included conditions with no slope, slopes of -6 or -14 dB, an infinite slope (pure tones).

Results

Preliminary data, collected online, show above-chance performance in all conditions. However, performance appears to be markedly better for conditions in which beats are available, even when they are attenuated by the presence of a spectral slope. There was also a trend for mistuning to be better detected when the upper voice was mistuned down (compressed intervals) than when it was mistuned up (expanded intervals) in all conditions.

Conclusions

The results suggest that beats may be used to detect mistuning between instruments in everyday musical situations.

W94. Impact of Different Components on Spoken Sentence Comprehension Revealed by Speech-Evoked Electroencephalography

Psychoacoustics

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Background

Verbal communication comprises the retrieval of semantic and syntactic information elicited by various types of words (i.e., parts of speech) in a sentence. Content words, such as nouns and verbs, convey essential information about the overall meaning (semantics) of a sentence, whereas function words, such as prepositions and pronouns, carry less meaning and support the syntax of a sentence. Recently, to investigate speech processing, electroencephalography (EEG) has been widely used because it can be utilized to measure brain activity with excellent temporal resolution. Traditional event-related potential (ERP) techniques rely on repeated measures of isolated sensory events (e.g., isolated syllables). Recent advances in EEG analysis have made it possible to parse speech-evoked EEG components elicited by individual segments (e.g., phonemes and word onsets in a sentence) from continuous speech stimuli. We propose a new approach to investigate the different contributions of certain types of words on overall sentence comprehension.

Methods

EEG signals were recorded using a 64-channel EEG system in 13 normal-hearing subjects. The participants were asked to watch a silent movie during the experimental session, while 10 sentences were presented through a loudspeaker; each sentence was presented 100 times. The ERP for each sentence was extracted by averaging the event-related EEG signals. The phoneme impulse train was set based on the phonemes' onset time in each sentence and was extracted for whole-sentence and component-exclusion cases. The components were examined in pairs as follows: (1) content vs. function, (2) noun vs. verb, and (3) object vs. subject. In the cases of component-exclusion, phoneme information related to the component was omitted in the whole-sentence phoneme impulse train. Speech-evoked EEG responses were calculated by computing the cross-correlation coefficients between the ERPs and phoneme impulse train. The Wilcoxon-signed rank test was used to compare whole sentences with each component-exclusion.

Results

Speech-evoked EEG responses of whole sentences and all component-exclusion cases revealed a pattern comparable to that of the auditory complex (i.e., P1-N1-P2) approximately 150–300 ms after stimuli onset in the cortical language circuit. When comparing the speech-evoked EEG responses of each component-exclusion pair with those of the whole-sentence cases, significant reductions ($P < .05$) were observed for the content word-, noun-, and object-exclusion pairs in many language-related brain regions (e.g., left inferior frontal and temporal regions) at time N1. In contrast, such differences were not revealed for the function word-, verb-, and subject-exclusion cases, respectively.

Conclusions

We evaluated the effects of noun, verb, object, and content word components in a sentence on sentence comprehension using speech evoked EEG. The results showed that content words, nouns, and objects are dominant in spoken sentence comprehension compared to function words, verbs, and subjects, respectively.

Supported by grants from the National Research Foundation of Korea (2017R1E1A1A01078409)

W95. Harmonicity Aids Hearing in Noise

Psychoacoustics

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Background

Hearing in noise is a core problem in audition, and a challenge for hearing-impaired listeners, yet the underlying mechanisms are poorly understood. We explored whether harmonic frequency relations, a signature property of many communication sounds, aid hearing in noise.

Methods

We measured detection thresholds in noise for tones and speech synthesized to have harmonic or inharmonic spectra.

Results

Harmonic signals were consistently easier to detect than otherwise identical inharmonic signals. Harmonicity also improved discrimination of sounds in noise. In contrast to other documented effects of harmonicity, harmonic detection advantages were comparable in musicians and non-musicians.

Conclusions

The results show that harmonicity is critical for hearing in noise, demonstrating a previously unappreciated aspect of auditory scene analysis. The consistency of the effect across synthetic and natural stimuli, as well as across musical expertise, suggests its importance in everyday hearing.

W96. FST is an Essential Co-Factor of LIN28B Mediated Supporting Cell Reprogramming and Hair Cell Regeneration in the Early Postnatal Cochlea

Regeneration

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Background

The loss of mechano-sensory hair cells within the inner ear cochlea is among the leading causes for hearing impairments and deafness in humans. In birds, fish and other non-mammalian vertebrate species, lost hair cells are regenerated by neighboring glia-like supporting cells. Prior to the onset of hearing, cochlear supporting cells in mammals have some limited regenerative capacity; however, the capacity of cochlear supporting cells to regenerate lost hair cells rapidly declines within the first postnatal week in mice. Previously, we found that reactivation of the RNA binding protein LIN28B restores the declining capacity of postnatal day 5 (P5) supporting cells to re-enter the cell cycle and generate hair cells and identified follistatin (Fst) as a downstream target of LIN28B. FST is a secreted protein known to antagonize Activin-type signaling. During cochlear development FST functions to prevent premature cell cycle exit and differentiation of hair cell and supporting cell progenitors (pro-sensory cells). Whether FST plays a role in hair cell regeneration is currently unknown.

Methods

To address the role of FST in hair cell regeneration we used stage P5 murine cochlear organoid and explant culture models. To overexpress FST and or LIN28B we used doxycycline inducible transgenes. To knockout/knockdown endogenous Fst expression we infected organoids with lentiviral particles containing

shRNA and CRISPR-Cas9 constructs. To identify downstream targets of LIN28B and or FST regulation we used bulk-RNA sequencing.

Results

Here, we identify FST as an important co-factor in LIN28B-stimulated hair cell regeneration. We found that co-activation of FST greatly enhanced LIN28B's positive effect on supporting cell proliferation and hair cell production, whereas knockout/knockdown of endogenous Fst in LIN28B overexpressing organoids/explants greatly attenuated LIN28B's positive effect on proliferation and hair cell generation. Furthermore, consistent with FST known inhibitory role in Activin/TGF β -type signaling, we found that higher than normal levels of TGF β -type ligands such as TGF β 2, ActivinA or BMP4 inhibited LIN28B-induced supporting cell proliferation and blocked hair cell formation. Global gene expression analysis uncovered that LIN28B and FST promoted supporting cell reprogramming through distinct mechanisms. We found that FST inhibited ectopic activation of mesenchymal and neuronal gene expression, whereas LIN28B activated pro-growth gene pathways and induced key pro-sensory and hair cell-specific genes. Furthermore, we found that LIN28B-mediated upregulation of pro-sensory and hair cell-specific genes was greatly enhanced by FST co-expression.

Conclusions

Our results identify FST as an important co-activator of cochlear hair cell regeneration. Our analysis suggests that FST acts downstream of LIN28B and that its main functions is to reduce TGF β -type signaling in supporting cells during supporting cell reprogramming. Future studies will examine whether reactivation of LIN28B and FST promotes hair cell regeneration in the mature cochlea.

W97. Type 1 Interferon/JAK/STAT Signaling Pathway in the Ignition of Supporting Cell Activation for Hair Cell Regeneration in the Avian Auditory Epithelium

Regeneration

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Background

The mammalian cochlea has a limited capacity for hair cell (HC) regeneration, while the avian cochlea, namely basilar papilla (BP), HC regeneration occurs by direct trans differentiation and mitotic division of supporting cells (SCs) throughout their lives. However, the critical molecules for the induction of SC activation have not been clarified in the avian BP. The aim of this study was to identify the candidate pathway for the ignition of SC activation in chick BPs and validate its role in Atoh1 upregulation after HC injury using chick BP explant cultures.

Methods

We used explant cultures of post-hatched one day chick BPs. To induce total HC loss in BPs, we exposed BP explants to streptomycin (SM). After 48 h-exposure to SM, BP explants were incubated in the culture media containing no SM. BP samples for bulk RNA sequencing (RNA-seq) were collected at four time points: SM0 (before SM exposure), SM24 (24-h exposure to SM, the time point for total HC loss), SM48 (48-h exposure to SM, the endpoint of SM exposure), and post-SM6 (6 h after SM treatment, 12 h before the emergence of converting HCs). Alterations in the RNA-seq data were validated by quantitative PCR (PCR) and in situ hybridization (ISH). A JAK inhibitor, ruxolitinib phosphate was used to block type1 interferon/JAK/STAT signaling.

Results

In the data set of RNA-seq, 1,219 genes were identified as differentially expressed genes (DEGs) in 19,238 genes. Atoh1 was upregulated from SM24 together with other signature genes that have been demonstrated involvement in HC regeneration in the avian and /or murine cochleae. Atoh1 upregulation was confirmed with qPCR and ISH. Among DEGs, Ifi6 was identified only one gene that exhibited upregulation from SM0 to SM24 and downregulation from SM24 to SM48 or post-SM6. Based on this, we focused on the type I IFN/JAK/STAT signaling pathway. We found 13 DEGs encoded molecules associated with Type I IFN/JAK/STAT signaling pathway. In ISH for Ifi6, virtually no expression was found in SM0 samples, while

both the SM24 and SM48 samples exhibited intense expression in some SCs, indicating the activation of non-canonical type I IFN/JAK/STAT signaling in SCs of damaged BPs. Supplementation of a JAK inhibitor to the culture media resulted in a significant reduction of Ifi6 expression in qPCR at 24 h after SM exposure. In addition, a trend to decrease Atoh1 expression levels by a JAK inhibitor was observed, although the difference was not statistically significant. Altogether, IFN/JAK/STAT signaling could be associated with Atoh1 activation in SCs of chick BPs in response to SM-induced damage.

Conclusions

The present results indicate the involvement of type I IFN/JAK/STAT signaling for the ignition of SC activation for HC regeneration in chick BP explant cultures.

W98. Characterizing the Flat Cochlear Epithelium as a Novel Model of Age-Related Hearing Loss

Regeneration

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Background

Neurosensory hearing loss is one of the most common sensory disorders, and its long-term effect is characterized by the flat epithelium present after hair cells have died in the organ of Corti (OC). Most people who suffer from age-related hearing loss undergo progressive deafness for several years. Cochlear implants and hearing aids are limited treatment options since they both require the presence of at least some spiral ganglion neurons and hair cells, respectively. As a result, it is important to understand what cell types are present and what genes are expressed in the flat epithelia in order to guide proper treatment.

Methods

Therefore, we sought to establish a model of the cochlear flat epithelia resulting from a single injection of diphtheria toxin (DTX) in the Pou4f3-DTR mouse line. Previously, the Pou4f3-DTR line has been shown to kill hair cells within the first postnatal week, but there is some hair cell regeneration soon after. Previous additional work in our lab has consistently generated a flat epithelium in the mouse cochlea by inducing hair cell death in the Pou4f3-DTR line during the late postnatal period.

Results

We also confirm that hair cell loss via this method is permanent until at least 6 months, with no evidence of spontaneous regeneration. By performing immunohistochemistry and H&E stains on whole mounts and frozen sections of cochleas, we were able to histologically characterize flat epithelium in these models and analyze the morphology of the cells that remain. Furthermore, by performing in situ hybridization and qPCR for a variety of sensory and non-sensory genes, we were able to better define the genetic expression of these cells.

Conclusions

This model provides a consistent flat epithelium phenotype that can be used as a baseline in regeneration studies.

W99. Survival of LGR5-positive Supporting Cells Following Ototoxic Trauma in the Adult Mouse Cochlea

Regeneration

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Background

Previous studies have shown that certain supporting cells in the mammalian cochlea express stem cell markers, including Leucine-rich repeat-containing G-protein-coupled receptor 5 (LGR5). It has been demonstrated that LGR5-expressing supporting cells from the mouse cochlea can differentiate into new hair cells in vitro via manipulation of Wnt and Notch signalling pathways. However, as these studies mainly focused on the neonatal

cochlea, it is less clear whether LGR5⁺ supporting cells are a potential stem cell source for hair cell regeneration in the adult cochlea. Therefore, the current study examines to what extent LGR5⁺ supporting cells are present in the adult mouse cochlea and whether these cells survive after ototoxic trauma in vivo.

Methods

Auditory brainstem responses (ABRs) were recorded in 1) adult (postnatal day 30) wild type, 2) normal-hearing Lgr5-eGFP-IRES-creERT2 heterozygous (Lgr5eGFP/[±]) and 3) deafened Lgr5eGFP/[±] littermates. Animals were deafened with a single dose of 100 mg/kg i.v. furosemide and 700 mg/kg s.c. kanamycin. Cochlear whole-mounts and cryosections were prepared and processed for immunohistochemistry in order to characterize the status of mature hair cells and LGR5⁺ supporting cells.

Results

In normal-hearing (ABR confirmed) adult mice, LGR5-EGFP expression was detected in the third row of Deiters' cells and, to a lesser extent, in the inner pillar cells in the organ of Corti. Seven days after the ototoxic trauma severe hearing loss was confirmed by ABR recordings. Immunohistochemistry showed extensive outer hair cell loss and limited inner hair cell damage following ototoxic trauma. Notably, there was substantial LGR5⁺ cell survival in these cochleas. In some of the deafened mice an increased EGFP signal was even observed, especially in the third row of Deiters' cells.

Conclusions

The presence of the LGR5⁺ cells in the cochlea of the adult mouse strongly suggests potential endogenous cochlear stem cells with regenerative capacities in adulthood. To our knowledge, this is the first study showing LGR5⁺ cell survival after an ototoxic trauma in vivo, even in adult mice with extensive hair cell loss. The increased EGFP signal in the deafened cochlea, which might be seen as a result of ototoxicity induced LGR5⁺ cell proliferation, needs to be further explored.

W100. Using Active Inference to Model Selective Attention During Cocktail Party Listening

Speech perception

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Background

Selective attention enables listeners to focus on a talker among a mixture of talkers. Yet, it does not appear to be all-or-none: when an attention-directing cue is presented, preparatory attention builds up over time. For example, reaction times progressively improve as an instructional cue is presented longer in advance of the target talker. Also, EEG activity increases in amplitude before the target talker speaks. The computational processes underlying this slow induction of attentional set are not fully understood.

Methods

Here, we take a theoretical stance, based on active inference (Friston et al., 2017). We introduce a new generative model of selective attention during cocktail party listening and treat selective attention as an inference problem. We model a simple paradigm in which two spatially separated talkers each speak a different colour and number word. A visual cue directs attention to the left or right talker, and the task is to identify the words spoken by the cued talker. We used this model to test competing hypotheses about how time-sensitive changes in precision affect simulated reaction times and EEG responses.

Results

Temporal changes in precision were unnecessary to explain the improvement in reaction times with longer cue-target intervals but were needed to explain the increase in EEG responses before the target talker speaks. An exponential-shaped increase in precision fit the EEG data better than alternative functional forms tested.

Conclusions

Overall, this work contributes to our understanding of selective attention. The model generates quantitative (testable) predictions about behavioural, psychophysical and electrophysiological responses, and underlying changes in synaptic efficacy.

W101. Relationships Between Subjective and Objective Measures of Continuous Speech Understanding Reveal Age-Related Deficits

Speech perception

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Background

Understanding speech in realistic listening conditions can be challenging. On one hand, naturalistic speech often contains contextual information that helps listeners. However, realistic listening environments also often contain multiple talkers that can mask the acoustic signal and limit such context benefits. Difficulties with understanding are exacerbated by other factors that commonly affect speech perception such as age-related hearing and cognitive declines. As a result of these conflicting factors, listeners may find it difficult to accurately assess and articulate the intelligibility of their everyday listening environment. It is therefore imperative to determine if subjective ratings of speech understanding reflect sentence recognition scores in more naturalistic speech environments and whether they can capture age-related effects on speech-in-noise perception. Based on previous findings comparing standardized measures, we hypothesized a more positive relationship between objective intelligibility scores and subjective ratings would be observed for younger adults compared to older adults, who have been thought to under-report hearing-related challenges.

Methods

Nineteen younger and fifteen older normal-hearing adults rated the intelligibility of minute-long naturalistic speech passages spoken by a single talker and presented in quiet or with a competing speaker that they were instructed to ignore. The target speaker was presented once in quiet, and three times each at two signal-to-masker ratios relative to the distractor speaker, 0 dB and -6 dB. For each presentation, listeners rated their understanding of the passage on an interval scale from 0 to 10. In a separate session, researchers presented 3-4 second isolated sentences that were matched in content and signal-to-masker ratio to the minute-long passages. Listeners repeated target speaker sentences aloud and were scored on the number of keywords they correctly recited.

Results

An ordinal mixed-effects regression examined the relationship between subjective intelligibility scores as a function of signal-to-masker ratio, age group, and objective speech recognition scores. The relationship between subjective speech intelligibility and objective speech recognition was more positively correlated for lower signal-to-masker ratios compared to higher ratios and the quiet condition. There was no effect of age on sentence recognition scores or intelligibility ratings; however, the correlation between sentence recognition scores and subjective ratings were more positive for younger adults compared to older adults as the signal-to-masker ratio increased.

Conclusions

Subjective ratings were predicted by objective sentence recognition scores in a limited set of conditions. This suggests that neither measure alone fully captures age-related effects on speech-in-noise perception in naturalistic speech environments. Rather, age-related differences may arise when looking at the strength of the relationship between the two measures. This finding has potential implications for an older patient's ability to provide a meaningful self-assessment in a clinical setting.

This work was supported by the National Institute on Aging of the National Institutes of Health under Award Number P01AG055365 (Simon).

W102. Predicting Aided and Unaided Speech Intelligibility of Hearing-Impaired Listeners Using an Auditory Model

Speech perception

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Background

The speech-based computational auditory signal processing and perception model [sCASP; Relano-Iborra et al., (2019), *J. Acoust. Soc. Am.*, 146(5), 3306–3317] has been shown to successfully account for the speech intelligibility of normal-hearing listeners in a wide variety of listening conditions, including speech degradations as well as non-linear speech enhancement algorithms. The model combines a non-linear auditory-inspired preprocessing with a back end based on the cross-correlation between the clean and the degraded speech representations in the modulation envelope domain. In the present study, the sCASP model was evaluated as a predictor of speech intelligibility data obtained with hearing-impaired (HI) listeners in a speech in noise task.

Methods

Data from two different experiments were used for the model evaluation. Experiment 1 focused on predicting aided speech intelligibility in the presence of a stationary noise. Speech reception thresholds (SRTs) were simulated for 68 HI-listeners originally measured in the study of Johannesen et al. (2016) [*Trends Hear.*, 20, 1–14]. The stimuli consisted of sentences from the Spanish Hearing in Noise Test (HINT) corpus presented at 65 dB sound pressure level (SPL) in the presence of a speech-shaped noise (SSN) masker. The listeners were provided with linear amplification and the input signals to the model were amplified correspondingly. Experiment 2 considered the speech intelligibility of unaided listeners in the presence of stationary and fluctuating noises. SRTs were modelled for 13 HI-listeners and compared to data collected in Christiansen and Dau (2012) [*J. Acoust. Soc. Am.*, 132(3), 1655–1666]. The stimuli consisted of Danish sentences from the CLUE corpus presented at 80 dB SPL in the presence of three different maskers: SSN, an 8-Hz modulated SSN and a nonsense-speech masker.

The model was tuned to the individual listeners' auditory profile, making use of their pure tone audiogram as well as estimates of cochlear compression and outer- and inner-hair cell loss. The model was evaluated in terms of its predictive power of the average listener data and as a predictor of the individual listeners' performance.

Results

The predictions obtained with sCASP reflected the general decrease in performance observed for the HI-listeners as compared to results from normal-hearing listeners. Furthermore, the model correctly predicted masker-type effects in the SRTs. Generally, the model accounted well for the trends observed at a group level, whereas reasonable correlations between the measured and predicted performance across the individual listeners were only found for experiment 2.

Conclusions

Overall, albeit promising, the results suggest that further investigations are required for the model to account for individual performance.

W103. Amplified Cortical Tracking of Word-Level Features of Continuous Competing Speech in Older Adults

Speech perception

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Background

Speech-in-noise (SIN) comprehension difficulties are a common complaint among the elderly population, yet traditional objective measures of speech perception are largely insensitive to this deficit, particularly in the absence of clinical hearing loss. In recent years, a growing body of magneto- and electroencephalographic (M/EEG) recordings in young normal-hearing adults has demonstrated that high-level features related to speech semantics and lexical predictability elicit strong centro-parietal negativity in the EEG signal around 400 ms following the word onset. Here we investigate effects of age on cortical tracking of these word-level features, and their relationship with self-reported difficulties with SIN understanding.

Methods

Younger (18-40 years, n = 19) and older (41-70 years, n = 20) adult participants with normal audiometric thresholds or mild high-frequency hearing loss (corrected for using amplification) participated in a noninvasive EEG recording from 64 surface electrodes, while attending to one of two diotically presented, spatially co-located audiobooks (65 dB SPL each). After each ~60 sec block, participants responded to several comprehension questions about the target story, and provided ratings of confidence for each response, general intelligibility, and state of attentiveness. Additionally, all participants filled out a modified speech, spatial and qualities of hearing (SSQ_m) scale to rate their subjective SIN perception difficulties.

Audiobook transcripts of both the attended and ignored stories were separately used to generate vectors with time-aligned impulses scaled by values of three features: 1) “semantic” dissimilarity of each word relative to the preceding context, 2) lexical surprisal for each word, and 3) overall word audibility, reflecting the average signal-to-noise ratio (SNR). The vectors were then simultaneously regressed against the EEG signal to estimate the strength of cortical tracking of the three features, as well as the corresponding temporal response functions (TRF) for both attended and ignored speech.

Results

Our results revealed robust cortical tracking of all three features for attended speech, with surprisal and word audibility showing significantly stronger contributions to neural activity than dissimilarity. Contrary to our initial hypothesis of weakened tracking of word-level features in older adults, we found that both surprisal and audibility showed significantly stronger tracking in older than younger adults, especially at frontal electrode sites, potentially reflecting increased listening effort. Similar trends were observed with TRFs for the three features, albeit to a weaker degree. Finally, neuro-behavioral analyses revealed trends of a negative relationship between SSQ_m and the overall model fits for attended speech, and a positive relationship between task performance and the overall model fit, indicating behavioral relevance of these measures.

Conclusions

Together, these results demonstrate the utility of modeling cortical responses to competing speech using complex, word-level features and the potential for their use to study changes in speech processing due to aging and hearing loss. [Supported by NIH grant R01DC015462].

W104. The Effect of Face Coverings on Children’s Speech Perception in Noise

Speech perception

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Background

Consistent access to quality speech is critical for the development of listening and spoken language among children who are deaf or hard of hearing (D/HH). It has been established that noise degrades the quality of

speech and interferes with children's access to spoken language in the classroom. Current guidelines from the Centers for Disease Control and Prevention promote the use of face coverings to mitigate the spread of COVID-19. Recent acoustic analyses suggest that face coverings affect the quality of speech. This implies that face coverings interfere with children's access to spoken language in the classroom, where noise is already a barrier to effective communication. It remains unclear to what extent face coverings affect the ability of children who are D/HH to understand speech in the classroom. This study investigates the effect of face coverings on the speech recognition abilities of school-age children who are D/HH.

Methods

Children ages 5-12 years of age completed a sentence recognition task in quiet and competing speech. One group of children were D/HH, enrolled in a listening and spoken language (LSL) program at the DePaul School for Hearing and Speech in Pittsburgh, PA. The other group of children had normal bilateral hearing. Target sentences were recordings of Bamford-Kowal-Bench (BKB) sentences spoken by a female talker in the absence of a face covering and in a variety of face covering conditions (e.g., cloth face covering, cloth face covering with transparent window, fully transparent face covering, and face shield). An adaptive tracking procedure was used to establish the signal-to-noise ratio (SNR) required for 70%-correct recognition of sentences in each condition. Preliminary results suggest an effect of face covering on intelligibility.

Results

Analyses will focus on the effects of age, hearing status, face covering condition, and noise on speech perception.

Conclusions

Preliminary results suggest an effect of face covering on speech intelligibility.

W105. Lip-Reading or Lip-Aiding? – Visually-Presented Acoustic Temporal Envelope Enhances Speech Perception in Noise

Speech perception

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Background

When the auditory signal is degraded due to the impoverished nature of the signal transmission, the visual cues of the talker's face may be crucial for better speech intelligibility. "Lipreading" is therefore considered as an essential skill, especially for hearing impaired (HI) listeners. However, the nature of the speech information that visual modality conveys and how auditory and visual modalities integrate during the speech perception is still debatable. In the classic Motor Theory perspective (Liberman et al., 1967), the visual input provides information about the speaker's articulations, integrated with the auditory input in a particular speech processing module. As an alternative theory, General Auditory approaches suggest that the visual information is not about articulations, per se, but about the correlation between the visual and auditory signals (Diehl et al., 2004). In this study, we hypothesized that integrating auditory and visual modalities is based on the temporal structure of the speech acoustic signals. In other words, instead of providing specific articulation gestures, dynamic movements of the mouth in a continuous speech are served as a visual analog of the acoustic temporal envelope.

Methods

20 normal hearing (NH) listeners and 10 HI listeners participated in speech-in-noise measurements for audio-only and audio-visual conditions. Target sentences consisted of the Harvard sentences (IEEE, 1969) spoken by two male and two female talkers presented at 65 dB SPL and embedded within background multi-talker babble noise. Prior to the audio-visual speech perception measurements, audio-only speech perception was tested in various noise levels (62 to 72 dB SPL with 2 dB steps) to estimate perceptually equalized signal-to-noise ratios (SNRs) across subjects. Individualized SNR of the speech stimuli was then generated afterward for the audio-visual conditions. Instead of actual lip movements, a visual sphere that increased and decreased in sync to the amplitude envelope of the speech signals as provided as visual stimuli in audio-visual conditions.

Results

The audiovisual benefits were calculated by the differences between audio-only and audio-visual speech perception accuracies. The group-averaged results show that 5 - 45 % (mean $\pm$ std = $28 \pm 11\%$) significant audiovisual benefits in NH listeners. There were more individual variations in HI listeners due to various subject factors (e.g., degree of hearing loss and onset of hearing loss).

Conclusions

Visual modality aids the transmission of the information of acoustic amplitude envelope and enhances speech perception in multi-talker listening environments for both NH and HI listeners. These findings suggest that temporal dynamic cues of lip-movements aid speech perception because they provide time-varying, rhythmic patterns of the speech rather than enhancing the phonological representation of the sound.

W106. Lexical Properties of Perceptual Errors Made by Younger and Older Adults Listening to Speech in Multitalker Babble

Speech perception

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Background

Large individual differences in recognition of speech in babble are often observed among older adults, even when audibility is ensured through appropriate amplification. To assess one potential source of variability, this study examined the patterns of word errors made when younger and older adults listened to sentences in babble. Previous literature has suggested that although older adults make more errors, they may use context better than younger adults to fill in final words in sentences. This may be related to older adults' larger and more diverse lexicon. That is, when compared to younger adults, perceptual errors by older adults may be less constrained by the target word, due to poorer perceptual encoding related to reduced audibility, but more reflective of the linguistic diversity of their lexicon.

Methods

To test this hypothesis, three groups of participants: younger normal-hearing adults, older normal-hearing adults, and older adults with hearing impairment (OHI) listened to low-context and high-context sentences in multitalker babble and identified the final word of each sentence. Each participant was encouraged to guess what they heard, even if it did not make sense to them. All participants were provided with spectral shaping appropriate to their individual hearing thresholds and were native speakers of American English. Participants also completed a standardized test of receptive vocabulary knowledge.

Results

Preliminary results show that although OHI listeners made more errors overall, they made fewer attempts at guessing the final word compared to both younger and older normal-hearing groups. Therefore, a lower propensity for making response attempts may be characteristic of poorer perceptual encoding related to hearing loss, and not due to aging. Incorrect responses for all groups in both contexts tended to words with higher rates of occurrence in the English language compared to correct responses to the target words.

Conclusions

Thus, listener errors contained more common words in the language under uncertainty, particularly with the greater contextual uncertainty of low context sentences. However, group differences indicate a potential contribution of vocabulary knowledge. Finally, OHI listeners also demonstrate large differences between error word frequency in low and high context sentences. Further discussion of lexical response error analyses will examine group differences in how listeners recruit lexical abilities to fill in ambiguous cues when listening to speech in babble. Outcomes will be interpreted within the context of the extent to which error profiles due to lexical as well as auditory abilities explain individual differences in recognition of sentences in babble.

W107. Attentional Reinforcement of Human Corticofugal System Aids Speech Perception in Noise

Speech perception

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Background

Perceiving speech-in-noise (SIN) demands precise neural coding between brainstem and cortical levels of the hearing system. Attentional processes can then select and prioritize task-relevant cues over competing background noise for successful speech perception. In animal models, brainstem-cortical interplay is achieved via descending corticofugal projections from cortex that shape midbrain responses to behaviorally relevant sounds. Attentional engagement of corticofugal feedback may assist SIN understanding but has never been confirmed and remains highly controversial in humans.

Methods

To resolve these issues, we recorded source-level, anatomically constrained brainstem frequency-following responses (FFRs) and cortical event-related potentials (ERPs) to speech via high-density EEG while listeners performed rapid SIN identification tasks. We used functional connectivity to measure the directed strength of coupling between levels and characterize “bottom-up” vs. “top-down” (corticofugal) signaling within the auditory brainstem-cortical pathway. Attention was varied with active vs. passive listening scenarios whereas task difficulty was manipulated with additive noise interference.

Results

Active listening (but not arousal-control tasks) exaggerated both ERPs and FFRs, confirming attentional gain extends to lower subcortical levels of speech processing. Attention strengthened brainstem-cortical connectivity bidirectionally; however, corticofugal transmission disengaged under passive (but not active) SIN listening.

Conclusions

Our findings (i) show attention enhances the brain’s nascent transcription of speech even prior to cortex and (ii) establish a direct role of the human corticofugal feedback system as an aid to cocktail party speech perception.

W108. Hyperacusis Masks Tinnitus Response Pattern of Sound-Evoked and Resting State BOLD fMRI

Tinnitus

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Background

Hearing loss is often aggravated by symptoms such as tinnitus, a constant ringing, or hyperacusis, a perception of moderate sounds as too loud or even painful. Tinnitus has a prevalence of 10 % to 20 % in the population, which enforces successful approaches for causative tinnitus therapies. We hypothesize that the success of tinnitus therapy highly varies with the co-occurrence of hyperacusis.

Methods

43 controls and 50 tinnitus patients with and without a co-occurrence of hyperacusis were compared on the basis of a questionnaire, tympanometry, acoustic reflex measurements, auditory brainstem potentials, pure tone audiometry, and evoked- and resting-state fMRI in anatomically predefined brain regions.

Results

Patient groups with tinnitus and patient groups with an additional comorbidity of hyperacusis showed significant differences in the tinnitus questionnaire (Goebel-Hiller-Score), ABR waves, sound-evoked and resting-state fMRI BOLD activity.

Conclusions

The results demonstrate an undiagnosed co-occurrence of hyperacusis in tinnitus patients as a major caveat for studies on the neural correlate of tinnitus. These results should guide medical tinnitus practice in the direction of personalized curative therapies using objective diagnostic markers identified for tinnitus and hyperacusis.

W109. Translational Study of Intratympanic Botulinum Toxin on Middle Ear Myoclonic Tinnitus

Tinnitus

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Background

Middle ear myoclonic tinnitus (MEMT) is a rare disease caused by an abnormal pulsatile movement of middle ear muscles (MEMs). Up to now, the management of MEMT is primarily limited to conservative therapy and few effective medications. To propose a breakthrough treatment for intractable MEMT, our research group previously conducted an animal study to investigate the safety and efficacy of intratympanic botulinum toxin (IT-BT) injection. This translational study introduces the latest results of our animal study as well as the results of a pilot clinical study of IT-BT on intractable MEMT patients.

Methods

Immunofluorescence of cleaved synaptosomal-associated protein of 25 kDa (SNAP-25) were detected from MEMs of Male Sprague-Dawley rats injected with 1 Unit/100 μ l IT-BT. Trachea, esophagus, and stomach tissues of IT-BT injected rats were evaluated for morphologic changes after IT-BT. The body weight changes of the control rat group and IT-BT injected rat group were observed in comparison for 2 weeks. For human trials, 9 intractable MEMT patients were enrolled for IT-BT injection. The first 3 patients were injected with 0.1 Unit/100 μ l BT, next 3 patients were injected with 0.5 Unit/100 μ l BT, and the last 3 patients were injected with 1 Unit/100 μ l BT. The hearing thresholds and the degree of tinnitus distress measured by visual analogue scale (VAS) and tinnitus handicap inventory (THI) were observed for changes at 2 weeks, 4 weeks, and 3 months after IT-BT injection. All patients were closely followed up for any immediate and delayed complications for up to 3 months.

Results

Cleaved SNAP-25 was strongly expressed in MEMs of IT-BT injected rats, especially at 1 week and 3 weeks after injection, which then became weak at 2 months. No significant morphologic changes were found in the respiratory and digestive organs of rats after IT-BT injection. Body weight changes did not differ between control and experimental rat groups. As for clinical trials, MEMT patients generally showed improvement of VAS and THI scores after IT-BT injection, indicating alleviation of tinnitus distress. There were no significant changes in hearing thresholds of all enrolled patients, and no major complications were noted during the follow-up period.

Conclusions

Human pilot trial was successfully performed based on sufficient evidence from the animal study that showed the safety and efficacy of IT-BT. A novel method of IT-BT injection for intractable MEMT patients before considering surgical management of middle ear tendon resection seems acceptable with excellent clinical results of efficacy and safety. A further clinical study in a large sample size will be necessary for in-depth investigation of IT-BT on MEMT patients.

W110. ²³Na MRI, a New Quantitative Method for the Detection of Tinnitus in an Animal Model

Tinnitus

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Background

The difficulty to detect tinnitus objectively in animal models means carrying out new quantitative methods is a key step in the development of new compounds. In the MRI field, MEMRI is often used for detection. To circumvent the toxicity of MnCl₂ and its non-translational method, a non-invasive protocol without a contrast agent injection was developed based on sodium MRI (²³Na MRI). Sodium plays an important role in metabolism and ²³Na MRI is a powerful tool for quantitatively assessing the distribution of tissue sodium (intracellular and extracellular spaces). In the literature, aberrant distribution in Na^v1.6 channels have been observed, which can lead to hyperexcitability in the auditory system associated with tinnitus. ²³Na MRI provides physiological information complementary to proton MRI (¹H MRI), therefore ²³Na MRI is applied to clinical applications (tumors, strokes, multiple sclerosis and muscular disease) to assessing diseases and therapies non-invasively.

Methods

MRI protocol:

Two series of Proton/Sodium acquisitions were performed on each rat under isoflurane anesthesia. The first series of acquisitions served as the baseline (before salicylate administration). An injection of salicylate (300 mg/kg/IP route) was then performed. Two hours after injection of the drug, a second series of acquisitions was performed.

Analysis of MRI images:

The distributions of ²³Na concentrations were determined by integrating the ²³Na MRI signals corresponding to short and long relaxing components (intra and extra cellular) in ROIs from the inferior colliculus (IC) and the cerebral cortices (CC).

²³Na MRI signal levels were referenced to the concentration (154mM) of NaCl tubes positioned close to the brain of the rat. Only the long NMR relaxation component is expected in the reference tubes. The distributions of ²³Na concentrations were determined by integrating the ²³Na MRI signals corresponding to short (5ms) and long (25ms) relaxing components (intra and extra cellular) in ROIs from the inferior colliculus (IC) and the cerebral cortices (CC).

Results

Increases of the ²³Na concentrations were observed in the right inferior colliculus (IC) for 5 rats except in one animal, in the right cerebral cortex (CC) for all rats and in the left CC of 3/5 rats. On the contrary, a decrease was found in the left IC for 3/5 rats. Finally, in the whole brain, an increase from 8,8 to 17,5% was demonstrated for all rats except in one animal.

Conclusions

Preliminary results revealed a potential increase of sodium in the volume of extracellular space in the inferior colliculus in the tinnitus animal model. However, this increase in extracellular space does not appear to be specific to the auditory system in the presence of sodium salicylate. In future ²³Na MRI experiments, it would be interesting to validate this technic on a noise-induced tinnitus model, a more translational tinnitus model.

W111. Ebselen Mitigates Tobramycin-Induced Threshold Shifts and Hyperacusis in Mice

Tinnitus

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Background

Aminoglycosides (AG) such as tobramycin are commonly used in CF patients with recurrent pulmonary infections and those infected with multi-drug resistant tuberculosis. AGs are highly ototoxic, resulting in

auditory dysfunction including hearing loss, hyperacusis, and tinnitus. Prior studies have shown that ebselen can prevent and treat sensorineural hearing loss in animals and humans. Traditionally AG animal models only assessed auditory brainstem responses (ABRs). Our group has recently shown that the acoustic startle reflex can assess both evidence of hyperacusis and tinnitus in an amikacin mouse model. The goal of this study is to further establish an ASR methodology for examining the effect of AGs on auditory dysfunction and the efficacy of ebselen in reducing tobramycin-induced ototoxicity in a clinically translatable mouse model.

Methods

CBA/CaJ mice three months of age were divided into three groups: Group 1 served as a control and did not receive tobramycin or ebselen, Group 2 received tobramycin (200 mg/kg/s.c.) and the vehicle (DMSO/saline/i.p.) daily for 14 continuous days, and Group 3 received the same dose/schedule of tobramycin as Group 2 and ebselen at (20 mg/kg/i.p.). ABR and ASR assessments of hearing were performed at baseline and 2, 6, 10, 14, and 18 weeks from the start of treatment. ASR tests included input/output functions to assess general hearing and hyperacusis, PPI (Prepulse Inhibition) Audiometry to assess hearing deficits, and GPIAS (Gap-induced Prepulse Inhibition of the Acoustic Startle) to assess tinnitus. Following hearing assessments, hair cell counts were obtained via cochlear histologic analysis.

Results

Following 14 days of tobramycin injections, 16 kHz thresholds increased from baseline over the 18-week recovery period with evidence of fluctuations. ABR wave 1 amplitude changes were also seen but varied in magnitude as a function of frequency. Input/output ASR functions revealed hyperacusis as exaggerated startle response magnitudes evoked from stimulus levels above 80 dB SPL. This phenomenon was significantly ameliorated by ebselen treatment. Gap detection deficits, representing tinnitus, were observed in a smaller subset of animals. Cochlear histology did not reveal significant changes in hair cell counts.

Conclusions

This work corroborates our previous findings that AGs can result in hearing dysfunctions. We show that a clinically comparable 14-day course of tobramycin treatment can cause hearing loss, hyperacusis, and to a lesser extent, tinnitus. Ebselen was able to ameliorate tobramycin-induced hearing loss and hyperacusis. These data support the clinical testing of ebselen as a potential treatment for hearing loss, tinnitus, and hyperacusis in patients receiving tobramycin.

W112. Onset-Offset Cortical Auditory Evoked Potential Indices of Tinnitus-Related Excitatory-Inhibitory Neural Plasticity

Tinnitus

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Background

The perception of tinnitus may be triggered by changes in the balance of excitatory-inhibitory neurotransmission in the central auditory system secondary to noise exposure or other peripheral damage. Because it has been suggested that responses to sound onset and offset may differentially reflect excitatory and inhibitory neural responses, onset-offset differences in cortical auditory evoked potential (CAEP) components may provide an objective index of whether such central auditory changes are associated with tinnitus perception in humans. The aim of this study was to assess whether onset-offset CAEP amplitude and/or latency differences indicating changes in central auditory function distinguish between a group with constant tinnitus and matched non-tinnitus controls, and whether tinnitus presence and/or other factors (e.g. age, hearing level) predicted onset-offset outcomes.

Methods

CAEPs were recorded at the onset and offset of a 3-second white noise burst presented at 50 dB SL (including a silent gap in the center, not analyzed for the current study) in individuals with chronic tinnitus and no-tinnitus counterparts matched for sex, age, and hearing thresholds (n = 13, 6 male in each group; age range 20-76, mean = 54 years). Onset-offset differences in area, amplitude, and latency for the P1, N1, and P2 components were compared between groups using independent t-tests. The relative predictive value (proportional

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reduction in error, PRE) of tinnitus, pure tone average (PTA), sex, age, and behavioral gap detection threshold on CAEP outcome variables that significantly differed between groups was analyzed using linear regression.

Results

Onset-offset differences in overall area ($p = 0.035$), P2 amplitude ($p < 0.001$), and P2 latency ($p = 0.024$) were significantly larger in the tinnitus group compared to controls. Presence of tinnitus was the best predictor of the P2 amplitude onset-offset difference (Partial PRE = 0.482). When the age range was limited to adults over the age of 50 years (excluding 7 subjects < 50 years), tinnitus presence explained more of the variance (Partial PRE = 0.707). Other characteristics were significant predictors (PTA for area difference, age for P2 latency difference), but did not account for as much of the variance as tinnitus presence for P2 onset-offset amplitude difference.

Conclusions

Onset-offset difference in individuals with tinnitus, particularly related to P2, were significantly larger compared to a group of matched no-tinnitus controls. This finding supports changes in central auditory excitatory and inhibitory function related to the perception of tinnitus. The results are consistent with the theoretical mechanism of tinnitus generation in which peripheral auditory insult triggers reduced inhibition and consequently, auditory hyperactivity. The preliminary finding that tinnitus was a better predictor of onset-offset differences in individuals 50+ years also suggests an interaction or moderating effect between age-related changes and tinnitus perception may exist.

W113. The Aging-Related Decrease in cVEMP Amplitude Cannot be Accounted for by EMG Rectification

Vestibular: Basic Research & Clinical

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Background

Cervical Vestibular Evoked Myogenic Potentials (cVEMP) is an electromyogenic measure commonly used in clinic to assess saccule function. The main parameters are peak-peak amplitude and interaural asymmetry ratio (IAR). Several non-vestibular factors may influence these parameters. Notably, a greater EMG contraction level prior to stimulation leads to an increased amplitude. As aging impacts both vestibular structures and muscle properties, it is still a matter of debate whether the decrease in cVEMP amplitude observed in normal aging is due to EMG differences prior to stimulation or to the effect of aging on the sacculo-colic reflex pathway. At the clinical level, understanding the effect of aging on the relationship between EMG activity and cVEMP response (amplitude, asymmetry ratio) and the effect of EMG rectification is crucial to improving the categorization of healthy versus pathological responses.

Objective: To investigate whether EMG rectification modifies cVEMP amplitude and asymmetry ratios differently in young and older healthy adults.

Methods

cVEMP recordings were conducted in 42 normal healthy participants divided in two age groups: young ($n=29$): mean=22.79 SD=1.66; and older ($n=13$): mean=69.00 SD= 3.61. Air-conducted cVEMP were recorded using Eclipse (Interacoustics, Denmark). The stimulus was a 95dBnHL tone burst (500Hz) with rise, plateau and fall time of 1ms. cVEMP were recorded only when EMG levels were between 50uV and 150uV, using the Eclipse (Interacoustic, Denmark) monitoring system.

Results

No significant differences were observed for prestimulus EMG levels between young and older participants ($F(1,83)=1.13$, $p=0.291$). However, significant differences between groups were observed for cVEMP non-rectified ($F(1,83)=14.78$; $p=0.000237$) and cVEMP rectified ($F(1,83)=21.85$; $p=0.000011$). A significant positive linear relationship between prestimulus EMG contraction level (RMS) and cVEMP non-rectified amplitude was observed in young participants ($r^2=0.234$; $p=0.00012$), but not in older adults ($r^2= -0.0144$;

$p=0.056$). Finally, no significant differences between young and older participants were observed for non-rectified asymmetry ratios ($F(1,41)=0.124$, $p=0.726$) or rectified asymmetry ratios ($F(1,41)=0.726$, $p=0.508$).

Conclusions

Our results suggest that when EMG is monitored and activation of the SCM is sufficient, the observed decline in cVEMP amplitude with normal aging does not seem to be caused by EMG differences and is therefore likely due to the known histopathological modifications of the vestibular system that occurs with normal aging.

W114. Identification of SARS-CoV-2 Pathway of Entry: The ACE-2 Receptor in Vestibular Endorgans From Normal (Autopsy) and Meniere's (Surgical) Patients

Vestibular: Basic Research & Clinical

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Background

Since the declaration of the COVID-19 pandemic (March 2020), reports of dizziness, vertigo, and vestibular disorders in patients with SARS-CoV-2 infection began to emerge. In a retrospective study of 214 COVID-19 patients from Wuhan, China from January 16 until February 19, 2020, 17% of the patients had dizziness (Mao et al., 2020). There are also isolated case reports of vestibular neuritis in young asymptomatic patients with SARS-CoV-2 infection (Pellitero and Ferrer-Bergua, 2020; Malayala and Raza, 2020). In the present study we localize the expression of the receptor and proteases used by SARS-CoV-2, angiotensin I converting enzyme 2 (ACE-2), transmembrane protease serine 2 (TMPRSS2) and furin within the human vestibular periphery.

Methods

Macula utricle were acquired at surgery from patients who required transmastoid labyrinthectomy for intractable Meniere's disease (N=3), and patients who required a translabyrinthine approach for vestibular schwannoma resection (N=2). Celloidin embedded sections containing the maculae utricle or saccule were obtained from temporal bones acquired at autopsy from individuals with no auditory or balance disorders (2 male, 1 female, ages 65 to 75 years old) (N=3). Immunofluorescence localization was conducted in cryostat or celloidin-embedded sections using mouse monoclonal antibodies against ACE-2, TMPRSS2 or furin. Fluorescent or HRP secondary antibodies against mouse were used for visualization.

Results

ACE2-IF was seen in the apical pole of transitional and dark epithelial cells located at the periphery of the utricular neurosensory epithelia. The apical pole of supporting cells also demonstrated ACE2-IF. Vestibular hair cells did not exhibit ACE2-IF. The stromal cells, nerve fibers, nerve terminals, and stromal vasculature also did not exhibit ACE2-IF. A similar pattern of ACE-2-IF was demonstrated in the saccular macula. TMPRSS2 and Furin were also immunolocalized in formalin-fixed cryostat and celloidin sections of the human normal macula utricle and saccule. TMPRSS2-IR localized to the transitional, dark and supporting cells of the macula utricle; vestibular hair cells were non-IR. Furin-IF was also seen in the apical pole of transitional and dark epithelial cells located at the periphery of the utricular neurosensory epithelia. The apical pole of supporting cells was also Furin-IF. Punctated Furin-IF was seen in the stroma. Nerve fibers and terminals and vascular tissue located in the stroma underlying the macula utricle sensory epithelia did not express TMPRSS2 nor furin. The utricular maculae from Meniere's disease and vestibular schwannoma exhibit similar patterns of immunolocalization of ACE2, TMPRSS2 and furin compared with normative.

Conclusions

To our knowledge this is the first study examining the localization of ACE2, TMPRSS2 and furin in normal and Meniere's macula utricle and saccule. The presence of these proteins suggests a potential port of entry of SARS-CoV-2 into the human peripheral vestibular system.

W115. Dizziness Following Traumatic Brain Injury: A Track-TBI Study

Vestibular: Basic Research & Clinical

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Background

Traumatic brain injury (TBI) is a leading cause of disability and global injury burden, with 27 million new cases in 2016. Dizziness affects 40-60% of patients after TBI, which can significantly impact their employment, social relationships, and overall quality of life. The objective of this study is to investigate the longitudinal incidence of dizziness following TBI and associated demographic factors, prior medical history, and other TBI symptoms.

Methods

1,514 subjects were identified from the Transforming Research and Clinical Knowledge in Traumatic Brain Injury (TRACK-TBI) study, which is a multi-center, prospective longitudinal cohort study. The primary indicator of dizziness was a self-report of “moderate” or “severe” dizziness at 12 months on the Rivermead Post-Concussion Symptoms Questionnaire (RPQ). Demographic factors, medical comorbidities, and injury type and severity were compared among those with and without dizziness. A univariate ordinal regression model was constructed to identify potential predictors of dizziness at 12 months following TBI.

Results

Of the total 1,514 subjects who had 12-month data, 143 (9%) reported moderate or severe dizziness. Compared to the subjects with mild or no dizziness, this cohort had fewer years of education (mean 12.7 [SD 2.6] vs. 13.8 [SD 2.7], $P < 0.001$) and a higher prevalence of hypertension (24% vs. 15%, $P < 0.05$) and Type II diabetes (15% vs. 6%, $P < 0.05$). No statistically significant differences were seen for history of hearing deficit, injury cause, Glasgow Coma Scale score at the emergency department, and extracranial injury. Potential predictors of dizziness included age (OR: 1.15; 95% CI: 1.08-1.22), female sex (OR: 1.76; 95% CI: 1.40-2.22), Patient Health Questionnaire-9 (PHQ-9) score (OR: 1.19; 95% CI: 1.16-1.21), history of migraines (OR: 2.17; 95% CI: 1.44-3.27), history of anxiety (OR: 2.09; 95% CI: 1.54-2.84), history of depression (OR: 1.97; 95% CI: 1.48-2.62), litigation intent (OR: 1.51; 95% CI: 1.16-1.97), worse hearing after injury (OR: 3.57; 95% CI: 2.64-4.82), headache (OR: 3.45; 95% CI: 2.92-4.07), nausea (OR: 4.43; 95% CI: 3.45-5.69), noise sensitivity (OR: 3.02; 95% CI: 2.54-3.59), light sensitivity (OR: 3.51; 95% CI: 2.91-4.23), fatigue (OR: 3.33; 95% CI: 2.83-3.90), irritability (OR: 2.46; 95% CI: 2.10-2.89), taking longer to think (OR: 3.17; 95% CI: 2.69-3.74), blurry vision (OR: 3.65; 95% CI: 3.01-4.44), and double vision (OR: 4.59; 95% CI: 3.50-6.03).

Conclusions

Moderate or severe dizziness persists in a significant percentage of patients with TBI at one-year follow-up. Our findings suggest that dizziness may be part of a larger symptom complex associated with TBI, rather than a unique symptom, which has implications for pathophysiology. By better understanding this common and debilitating post-concussive symptom, we hope to have identified possible avenues for treatment.

W116. Exploring the Roles of TMC1 and TMC2 in Hair Cells of the Vestibular End Organs

Vestibular: Basic Research & Clinical

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Background

Tmc1 and Tmc2 form sensory transduction channels in auditory and vestibular hair cells. Mice with targeted deletion of Tmc1 (Tmc1Δ) are deaf, while those without Tmc2 (Tmc2Δ) are phenotypically normal. Mice lacking both (Tmc1Δ;Tmc2Δ) lack sensory transduction, and exhibit deafness and vestibular dysfunction. Consistent with these morphological and functional findings, we recently found Tmc1Δ and Tmc1Δ;Tmc2Δ mice have abnormal inner hair cell (IHC) synapse counts relative to WT mice. These differences evident in

Tmc1Δ and Tmc1Δ;Tmc2Δ mice are consistent with a developmental shift from Tmc2 to Tmc1 that only occurs in the cochlea.

While Tmc2 is transiently expressed in the developing cochlea, its expression persists in vestibular hair cells of the mouse utricle. Despite being phenotypically normal and having no cochlear abnormalities, Tmc2Δ mice have attenuated vestibulo-ocular reflexes (VOR) and utricular type II hair cell sensory transduction currents. These findings suggest Tmc2 may play a more prominent role in the vestibular system.

The purpose of this study was to expand upon our cochlear findings and examine the roles of Tmc1/Tmc2 in the developing and mature vestibular end organs. Characterizing the vestibular phenotypes of Tmc mutant mice lacking either or both Tmc1/2 will enhance our understanding of the developmental shift from Tmc2 to Tmc1 in the cochlea and help to potentially reveal the roles of Tmc1 and Tmc2 in vestibular hair cell function.

Methods

Vestibular sensory evoked potentials (VsEPs) were collected from wild-type, Tmc1Δ, Tmc2Δ, and Tmc1Δ;Tmc2Δ mice at various time points (1 – 8 mo.). Following VsEP acquisition, utricles and saccules from these mice were harvested and immunostained with antibodies to C-terminal binding protein 2 (CtBP2), calretinin, myosin7a, and NF200. Calretinin immunostaining was used to differentiate striolar from extrastriolar regions. Confocal z-stacks of utricular/saccular striolar and extrastriolar regions were obtained. Imaris imaging software was used to generate 3-D projections, count average ribbon numbers, and track changes in vestibular afferents over time.

Results

Tmc2Δ mice demonstrated elevated VsEP thresholds at all timepoints relative to WT mice, consistent with the abnormal sensory transduction currents and VORs recorded from previous studies. Tmc1Δ;Tmc2Δ mice had absent VsEP thresholds at all timepoints. Decreased synapse counts were also evident in both Tmc2Δ and Tmc1Δ;Tmc2Δ mice at later timepoints.

Conclusions

Continued expression of Tmc2 in vestibular hair cells may be crucial in differentiating cochlear from vestibular hair cells. Assessment of balance function alone cannot be assumed to reflect the status of the vestibular periphery and additional assessments (i.e. VsEPs, VORs, immunohistochemistry) are necessary to accurately characterize the vestibular periphery in Tmc mutant mice.

W117. Music Listening Habits and Perception in Cochlear Implant Users

Auditory Prostheses

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Background

Cochlear implant (CI) users demonstrate limitations in music perception, therefore we designed a study to compare music listening habits in CI users and individuals with normal hearing (NH). The goal of this exploration of music listening habits was to improve our understanding of the impact CIs have on hearing restoration in an everyday, real-world context.

Methods

We conducted a survey-based cross-sectional study to examine music listening habits in CI users and NH individuals. A Qualtrics survey was distributed August 2019-2020 and advertised via online support groups (Cochlear, MED-EL, Advanced Bionics, Facebook), SurveyCircle, and physical bulletin board postings. Eligible participants included children (>14 years) and adults; we excluded CI users that were activated within three months of the survey. Our primary outcome measures were differences in music listening habits between NH individuals and CI users. Responses were analyzed by two-tailed, unpaired t-test.

Results

A total of 44 CI users (43 adults, 1 child) and 71 NH individuals (all adults) participated in this study, totaling 115 responses. Of these, 37 (84.1%) CI users and 65 (95.8%) NH individuals listened to music. We found that CI users utilized their implant's microphone (n=31, 49.2%), streamed wirelessly (n=20, 31.3%), and/or used direct-line audio input (n=12, 15.8%) for music listening. Less CI users (n=21, 53.8%) spent more than one

hour per day listening to music compared to NH individuals (n=54, 83.1%). The top three music genres CI users listened to were Classical (n=26, 59.1%) Rock (n=25, 56.8%), and Oldies (n=24, 54.5%) while NH individuals preferred Pop (n=48, 70.6%), Rock (n=46, 67.6%), and Hip-hop (n=43, 63.2%). Similar proportions of CI users and NH individuals enjoyed listening for the beat, melody, or timbre of a musical piece (p=0.20, 0.46, 0.47, respectively). Of those three musical elements, CI users (n=14, 43.8%) rated melody as the most enjoying (p=0.18). Both groups further displayed similar proportions of individuals who enjoyed listening to music at home, through a hand-held device, in a vehicle, and at a live performance. However, CI users (n=21, 58.3%) reported that listening to ambient music in public locations was perceived as less enjoyable than listening in a quiet-focused environment compared to NH listeners (n=18, 27.3%) (p=0.002). Finally, few CI users (n=5, 14.3%) demonstrated discomfort while listening at a live performance compared to NH individuals (n=40, 61.5%) (p<0.0001).

Conclusions

CI users demonstrate different genre preferences and spend less time listening to music compared to NH individuals. Interestingly, CI users demonstrate less auditory discomfort to live performance listening in comparison to NH listeners. These differences in music perception shed light on the limitations of CI-mediated listening, but also suggest that many CI users are regular participants in musical activities.

W118. Effect of Target Talker and Masker Familiarity on a Dichotic Speech Perception Task

Speech perception

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Background

Conversing with a partner in a noisy environment requires the listener to use strategies to pick out the important information (i.e. speech) from background noise. Speech understanding in a complex environment can be especially difficult for older adults with normal hearing (ONH), who are less able to extract the target signal and inhibit the competing noise than younger adults with normal hearing (YNH). One cue for speech segregation that improves speech understanding in noise for older adults is the familiarity of the target voice (FV). It may also be the case that the FV benefit can also correspond with a listener's ability to identify the voice of the target talker. The goals of this study were to determine: 1) if listeners experience a benefit on speech understanding in a complex auditory environment with one or two competing talkers when the target talker is familiar (FT); 2) if these effects persist when the masker is familiar (FM); 3) if the familiar benefit is dependent on whether the listener can identify the familiarity of the target voice (ID), and 4) if these effects vary as a function of listener age.

Methods

Participants included 15 YNH and 15 ONH adults who were recruited as familiar couples. Couples recorded sentences from the Boston University Gerald (BUG) corpus. Listeners were presented with sentences where the FV was either the target or the masker, and were instructed to report what was said using a matrix of word choices. Speech understanding performance was measured when the target and a single masker were routed to the same ear (monotic) at 0- and -5-dB SNR, and when the target was presented monaurally and two maskers were presented dichotically. Listeners were also prompted to identify the familiarity of the target talker at each trial.

Results

For the monotic conditions, when the ID was correct, YNH listeners had better speech understanding performance than ONH listeners across all conditions. However, when the ID was incorrect, the YNH listeners showed a greater decline in speech understanding performance from the FT to the FM conditions than the ONH listeners. While performance was significantly poorer in the dichotic than the monotic configuration for both groups, both groups had better speech understanding performance in the FT condition relative to all other dichotic conditions.

Conclusions

Across monotonic and dichotic listening configurations, the YNH and ONH listeners exhibited a familiarity benefit when the FV was the target talker. When the FV was the masker, listeners showed varying ability to segregate target from masker speech, depending on the number of competing talkers and familiar/unfamiliar talker identification accuracy.

W119. Age Related Changes in Signal-In-Noise Perception Assessed via Prepulse Inhibition of the Startle Reflex Response

Aging

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Background

The inability to comprehend speech in an environment with background noise is a chief complaint of the elderly and also in young adults with hearing loss in noisy environments such as concerts, construction work, and in crowds. Signal-in-noise (SiN) perception can be measured by using a simple paradigm where a short tone is played in a continuous background noise and the tone is varied in level to produce various signal-to-noise ratios (SNRs in dB). We measured SiN processing to determine the effects of age in a mouse model of age-related hearing loss. The purpose of this study is to determine the effects of age and hearing loss on signal-in-noise processing across the life span of the CBA mouse.

Methods

A total of 108 mice of both sexes ranging in age from 2 months to 24 months and categorized into; young (2-6 months old), middle (10-16 months old), and old (17-25 months old) groups. ASR test protocols included startle I/O functions, and pre-pulse SiN. SiN was assessed using 10,16 and 24 kHz, 50 ms tone bursts embedded in a continuous 65 dB SPL background noise and SNR varied from +0 to +12 dB SNR, in 3 dB steps. Control trials (N=30) were randomly interspersed through the session. Raw startle waveforms were then classified into true startles and non-startles using a machine learning algorithm. The algorithm scales each normalized waveform and classifies the response as a true startle or non-startle. PPI was calculated by $(1 - (\text{prepulse trial} / \text{control trial})) \times 100$. Each SNR consisted of 30 trials per session for a total of 180 trials each session and each mouse was run 3 times/week for a total of 540 trials.

Results

The goal of this project was to determine the effects of age on a SiN task using a high throughput behavioral paradigm, PPI of the ASR. Compared to young mice in which the majority (95%) could detect the SiN in the most favorable SNR conditions of +9 and +12, older mice had a wide range of performance outcomes based on PPI. Therefore, they were grouped into non-responders, and responders based on PPI. Further, responders were grouped into fair (PPI between 20 and 50%) and good (PPI above 50%). All groups, except the poor responders, showed a systematic increase in PPI with improved SNR. The 10 and 16 kHz signals resulted in the greatest PPI values with young mice showing mean PPI of >50% for SNRs > +6 dB. In contrast both fair and good old responders had PPI values < 50%. The 24 kHz signal showed the lowest mean PPI results for all groups.

Conclusions

Results indicate an overall age-related decline in the ability of old mice to detect signals in background noise.

W120. An Exploration Study to Characterise the Response of the Heart in Sleeping Infants to Detection and Discrimination of Speech Sounds

Speech perception

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Background

Behavioural assessment of speech discrimination in infants is challenging and thus not commonly measured in the clinical setting, though it is a useful measure that informs the outcome of amplification management in infants with hearing loss. Thus, there is a need for the development of an objective speech detection and discrimination measurement tool. One objective measure is heart rate, which has been shown to be affected by a change of state of alertness and vigilance in awake infants. This change of state evokes the general arousal system, which in turn regulates the acceleration or deceleration of the heart rate. This study explored the change in heart rate in response to speech sounds in sleeping infants. We have separately shown that speech detection and discrimination ability in sleeping infants can be measured using fNIRS to measure cortical brain activity; the fNIRS measurement may be made more robust for clinical use by combining brain activity and cardiac information.

Methods

fNIRS data were recorded from 24 sleeping infants with normal hearing using natural voice recordings of three consonant-vowel contrast pairs presented at 65 dB SPL in a habituation/dishabituation paradigm. The standard stimulus of a contrast pair was presented repeatedly to induce habituation, followed by the novel stimulus. The test was repeated until the infants awoke. The heart rate was extracted from the fNIRS raw data through a custom signal processing pipeline.

Results

The group average heart rate change response to the standard stimulus blocks showed a short deceleration response post-stimulus onset, possibly representing stimulus orientation phase, followed by an acceleration response above baseline level, which is consistent with what is observed in prior literature. The heart rate change response between the first and second five blocks of standard stimuli did not show observable difference. The heart rate change was larger with presentation of novel stimuli compared to standard stimuli, implying that heart rate change reflects speech detection and discrimination ability.

Conclusions

In this study, we have presented a signal processing pipeline that robustly extracts heart rate from fNIRS data. We have shown heart rate change response to be a useful feature that reflects speech detection and discrimination which might be useful when used in conjunction with fNIRS cortical brain response to increase the reliability of the objective test. This finding increases the diagnostic value of using fNIRS to measure speech discrimination ability in infants.

W121. Assessing of Listening Effort Modulated by Task Difficulty and Reward, Using Response Time While Performing a Listening Task

Speech perception

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Background

Multiple studies have shown that manipulation of task difficulty influences the amount of mental effort exerted to understand an auditory stimulus. It has also been shown that other factors such as the motivation of the individual influence the amount of mental effort a person is willing to exert to understand the stimulus. In this study, behavioral response time and brain activity measured with functional near infrared spectroscopy (fNIRS) were used during a listening task to evaluate the effect of task difficulty and motivation in the exertion of listening effort. The aims of this study were a) to determine if there was an effect of task difficulty and motivational reward on response time, and b) to determine the effect of task difficulty and motivational reward on the fNIRS responses in different areas of the brain. Here we report the response time results only.

Methods

Twenty-one participants with standard hearing thresholds were presented sentences in ICRA noise. After each sentence they pushed one of two keys on a keyboard to indicate whether a word flashed in a screen was or was not the last word of the last sentence heard. Difficulty was manipulated by changing the SNR and

motivation was manipulated by offering a monetary reward to the participants if they improved their performance with respect to the non-rewarded condition. While the participants performed the task, fNIRS signals were recorded.

The response time was measured between the offset of each sentence and the button press. A 3x2 experimental design was used, with difficulty levels: easy, medium and hard, and motivation conditions: rewarded and non-rewarded. A two-way repeated measures ANOVA was used to assess the effect of difficulty on response time, the effect of reward on response time and the interaction between difficulty and motivation on the response time.

Results

The results showed that there were significant effects of difficulty and motivation on response times, and no interaction. Post-hoc comparisons revealed that on average, response times for the medium and difficult conditions were significantly longer than for the easy condition. Also, the average response time for the difficult condition was significantly longer than the medium difficulty condition. Motivation increased response time, as participants may have weighted correctness above response speed in the motivated conditions.

Conclusions

Both task difficulty and motivation influence response time. The increase of response time with difficulty can be explained by the need of more time to retrieve the meaning from the heard words. The increase in response time with motivation for correct responses could be due the participants taking more time to be sure that their responses are correct.