FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Degeneration of bushy cell dendrites and the innervating auditory nerve synapses in the cochlear nucleus during age-related hearing loss**
Day:	Thursday
Time:	9:30am
Authors:	Meijian Wang, Chuangeng Zhang, Shengyin Lin, Ruili Xie*
Affiliation:	Department of Otolaryngology, The Ohio State University

Cellular morphology and synaptic configuration are key determinants of neuronal function that undergo changes during pathological conditions. In the cochlear nucleus (CN), the first neural station of the central auditory system, principal bushy neurons are renowned for their bush-like dendritic arborization and being innervated by large auditory nerve (AN) synapses on the soma called the endbulb of Held, which was shown to degenerate during age-related hearing loss (ARHL). It remains elusive how bushy cell dendrites change with age, and the innervation profile of AN synapses on these dendrites under normal hearing and ARHL conditions. We studied these questions in young (2-5 months) and aged (28-32 months) CBA/CaJ mice of either sex. Bushy neurons were filled with fluorescent dye in acute brain slices to characterize cellular morphology, followed by immunohistochemistry to identify and quantify different subtypes of AN synapses they receive using antibodies against vesicular glutamate transporter 1 (VGluT1) and calretinin (CR). We found that the bushy cell dendrites degenerate with simplified complexity during aging. These neurons do receive AN bouton synapses on their dendrites, which show increased synaptic density during ARHL with dendritic degeneration. In young mice, these dendritic synapses are predominantly non-calretinin-expressing regardless of the subtype-specificity of the somatic synapses. Such preference in dendritic synapse subtype was not observed in aged mice. The findings suggest that bushy cell dendrites and the innervating AN synapses play significant roles in auditory processing, and alteration of both during aging may contribute to the development of ARHL.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Spatial coupling of Ca2+ entry to Synaptic vesicles required for ultrafast synaptic transmission is not unique to presynaptic CaV2.1 channels**
Day:	Thursday
Time:	9:42am

Authors: Priyadharishini Veeraraghavan1*, Rene Oliver Goral1, Debbie Guerro-Given2, Connon Thomas2, Paula Valino Ramos1, Naomi Kamasawa2, Samuel M. Young, Jr1,3

Affiliation: 1.Department of Anatomy and Cell Biology, University of Iowa, Iowa City, Iowa
2.Max Planck Florida Institute for Neuroscience, Jupiter, Florida
3.Department of Otolaryngology, Iowa Neuroscience Institute, University of Iowa, Iowa City, Iowa

Sound localization requires temporally precise and reliable synaptic transmission at synapses in the auditory brainstem. The efficiency of synaptic transmission relies on the spatial coupling of voltage-gated Ca2+ channels (VGCCs) to synaptic vesicles (SV) at the presynaptic active zone (AZ). In mammalian central nervous system synapses, the CaV2 family of VGCCs, CaV2.1, CaV2.2 and CaV2.3 can mediate AP evoked release with CaV2.1 being the most efficient. Ultrafast-AP firing presynaptic terminals are CaV2.1 exclusive with SVs tightly coupled. Hence, the current paradigm is that tight coupling is dependent on CaV2.1 and not the other CaV2 subtypes. However, it is still unknown if tight coupling required for ultrafast AP evoked release is intrinsic to CaV2.1. Therefore, to test this we conditionally knocked out (CKO) CaV2.1 or both CaV2.1/2.2 using either CaV2.1fl/fl mouse or our novel mouse model, CaV2.1fl/fl/2.2fl/fl at calyx of held (calyx), a fast-firing auditory brainstem presynapse important in sound localization. We observed a partial compensation of calcium current (ICa) in both CaV2.1 and CaV2.1/2.2 CKO calyces that highly impacted AP evoked SV release without altering AZ ultrastructure. More importantly our preliminary data indicated that the compensated channels, predominantly CaV2.2 in CaV2.1 CKO calyces can still maintain the tight coupling required for ultrafast synaptic transmission. The reduction in AP evoked release could be simply due to reduced ICa and CaV2.2 biophysical properties. Hence, we propose that the fast SV release is not exclusive for CaV2.1 channels.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Early-Life Stress Impairs Temporal Processing across the Auditory Pathway**

Day: Thursday

Time: 10:10am

Authors: Yi Ye and Merri J. Rosen*

Affiliation: Hearing Research Group, Department of Anatomy and Neurobiology, Northeast Ohio Medical University (NEOMED)

During adulthood, stress can affect sound-evoked responses and synaptic elements in the auditory pathway. Yet despite increased plasticity of the ascending auditory system during critical periods of development, it is largely unexplored whether developmental stress (early-life stress, ELS) affects the periphery or central auditory system, and whether these effects last into adulthood. Our lab has shown that ELS impairs gap detection behaviorally and in primary auditory cortex, a region necessary for detection of short gaps. Yet ELS may have more widespread effects on the auditory pathway and even the auditory periphery; for example, a corticotropin-releasing factor signaling system is active in the cochlea (Vetter and Yee, 2018).

To study how ELS affects processing of complex sound stimuli across the auditory pathway and across development, we measured gap-in-noise ABRs (GIN ABR, reflecting auditory nerve to inferior colliculus inputs), middle latency responses (MLR, reflecting cortical responses) and frequency following responses (FFR, reflecting brainstem temporal synchrony) within the same animals at three ages across development. To induce ELS, gerbils were maternally separated and restrained for 2 hrs/day at unpredictable times from postnatal day (P)9 to P24. GIN ABRs, FFRs at several modulation frequencies (MFs: 35, 50, 125, 250, 500, and 1000 Hz), and MLRs were recorded in awake animals (sedated with dexdomitor) at three developmental time points: P25, P40 and P90. GIN ABRs were also measured at P40 in a separate group of ketamine-xylazine-anesthetized gerbils.

GIN ABRs revealed higher gap detection thresholds in ELS animals, driven by a reduced amplitude to the second sound burst in ABR waves I, II, and III (auditory nerve and brainstem). In FFRs, the response power across MFs matured gradually in both groups, with a better signal to noise ratio (SNR) and lower noise floor in adults (P90 vs both P40 and P25). This pattern was altered by ELS, which reduced SNR and increased the noise floor in comparison to controls, at all MFs and ages tested. ELS unexpectedly increased response power at slow but not fast MFs, which helped overcome the higher noise floor, and suggests central hyperactivity. Finally, the developmental pattern of middle latency responses was altered by ELS, which caused shorter latencies but lower amplitudes, indicating reduced response synchrony.

Our data reveal ELS effects on temporal processing in both the periphery and central auditory system, throughout development and into adulthood. This suggests that the known effects of ELS on learning, memory, and attention may arise in part from poor sensory input. It is possible that ELS may directly induce auditory temporal processing deficits in children, causing difficulty processing rapid transistions in speech, particularly in noisy environments.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Inner Hair Cell Damage and Cochlear Synaptopathy Differentially Impact Neural Envelope Coding of Modulations and Pitch**
Day:	Thursday
Time:	10:22am
Authors:	Andrew Sivaprakasam*, Ivy Schweinzger, Hari Bharadwaj, Michael Heinz
Affiliation:	Weldon School of Biomedical Engineering & Speech, Language, and Hearing Sciences at Purdue University

Background: The transduction of sound into neural impulses by the auditory periphery is undeniably altered by sensorineural hearing loss (SNHL). Identifying specific functional deficits and how they correlate with underlying cochlear anatomic damage (i.e., inner hair cell (IHC) damage and cochlear synaptopathy (CS)) is an ongoing area of study. Previous work suggests that these two pathologies result in temporal envelope coding deficits. Humans with SNHL likely exhibit mixed cochlear deficits, thus investigating modulation and pitch coding using controlled and validated chinchilla models to isolate these pathologies is helpful. We first investigated the effect of IHC damage and CS on Envelope Following Responses (EFRs) to three different types of modulations. We then collected pilot data to investigate how these hearing loss conditions may affect place and time coding of tone complexes as a function of harmonic rank.

Methods: In Experiment 1, eight young chinchillas (4 female) were randomly assigned to balanced, sex-matched Temporary Threshold Shift (TTS) and carboplatin (CA) exposure protocols inducing CS and IHC damage, respectively. In Experiment 2 we investigated the coding of pitch periodicity from 5 chinchillas (3 normal hearing, 1 TTS, 1 CA). Non-invasive OAEs, wideband MEMRs, and ABRs were collected as measures of auditory health. EFRs to sinusoidal and rectangularly amplitude modulated (Fc = 4 kHz, Fm = 100 Hz) tones (Exp. 1), and six different tone complexes (F0 = 103 Hz, 6 harmonics, alternating-phase) with harmonic ranks 3 to 13 (Exp. 2) were also collected. The TTS group was exposed to band-limited noise (1 kHz center frequency, 100 dB SPL, 2hrs). For the CA group, a 38 mg/kg injection protocol (shown to cause 10-15% IHC loss, significant IHC stereocilia damage) was used.

Results: Consistent with previous findings, OAEs remained similar after exposure, while MEMRs were reduced in the TTS group. In Exp. 1, a marked reduction in phase-locking value (PLV) to high harmonics of 100 Hz in sharply modulated stimuli was consistently observed in CA animals, and to a limited degree in synaptopathic (TTS) animals. The reduction was quantified using a ratio of summed upper (3-16) to lower (1-2) harmonics in the PLV spectra. In Exp 2. reductions were also found in responses to tone complex stimuli in CA animals. No apparent deficits were observed in the TTS chinchilla. A doubling in periodicity representative of a change in harmonic resolvability was observed with harmonic ranks greater than 7 for normal, TTS, and CA animals.

Conclusion: Dysfunction of the IHC transduction non-linearity and subsequent deficits in neural firing characteristics may manifest as reduced PLVs to upper harmonics in the recorded EFRs. EFRs to the chosen tone complex stimuli imply that, even with stereocilia damage, cochlear place representation is likely maintained in the presence of either CS or IHC damage, but that representation of timing is impaired with IHC damage. Coding of pitch periodicity by the auditory periphery appears relatively unaffected by synaptopathy. In summary, IHC damage and CS result in different neurophysiological effects. Future work aiming to diagnose or treat these pathologies must recognize these differences.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Group I mGluR-triggered temporally patterned spontaneous synaptic transmission in mouse MNTB neurons**
Day:	Thursday
Time:	1:50pm
Authors:	Huimei Wang*, Kang Peng, Rebecca J. Curry, Dong Li, Yuan Wang, Xiaoyu Wang, Yong Lu
Affiliation:	Northeast Ohio Medical University

Rhythmic action potentials are generated via intrinsic ionic mechanisms in pacemaking neurons, producing synaptic responses of regular inter-event intervals (IEIs) in their targets. In auditory processing, evoked temporally patterned activities are induced when neural responses timely lock to a certain phase of the sound stimuli. Spontaneous transmitter release, however, is a stochastic process, rending the prediction of the exact timing of the next synaptic event completely based on probability. Furthermore, neuromodulation mediated by metabotropic glutamate receptors (mGluRs) is uncommonly associated with patterned neural activities. Here, we report an intriguing phenomenon. In a subpopulation of MNTB neurons recorded under whole-cell voltage-clamp mode in acute mouse brain slices, temporally patterned glycinergic sIPSCs and glutamatergic sEPSCs were elicited by activation of group I mGluRs with 3,5-DHPG (200 μM). Knockout of mGluR5 (one member of group I mGluRs) largely eliminated these effects. Cell-attached recordings showed temporally patterned spikes evoked by 3,5-DHPG in VNTB neurons and AVCN bushy cells (potential presynaptic cells for synaptic inhibition and excitation to MNTB, respectively). Auto-correlation analyses revealed similar strength in the rhythmogenesis between spikes in the presynaptic cells and synaptic responses in MNTB neurons. 3,5-DHPG-induced rhythmic spike firing in VNTB neurons was strongly correlated with temporally patterned sIPSCs in MNTB neurons. Finally, immunocytochemical studies identified distinct subcellular localization of group I mGluRs in the AVCN-MNTB and VNTB-MNTB pathways. Our results imply a potential central mechanism underlying the generation of patterned spontaneous activity necessary for auditory circuit development.
Supported by NIH/NIDCD R01DC016054 (YL).

FULL ABSTRACT
PODIUM PRESENTATION

MIDWEST AUDITORY RESEARCH CONFERENCE
presented by the Kresge Hearing Research Institute

Title:	**Tinnitus emerges independently of inferior colliculus hyperactivity and thalamic dysrhythmia after noise trauma**
Day:	Thursday
Time:	2:02pm
Authors:	Calvin Wu*, Susan E. Shore
Affiliation:	Kresge Hearing Research Institute, Department of Otolaryngology, University of Michigan

The current pathophysiological model of tinnitus presumes increased spontaneous activity of auditory neurons as the mechanism that underlies auditory phantom perception. While evidence of "hyperactivity" has been reported in different nuclei and along the auditory pathway, its relations to tinnitus induction, the developmental time course, and specific characteristics of increased spiking in different regions remain unexplored. Contradictory findings arise when researchers investigate neural activity in different animal species, at different time scales, and using different methods of tinnitus induction and assessment. Thus, to achieve a coherent understanding of the neural underpinning of tinnitus, we set out to systematically investigation tinnitus correlates in a single animal model with extended temporal and spatial windows. Temporally, we look at the development of tinnitus from 0–10 hours after noise trauma. Tinnitus is inferred using a deep neural network classifier previously trained on behaviorally confirmed dorsal cochlear nucleus (DCN) single-unit recording. Spatially, we record multiple auditory regions simultaneously—cochlear nucleus, inferior colliculus (IC), and medial geniculate nucleus (MGN)—and look at their interdependence. Our results show: 1) while tinnitus-like activity emerged in DCN immediately after noise trauma, IC activity revealed no consistent pattern for either the effect of noise trauma or tinnitus; 2) MGN's increased spiking was not driven directly by that in DCN; 3) MGN's oscillatory activity was abolished by noise trauma in all cases, independent of tinnitus development. These findings dispelled the notion that hyperactivity is a general phenomenon associated with tinnitus, as well as questioned whether auditory thalamic dysrhythmia undergirds tinnitus perception.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Neural Population Activity in the Shell Inferior Colliculus Predicts Behavioral Outcomes**
Day:	Thursday
Time:	2:14pm
Authors:	Gunnar L Quass*, Meike M Rogalla, Alexander F Ford, Pierre F Apostolides
Affiliation:	Kresge Hearing Research Institute/Department of Otolaryngology, University of Michigan Medical School

Active listening requires not only correctly identifying primary sound features, but also predicting their behavioral relevance. Behaviorally relevant representations are well documented in the auditory cortex, but whether similar activity arises earlier in the central hierarchy is hotly debated. The dorsal and external nuclei of the inferior colliculus (shell IC) are important midbrain circuits that receive a variety of acoustic, multi-sensory and neuromodulatory signals (Gruters & Groh 2012). We tested the hypothesis that behavior and/or outcome signals are present in the shell IC during an auditory task using Ca2+-imaging, machine learning, and a reward-based discrimination task in mice.

We expressed the Ca2+-indicator GCaMP6f in shell IC neurons of 7 CBA/C57 Bl-6J mice. Animals were subsequently trained to discriminate the presence or absence of amplitude modulation in a bandpass noise stimulus using a GO/NOGO paradigm. Following training, we used 2-photon microscopy to record neural activity from the same neurons across 7 consecutive sessions as mice performed the task; modulation depth was varied to obtain psychometric functions. We analyzed the population activity at various epochs during the trial, and used a support vector machine (SVM) classifier to predict the trial outcome (mice's behavioral responses) from neural population activity.

Mice's modulation detection thresholds did not significantly change over the course of 7 days, indicating stable performance in our conditions. Likewise, the average neural trajectories and principal components did not change strongly over time, suggesting that population-level representations of task-related variables are largely stable in the shell IC. We used the fluorescence data from multiple shell IC neurons as training data for an SVM classifier to predict the outcome of each trial (hit, miss, false alarm, correct rejection). As expected, classification accuracy was highest after sound offset (~ 85%, chance: 40%). However, significant classification was achieved even if activity was integrated over the first (60%) or second (70%) half of the sound stimulus only. Remarkably, a similar accuracy was achieved when the SVM was exclusively trained on neural activity occurring prior to mice's behavioral response (70%). We can further conclude from the confusion matrices that this result is unlikely to simply reflect motor- or motor preparatory activity, because hits and false alarms can be reliably distinguished. Collectively, our data argue that neural population activity in the auditory midbrain reflects a mixed selectivity of predictive- and feedback information about behavioral outcome in response to behaviorally relevant sound features.

Supported by NIH R01DC019090, The Whitehall Foundation, and the Hearing Health Foundation.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Inhibition in the auditory tectothalamic pathway is shaped by NPY neurons**
Day:	Thursday
Time:	2:26pm
Authors:	Silveira, Marina A. *, Herrera, Yoani N., Drotos, Audrey C., Versalle, Trevor S., Roberts, Michael T.
Affiliation:	Kresge Hearing Research Institute, Dept. of Otolaryngology – Head and Neck Surgery, University of Michigan

Inhibitory synapses are essential to shaping auditory computations, impacting most aspects of auditory processing. The inferior colliculus (IC) contains many GABAergic neurons that project locally and/or to the auditory thalamus (MG). However, despite the important role of inhibition in the IC-MG pathway, the cellular organization of local and long-range inhibitory neural circuits remains largely unknown. The ability to selectively manipulate distinct classes of IC GABAergic neurons is key to understanding how inhibition shapes sound processing in the IC and MG. We recently identified Neuropeptide Y (NPY) expression as a marker for the first molecularly identifiable class of inhibitory neurons in the IC. NPY neurons are labeled in the NPY-hrGFP reporter mouse and send long-range inhibitory projections to the MG. Because most techniques for manipulating distinct neuron types target neurons that express Cre or FlpO transgenes, here we validate an NPY-FlpO mouse line by crossing NPY-IRES2-FlpO mice with Ai65F reporter mice, allowing us to visualize NPY neurons by the expression of tdTomato fluorescence. To validate the NPY-FlpO mouse we performed in situ hybridization using RNA scope. We found that 75.6% of tdTomato+ neurons express NPY and 92.9% express vGat. In agreement, using GAD67 immunolabeling, we found that 92.3% of tdTomato+ neurons co-label with GAD67. Additionally, tdTomato-expressing neurons exhibited sustained firing patterns and stellate morphology, consistent with the properties of NPY neurons labeled in the NPY-hrGFP mouse. Therefore, our data suggest that the NPY-FlpO x Ai65F mouse line labels the same population of NPY neurons previously identified in the NPY-hrGFP mouse. Next, we used a FlpO-dependent adeno-associated virus to selectively express the excitatory opsin Chronos in NPY neurons to investigate how the local and long-range projections of NPY neurons shape inhibition in the IC-MG pathway. To test if activation of NPY terminals using Chronos would evoke inhibitory postsynaptic potentials (IPSPs) in postsynaptic targets, we recorded from IC and MG neurons in acute slices, using flashes of blue light to activate Chronos-expressing terminals. Our data show that optogenetic activation of NPY terminals elicited IPSPs in postsynaptic neurons in the ipsilateral IC and MG, providing the first functional evidence of how a distinct class of GABAergic IC neuron provides local and long-range inhibition in the auditory tectothalamic pathway.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Immediate neural network impact after the loss of a semantic hub**
Day:	Thursday
Time:	3:40pm

Authors: Zsuzsanna Kocsis1,2*, Rick L. Jenison3, Thomas E. Cope4,5, Peter N. Taylor6,7, Ryan M. Calmus2, Bob McMurray8, Ariane E. Rhone1, McCall E. Sarrett9, Yukiko Kikuchi2, Phillip E. Gander1,10,11, Joel I. Berger1, Christopher K. Kovach1, Inyong Choi12

Affiliation: 1. Department of Neurosurgery, University of Iowa, Iowa City, IA, USA
2. Biosciences Institute, Newcastle University Medical School, Newcastle upon Tyne, UK
3.Departments of Neuroscience and Psychology, University of Wisconsin, Madison, WI, USA

The human brain extracts meaning from the world using an extensive neural system for semantic knowledge. Whether such broadly distributed systems crucially depend on or can compensate for the loss of one of their highly interconnected hubs is controversial. The strongest level of causal evidence for the role of a brain hub is to evaluate its acute network-level impact following disconnection and any rapid functional compensation that ensues. We report rare neurophysiological data from two patients who underwent awake intracranial recordings during a speech prediction task immediately before and after neurosurgical treatment that required disconnection of the left anterior temporal lobe (ATL), a crucial hub for semantic knowledge. Informed by a predictive coding framework, we tested three sets of hypotheses including diaschisis causing disruption in interconnected sites and incomplete or complete compensation by other language-critical and speech processing sites. Immediately after ATL disconnection, we observed highly specific neurophysiological alterations in the recorded fronto-temporal network, including abnormally magnified high gamma responses to the speech sounds in auditory cortex. We also observed evidence for rapid compensation, seen as focal increases in effective connectivity involving language-critical sites in the inferior frontal gyrus and speech processing sites in auditory cortex. However, compensation was incomplete, in part because after ATL disconnection speech prediction signals were depleted in auditory cortex. This study provides direct causal evidence for a semantic hub in the human brain and shows striking neural impact and a rapid attempt at compensation in a neural network after the loss of one of its hubs.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Investigating the neural correlates of harmonicity in auditory cortex in humans**

Day: Thursday

Time: 3:52pm

Authors: Anahita H. Mehta*, Emily J. Allen, Juraj Mesik, Kendrick N. Kay, Andrew J. Oxenham

Affiliation: University of Minnesota

Pitch and harmonicity are central perceptual properties of real-world sounds, including speech and music, and are crucial for the perceptual organization of such sounds in an auditory scene. However, there are still significant gaps in our basic understanding of how pitch is represented in auditory cortex. Previous work has suggested that regions near the anterolateral end of Heschl's gyrus are sensitive to pitch, as they show greater activation to resolved harmonic complex tones compared to unresolved harmonic complex tones and frequency matched noise. However, it is not clear whether this differential activation truly reflects the stronger pitch salience elicited by harmonically related tones, or whether these cortical regions are sensitive to other spectro-temporal differences between pitch-evoking complex tones and frequency-matched broadband noise, such as spectral density. In this study, we compare 3T fMRI responses for harmonic complex tones (resolved and unresolved) to inharmonic complex tones, which were matched to the harmonic tones in terms of spectral density and frequency range but do not elicit a clear unambiguous pitch. Additionally, we measure responses to frequency-matched broadband noise which has traditionally been used as a reference condition for localizing pitch sensitive regions. Lastly, we examine the effect of phase manipulation in the unresolved harmonics in order to investigate the responses driven by envelope fluctuations. We observed robust, bilateral, stimulus-driven activity in Heschl's gyrus and surrounding auditory regions for all stimuli. For complex tones with resolved components, preliminary univariate analyses indicate no systematic difference in activation within previously reported pitch sensitive regions for stimuli, with and without salient pitch percepts, when controlling for spectral density and bandwidth. For stimuli with only unresolved harmonics, we see strong activation based on amplitude modulation strength that is not restricted to the putative pitch sensitive regions. Our findings indicate that the regions near the anterolateral end of Heschl's gyrus may not be sensitive to F0 pitch per se, but might be sensitive to specific spectro-temporal properties that covary with sounds traditionally used as pitch stimuli. Future analyses will investigate if there are any differences in brain activation between harmonic and inharmonic tones that are not seen in univariate analyses.

Acknowledgments: NIH grant K99DC017472 (Mehta) and NIH grant R01DC005216 (Oxenham)

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Multi-unit neuronal responses to sound pitch recorded directly from human auditory cortex**
Day:	Thursday
Time:	4:25pm

Authors: Joel I Berger 1 *, Phillip E Gander 2, Yukiko Kikuchi 3, Sukhbinder Kumar 1, Christopher Kovach 1, Hiroyuki Oya 1, Christopher I Petkov 1,3, Hiroto Kawasaki 1, Matthew A Howard 1, Timothy D Griffiths 3

Affiliation: 1. Department of Neurosurgery, University of Iowa
2. Department of Radiology, University of Iowa. Biosciences Institute, Newcastle University, Newcastle upon Tyne, NE2 4HH UK

The perception of pitch requires the abstraction of stimulus properties related to the spectrotemporal structure of sound. Previous studies utilizing both animal electrophysiology and human imaging have indicated the presence of a center for pitch representation in the auditory cortex. Recent data from our own group - examining local field potentials (LFPs) in humans - indicate more widely distributed pitch-associated responses within the auditory cortex (Gander et al., 2019). To probe neuronal coding properties with cellular precision, we examined multi-unit neuronal spiking activity related to three different auditory stimuli in seven epilepsy patients who were implanted with clinical monitoring electrodes that contained high-impedance electrodes in auditory cortex necessary for recording neuronal activity. The stimuli were regular-interval noise (RIN) with a pitch strength that is related to the temporal regularity, and pitch value determined by repetition rate, and harmonic complexes with missing fundamentals. We demonstrated increases in spiking activity in 69 of 104 (66%) responsive multi-unit activity in auditory cortex due to pitch-associated stimuli. Importantly, these responses were distributed across the entire extent of Heschl's gyrus (HG), in both primary iand non-primary areas, rather than isolated to a specific region, and this finding generalized across the different pitch inducing stimulus conditions. These findings are the first multi-unit pitch responses recorded from humans, and they align with a recent study in a primate model (Kikuchi et al., 2019) demonstrating that both local field potential and unit responses to pitch-inducing stimuli are distributed throughout auditory cortex.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **A Language Independent Monosyllabic Word-Recognition Test**

Day: Thursday

Time: 4:37pm

Authors: *Kristina Mardlin, Rachael Kirby, Anthony T. Cacace, Robert H. Margolis

Affiliation: Wayne State University, Audiology Incorporated

Auditory processing of speech stimuli is primarily assessed via monosyllabic word stimuli in the language of the listener, often termed "word recognition testing." With this approach, difficulty often arises when linguistically diverse areas like Detroit and other big cities cannot accommodate test material needs for speech perception assessment. Besides English, languages spoken in the Detroit metropolitan area include Spanish, Arabic, German, Chinese, French, and others; worldwide, the number of languages and dialects are much more numerous. This complexity and diversity of languages poses distinct problems for audiologic speech assessment.
The linguistic content of word stimuli can be removed, while retaining their temporo-spectral characteristics by playing the speech stimuli "backwards." This process makes the monosyllabic speech stimuli "independent" of language. By adding varying amounts of distortion to the stimuli a discrimination task is created that requires the listener to distinguish between distorted and undistorted backward speech stimuli. A non-linguistic test of speech processing based on this approach was described by Margolis et al. (IJA, 2022).

Methods: Three experimental groups were studied: normal hearing young adults (21 females, 4 males, 22-47 years; normal hearing older adults, 8 females, 5 males, 54-64 years; and hearing-impaired adults (5 females, 2 males, 20-73 years). A 4-interval forced choice odd-ball paradigm was used to assess performance. Three compressive non-linear distortion conditions were used to ensure a sufficient level-of-difficulty for perception of the backwards stimuli.
Separate 2-way (2x3) repeated measures analyses of variance (ANOVA) were used to evaluate the effects of ear-of-stimulation and condition for each of the experimental groups. Comparisons were also made with standard monosyllabic word recognition in quiet and with the Words-in-Noise (WIN) test.

Results: Young normal hearing subjects failed to show significant main effects of ear ($F = 0.018$, $p>0.893$) or condition ($F = 0.418$, $p>0.659$) on test performance. There were no ear x condition interactions ($F = 0.005$, $p>0.995$).
However, older normal hearing subjects showed a significant main effect of condition ($F = 10.76$, $p <0.000$) but not ear ($F = 0.21$, $p>0.651$). The ear x condition interactions were not significant ($F = 0.19$, $p>0.823$). Monosyllables in quiet showed 100% performance for each of the normal hearing groups bilaterally.
Moreover, hearing-impaired subjects also showed a significant main effect of condition ($F = 3.49$, $p <0.043$) but not ear ($F = 0.008$, $p>0.930$). The ear x condition interactions were not significant ($F = 0.352$, $p>0.706$). Monosyllabic word performance in quiet was also depressed (left ear, 65.3%; right ear 75.4%).

Discussion: Initial assessment indicates that backwards presented speech appears to be a viable approach to assess speech perception in linguistically diverse areas.

Conclusion: Further work is needed to explore different linguistically diverse and pathologic populations, particularly in those conditions related to synaptopathy and neuropathy, where this methodology might be particularly valuable.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Location-Specific Facilitation in Marmoset Auditory Cortex**

Day: Friday

Time: 9:30am

Authors: Chenggang Chen*, Xiaoqin Wang

Affiliation: Johns Hopkins Univeristy

It has been well established that responses of neurons in auditory cortex are influenced by stimulus context. Contextual modulations can occur in spectral, temporal or spatial domain. However, comparing to spectral and temporal contextual effects, much less is known on spatial contextual effects in auditory cortex. In this study, we explored how spatial contextual modulations evolve over time by stimulating neurons in awake marmoset auditory cortex with sequences of sounds either randomly from various spatial locations or repeatedly from a single location.

To our surprise, instead of inducing adaptation as expected from well documented stimulus-specific adaptation (SSA) literature, repetitive stimulation from spatial locations away from the center of a neuron's spatial receptive field evoked lasting facilitation observed by both extracellular and intracellular recordings. Nearly half of the sampled neuronal population exhibited location-specific facilitation (LSF), irrespective of stimuli type and visibility of the test speaker. The extent of the LSF decreased with decreasing presentation probability of the test speaker. Intracellular recordings showed that repetitive sound stimulation evoked sustained membrane potential depolarization that was followed by firing rate facilitation. Computational models captured these findings and revealed two distinct neural mechanisms underlying LSF. Simulations of population responses to repetitive sound stimuli revealed two different groups of neurons: one exhibited LSF, and the other one exhibited SSA.

Taken together, our findings revealed LSF to repetitively presented sound stimuli in auditory cortex that has not been observed. This form of spatial contextual modulation may play a role in such functions as detecting the regularity, segregating the sound stream, and solving the cocktail party problem.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Tinnitus-related increases the activity of auditory cortical neurons: In vivo and in vitro studies**
Day:	Friday
Time:	9:42am
Authors:	Madan Ghimire*, Rui Cai, Lynne Ling, Kevin Brownell, and Donald Caspary
Affiliation:	Southern Illinois University, School of Medicine, Springfield, IL, 62702

Ringing of ears, commonly known as tinnitus, affects approximately 10-20 % of the total world population. Tinnitus is often associated with noise-exposure induced damage to the inner ear, resulting in partial loss of auditory input. This is found to instigate diverse permanent maladaptive plastic changes in neurons of central auditory neuraxis. The present study utilized LE rats, in a well-established noise-exposure induced behavioral tinnitus model. Tinnitus-related changes in the primary auditory cortex (A1) were examined in a series of in vivo and in vitro electrophysiological studies. Results: In vivo extracellular recordings in awake animals revealed a significant tinnitus-related increases in mean spikes/burst in spontaneous activity. Broadband noise (BBN, 200 ms long) rate-level functions (0-80 dB in 10 dB steps) evoked significantly greater firing rates (spikes/sec) in a subpopulation of A1 single-units from animals with behavioral evidence of tinnitus. In vitro whole-cell Patch-clamp recordings from layer 5 pyramidal neurons (PNs) from A1 slices showed tinnitus-related increases in spontaneous excitatory postsynaptic currents (sEPSCs) and decreases in spontaneous inhibitory postsynaptic currents (sIPSCs). We found that both these measures were directly correlated with the rat's behavioral tinnitus score. VIP neurons, part of an A1 local-circuit that can disinhibit layer 5 PNs, showed significant tinnitus-related increases in excitability that directly correlated with tinnitus severity. Collectively, the present A1 PN and VIP changes in a chronic tinnitus model are similar to findings from a somatosensory cortical chronic pain study model, suggesting common mechanisms for the generation of pathological fictive percepts.

FULL ABSTRACT
PODIUM PRESENTATION

MIDWEST AUDITORY RESEARCH CONFERENCE
presented by the Kresge Hearing Research Institute

Title:	**Enhanced stability of complex sound representations in the auditory cortex**
Day:	Friday
Time:	10:10am
Authors:	Harini Suri*(1) and Gideon Rothschild (1,2)
Affiliation:	1. Department of Psychology, University of Michigan 2. Kresge Hearing Research Institute and Department of Otolaryngology - Head and Neck Surgery, University of Michigan

Typical everyday sounds, such as those of speech or running water, are spectrotemporally complex. The ability to recognize complex sounds (CxS) and their associated meaning is presumed to rely on their stable neural representations across time. The auditory cortex is critical for processing of CxS, yet little is known of the degree of stability of auditory cortical representations of CxS across days. Previous studies have shown that the auditory cortex represents CxS identity with a substantial degree of invariance to basic sound attributes such as frequency. We therefore hypothesized that auditory cortical representations of CxS are more stable across days than those of sounds that lack spectrotemporal structure such as pure tones (PTs). To test this hypothesis, we recorded responses of identified L2/3 auditory cortical excitatory neurons to both PTs and CxS across days using two-photon calcium imaging in awake mice. Auditory cortical neurons showed significant daily changes of responses to both types of sounds, yet responses to CxS exhibited significantly lower rates of daily change than those of PTs. Furthermore, daily changes in response profiles to PTs tended to be more stimulus-specific, reflecting changes in sound selectivity, as compared to changes of CxS responses. Lastly, the enhanced stability of responses to CxS was evident across longer time intervals as well. Together, these results suggest that spectrotemporally CxS are more stably represented in the auditory cortex across time than PTs. These findings support a role of the auditory cortex in representing CxS identity across time.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Cell-type-specific roles of inhibitory interneurons in the rehabilitation of auditory cortex after peripheral damage**
Day:	Friday
Time:	10:22am
Authors:	Manoj Kumar*, Gregory Handy, Stylianos Kouvaros, Lovisa Ljungqvist Brinson, Brandon Bizup, Brent Doiron, and Thanos Tzounopoulos
Affiliation:	Pittsburgh Hearing Research Center, University of Pittsburgh

In all sensory systems, peripheral sensory organ damage leads to compensatory cortical plasticity that supports a remarkable recovery of perceptual capabilities. A major gap in knowledge is the lack of a precise mechanism that explains how this plasticity is implemented and distributed over the diverse excitatory and inhibitory cortical neurons. Here, we explored this mechanism in layer 2/3 of the primary mouse auditory cortex (A1). After peripheral damage, we found robust recovery in the sound-evoked activity of excitatory principal neurons (PNs) and parvalbumin (PVs) interneurons (INs), suggesting that PVs contribute to the stability of the A1 PNs. We found reduced activity in somatostatin-INs (SOMs), suggesting that SOMs allow for increased PN and PV activity. Finally, we observed robust recovery in vasoactive intestinal peptide-INs (VIPs), suggesting that VIPs may enable the recovery of PNs and PVs by inhibiting SOMs. These results highlight a strategic, cooperative, cell type-specific plasticity program that restores cortical sound processing and that will advance the field to a new level of understanding regarding cortical plasticity after peripheral organ damage.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Centrin-2 is a candidate light chain for Myosin-15 and a new member of the Elongation Complex in hair cells**
Day:	Friday
Time:	4:20pm
Authors:	Elli Hartig*, James Heidings, Zane Moreland, Jonathan Bird and Basile Tarchini
Affiliation:	Tufts University School of Medicine, The Jackson Laboratory, Gainesville Florida

Many deafness-associated proteins participate in the morphogenesis of the hair cell sensory organelle, a bundle of membrane protrusions, or stereocilia, organized in rows of graded heights. A group of five proteins, EPS8, WHRN, GPSM2, GNAI and their motor myosin-15 (MYO15), is referred to as the Elongation Complex (EC). The EC localizes to stereocilia tips where it promotes row 1 elongation and a graded height architecture across subsequent rows. Mouse mutants lacking EC proteins show defective stereocilia bundle morphogenesis, resulting in congenital deafness. Here, we identify a new EC member, centrin-2 (CETN2), a small calcium-binding protein with homology to myosin light chains. We propose that CETN2 acts as a binding partner and regulator of the critical hair cell myosin motor and EC member MYO15.

We used an Egfp-Cetn2 transgenic mouse line paired with immunostaining and confocal microscopy to track CETN2 and compare its localization to other EC members in wild-type and in Myo15 shaker-2 and Whrn whirler mutant mice. We used a trafficking assay in COS-7 cell filopodia and in cultured cochlear explants to assess the MYO15-CETN2 interaction. We engineered mice with deletions in Cetn2 and its paralog Cetn3 to assess hair cell morphology and Auditory Brainstem Response (ABR).

We first discovered that CETN2, although best known as a centriolar component, was also unexpectedly enriched at stereocilia tips. CETN2 precisely colocalized with MYO15 and EPS8, and was present at stereocilia tips in all rows at embryonic stages, gradually restricting to row 1 tips in postnatal stages. This profile is distinct from other EC proteins (WHRN, GPSM2, GNAI) which are limited to row 1. We found that MYO15 motor activity was required for CETN2 localization at stereocilia tips: Egfp-Cetn2 signal was absent at tips in Myo15 shaker-2 hair cells, and diminished but not eliminated in Whrn whirler hair cells, which have reduced MYO15 dosage. We established that CETN2 is a direct binding partner to MYO15 and that this interaction is dependent on the IQ3 domain of MYO15. We found that genetic ablation of Cetn2, but not Cetn3, resulted in elevated ABR thresholds. However, even double inactivation of Cetn2 and Cetn3 failed to phenocopy the stunted stereocilia observed in other EC mutants, preserving grossly normal stereocilia bundle morphogenesis.

In conclusion, we identify CETN2 as a first endogenous light chain associated with MYO15 in vivo. We postulate that Cetn2; Cetn3 knock-out mice escape severe stereocilia dysmorphology because other proteins may act redundantly with centrins as MYO15 IQ3-binding light chains. Ongoing work includes a detailed characterization of MYO15 behavior and stereocilia structure in centrin mutants, as well as the generation of a mouse model carrying a mutation in MYO15 IQ3 domain, a R1956W substitution reported in human patients.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**GRXCR2 function in hearing and deafness**
Day:	Friday
Time:	4:32pm
Authors:	Chang Liu, *Bo Zhao
Affiliation:	Indiana University School of Medicine

Recessive mutations in GRXCR2 cause deafness in both humans and mice (Imtiaz et al., 2014; Avenarius et al., 2018). In Grxcr2 null hair cells, the sensory receptors for sound in the inner ear, stereocilia are disorganized (Avenarius et al., 2018). Reducing the expression of taperin, a protein that interacts with GRXCR2 at the base of stereocilia, corrects the morphological defects of stereocilia and restores hearing in Grxcr2 null mice. To further validate this finding, we recently generated two novel taperin mutant mouse lines that exhibit progressive hearing loss. Then Grxcr2 null mice were crossed with one of these taperin mutant mice. The following morphological analysis revealed that reducing taperin expression indeed corrected stereocilia morphological abnormalities in Grxcr2 null mice. Functional analysis further confirmed that reducing taperin expression partially restored hearing in Grxcr2 null mice.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Mechanisms of Auditory Sensation in C. elegans**
Day:	Friday
Time:	4:44pm
Authors:	Can Wang*, Elizabeth A. Ronan*, Yuling Guo, Adam Iliff, Jianfeng Liu, X.Z. Shawn Xu
Affiliation:	Life Sciences Institute

Hearing is a fundamental sensory modality that has evolved independently several times in the Chordata and Arthropoda phyla, mainly driven by acoustic communication in species and acoustic detection of predators/prey. The sense of hearing allows animals to detect and transduce airborne vibrations into electrical signals. We recently reported that the earless roundworm C. elegans is able to sense and respond to airborne sound in the range of 100 Hz to 4 kHz and engages in aversive phonotaxis behavior. Specifically, we report that airborne sound physically vibrates the exterior surface of the worm, activating the specialized multidendritic mechanosensory neurons FLP and PVD which tile the head and body wall. Genetic screens uncover that the nicotinic acetylcholine receptor DEG-3/DES-2 is required for these neurons to sense sound. How is sound vibration transduced from external skin to neurons in order to trigger phonotaxis in C. elegans? Here, we report that worms sense sound from point sources. Specifically, localized sound waves that selectively vibrate one end of the worm generate a differential sound pressure gradient across the fluid-filled body cavity to activate auditory neurons and stimulate the phonotaxis response. Our findings are consistent with C. elegans neuroethology, as detection of localized sound emitted by small predatory insects may be important for survival. Importantly, C. elegans auditory sensation exhibits striking parallels to cochlear auditory sensation in vertebrates. Within the vertebrate inner ear, localized external sound pressure applies compression at one end of the fluid-filled cochlea via the oval window, with pressure relief occurring on the other end via the round window. This triggers a pressure gradient within the fluid-filled cochlea to activate hair cells. Broadly, our work reveals for the first time that C. elegans senses auditory stimuli, revealing the unexpected presence of this sensory modality in the lower phyla.

FULL ABSTRACT
PODIUM PRESENTATION

MIDWEST AUDITORY RESEARCH CONFERENCE
presented by the Kresge Hearing Research Institute

Title:	**Cochlear Neurotrophin-3 overexpression at mid-life prevents age-related cochlear synaptopathy and slows age-related hearing loss.**
Day:	Friday
Time:	4:56pm

Authors: Luis R. Cassinotti * ; Lingchao Ji; Beatriz C. Borges; Nathan D. Cass; Aditi Desai; David Kohrman; M. Charles Liberman and Gabriel Corfas

Affiliation: Kresge Hearing Research Institute and Department of Otolaryngology-Head and Neck Surgery, University of Michigan

Age-related hearing loss (ARHL), also known as presbycusis, is the most prevalent sensory deficit in the elderly, affecting a third of people over age 65 and half of those over 85 in the US. This progressive pathology, characterized by elevated hearing thresholds, is often associated with psychological and medical comorbidities, including social isolation, frailty, depression, and cognitive decline. Despite ARHL's enormous societal impact, no therapies to prevent or slow this process exist.

While the mechanisms of ARHL remain unclear, studies in mice and humans demonstrated that loss of synapses between inner hair cells (IHCs) and spiral ganglion neurons (SGNs), a.k.a cochlear synaptopathy, is an early event in ARHL, preceding hair cell (HC) loss. Furthermore, noise-induced cochlear synaptopathy in young mice accelerates SGN loss and threshold elevation. Thus, synaptopathy may be a trigger for progressive degeneration. and preventing or delaying it could be a powerful approach to treat ARHL.

Our previous studies showed the importance of neurotrophin-3 (Ntf3) in the formation of IHC-SGN synapses and their regeneration after noise damage. Ntf3 is expressed by IHCs and their supporting cells in the neonatal and adult organ of Corti. Using cell-specific inducible gene recombination, we also demonstrated that the neonatal Ntf3 level in IHC supporting cells regulates IHC-SGN synapse density, which is reflected in the amplitude of sound-evoked auditory nerve responses. Increasing supporting-cell Ntf3 expression immediately after a synaptopathic exposure results in synapse regeneration and functional recovery in young adult mice. Similar partial recovery of IHC synapses and cochlear function after noise exposure in young mice can be achieved by direct application of Ntf3 protein to the mouse round window and via virally mediated Ntf3 overexpression delivered through the posterior semicircular canal.

Here, we examine the effects of Ntf3 expression on ARHL starting at mid-life (60 wks of age) using a tamoxifen-inducible conditional transgenic mouse line that drives Ntf3 overexpression in inner border and inner phalangeal cells (Ntf3Stop:Slc1a3/CreERT). Ntf3Stop littermates were used as controls. ABRs and DPOAEs were measured at different time points for an additional 35 wks. After the final test, we evaluated Ntf3 expression by RT-qPCR, as well as HC numbers and IHC synapse counts by immunostaining.
We show that induction of supporting cell Ntf3 overexpression in mice at 1 yr. rapidly increases the amplitude of sound evoked potentials, indicating that Ntf3 treatment can enhance cochlear function acutely in mice with mild synaptopathy. Furthermore, Ntf3 overexpression slows the progression of ARHL, resulting in improved cochlear function and reduced age-related cochlear synaptopathy in mice approaching the end of their natural life.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Dual vector mediated gene therapy for restoration of STRC-related hearing loss**
Day:	Friday
Time:	5:08pm

Authors: Quynh-Anh Fucci, Madeline Barnes, Sarah Cancelarich, Tyler Gibson, Nivanthika Wimalasena, Yoojin Chung, XuDong Wu, Martin Schwander, Danielle Velez, Tian Yang, Leah Sabin , Ning Pan, Meghan Drummond, Lars Becker*

Affiliation: Decibel Therapeutics

Stereocilin (STRC) is a large, extracellular, structural protein expressed in outer hair cells of the cochlea. Functional outer hair cells amplify low level sounds within the ear, a process required for normal hearing sensitivity and frequency selectivity. STRC localizes to the stereocilia bundles in outer hair cells and is thought to facilitate bundle cohesion and connection to the overlying tectorial membrane. In humans STRC deficiency leads to autosomal recessive deafness (DFNB16) with patients showing complete loss of sound amplification. DB-AAV-104 aims at restoring hearing in DFNB16-patients. The affected population has been estimated to be the second most prevalent genetic auditory deficiency in the US and EU5.

To recapitulate the human phenotype and explore the feasibility of AAV gene therapy for DFNB16 in an animal model, we generated mice lacking STRC using CRISPR/Cas9. The animal model shows absence of DPOAE responses that are indicative of outer hair cell function, in addition to highly elevated sound detection thresholds, mimicking the patient phenotype. At the same time, outer hair cells remain intact to adult ages in the model, suggesting a potential long intervention window for gene therapies. For targeting the transgene precisely to the cell type of interest, we identified Strc regulatory elements that are selective for outer hair cells in organ of Corti explants and in mice in vivo. We then subsequently confirmed the specificity of the outer hair cell promoter in non-human primate ears. The size of the Strc transgene exceeds the packaging capabilities of AAV. Therefore, we relied on dual vector technology to re-express full-length STRC in our mouse model. We show hearing restoration after delivery of dual vector AAV and re-expression of STRC in hair bundles of outer hair cells. Our results indicate that dual vector gene replacement therapy re-expressing full length STRC can lead to auditory threshold improvements by allowing the attachment of ther tectorial membrane to the outer hair cells.

FULL ABSTRACT
PODIUM PRESENTATION

MIDWEST AUDITORY RESEARCH CONFERENCE
presented by the Kresge Hearing Research Institute

Title:	**Tip-Link Breakage During the Mechanotransduction-Dependent Remodeling of the Stereocilia Cytoskeleton in Mammalian Auditory Hair Cells**
Day:	Saturday
Time:	9:20am
Authors:	Sara Gonzalez-Velez*, Abigail K. Dragich, Gregory I. Frolenkov, A. Catalina Velez-Ortega
Affiliation:	Department of Physiology, University of Kentucky, Lexington, KY, USA

Auditory hair cells in the inner ear detect sound waves by the deflection of stereocilia, the microvilli-like projections at their apical surface. These actin-filled sensory organelles are precisely organized in a staircase-like structure and shorter row stereocilia are linked to their taller neighbors via tip-links. The long-term stability of the stereocilia cytoskeleton in mammalian auditory hair cells depends on a constant influx of calcium through the mechano-electrical transduction (MET) channels located at their tips, which are opened by tension build-up in the tip-links. We previously showed that blockage of MET channels in mouse inner and outer hair cells for 24 hours causes significant retraction (~200-500nm) in transducing (second and third row) stereocilia, yet tip-links and MET channel activity are still present (Velez-Ortega, et al. Elife, 2017). However, it is unknown whether tip-links are sliding down together with stereocilia, or if they constantly break and re-form during stereocilia retraction. Therefore, we exposed mouse organ of Corti explants to pharmacological blockers of MET channels for various periods of time and evaluated stereocilia morphology and tip-link counts via scanning electron microscopy. Our results indicate that, shortly after MET channel blockage (2 to 5 hours), there is a significant decrease in tip-link counts in the cochlear inner hair cells. Furthermore, existing tip-links often appeared abnormally short at these initial timepoints but recovered their normal appearance after 24 hours of MET channel blockage, which suggests the possible formation of “temporary” tip-links to maintain MET current. Therefore, we also started to evaluate MET currents in these preparations using whole-cell patch-clamp recordings. Preliminary data show prominent MET responses in hair cells after 24-hour exposure to the MET channel blockers. In conclusion, our data suggests that tip-links cannot easily slide down along the stereocilia shaft and are likely to break and re-form during MET-dependent stereocilia remodeling.

Supported by NIDCD/NIH (R01DC019054 to G.I.F. and R21DC017247 to A.C.V.)

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**The Potassium Channel Subunit Kv1.8 (Kcna10) Differentially Shapes Gain, Tuning, and Timing of Type I and II Vestibular Hair Cells**
Day:	Saturday
Time:	9:32am
Authors:	Martin HR*[1], Price SD[2], Lysakowski A[2], Eatock RA[1]
Affiliation:	[1] University of Chicago; [2] University of Illinois at Chicago

Background: Vestibular hair cells (HCs) convert head motions into receptor potentials (RPs) that drive synaptic transmission, generating extremely rapid gaze- and posture-stabilizing reflexes and providing a sense of gravity and spatial orientation. In amniotes, type I and II HCs (HC-I, HC-II) express voltage-gated potassium (Kv) conductances (g) with specific biophysical properties that shape RP gain, tuning, and timing. Previously, the molecular identities of the major Kv subunits in HC-I and HC-II were unknown. We investigated the roles in HC-Is and HC-IIs of Kv1.8, an understudied channel encoded by the Kcna10 gene. Kv1.8-null animals lack vestibular-evoked potentials normally evoked by transient head motions (Lee et al., Hearing Res 300 (2013),1). Kv1.8 forms conductive ion channels when heterologously expressed (Dierich et al., Cell Reports 32 (2020),1).

Methods: We stimulated HCs with voltages, currents, or hair bundle deflections and recorded HC whole-cell currents and voltages in intact utricles from Kv1.8-null mice and wildtype and heterozygous littermates (postnatal days 5-370). Sectioned utricular epithelia were stained with anti-Kv1.8 (Alomone).

Results: Kv1.8 immunoreactivity localized to the basolateral membranes of HC-Is and, to a lesser extent, HC-IIs. Kv1.8-null HC-Is lacked the large, low-voltage-activated K+ conductance of mature control HC-I (g-K,L). Kv1.8-null HC-Is expressed a much smaller (by 95%) K+ conductance that activated over 40 mV positive to g-K,L. As a result, input resistance was 20-fold greater in Kv1.8-null HC-Is than wildtype HC-Is, such that sinusoidal hair bundle deflections evoked larger and slower RPs. The low-pass corner frequency, f-c, of RP re: transduction current (I-MET) fell from ~400 Hz (control) to ~25 Hz (Kv1.8-null), and phase lag at 20 Hz increased by ~20-30 degrees (3.7 ms).

Wildtype HC-IIs typically express a fast-inactivating A-type conductance (g-A). In KV1.8-null HC-IIs, g-A was reduced by ~92% and the steady-state KV conductance by ~60%. Consequently, in Kv1.8-null HC-IIs, voltage responses to current steps rose more slowly, did not rebound, and, in some cells, showed electrical resonance. Accordingly, f-c of RP re: I-MET fell from ~70 Hz (control) to ~17 Hz (Kv1.8-null), and phase lag at 20 Hz increased by ~30-35 degrees (4.6 ms).

In Kv1.8-null HC-Is and HC-IIs, the residual K+ conductance (g-Res) lacked rapid inactivation and activated positive to resting potential (V-half range –30 to –40 mV). Block by XE991 (IC50 ~10 µM, n=20) suggests that Kv7 subunits mediate g-Res in both HC-I and HC-II.
Conclusions: We propose that Kv1.8 is a pore-forming subunit of K+ conductances modified by cell type-specific factors to have distinct voltage dependence and inactivation, producing g-K,L in HC-Is and g-A in HC-IIs. These Kv1.8-dependent conductances strongly affect response gain, tuning, and timing. Both g-K,L and g-A increase RP f-c and reduce phase lag at stimulus frequencies >5 Hz. Moreover, Kv7 subunits mediate the residual Kv1.8-independent delayed rectifier conductances in both HC-I and HC-II, suggesting that cell type-specific modulation of Kv1.8 is critical to shaping HC identity.

Supported by R01 DC012347 (RAE & AL) and NSF GRF (HRM). We thank S. Jones and T. Friedman for sharing Kv1.8 mutant mice.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Taperin deficiency increases nonlinearity of stereocilia bundle motion and transduction current-displacement relationship in mammalian auditory hair cells**
Day:	Saturday
Time:	9:44am

Authors: K. Sofia Zuluaga-Osorio1*, Isabel Aristizábal-Ramírez1, Rizwan Yousaf2, Elizabeth Wilson2, Sayaka Inagaki2, Shadan Hadi1, Thomas B. Friedman2, Inna A. Belyantseva2, Gregory I. Frolenkov1

Affiliation: 1.Department of Physiology, University of Kentucky, Lexington, KY, 40536, USA
2. National Institute on Deafness and Other Communication Disorders, NIH, Bethesda, MD, 20892, USA

Taperin is a protein located at the base of hair cell stereocilia (Rehman et al., 2010). Pathogenic variants of human TPRN are associated with nonsyndromic deafness DFNB79 (Rehman et al., 2010). Taperin seems to participate in the macromolecular complex at stereocilia bases (Salles et al. 2014; Zhao et al., 2016; Liu et al., 2018) but its exact role in hair bundle mechanics and mechano-electrical transduction (MET) is yet unknown. Here, we explored MET currents and mechanical properties of the stereocilia bundles in the young postnatal auditory hair cells of homozygous mice carrying a genomic deletion of amino acid position from 259 to 749 of Tprn (Tprntm1(komp)Vclg). We observed prominent MET responses in both inner (IHCs) and outer (OHCs) hair cells of homozygous Tprntm1(komp)Vclg mice, albeit their amplitude was approximately twice smaller than in heterozygous and wild type controls. Interestingly, relatively slow (50 ms) ramp-like deflections of the hair bundles with fluid-jet produced MET responses with highly non-linear current-force relationships in homozygous Tprntm1(komp)Vclg hair cells. However, this abnormal non-linearity was not observed with faster (2 ms) step-like deflections of hair bundles. High-speed video recordings of stereocilia bundle movements revealed that, in the control IHCs and OHCs, ramp stimuli produced generally linear movements of hair bundles, albeit slightly asymmetrical in positive and negative directions due to contribution of tip links and MET apparatus. In contrast, homozygous Tprntm1(komp)Vclg hair cells exhibited additional nonlinearity of the hair bundle displacements during positive bundle deflections, indicating dynamic changes of the stereocilia bundle stiffness. Ultrastructural examination of stereocilia insertions with FIB-SEM serial sectioning revealed rootlet abnormalities in the stereocilia of auditory hair cells of homozygous Tprntm1(komp)Vclg mice. These abnormalities are likely to be responsible for dynamic changes of stereocilia stiffness during their deflection by ramp stimuli and highly non-linear MET current-displacement relationships in Tprntm1(komp)Vclg hair cells.

Supported by NIDCD/NIH R01DC014658 and R01DC019054 to G.I.F. and in part by intramural NIDCD DC000039 to T.B.F.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **The Effect of Repetitive Linear Acceleration on Gravity Receptor Function**

Day: Saturday

Time: 9:56am

Authors: Syed Naqvi*, Rod Braun, Avril Genene Holt

Affiliation: Wayne State University

The vestibular system is crucial for posture, gait, and the perception of head and body position in space. Damage to this system can manifest as dizziness, imbalance, and poor postural control. Linear acceleration has been reported to result in measurable vestibular short-latency evoked potentials (VsEPs). The precise central neurons that contribute to the production of VsEPs are not well delineated. Manganese acts as a calcium surrogate, accumulating in active neurons. The paramagnetic nature of manganese permits visualization of these active neurons. Therefore, we have combined VsEP and manganese-enhanced magnetic resonance imaging (MEMRI) to assess vestibular function and visualize activity in central neurons responding to varying magnitudes of jerk stimulation (nonuniform linear acceleration).

Methods: Following anesthesia, each male Sprague Dawley rat (n=22) was attached to a mechanical shaker via a ceramic nut that had previously been attached with dental cement and centered on bregma. Each animal was then subjected to a jerk stimulation (either 640 g/s, 3,300 g/s, 5,100g/s or 8,700 g/s). Manganese chloride was administered just prior to stimulation. The stimulation was divided into three blocks. Each block consisted of five trials with 10-minute intervals between each block. For each trial, 200 jerk pair served as stimuli. Responses were recorded (CED power 1401 data acquisition system and Spike2 software) and analyzed using custom MATLAB scripts. Animals were also subjected to MRI (baseline, 24-hour post-, and 2-week post- stimulation) to assess manganese uptake in vestibular nuclei (lateral, medial, superior, and spinal vestibular nuclei).

Results: For each jerk stimulus, the P1 response latency was ~ 1 ms after the stimulus onset. While each jerk stimulus intensity resulted in VsEPs, the 640 g/s stimulus resulted in the least robust signal. The greatest signals were observed after moderate and intense stimulation. At each intensity, the P1 amplitude across the three blocks increased. The VsEP latency at P1 shortened then remained constant after 1000 jerk pairs. The P2 amplitude across the blocks was variable, while the latency was similar to P1. All the vestibular nuclei had elevated manganese uptake following stimulation versus baseline. Manganese uptake was least in animals after 640 g/s and 8,700 g/s stimulation. Greater manganese uptake was observed in vestibular nuclei of animals subjected to jerks of 3,300 g/s and 5,100 g/s.

Discussion: Irregular fiber activity (P1) appears to increase over time in response to repetitive linear acceleration. However, neuronal activity in the vestibular nuclear complex (P2) continued to decrease following each block. Our results demonstrate graded increases in manganese uptake in vestibular nuclei corresponding to increases in linear acceleration, particularly in the spinal vestibular nucleus. Manganese and VsEP are promising tools which can be used to non-invasively map vestibular activity.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**GluA3 Subunits are Required for the Appropriate Assembly of AMPA Receptors at Cochlear Afferent Synapses and for Presynaptic Modiolar-Pillar Features**
Day:	Saturday
Time:	11:50am

Authors: Mark A. Rutherford1*, Atri Bhattacharyya1, Maolei Xiao1, Hou Ming Cai2, Indra Pal2, María E. Rubio2,3,4

Affiliation: 1. Dept. Otolaryngology, Washington University School of Medicine, St. Louis, MO, USA; 2. Dept. Neurobiology, University of Pittsburgh, Pittsburgh, PA, USA; 3. Dept. of Otolaryngology, University of Pittsburgh, PA, USA; 4. Center for the Neural Basis of C

Background: All information about the acoustic environment is carried from the inner ear to the CNS by the afferent fibers of the cochlear nerve, called spiral ganglion neurons (SGNs). Rapidly gating, AMPA-type ionotropic glutamate receptors (AMPARs) comprised of GluA2, GluA3, and GluA4 subunits mediate excitatory transmission at the mature ribbon synapses between inner hair cells (IHCs) and SGNs. AMPAR-channels are tetramers that are typically heteromeric, assembled as dimers of dimers, although homomeric tetramers are possible. An individual ribbon synapse contains hundreds of AMPARs, on average. AMPARs typically contain at least one GluA2 subunit, making the channel relatively impermeable to Ca2+, while those lacking GluA2 are called Ca2+-permeable (CP-AMPARs). AMPAR-mediated glutamate excitotoxicity is implicated in the pathogenesis of hearing loss caused by noise trauma and ischemia. However, the contribution of each type of GluA subunit to AMPAR assembly, trafficking, and synaptic structure and function in the cochlea is unknown. We used global Gria3KO mice to ask what happens to IHC-SGN synapses in the absence of subunit GluA3. Results: Although they will later lose their hearing, 5-week-old GluA3KO mice have ABR thresholds and wave-1 amplitude similar to WT. Synapse ultrastructure is mainly intact, but modiolar-pillar specializations characteristic of WT are disrupted in GluA3KO mice. Ribbon synapse numbers are normal, but the GluA3KO mice have more lone ribbons and have paired synapses lacking GluA2. AMPAR volume per synapse is reduced overall in GluA3KO mice. IHC-SGN synapses of GluA3KO mice have less GluA2 but more GluA4, suggesting more Ca2+-permeable AMPARs.

Conclusion: Cochlear synaptopathy and subsequent threshold elevations in GluA3KO mice are preceded by alterations in AMPAR subunit expression causing an increase in Ca2+-permeable AMPARs relative to WT. This synaptopathic dysregulation of AMPA receptors in GluA3 KO mice demonstrates that cochlear afferent synapses seem to be extremely sensitive to alterations in subunit molecular anatomy.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Gata3 Expression in Inner Hair Cells is Required for their Long-Term Survival and Pillar Cell Development**
Day:	Saturday
Time:	12:02pm
Authors:	Paige V. Blinkiewicz*, Jeremy S. Duncan
Affiliation:	Western Michigan University

Mammalian HDR syndrome is characterized by hyperparathyroidism, sensorineural deafness, and renal disease and is caused by Gata3 haploinsufficiency. We have previously shown that early deletion of Gata3 in the inner ear of mice results in abnormal development of inner ear neurosensory epithelia. However, Gata3 expression begins in the otocyst and spans from early embryogenesis through adulthood. To better understand the function of Gata3 at later postnatal time points we conditionally knocked out Gata3 shortly after specification of the inner hair cells utilizing a Fgf8-cre mouse line. In the early postnatal, pre-hearing period, loss of Gata3 results in slightly disorganized pillar cells and a disrupted tunnel of Corti. When examined in adult mice, loss of Gata3 results in almost complete loss of inner hair cells and also some outer hair cell loss. The combination of these hair cell and supporting cell phenotypes results in shifted hearing thresholds in the homozygous mutants in mice over five months of age. This is the first study that suggests Gata3 regulates the diffusible morphogen Fgf8, and demonstrates conditionally knocking out Gata3 in one cell type, the IHCs can result in a drastic phenotype in the neighboring cells, the IPCs and inhibit functionality of the organ of Corti as a whole.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Single-cell analysis reveals cochlear and vestibular developmental trajectories in organoid-derived sensory cells**
Day:	Saturday
Time:	12:14pm

Authors: Joerg Waldhaus 1, Linghua Jiang*1, Liqian Liu 2, Jie Liu 2, R. Keith Duncan 1

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Inner ear organoids are novel model systems for studying development and disease, but the degree to which they mimic the complexity of the normal inner ear remains uncertain. To date, organoid sensory cells appear to default toward a vestibular fate, but these judgements have been based on a relatively small number of morphological and physiological markers. In this study, we sought to examine the complexity of inner ear organoids using single-cell RNA sequencing. Murine Atoh1/nGFP embryonic stem cells were used to generate inner ear organoids following standard protocols. Whole spheroids were collected after 20 days of culture and dissociated for flow sorting and downstream analysis. Reporter-positive cells were divided into GFP-high and GFP-low samples and libraries were generated on the 10X Genomics platform. The analysis pipeline included Seurat for clustering, Velocity to examine developmental trajectories, and AUCell to calculate and compare auditory and vestibular enrichment scores. Spheroids produced Pax2- and Six1-positive otic vesicles by culture day 12 and cystic organoids with MyoVIIa-positive hair cells and Sox2-positive supporting cells by day 20. In total, 6,339 single cells were captured and analyzed after stringent quality control. Overall, 13 clusters were identified and known marker genes used to annotate the clusters, revealing three hair cell clusters based on shared MyoVIIa expression and two supporting cell clusters based on Gjb2 expression. Analysis of developmental markers suggested that hair cell clusters represented distinct maturation stages. Differential expression of Atoh1 and Tmc1 outlined young versus more mature hair cells in two of the clusters, while the third cluster was annotated as a transient population owing to the detection of both markers. Based on trajectory analysis using an RNA velocity algorithm, two independent trajectories were predicted giving rise to two distinct types of hair cells in vitro. Joint analysis of the in vitro generated hair cells with postnatal day (P) 1 utricle and P2 organ of Corti revealed that the two trajectories predicted for in vitro generated hair cells segregated with the asymmetric distribution of auditory and vestibular lineages. Auditory versus vestibular hair cell enrichment scores were calculated and projected onto the in vitro data confirming differentiation of both cell types in inner ear organoids. Single-cell analysis reveals a complex ensemble of GFP-positive, Atoh1-expressing cell types in day 20 organoid cultures, including several classes of sensory hair cells that span several development stages with both auditory and vestibular fates. Notably, our approach required dissociation of a pooled sample of spheroids and thus eradicated information regarding aggregate-specific and vesicle-specific differentiation. Further study is required to determine whether organ-specific hair cells are intermingled within an organoid or segregated between individual vesicles or spheroids and to identify the signaling events that specify these fates.

FULL ABSTRACT
PODIUM PRESENTATION

MIDWEST AUDITORY RESEARCH CONFERENCE
presented by the Kresge Hearing Research Institute

Title:	**Opposing Gradients of Retinoic Acid and Sonic Hedgehog Signaling Specify the Tonotopic Axis in the Murine Cochlea**
Day:	Saturday
Time:	12:26pm
Authors:	Saikat Chakraborty1*, Shuze Wang1,2, Yujuan Fu2, Mary P. Lee1, Jie Liu2, Joerg Waldhaus1
Affiliation:	1) Department of Otolaryngology–Head and Neck Surgery, Kresge Hearing Research Institute, University of Michigan, Ann Arbor, Michigan, USA. 2) Department of Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, Michigan, USA

In the mammalian auditory system, frequency discrimination depends on morphological and physiological properties of the organ of Corti that gradually change along the longitudinal (tonotopic) axis of the organ and therefore shape the tuning properties of individual hair cells. At the molecular level, those frequency-specific characteristics are mirrored in gene expression gradients, which require tonotopic patterning of the cochlea, but molecular mechanisms that specify tonotopic identity remain poorly understood. To infer molecular mechanisms that pattern the organ of Corti along the frequency axis, we reconstructed the embryonic cochlear duct in 3D-space from single-cell gene expression data and proposed two hypotheses regarding spatial patterning. Analyzing two developmental time points suggested that morphogens, rather than a timing-related mechanism, confer spatial identity in the cochlea. Subsequently, retinoic acid signaling pathway was identified as a morphogen with a tonotopic gradient in the cochlear floor. Utilizing cochlear explants, functionality of the retinoic acid signaling cascade was confirmed and an inverse relation with sonic hedgehog signaling was bioinformatically predicted. Cell culture experiments indicated that sonic hedgehog signaling is involved in shaping the retinoic acid gradient via transcriptional regulation of Cyp26b1, which is a retinoic acid degrading enzyme. In summary, the findings suggest that retinoic acid and sonic hedgehog form opposing morphogen gradients, whereby retinoic acid patterns the base and sonic hedgehog the apex of the murine cochlea.