ABSTRACTS OF THE THIRTY-SECOND ANNUAL
MIDWINTER RESEARCH MEETING

ASSOCIATION FOR RESEARCH IN OTOLARYNGOLOGY

February 14-19, 2009

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Baltimore, Maryland, USA

ABSTRACTS OF THE THIRTY-SECOND ANNUAL MIDWINTER RESEARCH MEETING OF THE

Association for Research in Otolaryngology

February 14 – 19, 2009
Baltimore, Maryland, USA

Peter A. Santi, PhD
Editor

Association for Research in Otolaryngology
19 Mantua Road, Mt. Royal, NJ 08061 USA

CONFERENCE OBJECTIVES

After attending the Scientific Meeting participants should be better able to:

1. Understand current concepts of the function of normal and diseased ears and other head and neck structures.
2. Understand current controversies in research methods and findings that bear on this understanding.
3. Understand what are considered to be the key research questions and promising areas of research in otolaryngology.

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This book was prepared from abstracts that were entered electronically by the authors. Authors submitted abstracts over the World Wide Web using Mira Digital Publishing's PaperCutterTM Online Abstract Management System. Any mistakes in spelling and grammar in the abstracts are the responsibility of the authors. The Program Committee performed the difficult task of reviewing and organizing the abstracts into sessions. The Program Committee Chair, Dr. John Middlebrooks and the President, Dr. P. Ashley Wackym constructed the final program. Mira electronically scheduled the abstracts and prepared Adobe Acrobat pdf files of the Program and Abstract Books. These abstracts and previous years' abstracts are available at: http://www.aro.org.

Citation of these abstracts in publications should be as follows: **Authors, year, title, Assoc. Res. Otolaryngol. Abs.: page number.**

For Example:
Liberman, M. Charles, 2008, Connecting Hair Cells with Brain Cells: Afferent Responses and Efferent Feedback in Hearing and Deafness, Massachusetts Ear and Infirmary. Abs.: **715**.

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President's Message 2009

Welcome back to Charm City! We return to the Baltimore Marriott Waterfront at Inner Harbor East for the 32nd Annual MidWinter Meeting of the Association for Research in Otolaryngology. As you know, this will continue as our odd-year meeting site through 2015. So, you can begin to establish your favorite restaurants, watering holes and entertainments. But be aware that this district of Baltimore has continued its remarkable renaissance and so will look quite different even from our last visit.

This is a good thing, with even more shops and restaurants nearby, as well as a first-run cinema and Whole Foods Market (…barbecued 'soy riblets'?). It's still an easy walk to the core of the Inner Harbor, Little Italy and Fells Point, and a short cab ride to literally hundreds of restaurants throughout Baltimore, from Charles Village to Canton. The National Aquarium is close by and the Visionary Arts Museum and Science Center are around the Inner Harbor. The Walters Art gallery is downtown. A personal favorite is the extraordinary collection of Matisse paintings (part of the Cone sisters' collection, itself a unique treasure) at the Baltimore Museum of Art. This is next to the Johns Hopkins Homewood campus (about 3 miles from the hotel). And of course there are nearby music and dance clubs ranging from reggae to rock; not to mention the ARO's own 'Hair Ball' Wednesday evening in the Marriott ballroom.

The meeting this year is graced with another stellar collection of **Symposia**. These include: 1. Importance of Temporal vs Spectral Fine Structure for Pitch; 2. Mechanisms of Deafness Caused by Genetic Mutations: What Did We Learn From the Mouse Models? ; 3. Vestibular Compensation: New Clinical and Basic Science Perspectives; 4. From Psychophysics to Speech and from Physiology to Engineering: Jack Cullen's Contributions to Hearing Science; 5. New Scientific Developments in Auditory Processing Disorder; 6. Molecular Basis of Prosensory Specification in the Mammalian Cochlea; 7. Novelty Detection in the Auditory System: Correlating Animal and Human Studies. The Presidential symposium on Sunday is titled "Comparative Studies of the Ear - of (More Than) Mice and Men" and will provide views of inner ear function from moths to monkeys. The award of Merit winner, Dr. M. Charles Liberman will present his **Presidential Lecture** on Tuesday evening entitled "Connecting Hair Cells with Brain Cells: Afferent Responses and Efferent Feedback in Hearing and Deafness". Dr. David Ryugo will provide a synopsis of Charlie's career at the Awards Ceremony. Saturday's **Short Course** will cover Advanced Microscopy Techniques and **Workshops** will be presented by the NIDCD, the Patient Advocacy Committee, Media Relations Committee and the Animal Research Committee.

Remember to attend the **Business Meeting** Monday evening at 6. In addition to an update of the Association's affairs, new members of the nominating committee are chosen and other issues of concern are highlighted. Further, at this year's Business Meeting we will draw winners of the 'Exhibitor's Scavenger Hunt', prizes to include popular gizmos such as iPod, Wii-Fit, etc. So, please attend to play your part in Association business, and for the possibility of scoring cool toys.

The mid-winter meeting could not occur without the diligent and effective administration of Talley Management. Also, many members of the ARO dedicate hours of their time to program organization, symposium and workshop development, short courses and more. We are indebted to AAO-HNSF, DRF, AAAF and the Collegium Oto-Rhino Laryngologicum Amicitiae Sacrum -US Group, Inc, for their donations of travel funds for students and fellows. The collected efforts and generosity of all these deserve our recognition and thanks.

As I read through the program book I find the dilemma of choice more acute each year. There are just too many interesting titles to choose among. We continue to see remarkable growth in scientific diversity and depth. Our mid-winter meeting is a testament to the creativity and hard work that so many dedicate to understanding our related sciences. I hope you will enjoy this 32nd ARO as much as I will.

Paul A. Fuchs

M. Charles Liberman

2009 Award of Merit Recipient

M. Charles Liberman

2009 Recipient of the Award of Merit

The 2009 ARO Award of Merit will be given to Charlie Liberman for his many exceptional contributions to the field of auditory neuroscience. His research has spanned many aspects of hearing and deafness, including the effects of acoustic overstimulation on the inner ear, the subtypes of auditory nerve fibers and the correlation of their structure and function, and the role of the efferent innervation to the inner ear. Time and again, he has made significant and considerable advances in our knowledge. A hallmark of Charlie's work is his insightful and careful attention to detail and how these details evolve into significant and bedrock observations. Equally important is his remarkable ability to incorporate new concepts and techniques into his assault on old and new questions. He has been a leader in our field for much of his career.

Charlie comes from a family of scholars; his father, Alvin M. Liberman was Professor of Psychology at the University of Connecticut, Professor of Linguistics at Yale University, and President of Haskins Laboratories from 1975 - 1986. His mother, Isabelle Yoffe Liberman, was also a researcher at Haskins and a Professor at the University of Connecticut. His brother, Mark Liberman, is a Professor at the University of Pennsylvania in the Department of Linguistics and the Department of Computer and Information Sciences. And his sister, Sarah Ash, is an Associate Professor in the Department of Food Science at North Carolina State University.

Charlie's scientific career has taken place entirely in Boston: at Harvard and its medical school, at the Massachusetts Eye & Ear Infirmary, and within Harvard-MIT's program in Speech and Hearing Bioscience and Technology. Charlie's introduction to auditory physiology began when, as senior majoring in Biology at Harvard College, he took a readings class with Nelson Kiang at the Eaton-Peabody Laboratory of the Massachusetts Eye & Ear Infirmary. In the same lab as a graduate student, Charlie's Ph.D. work documented how acoustic overstimulation affected the inner ear and the responses of its nerve fibers (published as a supplement to Acta Otolarygologica in 1978). After narrow-band noise was used to damage hearing in a particular frequency region, he recorded responses of single auditory nerve fibers and documented their abnormal tuning curves. After characterizing the nerve's responses, he examined in detail the histopathology of the individual cochleas of each experiment. This made possible the most important aspect of these experiments: a correlation of the changes in the hair cells with the abnormal responses of the nerve. This structure/function relationship had never before been done with the precision of single-nerve fiber recordings. These studies answered questions like, "How is a mild loss of outer hair cells reflected in the tuning curve of an auditory nerve fiber?" Later studies by Charlie took this question to a finer level, examining how damage to the stereocilia on hair cells altered the responses of the nerve fibers. In addition to examining the stereocilia in the electron microscope, Charlie developed embedding and specimen-thinning techniques to enable their examination in the light microscope, a considerable technical feat. From the noise-exposure studies came the question of whether such damage could occur during a lifetime of "routine" exposure to sound. A study, now classic, used animals that had been reared in a low-noise chamber to prevent any significant exposure. Their nerve fiber responses showed exceptionally low thresholds, indicating that routine noise exposure in fact does take its toll on hearing. Charlie's investigational talents and the ability to pose such interesting research questions are his hallmarks.

Along with these studies of the damaged hearing organ, Charlie has made a host of contributions to normal anatomy and physiology of hearing. His work demonstrates the importance of the subgroups of nerve fibers as distinguished by their rates of spontaneous discharge, which correlates with other important properties such as threshold, point of contact with the inner hair cell, and central anatomy in the cochlear nucleus. Some of these studies originated in postdoctoral work with Sandy Palay at Harvard Medical School's Department of Anatomy, where serial-section electron microscopy was used to follow the peripheral terminals of auditory nerve fibers and demonstrate the types of synapses that they receive from the hair cell and from olivocochlear fibers. One of Charlie's most elegant contributions was to establish with precision the cochlear frequency mapping of auditory nerve fibers. For this, he brought the technique of single-unit labeling to the auditory system - after obtaining the nerve fiber's tuning curve and characteristic frequency, the fiber is injected with a neural tracer that could be followed to the point of contact with the inner hair cell along the cochlear spiral. Fibers of all spontaneous rates share a common "tonotopic" mapping, which is continued in the central auditory pathway as a fundamental organizing principle.

Charlie has greatly advanced our knowledge of the olivocochlear system, which sends messages from the brain out to the organ of Corti. His work shows the large differences in responses and innervation patterns for olivocochlear neurons compared to auditory nerve fibers. For example, the olivocochlear neurons are "jazzed up" by previous sound exposures. Importantly, they protect the ear from acoustic overstimulation and lessen the effects of noise masking. His current work is beginning to untangle the possible roles and actions of the lesser-known subgroup, the lateral olivocochlear neurons. In the most recent decade, Charlie has pioneered the use of genetically engineered models in the study of hearing. He and colleagues tested the mouse lacking the gene for the alpha 9 cholinergic receptor, the receptor that normally

mediates the effects of olivocochlear neurons on outer hair cells. This “knockout” mouse lacks the usual effects of olivocochlear stimulation and is thus functionally de-efferented. He and colleagues showed that outer hair cells from the Prestin “knockout” mouse lack electromotility, and that without this molecular motor there is a hearing loss of 40-60 dB. Recent tests of the alpha 9 cholinergic receptor “knockin” show that it has exceptional olivocochlear effects and has exceptional resistance to acoustic overstimulation.

These accomplishments are remarkable, and along with them Charlie’s talents are displayed in remarkable teaching and administration. He is an exceptional teacher, having sponsored numerous graduate students and fellows, and directing the graduate course on the peripheral auditory system for over 15 years. He was the president of ARO (1996-7). In 1998, after the retirement of Nelson Kiang, Charlie became the Director of the Eaton-Peabody Laboratory and recently became the first Harold Schuknecht Professor of Otology and Laryngology at Harvard Medical School. As a lab director, he creates an exceptionally conducive environment for research and as a colleague, he takes a personal interest in our grants and manuscripts. As a scientific role model, he sets the bar high in terms of scientific rigor, thoroughness, and clarity in thought and writing. He is held in universally high regard by his colleagues.

This award of merit is a richly deserved symbol of recognition for Charlie Liberman, and on behalf of the ARO, we congratulate him for it.

M. CHRISTIAN BROWN

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1 Comparative Studies of the Ear – of (More Than) Mice and Men

Paul A. Fuchs[1]
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Genetic analysis and genomic sequencing have added dramatically to our understanding of the molecular mechanisms underlying hearing and balance. The study of 'deafness genes' in transgenic mice has taken a particularly prominent role in recent years.

At the same time, natural selection has provided a variety of functional solutions in different species. Probing those varied solutions offers insights into fundamental biological mechanisms, and can provide sometimes surprising clues to conserved molecular mechanisms. This symposium will illustrate the breadth of comparative studies in hearing and balance, and highlight both similarities and differences that inform our understanding.

2 Adaptive Evolution in Mammalian Proteins Involved in Cochlear Amplification

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The remarkable high-frequency sensitivity and selectivity of the mammalian auditory system has been attributed to the evolution of mechanical amplification. Somatic electromotility in cochlear outer hair cells, as the basis for cochlear amplification, is a mammalian novelty and is largely dependent upon rapid cell length changes proposed to be mediated by the motor-protein prestin, a member of the solute carrier anion-transport family 26. Thus, one might predict that prestin has specifically evolved in mammals to support this unique mammalian adaptation. Using codon-based likelihood models we show evidences for positive selection in the motor-protein prestin only in the mammalian lineage, supporting the hypothesis that lineage-specific adaptation-driven molecular changes endowed prestin with the ability to mediate somatic electromotility. Moreover, signatures of positive selection are also observed on the α10, but not the α9, nicotinic cholinergic receptor subunits. An α9α10-containing nicotinic cholinergic receptor mediates inhibitory olivocochlear efferent effects on hair cells across vertebrates. Our results suggest that evolution-driven modifications of the α10 subunit probably allowed the α9α10 heteromeric receptor to serve a differential function in the mammalian cochlea. Thus, we describe at the molecular level signatures of adaptive evolution in two outer hair cell proteins only in the lineage leading to mammals. These findings will be discussed in relation to the roles these proteins play in somatic electromotility and/or its fine tuning.

3 Your Inner (Fly's) Ear

Ron Hoy[1]
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The comparative, evolutionary approach to the science of hearing and auditory behavior has long yielded foundational insights. From Glen Weaver to Jim Hudspeth and Mark Konishi; from Peter Dallos to Jim Simmons and Nobuo Suga, studies of the auditory apparatus and audition of bullfrogs and owls, as well as bats, hamsters, mice, and monkeys have provided key information about human hearing. Insects also hear and communicate through audition. But _What_, you might reasonably ask, could studying bug ears tell us about hearing in "real animals?" A small but devoted band of entomological audiophiles have probed and poked insect ears, inside and out, with surprising results--some more, others less. For example, on the outside and in the realm of macro-hearing, the auditory job of sound localization depends on the same principles of acoustical physics to extract interaural difference cues, in elephants or gnats. But bugs can be very small and their microscale ears generate micro-and nano-scale time and intensity difference cues. Yet flies localize sounds just fine. On the inside of hearing and in the realm of micro-hearing, in mosquitoes, it is known that metabolically-dependent processes affect the sensitivity and tuning of auditory receptor cells, possibly implicating molecular motors, suggested by recent identification of prestin-like proteins in the ears of flies. My aim is to point out the phylogenetic unity of auditory processes at some scales of biological organization, especially molecular, even as novel mechanisms for auditory processing emerge at other scales of organization.

4 The Vestibular System of a Teleost Fish, the Toadfish, Opsanus Tau

Stephen Highstein[1,2]
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The peripheral vestibular system of the toadfish evolved early and relatively completely, resembling those of other vertebrates including mammals. Results of experiments performed upon fish can therefore imply functional information about labyrinths in other vertebrates and may eventually lead to therapies for labyrinthine dysfunction that affect the human condition. The toadfish was chosen as an experimental subject because of its broad, flat head that allows unprecedented access to the peripheral vestibular labyrinth, as the end organs lie almost completely exposed within the cranial cavity. Further, the efferent vestibular system is equally amenable to study as it can be visualized and is accessible in-vivo. The transduction cascade that leads to the formation of the neural frequency code carried by VIIIth nerve afferents to the brain will be reviewed as will the efferent vestibular systems' modification of this code. The experiments reported today will attempt to bridge the gap between the in-vitro experiments conducted upon isolated hair cells and isolated epithelia and the in-vivo experiments conducted upon intact animals. It is thus hoped to provide insight into the function of the vestibular labyrinth.

5 A Neuroethological Approach to Auditory Function: The Amphibian Model

Peter Narins[1]
[1]*UCLA*

Anurans (frogs and toads) have oft proven to be appropriate subjects for studies of the neural mechanisms underlying auditory and seismic behavior. For example, saccular hair cells are exquisitely sensitive to substrate-borne vibrations, but also respond to high-level, low-frequency airborne sound. Nevertheless, anurans are unique among vertebrates in that they possess two distinct organs specialized to detect airborne sounds: the basilar papilla (BP) and amphibian papilla (AP). The BP functions as a single auditory filter and its ca. 60 hair cells make synaptic contact with auditory nerve fibers tuned as high as 8 kHz. In the bullfrog, the AP contains roughly 1000 hair cells. There is no analog of the basilar membrane in this organ; instead, shearing forces necessary for displacement of the stereovillar bundles result from the differential movement of the tectorial membrane (TM) relative to the stationary hair cell receptors. The TM itself is a highly fenestrated, acellular structure which overlies the hair cells and is coextensive with the AP. Intracellular dye-injections of physiologically-identified AP fibers have revealed a rostrocaudal tonotopic organization, with low- and mid-frequency fibers innervating rostral and caudal hair cells, respectively. The former fiber population exhibits two-tone rate suppression, whereas the latter group does not. Frog hair cells have served as a remarkably successful model for understanding fundamental cellular processes including forward and reverse transduction, adaptation and amplification. The recent discovery suggesting that some frogs are sensitive to ultrasound (up to 38 kHz), however, highlights the fact that that we know less about the high-frequency behavior of auditory periphery in the frog than previously believed. The frog inner ear thus provides a rich substrate for the examination of the mechanics, transduction and neural function subserving vertebrate hearing. (Supported by NIDCD grant no. DC-00222).

6 Birds – Same Thing, But Different?

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Recently, biologists' fascination with the evolution and diversity of auditory systems has uncovered some remarkable examples of convergent evolution that make comparisons between birds and mammals especially interesting and instructive. This talk will highlight two areas in cochlear and brainstem physiology where birds continue to be informative comparative animal models.

1. The parallel evolution of cochlear hair-cell subtypes and their efferent control.

Mammalian outer hair cells and avian short hair cells both appear to be specialized for a local (probably amplificatory) function within the cochlea that is modulated by efferent feedback. They contribute little or no afferent input to the brain. The more classical sensory role is instead carried out by mammalian inner hair cells and avian tall hair cells and is modulated by different sets of efferent neurones. Most remarkable of all, this division of labour between different hair-cell types has evolved independently in birds and mammals and thus likely reflects common, profound advantages in the cochlear processing of higher frequencies.

2. How to best build a brainstem circuit to extract interaural time differences (ITDs) for sound localization.

ITDs are an important cue for localizing low-frequency sounds in azimuth. Research on the barn owl has revealed a dedicated brainstem circuit for extracting ITDs and many of its cellular specialisations associated with temporal processing on an impressively fast time scale. However, small mammals which use low frequencies, such as guinea pig and gerbil, show salient differences to that classic model. It is currently unclear whether this reflects basic differences between birds and mammals or is rather more related to physical or ecological factors such as head size or required localization accuracy. Comparing different avian and mammalian species should provide insights into these questions.

7 A Bird Brain's View of Auditory Processing and Perception

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Although songbirds are often used to study vocal motor learning, they also excel at auditory learning and discrimination of the songs of others. This makes them a very useful model for studying neural processing of complex sounds and relating that processing to perception. We used neural recording in awake birds and behavioral studies of birds' auditory perception to ask how complex sounds are represented in the avian equivalent of primary auditory cortex, field L, and what parts of this representation birds might use to decide what song they have heard.

The use of a spectro-temporally rich stimulus based on properties of natural sounds revealed a surprisingly systematic organization of spectro-temporal encoding in field L, with individual cells sensitive to only a subset of acoustic features: neurons were narrowly tuned along either the spectral dimension, the temporal dimension, or both; broadly tuned and strongly orientation-sensitive cells were rare. Moreover, spectrally- and temporally-specialized cells segregated in different areas within field L, raising the possibility that an initial layer of cells tuned for both time and frequency gives rise to separate pathways of cells devoted to either spectral or temporal information.

We probed the relationship between the representation of complex sounds in field L and behavioral categorization of song, by training birds to classify different songs, and then systematically altering these songs along dimensions important in field L. We found that birds generalized well to songs of different duration, but not to songs shifted in pitch, suggesting that the spatial pattern of neurons activated by song, rather than the temporal pattern of neuronal activation, is important for this classification.

These results from songbirds, possible in part because of their complex behavior, raise the question of the degree to which similar principles of auditory organization and perception are shared across other vertebrates.

8 What Can Marmoset Teach Us About Neural Mechanisms Underlying Hearing and Vocal Communication?

Xiaoqin Wang[1]
[1]*Johns Hopkins University*

The common marmoset is a highly vocal small primate and has a rich vocal repertoire. Marmosets have a high reproductive rate and can be breed easily in captivity. Unlike many non-human primate species, marmosets remain vocal in captivity if properly housed in a social environment. The hearing range of marmosets is similar to that of humans, and it covers the frequency range beyond that of their species-specific vocalizations. These characteristics make the marmoset an attractive model for behavioral and neurophysiological studies of hearing and vocal communication. Our recent studies have shown that marmoset vocalizations contain exquisite information on call type, gender and caller identification, similar to what is known in human speech sounds. Our neurophysiology studies show that the auditory cortex of marmosets contains representations of complex sounds, including species-specific vocalizations and pitch of harmonic complex sounds. We also show that the auditory cortex of marmoset is involved in processing auditory feedback during vocalizing which likely contributes to vocal control and self-monitoring. These studies show that the auditory system of the marmoset has evolved to be an open system that processes not only its own species-specific vocalizations but also a wide range of complex sounds from the acoustic environment and other species. Findings from the studies of the marmoset brain have important implications for how the auditory cortex operates and provide insights into speech and language processing mechanisms in the human brain. (Research supported by NIH grants DC03180 and DC005808)

9 Importance of Temporal vs. Spectral Fine Structure for Pitch and Speech

Robert V. Shannon[1]
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The mechanism for coding harmonic pitch (missing fundamental pitch, residue pitch) has been a contentious issue for more than 100 years. Early pitch models focused on the place theory with models of template matching for harmonics. In the 1970's the zeitgeist shifted to temporally based models using neural phase locking mechanisms. The actual mechanisms by which harmonic pitch is coded has taken on a more practical urgency for coding of pitch in cochlear implants. It appears that cochlear implant listeners can detect and discriminate pitch based on temporal periodicity only up to about 300-500 Hz, similar to normal hearing listeners without spectral cues (modulated wide-band noise). For pitch above about 2000 Hz the primary mechanism appears to be primarily based on the place of excitation, but this is a weak form of pitch. The frequency region 500-2000Hz is a critical frequency region for coding harmonic pitch in speech and music. Recent data suggest that temporal cues alone are not accessible for complex pitch unless the spectral distribution is appropriate as well. A new debate has arisen regarding the relative importance of temporal fine structure vs spectral fine structure in the coding of harmonic pitch. This session includes speakers with different viewpoints to highlight the recent results and the issues.
Supported by NIDCD

10 Representing Temporal Fine Structure for Pitch and Speech

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Acoustic temporal fine structure information seems to be important for many aspects of pitch and speech perception, but how is this fine structure represented in the auditory system? In principle, acoustic temporal fine structure information could be extracted from the auditory nerve by either a rate-place (tonotopic) code or a temporal-interval (timing) code, or a combination of both. At higher levels of the auditory system, pitch is almost certainly coded by a rate-place or population code, as temporal synchrony is not usually observed beyond a few hundred Hertz. In the periphery, certain constraints limit the potential use of either place or time code: the place code will break down once components become unresolved in the auditory periphery – a relative frequency constraint – and the time code will function only if neurons can phase-lock to the relevant frequency – an absolute frequency constraint. This talk will review recent psychophysical data examining these constraints, as they apply to the pitch of single and multiple complex tones, and to the use of pitch cues in understanding speech in complex environments. The results suggest that in some circumstances, accurate pitch perception can occur even in the absence of resolved harmonics, in contradiction of a purely place code, but that accurate pitch is also possible even with resolved components well above 6 kHz, in contradiction of a purely temporal code with limited phase-locking beyond about 4 kHz. Finally, for speech perception, the results suggest that accurate temporal fine structure information may not be as critical in dealing with fluctuating backgrounds as has recently been proposed; in contrast, any decrease in acoustic information may lead to similar difficulties in fluctuating complex maskers. [Supported by National Institutes of Health grant R01 DC 05216.]

11 Temporal Pitch Perception by Cochlear-Implant and Normal-Hearing Listeners

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Two methods of studying "purely temporal" pitch perception are to present bandpass filtered acoustic pulse trains to normal-hearing listeners, and to stimulate one electrode of a cochlear implant (CI) with an electric pulse train. At low (< 300 Hz) repetition rates, this code supports musical performance, and, in both CI and NH listeners, is

strongly influenced by refractory effects at the level of the auditory nerve (AN). For CI users, discrimination of faster repetition rates is usually much worse, and the "upper limit" varies across patients, and, to some extent, electrodes. However, it probably does not arise at the AN, as it is impervious to a range of manipulations that would be expected to markedly affect the temporal pattern of AN activity: these include the number of electrodes stimulated concurrently, and the addition of high-rate "desynchronising" pulse trains. Furthermore, the variation in performance for single-pulse-per-period pulse trains correlates, across listeners, with discrimination of different rates of sinusoidal amplitude modulation imposed on a 5000-pps carrier – suggesting a more-central limitation. The inability of CI users to process fast fluctuations is not specific to tasks based on pitch judgements: in bilaterally implanted patients, rate discrimination can be improved by presenting a constant-rate stimulus to the contralateral ear, such that changes in pulse rate can be detected via a change in spatial percept, but this is only true at low pulse rates. For NH listeners, measurement of the upper limit of temporal processing is affected by issues arising from cochlear filtering, but the fastest repetition rate that can be discriminated based on purely temporal cues is at least twice that for the majority of CI patients. The implications of the results both for basic models of pitch and for attempts to introduce temporal fine structure cues into CIs will be discussed.

12 Importance of Temporal Fine Structure Cues in Speech for Normal-Hearing and Hearing-Impaired Listeners

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A wide range of evidence has been presented to support the idea that, for normal-hearing listeners, temporal fine structure (TFS) cues play a role in speech identification in complex backgrounds, especially for "glimpsing" speech in the temporal minima of fluctuating backgrounds. There is also evidence that cochlear damage associated with mild to moderate hearing loss may severely degrade the ability to use TFS cues in speech sounds. This is consistent with the relatively preserved ability of hearing-impaired listeners to identify speech in quiet when audibility is controlled for, and the substantial deficits observed for these listeners when speech is masked by fluctuating background sounds. Further work is now required to quantify and predict the reception of TFS cues for normal-hearing and hearing-impaired listeners in various listening conditions. Various approaches attempting to quantify TFS reception will be presented and discussed.

13 The Relative Importance of Temporal Envelope and Fine Structure Cues for Pitch and Speech Perception in Noise

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Recent studies have shown that lack of access to the temporal fine structure cue is a major reason for the difficulty in speech perception in noise by hearing-impaired listeners. To further understand the role of temporal fine structure, we need to define the temporal fine structure and to delineate its relationship to the temporal envelope in both acoustical and perceptual domains. I will examine the relationship between temporal envelope and temporal fine structure in signal processing terms and then relate it to speech production and perception. Acoustically, the temporal fine structure primarily contributes to changes in fundamental frequency, harmonics, and formant transition. Perceptually, while the temporal fine structure can contribute to speech intelligibility via the formant transition cue, it contributes to speech perception in noise by enhancing auditory objection formation rather than increasing speech intelligibility directly.

14 Impact of Recent Experimental Findings on Pitch Theory

Alain De Cheveigné[1]
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New experimental results have emerged that challenge what we thought we knew about pitch. Pitch may extend beyond the classic upper limit (4-5 kHz), both for pure tones and for individual partials involved in periodicity pitch. Harmonics of low rank that dominate pitch are also spectrally resolvable by the cochlear filter, but rank rather than resolvabililty seems to be the determining factor. Pitch may arise from a stimulus that is periodic only within a restricted spectral region, implying that the ear can focus on one region and ignore others, but pitch within one region can nevertheless degrade pitch within another region, suggesting interference within the pitch (as opposed to spectral) domain. In certain situations listeners can hear pitch changes but not the pitches themselves, as if the ear were equipped with pitch-change detectors. I will attempt to work out what these new results imply in terms of theories currently used to explain pitch: place, time, pattern matching, autocorrelation, unitary and multiple-mechanism models. I will also look at how they relate to what is known of the physiology of neural circuits that might be involved in processing pitch.

15 Hydrogen Rescues Auditory Hair Cells from Reactive Oxygen Species

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The loss of auditory hair cells is one of major causes for sensorineural hearing loss. Previous studies have demonstrated involvement of reactive oxygen species (ROS) in mechanisms for degeneration of auditory hair cells due to various causes including aging, excessive noise and ototoxic drugs. Therefore, scavenge of generated ROS is a highly potent strategy for the treatment of sensorineural hearing loss. Hydrogenation is a fundamental reduction reaction of life. Recent experiments have demonstrated that hydrogen can protect neurons and hepatocytes from ROS-induced damage. This study was aimed to examine the potential of hydrogen to protect auditory hair cells from ROS-induced damage

using explant culture systems. We used antimycin A to generate ROS in explant cultures of auditory epithelia obtained from P2 mice, and examined the protective effect of hydrogen on hair cells against ROS-induced cell death. Antimycin A generated ROS and subsequent lipid peroxidation in auditory epithelia, resulting in hair cell loss depending on a concentration of antimycin A. Supplement of hydrogen into the culture medium significantly reduced ROS generation and lipid peroxidation in auditory epithelia, leading to increase surviving hair cells. These findings demonstrate the potential of hydrogen for protection of auditory hair cells from ROS-induced damage, suggesting therapeutic potential of hydrogen for sensorineural hearing loss.

16 Use of L-NAC as a Noise Otoprotectant in Mice

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Noise-induced hearing loss (NIHL) is one of the most common occupational injuries in the United States. It would be extremely valuable if a safe, inexpensive compound could be identified which protects worker hearing. In a series of experiments, Kopke has shown that the compound N-acetyl-L-cysteine (L-NAC) can protect the hearing of chinchillas from the effects of a single exposure to noise. L-NAC is a glutathione precursor which crosses the blood-cochlea barrier, is used in clinical medicine and is very safe. Although L-NAC appears to be promising, it has not been successful in other studies (Kramer et al., 2006, Hamernik et al., 2008).The present study was undertaken to demonstrate that L-NAC could protect C57BL/6J mice from the permanent effects of noise. Five mice were injected with 300 mg/kg L-NAC approximately 1 hr prior to a 104 dB broadband noise exposure and again immediately after the exposure. A control group (N=6) was exposed the same but injected with sterile saline. Auditory brainstem response measurements were made at 4, 8, 16 and 32 kHz prior to and 12 days after exposure.

There were no statistically significant differences in ABR threshold shifts between the mice receiving L-NAC and the control mice. This indicates that L-NAC was not effective in preventing permanent threshold shift in the mouse model of NIHL. Future work will examine higher concentrations L-NAC for otoprotection.

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17 Deletion of CGMP-Dependent Protein Kinase CGKI Increases Sensitivity for Noise-Induced Hearing Loss

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Noise induced hearing loss (NIHL) is a major reason for hearing disorders like hypacusis and tinnitus. Otoprotection and adequate cellular response to trauma is therefore an essential need in a noisy environment.

The nitric oxide (NO)-cyclic GMP (cGMP) signaling pathway is proposed to play a crucial role for normal cellular function and for response to trauma. The cGMP-dependent protein kinases (cGKs) are central players in NO-activated second messenger pathways. cGKs have a regulatory function for ion channels in e.g. kidneys and retinal bipolar cells (Snellman and Nawy 2004) and play a role in cerebellar LTD and motor learning (Feil et al. 2003). In the cochlea, excessive NO production could be observed in inner ear disorder.

We detected cGKI in inner (IHC), outer hair cells (OHC), spiral ganglia neurons, and vestibular ganglia, indicating that the cGMP-signaling pathway may determine the trauma response via the activation of cGKI. Here, we present a knockout mouse model for cGKI-deficiency to clarify cGKI importance in trauma response. Knockout mice had nearly normal hearing function, active cochlear mechanics and cochlear structure. cGKI deletion, however, led to significant higher noise vulnerability. Exposure to traumatic noise resulted in a substantial hearing loss 7 days post acoustic trauma. The hearing loss was accompanied by a decrease of distortion product otoacoustic emission (DPOAE) and was linked to a loss of expression of distinct OHC ion channels (BK, KCNQ4), suggesting OHC dysfunction and the pending death of hair cells. The importance of NO-cGMP-cGK pathway in trauma response and promising therapeutical intervention are discussed.

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18 Cochlear Cytokine Gene Expression Following Systemic and Intratympanic Steroid Treatment in Mice

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Intratympanic steroid delivery for hearing loss is increasing in popularity because of the ability to get higher concentrations of drugs into the inner ear and avoid systemic side effects. However, little is known of the

actual impact of steroids on gene expression in the inner ear and how these functions are altered by middle ear delivery. Therefore, preliminary studies were conducted to determine if cochlear cytokine gene expression is different between systemic and intratympanic delivery methods. Autoimmune MRL/lpr mice were given prednisolone or dexamethasone either systemically or intratympanically in therapeutic doses for equivalent clinical effects. After 24 hours, cochleas were harvested, the total RNA isolated (RNAeasy Kit, Qiagen), and RNA assessed by gene arrays (SuperArray). Arrays were assessed for the number of cytokine genes upregulated or downregulated. Comparison of gene expression with untreated mice in the two delivery methods showed intratympanic delivery of prednisolone impacted 15 genes (10 upregulated, 5 downregulated) compared to 47 genes (21 up, 26 down) affected by systemic delivery. On the other hand, intratympanic delivery of dexamethasone impacted more genes (29: 14 up, 15 down) than it did systemically (15: 5 up, 10 down). The total cytokines affected were significantly different with the two delivery methods (Chi-square = 18.5; $p < 0.001$). These studies suggest a potential difference in efficacy of systemic versus middle ear delivery, depending on the steroid used. Dexamethasone may actually have a greater effect on cochlear cytokine expression than prednisolone if given transtympanically, while prednisolone may have greater impact in the cochlea if given systemically. More extensive studies are ongoing to quantify cochlear gene expression to evaluate relative drug x delivery impact.
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19 JAK-STAT Signaling Is Critically Required Forcisplatin-Mediated Proinflammatory Cytokine Production

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In our previous study, we clearly demonstrated the roles of pro-inflammatory cytokines on cisplatin-induced ototoxicity. The Janus kinase (JAK) / Signal transducer and activator of transcription (STAT) pathway represents the main effector for many cytokines. Therefore, we investigated the role of JAK and STAT proteins in cisplatin-mediated ototoxicity and proinflammatory cytokine production. The phosphorylation of JAK3 and STAT6 were increased by cisplatin in HEI-OC1 cells and wild-type mouse embryonic fibroblasts (WT-MEFs). JANEX-1, a JAK3 inhibitor attenuated cisplatin-induced proinflammatory cytokine production and toxicity. Furthermore, transfection of JAK3 and STAT6 siRNA markedly reduced the production of proinflammatory cytokine and toxicity by cisplatin in HEI-OC1 cells. In addition, cisplatin-mediated proinflammatory cytokine production was markedly reduced in STAT6-deficient knock-out (KO) MEFs. Immunohistochemistry revealed that the production of proinflammatory cytokines was ubiquitously expressed in the cochleae of cisplatin injected WT and STAT4 KO Mice, whereas dramatically reduced in the cisplatin injected STAT6 KO mice. These results indicate that the JAK-STAT pathway plays an important role in cisplatin-mediated proinflammatory cytokines production. Thus, we suggest that the inhibition of JAK-STAT pathway may be a therapeutic target for cisplatin-induced ototoxicity.
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20 The Role of STAT1 in Cisplatin-Induced Vestibular Hair Cell Death

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Oxidative stress, DNA damage, and inflammatory cytokines have all been implicated in cisplatin-induced hair cell death. The transcription factor STAT1 is an important mediator of cell death and can regulate all of these processes in other cell types. However, the role of STAT1 in cisplatin-induced hair cell death is unknown. We first determined the dose-response relationship of hair cell death following exposure of mature Swiss Webster mouse utricles to cisplatin for 24 hours *in vitro*. Exposure to 80 µg/ml cisplatin reliably killed approximately ½ of the extrastriolar and stiolar hair cells after 24 hrs of exposure. We next used immunocytochemistry in these adult mouse utricles to show that STAT1 phosphorylation is an early event in hair cells and supporting cells following exposure of utricles to moderate-dose cisplatin (40 µg/ml) or the cytokine interferon-γ. Phosphorylation peaked at 4 hours after the initiation of exposure and returned to control levels by 8 hours of exposure. The STAT1 inhibitor epigallocatechin gallate (EGCG) attenuated STAT1 phosphorylation in cisplatin-treated utricles and resulted in dose-dependent increases in hair cell survival after 24 hours of continuous cisplatin exposure. Furthermore, utricular hair cells from STAT1-deficient mice were found to be highly resistant to cisplatin-induced hair cell toxicity compared to utricular hair cells from wildtype mice. EGCG failed to provide additional protection from cisplatin in these STAT1-deficient mice, further supporting the hypothesis that the protective effects of EGCG are due to STAT1 inhibition. These results show that STAT1 is required for maximal cisplatin-induced hair cell death *in vitro*. Future experiments will determine the role of STAT1 in cisplatin-induced hair cell death *in vivo* and if pharmacological inhibition of this pathway is a useful strategy for preventing the ototoxic effects of cisplatin.
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21 c-Myc Regulates Expression of Mitochondrial Peroxiredoxin in Mouse Cochlear Hair Cells

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Peroxiredoxins are components of cellular antioxidant defenses and play a critical role in maintaining the intracellular redox balance and mitochondrial membrane potential. Peroxiredoxin 3 (Prx3) is a 2-Cys subclass, mitochondrion-specific enzyme required for normal mitochondrial function. Depletion of Prx3 results in increased intracellular levels of H2O2 and sensitizes cells to apoptotic signaling.

We have previously reported that Prx3 protein initially increases in the mouse cochlea in vivo in response to aging and aminoglycoside treatment but subsequently decreases, followed by hair cell death. We now investigate the regulation of expression of Prx3 in CBA/J mouse cochlear explants (postnatal day 3), challenged with 0.2 mM gentamicin which causes significant hair cell death after 20 h. Expression of mRNA for Prx3 increased significantly after gentamicin treatment and protein levels of Prx3 were increased at 8 h but reduced by 16 h in outer hair cells. Consistent with the mRNA expression, protein levels of c-Myc, a transcription factor that targets the Prx3 gene, and its phosphorylation at Thr58 and Ser62 was also increased after 8 h of treatment. Supporting a connection between c-Myc and Prx3, 10058-F4, an inhibitor of c-Myc, prevented the early rise of Prx3 protein levels after 8 h of gentamicin treatment.

These results demonstrate that the initial response of hair cells to oxidative stress is an upregulation of Prx3 which, at least in part, depends on the transcription factor c-Myc. Down-regulation of Prx3 in outer hair cells is then followed by cell death.

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22 Oral D-Methionine (MRX-1024) Significantly Protects Against Cisplatin-Induced Hearing Loss: A Phase II Study in Humans

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D-methionine (D-met) protects against cisplatin-, aminoglycoside- and noise-induced hearing loss in multiple animal studies. Further, D-met protects against radiation-induced oral mucositis. We are currently preparing for clinical trials for D- met protection against noise-induced and aminoglycoside-induced hearing loss. Phase II clinical trials results for D-met protection from radiation-induced oral mucositis are currently being prepared for publication.

This study represents the first clinical trial results for D-met protection from cisplatin-induced ototoxicity in humans. In this double blind randomized pilot study, 14 adult patients received 100mg/kg dose of an oral orange flavored suspension of D-met (MRX-1024) and 13 adult patients received flavor matched placebo in equivalent volume prior to each dose of cisplatin. Mean cumulative cisplatin dosing was 263.57 (SD 74.79) in the experimental group and 253.85 (SD 56.94) in the control group. Primary tumor sites ranged from genitourinary tract to head and neck cancers. Six patients in the experimental group and four patients in the placebo group also received radiation to the head and/or neck area for primary tumors in that region. Auditory thresholds were tested bilaterally at 8, 10, 11.2 and 12.5 kHz with a GSI 61 audiometer using a modified Hughson-Westlake technique. Significant threshold protection at was obtained for the frequencies of 10 kHz and above. No difference in tumor regression was noted between groups. We are encouraged that significant otoprotection was observed even with a small group of patients receiving fairly low cumulative cisplatin dosing.

23 Efficacy of STEALTH-Nano-Particles Encapsulating Betamethasone for Attenuation of Noise Induced Hearing Loss in Mice

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The use of high dose steroid is a standard treatment for acute sensorineural hearing loss. Systemic injection of steroid can cause several adverse effects such as hypergclycemia or gastric ulcer. Therefore, we developed biodegradable sustained-release materials, namely STEALTH-nano-steroid, which are nano-size particles made of poly-lactic acid (PLA) polymer encapsulating betamethason phosphate (BP) and coated with poly-ethylene glycol (PEG). PEG coating can cause that stealth provides better tissue delivery by reduced liver entrapment. In this study, we examined the effect of STEALTH-nano-steroid on damaged inner ear after acoustic trauma. Methods: Animals (male CBA mice, n= 12) were separated into 3 groups, STEALTH-nano-steroid, BP, and saline groups. Each drug was injected intravenously followed by noise exposure (8 kHz band noise at 120 dB for 2 hours). Thresholds of ABRs were measured on day 3, 7 and 14 for cochlear function, and cochleae were collected on day 14 for histological analysis. To examine the distribution of STEALTH-particles, we injected rhodamine B or STEALTH-nano-particles encapsulating rhodamine B intravenously followed by noise exposure (n=12 each). At 15 min, 12 and 24 hours after injection, brains, cochleae, spleens and livers were excised. To evaluate the cumulative steroid release in each tissue, we injected 3 drugs into 9 animals respectively. At 1, 12 and 24 hours after injection, tissues were collected.

Results: STEALTH-nano-steroid significantly reduced the elevation of ABR thresholds compared to BP and saline group. Histological analysis revealed the STEALTH -nano-steroid significantly inhibited the loss of outer hair cells. STEALTH-nano-steroid will be a realistic therapeutic tool for sensorineural hearing loss.

24 Towards the Development of a Laboratory Model of Noise-Induced Hearing Loss with Real-World Relevance for Human Subjects

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A variety of antioxidants and other agents have been shown to reduce noise-induced hearing loss in animal studies; several will soon be evaluated in human clinical trials. The specific trial designs are largely constrained by investigator-specific access to unique subject populations. Thus, it is challenging to compare efficacy of different agents across human studies. One solution is identification of real-world-relevant exposures that can be reliably structured, analyzed and defined, and reliably reproduced across clinical/laboratory environments, and to which subjects will volunteer to listen (given appropriate level and duration, to minimize risk to subjects). Here, we describe procedures for manipulating digital music files such that subjects can listen to music level-equated both within and across songs. Exposures are real world, yet consistent across time, more pleasant to listen to than pure tones or shaped noise, and closely follow music exposures subjects may experience under normal listening conditions. The manipulated digital music files have no appreciable decrease in sound quality. To develop these procedures, we purchased 326 rock and pop songs (21.7 hours). All songs were digitally manipulated using Matlab programs, during which the music files were broken into 50 msec windows, filtered using an A-weighted function, and then level equated. Prior to Matlab processing, the average level of individual songs had a 16-dB range and average level variation within each song was 4.6 dB. Subsequent to Matlab processing, the average level of individual songs had a 3-dB range and within-song variation was reduced to 2.3 dB. Taken together, overall levels were held significantly more constant across time. Moreover, kurtosis, which has had a considerable effect on threshold changes in animal noise studies, was reduced. The development of routine exposure parameters that facilitate direct comparisons across human clinical trials would be of significant benefit to clinicians who use evidence-based practice in counseling their patients.
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25 The Sound-Induced Motion of the Human Tympanic Membrane and Stapes Determined by Opto-Electronic Holography and Laser Doppler Vibrometry

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Computer-assisted opto-electronic holography (OEH) is used to measure the sound-induced motion of the tympanic membrane (TM) in human temporal bones. The holographic measurements are performed in 'time-averaged' mode for fast characterization of frequency dependent TM vibration patterns and stroboscopic mode for determination of the magnitude and phase of motion of the entire surface of the membrane. Immediately following OEH, the stapes velocity is measured by laser Doppler vibrometry (LDV). The time-averaged holograms (TAHs) of TM motion show 'simple', 'complex' and 'ordered' fringe patterns as sound frequency increases from 500 to 20000 Hz. At each frequency, as stimulus intensity increases, the pattern of fringes remains the same, but the number of visible fringes increases, which is proportional to the amplitude of the motion of the membrane. The volume velocity and input impedance of the TM are calculated from the complex velocity of the surface of the TM measured by stroboscopic holography (SH). The measured input impedance of the human TM is compared with published data in the literature. The results of qualitative (modal patterns from TAH) and quantitative (complex velocity from SH) motion of the TM together with stapes velocity should help us better understand how the TM couples sound to the ossicular chain. These techniques will be used to quantify relationships between changes in TM motion and stapes velocity in ears with various manipulation induced pathologies of the ossicular chain and eardrum (see the poster by Aarnisalo et al.).
[Work supported by F32 & R01 from NIDCD and a donation from L. Mittal.]

26 The Motion of the Tympanic Membrane Evaluated with Time-Averaged Holography After Ossicular Chain Pathologies and Cartilage Placement on the Tympanic Membrane

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Computer-assisted Opto-Electronic Holography (OEH) is used to produce time-averaged holograms (TAH) that describe the magnitude of the sound-induced motion of the

tympanic membrane (TM). TAHs are gathered in (i) normal cadaveric temporal bones, (ii) after malleus fixation, (iii) after reversal of the fixation, (iv) after stapes fixation and (v) after incudostapedial joint interruption. Stapes velocity (Vs) is measured in each condition with a laser Doppler vibrometer. After ossicular fixation, with low-frequency sound stimulation (≤ 1 kHz), higher than normal sound pressure levels are required to see TAH fringe patterns. This increase is reversed after reversing malleus fixation. Little difference in fringe pattern is seen between the fixed and normal states at frequencies above 3 kHz. Vs was decreased at 500 Hz and 1 kHz after either malleus or stapes fixation. The reversal of the malleus fixation increased Vs back to the baseline level. Interruption of the inducostapedial joint alters fringe patterns at all studied frequencies and reduces Vs to artifact levels.

In other temporal bones, either a 0.5 mm or 1 mm thick oval piece (0.6 x 0.3 mm) of conchal cartilage is placed on the postero-superior part of the medial surface of the TM. The cartilage is rotated so that it is either in contact with the bony rim or not. At frequencies above 4 kHz the cartilage reduces the fringe patterns on the adjacent TM surface, i.e. higher levels were needed to produce fringes. The reduction in fringes is more obvious with the 1mm thick cartilage. The position or thickness of the cartilage have little effects on Vs.

TM surface displacements measured with OEH can identify how changes in ossicular load or cartilage placement affect the motion of the TM over a broad frequency range. OEH is a new promising technique to evaluate the causes of conductive hearing loss and optimize techniques of tympanoplasty. [Work supported by NIDCD and a donation from L. Mittal].

27 Tympanic-Membrane Boundary Conditions in Human and Gerbil Derived from Static Displacements Observed with Computerized Tomography

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To understand middle-ear function, mathematical models have been used for many years now. The middle ear is too complex a system for its function to be fully understood with just simple descriptive models. Realistic mathematical models must be used in which structural elements of the ear are represented by a geometrically correct 3-D model, and assigned correct physical parameters. Simplifications are nevertheless always introduced to make the models easier to handle. A common assumption is to model the tympanic membrane as a thin plate, either *simply supported* or *fully clamped* at its boundaries with the annulus and manubrium. The choice of boundary condition could not be made based on experimental evidence as no clear-cut data were actually available. We studied the deformation of the tympanic membrane at its boundaries using micro x-ray CT in human and gerbil while static pressure was applied to the ear canal. 3D models of the tympanic membrane and its bony attachments were carefully made and used to measure the deformation of the tympanic membrane, with focus on the periphery and the manubrium attachment. For the pars flaccida of the gerbil it was clear that the boundary condition could be described as simply supported. For the human pars flaccida the situation is more complicated: superiorly the membrane contacts the underlying bone more and more when pushed further inward, and it gradually detaches from the wall when sucked outward. In gerbil we found that the attachment of the tympanic membrane to the manubrium can be described as simply supported. In human the manubrium is attached underneath the tympanic membrane via the plica mallearis and the contact of the tympanic membrane with the bone is indirect. For both human and gerbil the choice of peripheral boundary condition for the pars tensa could also not readily be made; the thickness of the tympanic membrane varies rapidly close to the peripheral edge and then the membrane gradually continues into the annulus fibrosis which finally makes contact with the bone. To investigate how the membrane-to-bone attachment should be described in a complete middle-ear model in order to replicate the experimental deformation at the edges, a separate model with nothing but these structures could be very useful.

28 Laser-Doppler Inertial Vibrometry for Non-Contact Elasticity Analysis of Stapes Foot Plate

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Although correct evaluation of the mobility of stapes footplate is crucial for successful ossicular chain reconstruction or stapes surgery, objective measurement has been difficult. Currently available vibrator with force measurement should be directly attached on stapes, inherently containing risks for dislocating stapes during measurement. Thus, non-contact measurement of footplate mobility is anticipated.

We have utilized a laser Doppler vibrometry during passive movement of stapes. We assumed finite aqueduct model of stapes, cochlea and round window containing fixed amount of perilymph. Sound conduction would be defined as a vibration conducted to the perilymph. Vibration of the temporal bone can also generate force to the perilymph resulting in inertial vibration of stapes. Phase delay and resonant frequency of the inertial vibration of stapes are directly dependent on the mass and the elasticity of the stapes footplate.

We evaluated this system in cadaveric studies. A custom-made vibrator was attached on the exposed temporal bone to generate inertial stapes movement. Laser Doppler vibrometry revealed phase delay and resonance between the temporal bone - stapes vibration. When a weight was attached on the stapes to mimic mass effect, resonance was observed in smaller frequency. When stapes was glued to temporal bone to mimic stapes fixation, phase delay and resonance were barely observable, if any in the

very high frequency outside our measurement range. The estimated modulus of elasticity and total mass of the aqueduct agreed with previous reports. Our study indicates that non-contact stapes vibrometry can provide safe and objective measurement of stapes footplate mobility.

29 Finite Element Modeling to Determine Cetacean Middle Ear Function

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How sound gets from the water to the cetacean cochlea is not well understood. The cetacean middle ear ossicles and muscles are massive compared to their counterparts in terrestrial ears and yet many cetaceans have exceptional high-frequency hearing. For example, the high-frequency cutoff for the bottlenose dolphin (*Tursiops truncatus*) audiogram is eight times higher than that for human while the mass of the dolphin malleus is eight times that of the human malleus. In spite of these differences, the dolphin and human cochleae are similar in size.

In order to better understand the sound conduction pathway to the cochlea in cetaceans, a linear finite element (FE) model of the bottlenose dolphin middle ear was developed. Micro-CT scans (36 micron cubic voxels) of a thawed ear sample were segmented and meshed using Amira software. Soft tissue dimensions and orientations were directly measured from dissected samples and added to the model. The mesh was imported into the COMSOL finite element solver. Human values for tissue density and Young's modulus were used. A force was applied to the stapes footplate and the motion of the ossicular chain was computed in order to mimic our previously published experimental work (Miller *et al*., 2006, IEEE J. Oceanic Eng. 31:87-94).

The predicted motion of the malleus-incus complex has both bending and rotational components with the rotational component being the dominate one. The rotation of the malleus-incus complex occurs about the axis between the anterior process of the malleus and the short process of the incus. This axis of rotation is similar to that observed in terrestrial mammals suggesting that cetacean middle-ear function follows the typical mammalian pattern. The anterior process of the malleus appears to act as a torsional spring which stiffens the middle ear in a manner similar to that observed in high-frequency terrestrial mammals such as bats and mice. We conclude that, even though the cetacean ossicular chain is much more massive than that found in terrestrial mammals, the fundamental features of cetacean middle-ear function are the same as those found in other mammals.

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30 Conductive Hearing Loss in Mice

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It is common for gene mutations in mice to produce mixed hearing loss with both conductive and sensorineural loss components. It is also commonly observed that certain mutant mice are more susceptible to middle-ear disease with subsequent hearing loss. Up till this time, most studies that identify conductive hearing loss in mice do so by identifying some gross structural abnormality of the middle ear. We are working at methods to better define and understand conductive hearing loss in different mouse phenotypes to improve our understanding of the cause of hearing loss in different mouse populations. In the past our tool of choice has been the measurement of sound-driven tympanic membrane (TM) velocity using laser-Doppler vibrometry, together with anatomical analyses of the ossicular chain and middle-ear air space. These techniques shed light on the increased incidence of middle-ear disease in 129S6/SvEv mice and on the conductive component of hearing loss in alpha retinoic-acid knockout mice. The general conclusion is that signs of middle-ear infection correlate well with reduced TM velocity, but that changes in TM velocity by itself probably underestimate the conductive hearing loss. In order to define a more generally available tool for separating the component of mixed hearing, we are investigating the differential effects of conductive pathology on auditory brainstem response (ABR) and distortion-product oto-acoustic emission (DPOAE) thresholds. Our results so far are consistent with conductive hearing loss having a bigger effect on DPOAE response thresholds than on ABR thresholds. We wish to quantify the relationship between the two in order to better estimate conductive hearing loss. We have also begun to test bone-conducted ABR as an independent test to separate conductive and sensorineural hearing loss in mice. [Supported by the NIDCD]

31 Evaluating Ossicular Discontinuity and Repair Using Wideband Energy Reflectance in Human Cadaver Ears

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The purpose of this study was to evaluate the use of wideband energy reflectance (ER) to evaluate an ossicular disarticulation in human cadaver temporal bones. Measurements were obtained on 5 temporal bones at ambient pressure in three conditions: 1) intact ossicular chain; 2) with the ossicular chain cut using an argon laser; and 3) repaired ossicular chain. Laser Doppler vibrometry (LDV) of stapes footplate velocity was used to monitor the effect of the experimental conditions. Disarticulation resulted in a low-frequency drop in ER in a narrow notch below 1000 Hz. The average reduction in ER was 31% at a mean frequency of 630 Hz. The low-frequency notch in ER was eliminated following repair of the ossicular chain with dental cement. LDV measurements confirmed the effects of the disarticulation and repair. A simple series

impedance model of the middle ear provided a good description of the response at frequencies below 2000 Hz. It appears that a disarticulation of the ossicular chain produces a low-frequency notch in ER that recovers after repair of the disarticulation. These results suggest that ER has potential for use in the diagnosis of ossicular discontinuity and may be useful to monitor the status of the post-surgical ear. Additional data are needed from patients undergoing surgery for ossicular discontinuity to further study the usefulness of ER in diagnosis and evaluate the simple series impedance model of the middle ear.

32 Evaluation of Round Window Stimulation in Human Cadaveric Temporal Bones

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Round window (RW) and oval window (OW) sound stimulation produce similar cochlear potentials, auditory-nerve potentials and brainstem responses in animals (Wever & Lawrence 1950, Voss et al 1996, Dumon et al 1995, Spindel et al 1995). Colletti et al (2006) implanted an electromagnetic middle ear implant, the floating mass transducer (FMT) (MED-EL Vibrant Soundbridge), to stimulate the RW in 7 patients with various hearing losses and prior tympanoplasties and reported improvements in their speech intelligibility. The present study examines the mechanics of RW stimulation. We report measurements of stapes velocity and intracochlear sound pressures in scala vestibuli and scala tympani to evaluate FMT stimulation of the RW in cadaveric temporal bones.

Measurements of stapes velocity in response to FMT-RW stimulation were used to optimize FMT insertion. The optimum coupling between the FMT and RW was achieved with the placement of a thin piece of fascia between the RW and the FMT. Bracing the free end of the FMT against the hypotympanic wall with dental impression material also improved coupling.

The effect of RW stimulation on hearing was estimated by simultaneous measurements of intracochlear pressures in both cochelar scalae, allowing us to quantify the complex differential pressure across the cochlear partition. Our results indicate that FMT-RW stimulation provides cochlear stimulation comparable to sound-induced OW stimulation above 1 kHz. However, below 1 kHz the FMT is less able to produce differential pressure.

We can use these techniques to study the applicability of FMT-RW stimulation in cases with disarticulated middle ears, ears with stapes fixation, third-window lesions, and other clinically relevant conditions. Furthermore, basic questions regarding bone conduction and other modes of cochlear stimulation can be elucidated. [Work supported by the NIDCD].

33 Electrocochleographic Assessment of Mechanical Stimulation of the Round Window

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Middle ear implants (MEIs) have applications in sensorineural, conductive and mixed hearing loss. Benefits have been achieved primarily via direct mechanical stimulation of the ossicular chain. Recent clinical reports have shown mechanical stimulation of the RW provides functional benefit in cases with an altered ossicular chain. Despite successful outcomes in humans, little detailed objective data is available in terms of the physiologic performance of MEIs. This study explored the physiologic responses to RW stimulation induced by a MEI (Otologics MET, Boulder CO USA). Measurements of cochlear microphonic (CM), compound action potential (CAP) and auditory brainstem response (ABR) were made in 4 chinchillas with acoustic stimulation and with application of the MEI to the RW using pure tone stimuli (0.25-20 kHz) presented at different intensities (-20-80 dB SPL vs. 0.01-1000 mV). Waveform morphologies of the CM and the CAP and ABR peaks were remarkably similar between acoustic and round window stimulation. CM (CAP) thresholds were frequency-dependent with acoustic stimulation ranging from 5 (10) to 60 (55) dB SPL, while thresholds of the RW stimulation ranged from 0.3 (3) to 10 (200) mV. The sensitivities (μV amplitude/dB SPL or dB mV) of the CMs were virtually identical between the two stimulation methods as was the CM amplitude dynamic range. Equilibrating the stimulus amplitudes required to achieve CM threshold between the two methods yields an estimate of the equivalence of dB SPL at the tympanic membrane and dB mV to the MEI. The CAP and ABR as a function of stimulus frequency demonstrated similar decreasing amplitudes and increasing latencies with decreasing intensity (dB SPL vs. dB mV). Our results demonstrate that RW stimulation provides mechanical inputs to the cochlea that are functionally equivalent to those provided by acoustic stimulation. Support: Otologics Education Grant.

34 Estimating Wideband Eardrum Sound Levels in Humans

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Controlling stimulus levels at the eardrum of human subjects is important for both research and clinical applications and is especially difficult at frequencies above 8 kHz. Three methods of estimating the eardrum sound levels were compared, using behavioral thresholds as the "gold standard". It is reasonable to assume that threshold represents a fixed and reproducible input level to the ear. The accuracy of methods to estimate eardrum stimulus levels then can be judged by comparing the variance of either repeated test-retest measures of thresholds in individual subjects, variance of thresholds in a population of subjects, or repeated measures of thresholds with

systematic variation of the insertion depth of a stimulus probe (i.e., Neely and Gorga, JASA 104:2925-2934, 1998). The first method selects a calibration response from a family of measurements in an IEC-711 ear simulator with differing insertion depths to match the insertion depth in the subject (Gilman and Dirks, JASA 80:783-793, 1986) based on the frequency of the first half-wave resonance. The second method predicts the eardrum SPL using the pressure response measured with an Etymotic Research ER10-B+ otoacoustic emission probe. The third method uses the forward pressure calculated from the measured ear canal pressure response after calibrating the Thévenin equivalent source characteristics of the probe (Scheperle, et al., JASA, 124:288-300, 2008).
All three methods hold promise to minimize the systematic errors caused by standing waves in the occluded ear canal. Considering that forward pressure is consistently 3-6 dB below the total pressure at the eardrum, the three methods yield similar estimates of the input to the ear in our preliminary findings. Quantitative evaluation of relative performance awaits a larger sample size. The theoretical and practical advantages of each of the three methods will be presented and evaluated using experimental data.
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35 Feature Selection in HRTF Synthesis

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For many applications, it is ideal to posses individual head-related transfer functions (HRTF)-as in the presentation of virtual spatial audio. Direct acoustic measurement of individual HRTFs is often not possible (e.g. due to the duration of the measurement or the availability of a measurement system). Methods for determining an individual's HRTF without performing additional acoustic measurements have been described. In our study, a large database of HRTFs and corresponding measurements of head, torso, and pinna geometry are used as a basis for predicting / synthesizing the HRTF of an individual. The analysis is achieved through a tensor decomposition which is computed by higher-order singular value decomposition (HOSVD) of the mutlimodal data. We go on to describe a regression method for synthesizing an individual's HRTF from a limited set of the most significant anthropometric measurements. Synthesis results are shown for several individuals. Inconsistencies between synthesized and measured HRTFs are shown. In addition, a perceptual spectral distance is proposed and issues regarding the use of distance measures on HRTFs are discussed. Sound localization tests are performed using human listeners and the synthesized HRTFs.

36 Functional Consequences of Adaptive Evolution of the Mammalian α9α10 Nicotinic Acetylcholine Receptor.

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The $\alpha9\alpha10$ nicotinic acetylcholine receptor (nAChR) mediates efferent inhibition of cochlear hair cells in mammals and birds. This inhibition results from activation of a calcium-dependent potassium current thought to depend on calcium entry through the activated nAChR. Sequence analysis of the *CHRNA10* genes (but not of *CHRNA9*) of different species revealed signs of adaptive evolution in the mammalian lineage (Franchini and Elgoyhen, 2006). Therefore, one could propose that the mammalian $\alpha9\alpha10$ receptor (i.e., from *R. norvegicus*) would have functional properties different from those of the avian receptor (i.e., from *G. gallus*) as a result of specific, non-synonymous substitutions within the *CHRNA10* gene.
To begin to test this hypothesis, we analyzed the properties of the recombinant chicken $\alpha9\alpha10$ receptor, using the two-electrode voltage-clamp technique in *Xenopus laevis* oocytes expressing these subunits. The sensitivity to ACh of the *G. gallus* receptor was lower than that of the *R. norvegicus* receptor (EC_{50}=21.7±1.2μM and 13.8±1.7μM, respectively). In addition, the *G. gallus* $\alpha9\alpha10$ receptor did not desensitize significantly, in a manner similar to that of the homomeric $\alpha9$ from rat, and different to the strong desensitization of the heteromeric $\alpha9\alpha10$ *R. norvegicus* receptor. Perhaps most notably, the oocyte's endogenous calcium dependent chloride current stimulated by rat $\alpha9\alpha10$ was not activated by the *G. gallus* $\alpha9\alpha10$ receptors, suggesting that calcium permeability of the avian receptor is substantially lower than that of the mammalian receptor.
These results indicate that the mammalian $\alpha9\alpha10$ receptor has acquired new functional properties which are different from those of non-mammalian species. This most likely results from the positive selection of non-synonymous substitutions in the $\alpha10$ subunit during the evolution of this lineage. Supported by NIH, HHMI, UBA and ANPCyT.

37 Constitutive Expression of the α10 Nicotinic Receptor Subunit Alone or in Combination with α9 Overexpression Fails to Maintain Cholinergic Responses in Inner Hair Cells, After the Onset of Hearing

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Efferent inhibition of cochlear hair cells is mediated by $\alpha9\alpha10$ nicotinic cholinergic receptors (nAChRs)

functionally coupled to calcium-activated, small conductance (SK2) potassium channels. Before the onset of hearing (about day 12 in rats and mice) efferent fibers transiently make functional cholinergic synapses with inner hair cells (IHCs). The retraction of these fibers after the onset of hearing correlates with the cessation of transcription of the *Chrna10* (but not the *Chrna9*) gene in IHCs. To further analyze this developmental change, we generated a strain of mice whose IHCs constitutively express α10 into adulthood by expressing the α10 cDNA under the control of the rat *Pou4f3* gene promoter. *In situ* hybridization showed that the α10 mRNA is expressed in IHCs of 8-wk old transgenic mice, but not in wild type mice. Moreover, this mRNA is translated into a functional protein, since IHCs from P8-P10 (n=3) α10 transgenic mice backcrossed to a *Chrna10*$^{-/-}$ background (whose IHCs have no cholinergic function), displayed normal synaptic and acetylcholine (ACh)-evoked currents in patch-clamp recordings. Thus, the α10 transgene restored nAChR function. However, in the α10 transgenic mice no synaptic or ACh-evoked currents were observed in P16-17 (n=4) IHCs, indicating developmental down-regulation of functional nAChRs after the onset of hearing, as normally observed in wild type mice. Moreover, the endogenous level of expression of the *Chrna9* gene was not a limiting factor, since in double α10 and α9 transgenic mice (constitutively expressed under the *Pou4f3* promoter) no synaptic or ACh-evoked currents were observed in P19-20 (n=5) IHCs. The lack of functional ACh currents correlated with the lack of SK2 currents. These results indicate that multiple features of the efferent postsynaptic complex to IHCs, in addition to the nAChR subunits, are down-regulated in synchrony after the onset of hearing, leading to lack of responses to ACh.

38 Cholinergic Signaling in Frog Auditory Hair Cells

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We have utilized fluorescence and confocal microscopy to investigate the dynamics of acetylcholine-induced calcium increases in hair cells of the amphibian papilla (AP) of *R. pipiens pipiens*. The characteristics of ACh-induced calcium increases in these hair cells appear to correspond to the pattern of efferent innervation reported previously (Simmons *et al*., Auditory Neuroscience 1:183-193, 1995). Namely, ACh-induced calcium increases are seen mostly in tall hair cells from rostral region compared to somewhat shorter hair cells presumably from medial and caudal areas of the AP. Furthermore, it appears that ACh generates different patterns of calcium increase in rostral hair cells: 1) a diffused calcium increase limited to the basal portion of the hair cell, 2) a finite number of localized domains (hot spots) near the basal membrane in which the calcium increases are distinctly larger than the diffuse background, and 3) in a few cells, a modest global increase in calcium throughout the cell. For comparison, ionomycin-induced calcium increases in these same cells are generally global (i.e., uniform throughout the cell) with no indication of any "hot spots." So far, we have been able to record small and diffused calcium increases in response to ACh in only a few shorter hair cells of the AP, which readily responded to ionomycin with large increases in calcium. These findings suggest that rostral hair cells have at least nicotinic cholinergic receptors. ACh-induced calcium increases with somewhat similar features have previously been reported for mammalian outer hair cells (Doi and Ohmori, Hearing Research, 67:179-188, 1993). We are currently using α-bungarotoxin to localize the nicotinic receptors in AP hair cells.

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39 Early Onset Hearing Loss and Efferent Auditory Deficits in Mice Lacking α-Synuclein

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Synucleins are a family of 3 proteins (α, β, and γ) expressed throughout the central nervous system. They are thought to be integral to synaptic function though in what precise role remains to be defined. Previously, we have shown that all three synuclein family members are found within the cochlea and localize predominantly to the efferent auditory synapses in the base of the outer hair cells (OHCs). To further elucidate the role of synucleins in cochlear function, we investigated the inner ear of α-synuclein knock-out (KO) mice.

Auditory brainstem response (ABR) recordings demonstrated that α-synuclein KO mice develop a high frequency hearing loss from P2mo onwards. Further, distortion product otoacoustic emissions (DPOAE) studies demonstrate that OHC function is abnormal in mice from P2mo onwards. Basic histological studies and scanning electron microscopic study of the cochlea demonstrate degeneration of organ of Corti predominantly in the basal and mid turns, which mostly included OHC loss or dysmorphology in α-synuclein KO mice. Whole mount cochlea immunohistochemistry with synaptophysin showed α-synuclein KO mice had the abnormally larger but fewer efferent terminals than those in wild type littermates.

Together, these studies provide strong evidence that α-synuclein plays a role in either the development or maintenance of synaptic connections between efferent medial olivocochlear nerve fibers and outer hair cells. Further, ablation of α-synuclein may alter the synaptic function of efferent nerve endings predisposing to early onset hearing loss.

40 Real Time Synaptic Release Measurements from Auditory Hair Cells Using Dual Sine Wave Capacitance Measurements

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The ability to detect changes in cell surface area using capacitance measurements has been a powerful tool to study the dynamics of neurotransmitter release. A major limitation of existing single sine capacitance assessment techniques is the inability to measure release during conductance changes. We have developed a two sine capacitance method that measures synaptic release in real time. Capacitance changes measured with the two sine method had two components; a fast offset that changed with channel gating and a second Ca^{2+}-dependent component corresponding to synaptic release. The release component was removed by elevating intracellular Ca^{2+} buffering, allowing for the isolation and characterization of the first component. The first component correlated with Ca^{2+} current activation and was blocked by cadmium. Manipulation of the Ca^{2+} current revealed a correlation with gating charge and not current. Bay K 8644 increased the Ca^{2+} current, while slowing gating kinetics, thereby reducing the capacitance response. Ba^{2+} substitution increased the current amplitude but had no effect on gating charge and thus no effect on the capacitance response. The gating charge component was larger for high frequency cells than low presumably due to the greater number of Ca^{2+} channels in these cells. The first component was greatly reduced by increasing the sine wave frequency so as to make the Ca^{2+} channels insensitive to the stimulus while having no effect on the second, release component of the capacitance measurement. Similar results were obtained in turtle and mouse auditory hair cells. Release measurements with the two sine method are similar between sine wave frequencies and with single sine data. With the two sine method we have observed multiple kinetic components of release including depletion of the rapidly releasable pool. (Supported by NIDCD grants DC 000273 to JSS, DC003896 to AJR and DC008115 to AJR and JSS)

41 Characterizing Hair Cell Exocytosis in the Mammalian Utricle

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We here characterize depolarization induced exocytosis from hair cells in the rat utricle (P0-P6), using a semi-intact preparation. Hair cells were whole-cell voltage-clamped and the membrane capacitance (Cm) was determined from impedance measurements using a single sine wave of 1.5 kHz, 15 mV_{rms} amplitude superimposed onto the holding potential of -85 mV (baseline Cm: 6.0 ± .09 pF). Depolarizations elicited small, step-like increases in Cm and little endocytosis over a 10-30 second period. These Cm increases are calcium-dependent. Lowering the extracellular calcium concentration to 50 µM reversibly abolished exocytosis, and nifedipine (10 µM) also reduced exocytosis. Depolarizing pulses of varying amplitude (-70 to +60 mV) and constant duration revealed that the changes in Cm mirror the peak calcium current (maximal current at -10 mV: -70 ± 6 pA). Interestingly, the Cm increase is linearly related to the calcium current, suggesting that release at this hair cell synapse might involve calcium nanodomains, as previously observed in lower vertebrate and mammalian auditory hair cells. Altering the duration of the depolarization at a constant amplitude revealed two components of exocytosis: i) a fast releasable pool of 2-5 fF that was exhausted with a time constant of τ = 22-56 msec, followed by ii) a slower, sustained phase of ~ 17 fF/s. Our results demonstrate that mammalian vestibular hair cells obtained from young animals do indeed show calcium- and time-dependent exocytosis. The relatively low amplitude and slow kinetics of exocytosis in these hair cells might be due to the relatively low number of ribbon synapses, the age studied or intrinsic differences in the release process. Our next goal is to extend the study of vestibular hair cell exocytosis to an age when type I and type II hair cells can be clearly distinguished and the vestibular system is mature. [NIDCD: DC007678]

42 Vesicle Dynamics at the Ribbon Synapse of Mouse Inner Hair Cells Measured in Situ

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It has been previously shown that the styryl dye FM1-43 can be used to follow the dynamics of vesicle release at the ribbon synapses of cochlear inner hair cells (IHC) in guinea pig (Griesinger *et al.*, 2005). We have now applied similar techniques to study mouse IHCs in order to permit access to a wide range of transgenic models. Mouse temporal bones were attached to the base of a chamber superfused with artifical perilymph, and a small opening was made in the apical turn to expose the 10-20kHz region of the organ of Corti. There is no restriction on the age of the mouse. FM1-43 (2µM) was added to the bath whereupon it was taken up rapidly by hair cells from their intact apical surface, and could be imaged with 2 photon (2P, 840nm) laser scanning microscopy. Uptake was not significantly affected by agents designed to disrupt transduction, or in myo6 -/- mice in which transduction is compromised. Hotspots at sites corresponding to trafficking of dye to the synaptic ribbons on the basolateral membrane were identified after 300s and these spots were imaged with frame series at up to 40Hz and lines scans at 500Hz. On stimulation with extracellular current designed to depolarise the terminal, spots destained by up to 15% during 100ms 160µA pulses. The response was graded with amplitude and duration and followed a similar dynamics as previously recorded in the guinea pig, but depended critically on electrode placement. To investigate whether extracellular current was modulating the 2P fluorescence, IHCs were also recorded in whole cell mode *in situ* using 140 mM CsCl in the pipette to reduce the large outward K^+ currents present in these cells. Peak

inward calcium currents of 60-100pA (at -20mV) recorded in IHCs in 2mM extracellular Ca^{2+}, suggest that the cells were not compromised. Simultaneous FM1-43 2P imaging of presumed ribbon release sites held under voltage clamp further suggests that about 10-30 vesicles are released per site by a 100ms depolarizing command to -20mV delivered to the IHC. *Supported by Chaires Blaise Pascal and Eurohear LSHG-CT-20054-512063*.

43 Divergent Presynaptic Ca^{2+} Signaling Within Single Inner Hair Cells: A Candidate Mechanism for Heterogeneous Spiking Properties of Spiral Ganglion Neurons

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Transmitter release at the ribbon synapse of cochlear inner hair cells (IHCs) is regulated by Ca^{2+}. Here, we used fast confocal microscopy of Fluo-5N loaded murine apical IHCs to visualize Ca^{2+} signals of individual synapses. Hotspots of Ca^{2+} indicator fluorescence built up and collapsed within few milliseconds upon stimulus onset and cessation. These Ca^{2+} microdomains resulted from $Ca_V1.3$-mediated Ca^{2+} influx at ribbon-type active zones. Interestingly, the synaptic Ca^{2+} indicator fluorescence signals showed pronounced variability across different synapses within one IHC, with the largest scatter in the signal's amplitude. We explored putative mechanisms for the observed variability including differences of Ca^{2+} channel number, Ca^{2+}-induced Ca^{2+} release (CICR) and mitochondrial buffering. While differences in Ca^{2+} channel number can explain some of the variability, our data argue against a discernible contribution of CICR to the synaptic Ca^{2+} signals in mature mouse IHCs. We conclude that Ca^{2+} channel number and function are differentially regulated among active zones of a given IHC and propose this as a candidate mechanism for the divergent discharge properties observed in spiral ganglion neurons with comparable characteristic frequency.

44 Changes in the Exocytotic Calcium-Sensitivity at Inner Hair Cell Ribbon Synapses

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Synaptic vesicle fusion at IHC presynaptic active zones is triggered by Ca^{2+} entry through L-type ($Ca_V1.3$) Ca^{2+} channels (Platzer *et al* 2000, *Cell* 102:89-97) in response to either spontaneous Ca^{2+} action potentials (APs), which are intrinsic to pre-hearing cells, or sound-induced graded receptor potentials in adult animals (Kros *et al* 1998, *Nature* 394:281-4). In order for the synaptic machinery to follow reliably the different IHC receptor characteristics, ribbon synapses become more sensitive to Ca^{2+} upon maturation (Moser *et al* 2006, *JPhysiol* 576:55-62). We looked for changes in the functional and molecular composition of the synaptic machinery during development. Experiments were performed using both mouse and rat cochleae during pre-hearing and adult stages. Immunolabelling results indicate that the classical Ca^{2+}-sensing synaptotagmins are present in developing hair cells. Synaptic vesicle exocytosis was measured as an increase in cell membrane capacitance (ΔC_m) using the whole cell patch-clamp technique and under near physiological recording conditions (body temperature and 1.3 mM extracellular Ca^{2+}). Neurotransmitter release was directly modulated by synaptotagmin because of its effect on the Ca^{2+} sensitivity of vesicle exocytosis in adult hair cells. Our findings suggest that the different exocytotic Ca^{2+} sensitivity observed in mammalian cochlear hair cells is determined by synaptotagmins, which are differentially expressed as function of age and cochlear position.
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45 Otoferlin and Calcium-Dependent Exocytosis at Cochlear Hair Cell Ribbon Synapses

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Mutations in the gene OTOF, encoding otoferlin, underlie the non-syndromic prelingual deafness disorder DFNB9 (Yasunaga et al., 1999). This genetic disorder has been demonstrated to originate from a synaptic deficit at the inner hair cell (IHC) synapse (Roux et al. 2006). Indeed mice presenting a null mutation for otoferlin are deaf and show cochlear IHC lacking in calcium-evoked exocytosis. This large calcium binding protein with several C2 domains displays calcium dependent interactions with the SNARE complex proteins syntaxin-1 and SNAP25. Although the precise function of otoferlin remains to be elucidated, these results strongly suggest otoferlin as a calcium sensor, synaptotagmin-like, specific for cochlear IHCs. In recent experiments, we observed that otoferlin is also essential in outer hair cells (OHCs) afferent ribbon synapses that occur transiently during development (Beurg et al 2008). In the present study, we study in detail the kinetics and the calcium efficiency of vesicle exocytosis in both types of cochlear hair cells. The differential role of otoferlin in IHCs and OHCs will be discussed.

46 Otoferlin and Myosin VI, a Cross-Talk Between Two Proteins Involved in Human Hereditary Deafness, at the Auditory Hair Cell Ribbon Synapse

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The auditory inner hair cell (IHC) ribbon synapse operates with an exceptional temporal precision and can maintain a high level of neurotransmitter release in response to sustained stimulation. We have previously shown that otoferlin, a predicted C2-domain transmembrane protein, defective in a recessive form of human deafness is essential for a late step of synaptic vesicle exocytosis. We proposed that otoferlin may act as the major Ca^{2+} sensor triggering membrane fusion at the IHC ribbon synapse. Here we report the interaction of otoferlin with myosin VI, also known to be defective in different forms of human deafness. The interaction between otoferlin and myosin VI was revealed by a yeast two-hybrid screen and confirmed by coimmunoprecipitation experiments identifying these proteins in the same complex in cochlear sensory neuroepithelium extracts. In addition, using *in vitro* binding assays, we showed that this interaction was direct and Ca^{2+}-independent. Using confocal laser microscopy and immunogold electron microscopy, we showed that otoferlin and myosin VI were both present in the IHC synaptic region. Furthermore, we demonstrated that both synaptic maturation and Ca^{2+}-dependent exocytosis of the IHC were affected when myosin VI was absent. These data bridge otoferlin and myosin VI and led us to establish that myosin VI plays an important role in the IHC synaptic maturation.

47 Secondary Structure of Otoferlin Reflects Calcium-Dependent and -Independent Functions at the Hair-Cell Synaptic Complex

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Otoferlin is hypothesized to be a Ca^{2+} sensor within the hair-cell synaptic complex, potentially acting through Ca^{2+}-dependent binding to the target-SNARE proteins syntaxin 1A and SNAP-25, and to the $Ca_v1.3$ voltage-gated calcium channel (Ramakrishnan et al., Assoc. Res. Otolaryngol. Abstr. 31: 204, 2008). A new rabbit antibody to otoferlin (Kelley and Morley, Soc. Neurosci. Abstr. 32: 140.4, 2006) and goat antibody to $Ca_v1.3$ elicit immunofluorescence at overlapping sites within the basal region of inner hair cells, consistent with a functional interaction. Otoferlin contains six Ca^{2+}-binding domains, C2A-C2F, of which C2F appears to be most important for Ca^{2+}-dependent binding to syntaxin 1A. As measured by surface plasmon resonance (SPR), binding is absent without Ca^{2+}, but increases in 30 μM free Ca^{2+} to a maximum at 61 μM Ca^{2+}, then decreases by ~50% at 95 μM Ca^{2+} and remains reduced up to 371 μM Ca^{2+}. The mechanism for decreased C2F binding at higher Ca^{2+} concentrations is unknown, but may reflect electrostatic screening by Ca^{2+} that can change the local molecular environment. We observed that a Pro1825Ala mutation in C2F (termed C2Fm), known to cause DFNB9 deafness, almost completely abolishes interaction with syntaxin. Secondary structure of C2F indicates that Pro1825 is situated between β sheets 7 and 8, a location near the presumptive Ca^{2+}-binding region in the molecule. The tryptophan fluorescence spectrum of native C2F shows enhancement with Ca^{2+}, whereas that of C2Fm shows no Ca^{2+}-dependent enhancement. Since addition of Ca^{2+} does not increase the fluorescence intensity for C2Fm, the latter protein may not fold properly to bind Ca^{2+} and thus not bring tryptophan residues into optimal register. In contrast to the C2F results, SPR binding of the C2A domain to syntaxin 1A shows that Ca^{2+} is not necessary for interaction, suggesting that C2A, unlike C2F, may not contribute directly to Ca^{2+}-sensitive exocytosis at the mammalian hair-cell synapse.

48 Ca^{2+} Currents and Exocytosis in Inner Hair Cells of Otoferlin- And Myosin 6-Deficient Mice

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Mutations in the otoferlin gene lead to nonsyndromic autosomal recessive deafness DFNB9, and recent studies using an otoferlin knockout mouse model proposed the synaptic vesicle protein otoferlin to be the Ca^{2+} sensor for inner hair cells exocytosis (Roux et al., Cell, 2006). Myosin 6, a deafness gene itself in mice (Snell's waltzer mice; Avraham et al., Nature Genetics, 1995) and man (DFNA22; Melchionda et al., Am J Hum Genetics, 2001; DFNB37; Ahmed et al., Am J Hum Genetics, 2003) has been identified as an interaction partner of otoferlin in inner hair cells (Heidrych et al., in preparation). To test if lack of myosin 6 corrupts exocytosis, we analyzed voltage-activated Ca^{2+} currents and exocytosis in IHCs of myosin 6-deficient mice (Jackson Laboratories). For comparison, similar studies were performed in a new mouse model for otoferlin deficiency (Longo-Guess et al., Hear Res, 2007; obtained from Jackson Laboratories). Finally, immunohistochemistry was performed to analyse Ca^{2+} channels and synaptic proteins (e.g. the ribbon synapse protein CtBP2) in both types of mutants. These results are discussed in the context of the suggested role of otoferlin as the Ca^{2+} sensor for exocytosis.

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49 Otoferlin Interaction Partners

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One of the genes underlying hearing impairment in mice and humans is otoferlin. Mutations within OTOF lead to the recessive non-syndromal deafness DFNB9. Several studies have indicated that otoferlin is associated with ribbon synapses of cochlear sensory hair cells. Otoferlin was proposed to be the Ca2+ sensor for exocytosis in IHCs, as exocytosis was absent in otoferlin-deficient mice (Roux et al., Cell 2006). Other studies have shown that otoferlin is also present in various neurons, nerve fibers and in the entire cytoplasm of hair cells, suggesting a more ubiquitous function (Schug et al., Eur J Neurosci 2006). We recently noted otoferlin's absence in spite of functional exocytosis in hypothyroid animals posing the question if other Ca2+-sensing proteins were upregulated under hypothyroid conditions (Brandt et al., J Neurosci 2007). Molecular studies were therefore performed to identify possible candidates substituting for the supposed Ca2+ sensor function of otoferlin in hypothyroid conditions. These included the search for otoferlin binding partners using a yeast two-hybrid screen and mass spectroscopy. We identified Rab8b (Heidrych et al., Hum Mol Genet 2008) and myosin VI as otoferlin interaction partners, and the interactions were verified by co-expression, co-localization and co-immunoprecipitation studies. Results will be discussed in the context of the presumptive role of otoferlin for exocytosis in inner hair cells.

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50 Developmental Changes in Exocytosis from Apical and Basal IHCs of the Gerbil Cochlea

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Immature inner hair cells (IHCs) of the mammalian cochlea fire spontaneous action potentials whereas mature cells respond to sound with graded receptor potentials. IHC depolarization leads to the activation of $Ca_v1.3$ L-type Ca^{2+} channels (Platzer *et al* 2000, *Cell* 102:89-97) that cause the Ca^{2+}-dependent release of synaptic vesicles (Moser & Beutner, 2000, *PNAS* 97:883-8). Since IHCs respond differently before and after the onset of hearing and as a function of frequency position, we investigated whether the properties of synaptic vesicle exocytosis change accordingly.

Exocytosis was studied by monitoring changes in membrane capacitance (ΔC_m) in IHCs along the gerbil cochlea (P5-P69) using whole cell patch-clamp in near physiological recording conditions. Immature apical and basal IHCs showed a similar high-order (power of 4) Ca^{2+}-dependence of exocytosis. Following functional maturation adult high-frequency IHCs showed ΔC_m that increased linearly with the Ca^{2+} current, as previously described in the mouse (Johnson *et al* 2005, *J Physiol* 563, 177-91), while in low-frequency cells the Ca^{2+} dependence was significantly more non-linear (power of ~2). This tonotopic difference seemed to be correlated with ribbon synapse morphology (spherical in apical and ellipsoid in basal IHCs) but not with the spatial coupling between Ca^{2+} channels and active zones.

The results presented here provide the first evidence for a position-dependent difference in the development of IHC synaptic transmission. A possible reason for a tonotopic difference in the Ca^{2+}-dependence of vesicle exocytosis in mature mammalian IHCs could be to support the different receptor characteristics of the cells upon sound stimulation.

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51 Serotonin 5-HT3 Receptor Expression in the Mammalian Cochlea

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A role for serotonin as a neurotransmitter at the auditory periphery was originally predicted from the determination that mRNA for multiple 5-HT receptors is expressed in microdissected organ of Corti and spiral ganglion cochlear subfractions (Oh et al., Mol. Brain Res. 70: 135-140, 1999). We and others have obtained evidence of serotonin-positive nerve fibers passing within the organ of Corti ascribed to olivocochlear lateral efferents (Bartolomé and Gil-Loyzaga, Int. Tinnitus J. 11: 119-125, 2005) which, in addition to making contact with type I afferent dendrites, cross the tunnel of Corti to the outer hair cells. The focus of the present study was to determine the expression and localization in the cochlea of the 5-HT3 receptor, the only ligand-gated serotonin receptor, characterized by a pharmacological profile overlapping that of the α9/10 nicotinic receptor. Message for 5-HT3a but not 5-HT3b was found, by RT-PCR, in both the organ of Corti and spiral ganglion microdissected cochlear subfractions, consistent with expression of a homomeric 5-HT3a receptor/channel by cells of the organ of Corti and by afferents in the spiral ganglion. These experiments did not address whether there is additional expression in efferent nerve fibers with mRNA residing in cell bodies at a distance from the organ of Corti. 5-HT3 protein has been immunolocalized within the organ of Corti to neural sites above the habenula perforata and at the base of the outer hair cells. Immunofluorescence appeared in small-diameter nerve fibers associated with the reticular lamina close to, but distinct from, supranuclear expression of the serotonin transporter (SERT), a presynaptic serotonergic marker. Therefore, 5-HT3 may be a post-synaptic target of 5-HT, present in nerve fibers with an appearance not dissimilar to that of adrenergic fibers containing dopamine β-hydroxylase, the enzyme of synthesis of norepinephrine (Drescher et al., Neuroscience 142: 139-164, 2006).

52 A Presynaptic Glutamate Transporter at Vestibular Calyx Synapse

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Glutamate clearance from synaptic zones is crucial for shaping synaptic transmission and preventing excitotoxicity. This is particularly true in sensory organs that use synaptic ribbons to facilitate continually high rates of neurotransmitter release. In the case of most hair cells, clearance is the responsibility of the glutamate transporter EAAT1 (GLAST) expressed by the supporting cells. A similar mechanism cannot work in type I hair cells because the calyx ending separates the synaptic zone from supporting cells. Based on this arrangement, it has been conjectured that a glutamate transporter must be present in the type I hair cell, the calyx ending, or both. By combining RT-PCR and immunocytochemistry, we demonstrate that EAAT5, a glutamate transporter previously only found in the retina, is selectively expressed in type I hair cells. Its presence is confirmed electrophysiologically by whole-cell recordings from type I hair cells of a non-stochiometric chloride current that is blocked by the non-transportable EAAT antagonist, DL-TBOA. There is a striking similarity of our findings to those in the retina, where EAAT5 is localized presynaptically in association with ribbon synapses. Because EAAT5 is expressed in the vestibular endorgans but not in the cochlea, it may offer novel opportunities for controlling vestibular transmission without affecting auditory function. (Supported by grants from the Centre National d'Etudes Spatiales, the French Ministry of Research and New Technologies, and the NIDCD).

53 Characterization of the Alpha Tectorin C1509G Mutation in Mice

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The C1509G mutation in tectorial membrane protein alpha-tectorin results in autosomal dominant partial congenital hearing loss in humans. To identify the pathophysiology, we recreated this mutation (knock-in) in the mouse Tecta gene. Plastic-embedded cochlear cross-sections demonstrated that the tectorial membrane was shorter in heterozygotes and was elevated off of the epithelium in homozygotes. Imaging of living excised whole cochleae revealed that the tectorial membrane overlaid all rows of outer hair cells (OHCs) in wild-types but only one row in heterozygotes. Auditory brainstem responses demonstrated a 25-40 dB threshold elevation in heterozygotes and a 30-50 dB elevation in homozygotes. Distortion product otoacoustic emission (DPOAE) studies revealed a 20 dB threshold elevation between 10-40 kHz in heterozygotes. Homozygotes had no reliable DPOAE to equipment limits. Noise-induced hearing loss studies showed a recoverable DPOAE threshold shift in wild-types, but a permanent threshold shift in heterozygotes at 40-55 kHz. Cochlear microphonic (CM) responses to 6 kHz sine wave stimuli were reduced by 10 dB in heterozygotes and by 35 dB in homozygotes. Additionally, the CM response was in-phase with the stimulus in wild-type mice, but led the stimulus by 90 degrees in the homozygote mice. In heterozygotes, the CM response was in-phase with the stimulus at lower intensities, but developed a progressive phase lead as the intensity increased. We conclude that the C1509G mutation affects tectorial membrane-OHC interactions; this is consistent with a model whereby the heterozygote tectorial membrane attaches to one row of OHCs, and the homozygote tectorial membrane does not attach to any rows. As a result, the heterozygote mouse shows congenital hearing loss and noise-induced progressive hearing loss that is consistent with the human phenotype.
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54 The Identity of the Cochlear Amplifier in Mammals

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Cochlear amplification is the mechanism responsible for the high sensitivity, sharp tuning and wide dynamic range of the mammalian cochlea. The basis of this phenomenon is mechanical feedback performed by the sensory-motor outer hair cells (OHC). The feedback amplifies low-level and compresses high-level basilar membrane (BM) vibrations at a characteristic frequency (CF) for each location along its length. The two candidates for the cochlear amplifier are prestin-based somatic motility and Ca++-dependent hair-bundle motility. The later is a ubiquitous process in vertebrate auditory hair cells, while the former is unique to the mammalian OHCs. We aimed to identify the relative contribution of these two mechanisms to cochlear amplification in mammals, a question that has been under debate in the field for more than 20 years. We measured acoustically- and electrically-evoked BM displacements in wild type and TectadeltaENT/deltaENT mice by focusing the beam of a self-mixing laser interferometer through the round window in the 60-65 kHz region of the BM. TectadeltaENT/deltaENT mice have a vestigial tectorial membrane detached from the OHC hair bundles, precluding the bundles from introducing force into the vibrations of the BM at low levels of stimulation. Sound-evoked BM vibrations in TectadeltaENT/deltaENT mice are insensitive and can drive the OHC bundles only through fluid coupling at high levels of stimulation. However, extracochlear electrical stimulation of TectadeltaENT/deltaENT cochleae, directly driving OHC motility, generates BM responses that are similar in sensitivity and frequency tuning to those of their wild-type siblings in response to either acoustic or electrical

stimulation. Electrically-evoked BM responses are suppressed by the prestin blocker salicylate in wild type and TectadeltaENT/deltaENT mice. We conclude that prestin-based somatic motility and not hair-bundle motility is the mechanism underpinning amplification in the basal region of the cochlea in mammals.

55 Phase Differences of Distortion Products Between the Basilar Membrane and Stapes

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Direct measurements of basilar membrane (BM) vibration as a function of the longitudinal location show that the phase of the two tone-induced distortion product (DP) decreases with the distance from the cochlear base. BM and stapes vibrations measured as a function of frequency indicate that the DP arrives at the stapes earlier than at the measured BM location. Data also show that the delay of the DPs at the stapes is equal to or smaller than the forward traveling wave delay. These observations indicate that the DPs propagate from their generation locations backward to the stapes dominantly as compression waves through the cochlear fluids. A recent study, however, showed that the delay of the DP otoacoustic emission is greater than that of the intra-cochlear DP pressure, which has been considered as supporting evidence for the backward traveling wave theory. To address this discrepancy in the experimental data, DPs were measured as BM and stapes vibrations and sound pressure in the ear canal in this study. Two tones at frequencies f1 and f2 (f2>f1) were presented to sensitive living gerbil cochleae when the f2/f1 ratio kept constant. Phase differences between the BM and stapes vibrations show that DPs arrive at the stapes and in the ear canal earlier than at the measured BM locations. In addition, the pattern of the DP phase transfer function from the stapes to the BM is very similar to that in response to external tones.

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56 Wave-Interference Patterns on the Basilar Membrane: Testing Models of OAE Propagation

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At low stimulus levels, measurements of basilar-membrane (BM) mechanical transfer functions reveal a quasiperiodic rippling pattern (Rhode, 2007; J. Acoust. Soc. Am. 121, 2792) suggestive of wave interference, perhaps due to otoacoustic emissions (OAEs). Both slow- and fast-wave models of OAE propagation predict that BM interference patterns result when an evoked stimulus-frequency (SF) OAE on its way to the ear canal reflects off the stapes, launching a secondary forward-traveling wave that combines with the primary wave produced by the stimulus. Frequency-dependent phase differences between the two waves then create a characteristic rippling pattern measurable on the BM. The BM rippling pattern is analogous to the acoustic interference pattern seen in ear-canal pressure when measuring SFOAEs.

The fast-wave model predicts that the frequency spacing between adjacent BM interference maxima should be approximately equal to the reciprocal of the near-CF BM delay. The slow-wave model, by contrast, predicts a substantially narrower frequency spacing due to the additional delay incurred by reverse slow-wave propagation. We test these predictions, and the otoacoustic origin of the BM rippling pattern, using near-simultaneous measurements of BM motion and ear-canal OAEs in the same animals.

Our preliminary measurements suggest that the interference ripples are spaced at intervals significantly narrower than predicted by the fast-wave model. This conclusion is supported by an unmixing analysis that disentangles the two putative interfering components.

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57 Responses to "Local Damaged" Gerbil Cochlea Support Two-Component Distortion Product Otoacoustic Emission Frame Work

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Distortion product otoacoustic emissions (DPOAEs) originate in cochlear nonlinearity. DPOAEs exhibit fine structure in amplitude, and a phase-vs-frequency slope that can be either flat or steep. These characteristics have led to a two-component model for the source of DPOAEs: nonlinear generation and linear reflection. With stimulation at fixed f2/f1 ratio, the two DPOAE components can be differentiated by their phase-vs-frequency slopes: Nonlinear generator DPOAEs have a flat phase, which in cochlear theory is traced to local scaling invariance. Reflection source DPOAEs show a phase that changes rapidly with frequency, and in theory are caused by reflections from a random pattern of pre-existing micromechanical impedance perturbations on the cochlear partition. Several emission studies have attempted to un-mix the two components and the results generally support the two-component framework. However, some results have been less supportive, warranting further exploration of the source of DPOAEs.

The present study explores intracochlear sources of DPOAEs via both ear canal and intracochlear pressure measurements. A locally damaged gerbil cochlea was produced by indenting the cochlear partition with the pressure sensor, which led to the diminishment of local cochlear nonlinearity and significant elevation of the CAP thresholds around the best frequency (BF) of the damaged region. The comparison of ″healthy″ to ″locally damaged″ cochlear conditions showed: (1) In the broad frequency region corresponding to the locally damaged BF, DPOAEs often had a bimodal change, decreasing in a lower frequency band and increasing in a band just adjacent and higher. (2) In the damaged cochleae DPOAE phase often varied more rapidly versus frequency (reflector type). These changes suggest that the local damage introduced a relatively large mechanical perturbation, which led to an increase in reflector emission, even though the damage caused a decrease in nonlinear generation.

58 Measurement of the Mechanical Properties of Soft Samples at Acoustic Frequencies

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The tectorial membrane (TM) has been shown to have a critical role in hearing sensitivity and frequency selectivity. The main role of the TM in hearing is determined by its mechanical interaction with the tips of the stereocilia of the OHC. Therefore, a number of studies have used specialized probes such as Atomic Force Microscopy (AFM) or Magnetic Tweezers to characterize the mechanical properties of the TM. However, all these studies have been performed at quasi-static or moderate frequency regimes (0.1 to 9 kHz), only partially overlapping the frequency range of mammalian hearing. Moreover, these studies disregarded the fact that different parts of the TM are excited at different frequencies. The aim of this work is to develop a novel technique to measure the mechanical properties of samples at acoustic frequencies. This new approach consists in oscillating a micron-sized sphere in close proximity to a compliant surface. The oscillation displays resonance peaks at several frequencies. These peaks exhibit a small frequency shift when the sphere is moved away from the compliant surface. In this study, we analytically prove that this frequency shift depends on the Young's modulus (E) of the sample. The initial part of the study has been performed on polyacrylamide gels with E ranging from 1KPa to 50KPa. Oscillation peaks at ~30 KHz were tracked. When the oscillating sphere was displaced 100 nm in the vertical direction, frequency shifts were measured, ranging between 0.1 Hz/nm for the softest gel to 0.54 Hz/nm for the stiffest gel. Agreement between E values at acoustic frequencies and quasi-static frequencies was assessed by standard AFM force-indentation measurements. This technique will be used to compute the Young's modulus of the TM at acoustic frequencies. Furthermore, the resonance peaks will be chosen to reproduce the frequencies at which each part of the TM is excited during hearing.

59 Regulation by the Tectorial Membrane of Calcium Concentration in the Subtectorial Space

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The mechanical properties of the tectorial membrane (TM) are known to be crucial for normal function of the cochlear amplifier. Being a polyelectrolyte gel, the chemical, mechanical, electrical and osmotic properties of the TM have been shown to be inextricably linked. It has also been proposed that the TM might act as an ionic buffer. Since mechanoelectrical transduction (MET) in the hair-cell stereocilia is strongly dependent on $[Ca^{2+}]$, it is not inconceivable that the TM might act as a $[Ca^{2+}]$ buffer. Therefore, here, we present a model of $[Ca^{2+}]$ dynamics in the subtectorial space which highlights the possible role of the TM as a regulator of extracellular $[Ca^{2+}]$ in the region of the stereocilia.

For $[Ca^{2+}]$ dynamics in the stereocilium, the one-dimensional diffusion model introduced by Lumpkin and Hudspeth (1998) was modified to represent the stereocilium of an outer hair cell. Extracellular $[Ca^{2+}]$ dynamics in the surrounding subtectorial space was simulated in three dimensions using Fick's law of diffusion in a cylindrical coordinate system. A calcium buffer was placed at the lower surface of the TM, adjacent to the subtectorial space. Local $[Ca^{2+}]$ transients were produced in this extracellular space by modulation of the open probability of the MET channel in the stereocilium. Using buffer concentrations and rate constants for the stereocilium similar to those in the LH-model and an ambient extracellular $[Ca^{2+}]$ of 30 μM, the extracellular transient in the region of the channel can be as much as 7 μM in the absence of a TM buffer, but an order of magnitude smaller in the presence of the buffer. We found an inverse relation between the dissociation constant of the TM buffer and its concentration that yielded an ambient $[Ca^{2+}]$ of 30 μM.

In conclusion, we show that during transduction a TM calcium buffer, together with a stable $[Ca^{2+}]$ within the TM, can maintain the $[Ca^{2+}]$ in the extracellular fluid near its ambient level.

60 Cochlear Responsiveness to Frequency-Independent Constant-Velocity Direct Mechanical Stimulation of the Round Window (RW) with the Otologics MET-V Transducer

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Appropriately-delivered mechanical stimulation of the cochlea via the RW may elicit physiological responses similar to acoustic stimulation. We examine here whether cochlear responses to direct RW stimulation by the Otologics MET-V (MET) transducer was linear and frequency independent and solely determined by the physical drive applied (volume velocity). In 6 adult chinchillas, the MET (0.5mm ball-tip) was loaded onto the RW via micromanipulator. Cochlear microphonic (CM) and compound action potential (CAP) were measured after calibrated sinusoidal stimuli from 0.25-14 kHz were delivered via insert earphone. Stapes and MET ball-tip velocities were measured by laser Doppler velocimetry. Frequency-dependent CM/CAP thresholds ranged from 16-50 dB SPL for acoustic and 0.2-56 mV for RW stimulation. For acoustic stimulation, stapes motion showed typical velocities for chinchilla with maxima at 700 Hz and a notch at 2.65 kHz. Stapes velocity demonstrated a maximum at 2-3 kHz when either the malleus or RW was driven with the MET. When normalized for stapes velocity, CM and CAP thresholds where nearly independent of stimulus frequency for both acoustic and for stimulation of the malleus with the MET. However, for MET stimulation

of the RW, thresholds decreased by ~10 dB above 4 kHz despite normalization by ball-tip velocity. When a constant ball-tip velocity for all frequencies was produced by applying a FIR filter to the driving voltage, the decrease in CM thresholds above 4 kHz remained. Stapes velocity remained constant at all test frequencies confirming the filter-dependant performance of the transducer tip velocity and the linear relationship between stapes and ball-tip velocity. The dynamic range of the MET transducer and the increased sensitivity of the cochlea to high frequency RW drive are likely to be important properties for the clinical application of the device in mixed hearing-loss patients.

61 Surgical Approaches to the Middle and Inner Ear of Laboratory Rats: Advantages of Retroauricular vs. Ventral Approach

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Background: The rat has become a popular species for middle ear and inner ear research due to its well-characterized genome that contains homologous genes in common with those of the human. A successful surgical approach to the rat ear would help substantially the development of new method of drug delivery and hearing preservation. However the rat ear is difficult to access surgically because of the small size of the middle and inner ear. The objective of this study is to describe the retro auricular and ventral approaches to the rat ear and illustrate the advantages and disadvantages of each method.

Material and Methods: All surgeries are perfored under ketamine and xylacine intramuscular injection.The surgeries are perfomed under operating microscope. Retro auricular and ventral dissections of rat middle and inner ear were completed and photographed. The difficulty, accessibility to middle and inner ear structures, and anatomic relationships were compared between the two approaches.

Results: The retro auricular approach is an easier method of access to the inner ear. The cochlea turnes in a clockwise manner when approached by this method. This is helpful in surgeries that involve the inner ear, such as cochlear implantation. The ventral approach to the bulla is the more challenging approach because we need to retract more anatomical structures before we access the bulla, but allows better exposure of the rat middle ear contents. This approach is better for surgeries involving the middle ear structures.

Conclusions: We can access the bulla and ear structures of the rat through the retro auricular or ventral approach. The former is preferable for inner ear surgery and the latter is better for middle ear surgery.

62 Inverse-Solution Method for a Feed-Forward Cochlear Model

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Measurements of Distortion-Product waves (DP waves) inside the cochlea have shown dominance of a forward over a reverse wave. This finding is at variance with the “classical” type of cochlear model where both types of wave are equally important. Of the alternatives that have been proposed to remedy this situation the feed-forward model is one of the most promising. This poster describes a method to apply the inverse solution to this model, with the aim to attain a feed-forward model that accurately reproduces a measured response. It is demonstrated that this approach is highly successful both for single tones and for DP waves generated by double tones. It is shown that in a feed-forward model with nonlinear distortion DP waves are almost exclusively forward-traveling waves which property agrees with the nature of experimental findings. Finally, it is shown how the solution method can be generalized to models with more complex BM dynamics. A remaining problem is how to integrate the extended feed-forward model with the principle of coherent reflection.

63 An Extended Three-Dimensional Model of the Basilar Membrane of the Guinea Pig and Implications for Cochlear Mechanics

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Within the last decades, many models of the basilar membrane (BM) of different species have been developed. Starting with von Bekesy’s beam model to represent a radial cross section of the BM up to complex plate models which represent a longitudinal section of the BM, including orthotropic material properties and surrounding fluid, have been investigated. Here, a more complete finite-element model of the entire BM is presented.

The model, developed for the guinea-pig cochlea, is based on anatomical and static mechanical measurements. It includes the complex coiled structure from base to nearly the apex and is based on micro-CT scans and histological slices. Furthermore, the arcuate zone (AZ) and the pectinate zone (PZ) have been treated individually; the gradients of width and thickness of collagen fibre bundles within the PZ have been taken into account.

The model has been successfully tested by showing a good agreement with experimental stiffness data (Gummer et al., 1981; Naidu & Mountain, 1998).

By including micro-structural features, a change in the characteristics of the radial stiffness profiles from base to apex can be observed. Whereas the minimal stiffness in a radial profile in the basal zone of the BM is found close to

the basal ends of the outer pillar cells, this value is shifted to the midpoint of the PZ in the apical part of the BM. This shift occurs close to the 1-kHz characteristic place. This observation leads to the prediction that the mechanical excitation mechanism in the apical region of the organ of Corti is different to that in the basal region. Evidence for a base-apical dichotomy in mechanical excitation mechanisms has already been found experimentally in neuronal (Tsuji & Liberman, 1997) and vibration (Zinn et al., 2000) data.

64 A Cochlear Wave Propagation Model Including Fluid Flow Within the Organ of Corti

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A common approximation in cochlear (inner ear) studies is to treat the organ of Corti (OC) as a single structure called the cochlea partition. The OC, however, is in reality a very complex structure made up of many different cell types and contains significant extracellular fluid-filled spaces. It is likely that fluid flow within these spaces plays an important role in cochlear function [Karavataki and Mountain, Biophys. J., 92:3284, 2007].

In order to access a realistic in-vivo mechanism within the OC it is desirable to represent the detailed structural arrangement within it. We have developed a reduced 3D hybrid numerical-analytical model for wave propagation in the cochlea based on the periodic arrangement of cells within the organ of Corti. Floquet's theory [L. Brilluoin, Wave propagation in periodic structures (Dover, New York, 1953)] of wave propagation in periodic structure is used to capture the fast scale variations while a WKB analysis of wave in media with slowly varying properties is used to resolve slow longitudinal variations. The model is aimed at investigating both the passive and the active in-vivo mechanics of the OC, especially the importance of transverse fluid flow within it for cochlear amplification.

We present and discuss the intricacy of the model and preliminary results from selected 3D short section solutions for the basal, middle and apical turn of a gerbil cochlear model. The resulting location-dependent dispersion relations and wave modes are used to form more general wave solutions within the entire cochlea.

65 Simulating the Effects of Experimental Conditions, Viscosity, and Compressibility on Cochlear Mechanics Measurements

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Experimental measurements of basilar membrane velocity using laser interferometry typically require creation of an optical path to the measurement location through either a hole in the bony wall or by opening the round window (Robles *et al.*, *Physiol. Rev.*, **81**(3)). Such interventions change the boundary conditions of the cochlear channels – do they affect the basilar membrane response to acoustic stimulation? A 3-dimensional finite element method (FEM) model including an opening on the boundary has been used to simulate the experimental conditions. Results show that the existence of a small hole will reduce the total amplitude and phase accumulation of the intracochlear pressure but does not change the decay pattern of the pressure as a function of height above the basilar membrane (BM) as studied experimentally by Olson (*J. Acoust. Soc. Am.*, **103**(6)). A hole located at the base is found to have large influence on the fast wave motion but not on the slow wave or the BM velocity. A hole located at the apex is found to have significant effects on both the fast and the slow waves, consistent with difficulties found in taking measurements at the apex (e.g., Zinn *et al., Hear. Res.,* **142**). We studied the importance of the viscous boundary layer at the BM and walls of the cochlea. The viscous boundary layer is found to have a greater impact on the BM velocity at the apical rather than basal locations. Compressibility is found to influence the fast wave most and have little effect on the slow wave. Hence the fluid compressibility does not appear to affect the BM velocity.

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66 Mechanical Properties of Outer Pillar Cells of the Organ of Corti

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Outer pillar cells (OPC) are important mechanical elements of the organ of Corti. To understand the role of the OPCs in the vibration response of the organ, we have developed a finite-element model of these cells.

There are two basic problems associated with this endeavor. First, the cytoskeleton of the OPC containing bundles of microtubules forms a three-dimensional anisotropic material. Second, being such a structure, the calculated displacement responses can depend on both the loading condition and assumptions regarding anisotropy. For example, although it is possible to choose material parameters such that isotropic and anisotropic materials yield the same responses for axial loading, the responses can differ by up to two orders of magnitude for other loading conditions, such as fluid pressure, momentum or transverse forces. In other words, a three-dimensional anisotropic model is required that is valid for the different loading conditions in the cochlea.

Clearly, it is not possible to represent the microtubules with a finite-element model at the molecular level. We have solved this problem by developing an overall homogenous, anisotropic material model of the OPC. We used the method of homogenization to describe the anisotropic material. This method makes use of the equality of the intrinsic strain energy of a periodical representative volume to calculate anisotropic material parameters for an effective, homogenous material from basic strain states.

We idealize the OPC as a cylinder with z-axis in the axial direction and calculate material parameters close to transversal isotropy E_z=523MPa, E_x=E_y=0.24MPa, G_{xy}=0.59MPa, G_{xz}=G_{yz}=0.12MPa, v_{xy}=0.92 and v_{xz}=v_{yz}=0.32, where E are the Young's-moduli, G the shear-moduli and v the Poisson ratios, respectively.
We successfully tested the model by showing that it yields stiffness values that are in the range of those measured by Tolomeo and Holley (1997).

67 Predicting the Role of Hair Bundle Motility in Cochlear Mechanics

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Recent experimental findings from Fettiplace *et al.* (*Nature,* **433**) support the existence of force generation by the hair bundle (HB) of the mammalian outer hair cell (OHC). The hypothesis that hair bundle force generation contributes to cochlear amplification challenges the prevailing theory that somatic outer hair cell motility is the only force generator. We investigate the potential role of hair bundle motility as a mediator of cochlear amplification. A nonlinear model describing the motion of active hair bundle is linearized for small harmonic motion around the operating point of the transduction channel. In this linear model, the transduction channel acts as a frequency filter and the HB generates a frequency dependent force described by an active stiffness and an electromechanical coupling coefficient. The parameters of the model are estimated based on experimental measurements of active hair bundle motion. HB force generation is then incorporated into a macroscopic mathematical model of the guinea pig cochlea based on a 3D-fluid representation coupled to an electromechanical model of the cochlea including OHC somatic motility (Ramamoorthy *et al., J. Acoust. Soc. Amer.* **121**(5)). The effects of both hair bundle motility and OHC somatic motility can be tested independently in the model by predicting the response to acoustic stimulation. Our results show that HB motility can work in synergy with the OHC somatic motility to boost the basilar membrane response but do not possess sufficient authority to drive cochlear amplification alone.

68 Bias-Tone Effects on Auditory-Nerve Responses Reveal Three Mechanical Drives, Two Dependent on Outer-Hair-Cell Motility and One Passive

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The processes by which bulk movements of the organ of Corti, e.g. the traveling wave (TW), bend inner-hair-cell stereocilia and excite auditory-nerve (AN) fibers are poorly understood. Recent evidence indicates that AN initial peak (ANIP) responses to moderate-level clicks are inhibited by medial olivocochlear (MOC) efferents. At click levels <100 dB SPL, rarefaction clicks evoke the ANIP response (ANIPr response), but at higher click levels condensation clicks evoke ANIP responses (ANIPc response). MOC efferents inhibit ANIPr responses but not ANIPc responses. To gain insight into the production of ANIP responses, we used low-frequency tones "bias" tones.
"Calibrated" bias-tone levels were chosen that produced twice-a-bias-tone-cycle suppression of responses to low-level clicks (levels where the TW cochlear-amplifier (CA) is operating). Presumably, these bias tones reduced CA gain by bending outer-hair-cell (OHC) stereocilia into low-slope saturation regions. Twice-a-bias-tone-cycle suppressions are taken to indicate that the response depends on the slope of the OHC receptor-current to stereocilia-angle ratio, not the stereocilia angle, and that stereocilia-modulated OHC motility is involved. "Calibrated" bias tones produced twice-a-cycle suppression of ANIPr responses (so OHC motility is involved), but produced a sinusoidal modulation of ANIPc responses (consistent with passive sinusoidal motion modulating the response). These data are consistent with AN fibers being excited by three mechanical drives: (1) amplified traveling waves, as shown by MOC inhibition and bias-tone twice-a-cycle suppression of low-level click responses, (2) an ANIPr drive, which also shows MOC inhibition and bias-tone twice-a-cycle suppression, but is not accounted for by the TW because the TW first peak, as shown by basilar-membrane motion, is not MOC inhibited, and (3) an ANIPc drive, whose properties are consistent with it being passive.
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69 Medial Olivocochlear Efferent Stimulation by Contralateral Noise Induces Similar Changes in Stimulus-Frequency and Click-Evoked Otoacoustic Measurements of Auditory-Filter-Related Delays

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It has been reported that stimulating medial olivocochlear (MOC) efferent neurons with contralateral noise reduces the delay in the fine-structure of human click evoked otoacoustic emissions (CEOAEs). System theory states that widening a filter decreases its phase-gradient delay, or equivalently in time, its narrow-band input-output delay. Thus, the observed MOC-induced reduction of CEOAE fine-structure delays may be due to the widening of auditory filters from an MOC-induced reduction of cochlear amplification. Similarly, these changes should be reflected in stimulus frequency otoacoustic emission (SFOAE) phase-gradient delays. To test this, we recorded SFOAEs with and without 60 dB SPL contralateral broad-band noise from 16 subjects. SFOAE phase-gradient delays were obtained from the negative slope of a linear fit to the SFOAE phase-versus-frequency functions. To make a comparison with the effects of contralateral noise on auditory filter outputs in the time-domain, we measured CEOAE envelope delays from 21 subjects (including all SFOAE subjects) with and without contralateral noise. To

obtain good spectro-temporal resolution, a wavelet analysis of the CEOAEs was used to extract envelopes from 500 Hz-wide frequency bins. Delay changes were acquired by cross-correlation of the frequency-binned CEOAE envelopes with and without contralateral noise. We found that the CEOAE temporal delays and SFOAE phase-gradient delays were similarly affected by contralateral noise: above 1.5 kHz both delay changes were distributed around zero, while below 1.5 kHz the change distributions indicated a small reduction in delay. These data suggest that auditory filter bandwidths at low characteristic frequencies may be more sensitive to MOC stimulation by contralateral noise than filters at higher characteristic frequencies.

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70 Effect of Contralateral Acoustic Stimulation in Cochlear Sensitivity in *Chinchilla Laniger*

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Outer hair cells of the mammalian cochlea receive a significant efferent innervation through the medial olivocochlear (MOC) system, comprising crossed and uncrossed fibers that originate in the region of the superior olivary complex. Significant species differences have been found in the distribution of crossed and uncrossed MOC fibers; with the highest ratio of crossed/uncrossed fibers (4:1) found in chinchillas among rodents. Besides this anatomical evidence, absence of suppressive effects of contralateral acoustical stimulation has been reported in this species. We have measured the effect in cochlear sensitivity of the activation of olivocochlear efferents, through acoustic stimulation of the contralateral ear, in 10 adult chinchillas (*Chinchilla laniger*) anesthetized with xilazine/ketamine. VIIIth-nerve compound action potentials (CAP) were acquired with a round-window electrode in response to acoustic stimuli at different frequencies and intensities. Stimuli were presented in 3 consecutive series of 64 trials, first ipsilateral stimulation alone, then contralateral stimulation (500 ms) followed (after 5-10 ms) by ipsilateral stimulation (15 ms) and finally ipsilateral stimulation alone. Contralateral acoustic stimulation produced amplitude reductions in CAPs elicited by ipsilateral tones at frequencies ranging from 1 to 9 kHz that reached up to 14 dB. Contralateral acoustic stimulation also produced concomitant increments in CAP latencies. In all cases ipsilateral CAP reductions caused by contralateral acoustical stimulation were frequency tuned to the ipsilateral stimulus frequency; that is, CAP reductions were maximal for contralateral stimulus frequencies close or equal to those of the ipsilateral tone. These results show the existence of a tonotopic distribution of efferent olivocochlear fibers that reach most of the extent of the chinchilla cochlea.

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71 Effects of Medial Olivocochlear Efferent Activation on Cochlear Potentials of the Chinchilla

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The mammalian cochlea has two types of sensory cells, inner hair cells, which receive VIIIth-nerve afferent innervation, and outer hair cells, innervated by efferent axons of the medial olivocochlear (MOC) system. The role of the MOC innervation in hearing is still controversial. Recently we demonstrated -by recording cochlear potentials in behaving chinchillas- that one of the functions of the efferent system is to reduce cochlear sensitivity during attention to visual stimuli (Delano, PH, Elgueda, D., Hamame, C.M. and Robles, L. J. Neurosci. 27:4146-4153, 2007). In spite of these compelling results, the physiological effects of electrical MOC activation on cochlear potentials have not been described in detail in chinchillas. Here we activated the MOC efferent axons in 12 chinchillas by applying current pulses with a bipolar electrode placed at the fourth-ventricle floor. VIIIth -nerve compound action potentials (CAP) and cochlear microphonics (CM) were acquired in response to clicks and tones of several frequencies. Electrical efferent stimulation produced CAP-amplitude suppressions reaching up to 12 dB along with latency increments of up to 20%, which were higher for low to moderate sound levels and for larger current pulses. Additionally, CM amplitude increments (<= 2.5 dB) and phase lags up to 8 degrees were found, both changes largest for low intensity tones. CAP-suppressions were present at all stimulus frequencies, but were greatest for 2 kHz, while CM-increments were highest for low frequency tones and almost non-existent at high frequencies. At the 3 Hz trial-repetition rate both, CAP and CM, amplitude effects generally showed a progressive decay, which was not observed at the lower 1 Hz rate. We conclude that the electrical activation of efferent fibers in anesthetized chinchillas produced similar effects to those seen in awake animals (Delano et al., 2007).

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72 Immunization of Mice with RHuCTL2/SCL44A2: ABR Assessment and Antibody Detection

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The monoclonal antibody KHRI-3 binds to a carbohydrate residue on CTL2 (SLC44A2), a membrane glycoprotein of the solute carrier family expressed on supporting cells in the inner ear. Hearing loss develops in KHRI-3 hybridoma-carrying mice. KHRI-3 infused into the inner ear bound to supporting cells and led to hearing loss in guinea pigs. CTL2 is also recognized by antibodies from patients with autoimmune hearing loss. The purpose of this study

was to determine if immunization of C57BL-6 mice with CTL2 will lead to immune responses and hearing loss. Human CTL2 cDNA was cloned and expressed in Sf9 insect cells. Escalating dose schedules using the purified protein (rHuCTL2) were used for immunization. Protein was emulsified in Freund's complete adjuvant and injected subcutaneously, followed by secondary immunizations in incomplete adjuvant. Two immunization schemes were used: one with 10 ug and another with 50 ug of rHuCTL2, given every two weeks for a total of three immunizations. Auditory brainstem response (ABR) testing was carried out prior to and two weeks after immunization. Pre- and post-immune sera were assessed for antibody to CTL2 on western blots. Positive sera were further tested on guinea pig inner ear surface preparations. No antibody to rHuCTL2 was observed with the 10 ug immunization. With 50 ug of rHuCTL2, one of two mice produced antibodies. The post-immune serum had antibodies to mouse inner ear supporting cells while the pre-immune control serum did not. Thus far, no hearing deficits have been observed in the immunized mice. These results indicate that rHuCTL2 is immunogenic in mice and that the antibodies recognize a mouse inner ear antigen. Additional experiments using longer immunization schemes, higher protein concentrations, and importantly, whole cells expressing rHuCTL2 could lead to a robust immune response adequate to assess possible effects on auditory function.

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73 Prolonged Basilar Membrane Displacements During Gel Injections Into the Cochlear Apex and Increased Sensitivity to Infrasound Following the Injections

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Viscous Healon (1% sodium hyaluronate) gel was injected into the apex of guinea pig cochleae at rates up to 250 nl/min. Boltzmann analysis of basal turn cochlear microphonic (CM) during injection showed a sustained increase in operating point, consistent with displacement of the basilar membrane resting position toward scala tympani. Simultaneously, endocochlear potential (EP) was increased, in some cases by more than 10 mV, the summating potential (SP) to high-frequency tones was increased, and sustained changes in distortion product levels were observed. Following the injection of 1 – 2 µL of Healon into the apex, basilar membrane position, EP, SP and distortion products returned close to pre-injection levels. Conversely, following a Healon injection, cochlear sensitivity to low-frequency tones (<30 Hz) was increased by up to 30 dB, as estimated by CM threshold measurements and by response biasing with 4.8 Hz tones. Increased sensitivity to very low frequency (including infrasonic) stimuli can be accounted for by the gel impairing the low-frequency shunt function of the helicotrema. The potential to change basilar membrane position or EP for prolonged periods of 20 minutes and longer provides a useful tool to investigate mechanisms underlying regulation of EP and basilar membrane resting position. Furthermore, the increased sensitivity to infrasound after helicotrema blockage was similar to an increased sensitivity to infrasonic bias tones found in animals with endolymphatic hydrops induced by surgical ablation of the endolymphatic sac and duct. This suggests that some of the functional changes in the hydropic ear might result from a reduced low-frequency shunt in the cochlea caused by hydrops occluding the perilymphatic spaces, and may account for some symptoms of Ménière's Disease.

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74 Quantitative Evaluation of the Cochlea and Cochlear Nucleus After Auditory Nerve Compression

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Insults to peripheral or cranial nerves cause damage to the nerves and eventually to the brainstem via trans-synaptic mechanisms. Using a controlled mechanical force, auditory neurons in the cerebellopontine angle of adult Sprague-Dawley rats (n=5) were selectively injured while preserving the labyrinthine artery as reported elsewhere (Matsumoto et al., 2008). This procedure allows confounding factors that lead to difficulties in interpreting the results to be excluded. The pathophysiological changes that result from nerve compression were studied using physiological measures of auditory function, as well as, morphological and biochemical analyses of the cochlea and cochlear nucleus. Five weeks post-compression revealed a loss of Type I ganglion neurons in the base (70%), lower middle (72%), upper middle (65%), and in the apical (50%) turns in comparison with controls. The auditory brainstem response (ABR) indicated that wave II and the following waves of the auditory evoked brainstem responses (ABR) were significantly reduced in amplitude immediately post-compression. Wave I, generated from the distal portion of the auditory nerve, was preserved immediately after compression, and at the 5th week post, all waves were abolished. Using unbiased stereological methods the total number of neurons in the cochlear nucleus was compared at 5 weeks post-operation. The total number of neurons in the compressed and intact side of the DCN were 7,021 (1,110, sd) and 11,330 (362); PVCN were 7,119 (1,164) and 14,625 (2,773); and the AVCN were 40,369 (295) and 40,709 (216), respectively. In conclusion, using a quantifiable and highly reproducible experimental model in which only auditory neurons are selectively injured, we show how the auditory nerve

threshold responses are elevated and spiral ganglion neurons degenerate. The cochlear nucleus demonstrated region-specific loss of neurons affecting the DCN and PVCN, but not the AVCN. These findings are characterizing how peripheral insults influence central auditory structures and illustrate a vitally important issue in the application of technologies which require knowledge on auditory degeneration processes, such as in cochlear or auditory brainstem implants.

75 The Differential Effects of Neurotrophin Support on Survival and Neuritogenesis of Mouse Type I vs. Type II Spiral Ganglion Neurons

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The cochlea contains two populations of spiral ganglion neurons – type I (SGNI) and type II (SGNII). While the peripheral neurites of SGNI form the inner radial fiber innervation of individual inner hair cells, the SGNII neurites form the outer spiral bundles innervating the outer hair cells in an en passant fashion. This dual afferent innervation pattern is established within a few days of birth in the rodent cochlea. We investigated whether the growth properties of type I and type II neurons differed during this critical developmental window. Using SG explants at postnatal day 1 (P1) and day 7 (P7), we distinguished SGNI from SGNII on the basis of confocal imaging of immunofluorescence for ß-tubulin, which labeled all neurons, and peripherin, which labels just SGNII in this model. Quantitative analysis showed that in our cultured explant model (48 hours), SGNII were more resilient to our culture conditions that SGNI, with proportionately less neuronal cell loss. Total cell numbers were compared with neuronal densities in age-matched whole-mounts from fixed cochleae. When neurotrophic support was provided (100ng/ml BDNF), this enhanced SGNI survival in P1 tissues, but had no effect on SGNII survival. The effect of BDNF on SGNI survival was reduced at P7, while an absence of effect on SGNII survival continued. BDNF produced a significant increase in neurite formation and outgrowth in both SGNI and SGNII at P1 and P7. In control experiments, lacking BDNF, SGNII could be distinguished from SGNI on the basis of greater neurite extension and frequency and angle of turning. These data indicate that SGNII differ from SGNI in their fundamental growth properties and response to neurotrophin signaling, contributing to the differential afferent innervation of the cochlea. Supported by the Royal Society NZ (Marsden Fund and James Cook Fellowship (GDH), and UNSW Faculty funding.

76 Rho Kinase Inhibitor Increases Neurite Length and the Number of Bipolar Neurons in Dissociated Cultures of Spiral Ganglion Neurons

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Both positive and negative regulation of the cytoskeleton underlie cell shape changes in all cell types. This raises the possibility that interventions to control cytoskeletal dynamics may be one way to induce and control neurite regeneration from spiral ganglion neurons. We have demonstrated that compared to controls, exposure of dissociated spiral ganglion cultures to BMP4 increases neuronal survival with increased numbers of monopolar neurons and neurite free neurons and little or no change in the number of bipolar neurons. On the other hand, exposure to LIF increases neuronal survival with increased numbers of bipolar neurons and little or no change in the numbers of monopolar neurons and neurite free neurons. We are using this model system to investigate biochemical mechanisms that can convert neurite-free and monopolar neurons into the usual bipolar shape of spiral ganglion neurons. BMP4 binding to BMP receptor II can link BMP4 signaling to effects on actin through LIM Kinase. Once released from the BMP receptor, LIM kinase can be activated by Rho kinase to phosphorylate cofilin and interfere with actin depolymerization. To determine whether Rho kinase activity interferes with the development of bipolar spiral ganglion morphology and neurite length, we exposed 18 hour BMP4 or LIF cultures to the Rho Kinase inhibitor H-1152 and measured survival, neuronal morphology and neurite length in cultures fixed 24 hours later. At concentrations up to 10 µM, H-1132 had no effect on neuronal survival. However, in BMP4 cultures, exposure to H-1152 increased the proportion of bipolar neurons and decreased the proportion of neurite free neurons in the culture dish. In LIF cultures, exposure to H-1152 increased the proportion of monopolar neurons and decreased the proportion of neurite free neurons in the dish. In both types of cultures, exposure to H-1152 increased the length of neurites from monopolar neurons. These observations begin to address the possibility of controlling neurite regrowth from spiral ganglion neurons with pharmacologic agents aimed at cytoskeletal regulatory mechanisms. Supported by the Birtman Fund (jointly by the American Hearing Research Foundation and Northwestern Memorial Hospital), the Hugh Knowles Leadership Fund, and the Department of Otolaryngology, Feinberg School of Medicine, Northwestern University.

77 c-Jun N-Terminal Kinase (JNK) Is Required for Neurite Growth in Dissociated Spiral Ganglion Neurons

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Regrowth of spiral ganglion neuron (SGN) peripheral processes following deafening carries critical implication for cochlear implantation as well as reinnervation of restored hair cells. c-Jun N-terminal Kinase (JNK) is a member of the Mitogen-Activated Protein Kinase family of proteins and contributes to neuronal apoptosis, including SGN loss following withdrawal of trophic support. JNK has also been implicated in regulating neurite growth and inhibition of mixed lineage kinase, an upstream JNK activator, blocks SGN neurite growth. Here we specifically examined the contribution of JNK signaling to SGN neurite growth *in vitro*. Transfection of the constitutively active MKK-JNK-1 isoform promotes SGN neurite growth whereas SP600125, a small molecule kinase inhibitor, strongly inhibits SGN neurite growth. In compartmented cultures that separate the neuronal somata from their growth cones, SP600125 inhibits neurite extension when applied to the growth cone, but not when applied to the somata, suggesting that JNK promotes neurite growth by modulating the growth cone and not cytoplasmic or nuclear events. Co-transfection of SGNs with dominant negative (dn) isoforms of JNK1 and JNK2 significantly reduced SGN neurite growth, whereas transfection of either dnJNK1 or dnJNK2 singly failed to reduce neurite growth. Thus, JNK1 and JNK2 may serve redundant functions in stimulating SGN neurite growth. In contrast to the ability of SP600125 and dn JNK isoforms to inhibit neurite growth, treatment with I-JIP, a cell permeable peptide that blocks JNK binding to the JIP-1 (JNK interacting protein) scaffolding protein required for JNK activation, failed to inhibit SGN neurite growth. The failure of I-JIP to inhibit neurite growth raises the possibility of that additional JNK scaffolding proteins, other than JIP-1, function at the SGN growth cone. Our results demonstrate that JNK functions to promote SGN neurite growth, probably by phosphorylating targets within the growth cone.

78 Modulation of Spiral Ganglion Neurons Using Patterned Electrical Stimulation

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Spiral ganglion neurons (SGNs) are the primary afferent limb that carries auditory information to the central nervous system. SGN survival depends critically on hair cell integrity and neural activity. Hair cell loss leads to degeneration of the peripheral afferent fibers and gradual loss of SGNs. Survival of SGNs in vitro and in vivo in the absence of trophic support by hair cells requires exogenous neurotrophins or membrane electrical activity by a stimulating electrode or chronic depolarization. Regeneration of peripheral afferent fibers should improve spatial and spectral resolution and reduce power requirements of cochlear implants. Previous studies have demonstrated that chronic membrane depolarization promotes SGN survival but limits neurite growth, even in the presence of neurotrophins. These studies utilized changes in extracellular potassium to alter membrane potential and induce a non-physiologic tonic depolarization. We hypothesized that patterned electrical stimulation using an applied electric field would promote neuronal survival and modulate neurite growth. We have developed an experimental system using an electrotactic chamber (Warner Instruments RC-47SLP) to apply a patterned electrical field to SGNs in culture. We have used field strengths from 500 mV to 2.5 V applied at 10 Hz for 20 msec. In comparison to control cultures, SGN in the presence of the applied field demonstrated a nearly two fold increase in neuronal survival after 24 hrs. This survival advantage was partially ameliorated by 10 µM verapamil, suggesting that it depends, at least in part, on Ca2+ entry through L-type Ca2+ channels. This experimental system can be used to develop stimulation protocols that optimally modulate SGN growth and survival.

79 Calmodulin Is a Potential Interacting Partner of Cadherin 23

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The mechano-electric transducer (MET) apparatus, located near the top of the stereocilia, plays a crucial role in hearing. Cadherin 23 (CDH23) was found to be a component of the tip link, associated with the MET apparatus. It is also known that mutations in the gene encoding CDH23 cause deafness. These important discoveries led us to seek CDH23-associated proteins. By using a membrane-based yeast two-hybrid system to screen a newly built cDNA library, made predominantly from OHCs, we found that the most abundant group of CDH23 prey (55%) comprises proteins with calcium-binding domain including calmodulin (CaM). This unexpected result is particularly intriguing since the intracellular domain of CDH23 is located where calcium concentration is highly regulated, and CaM is observed at both ends of the tip link and is known to play an important role in MET function. Yet, it has never been shown that CaM is associated with CDH23. Because yeast and mammalian cells differ in many ways, the detection of an interaction between CDH23 and CaM does not necessarily mean that the same interaction will occur in mammalian cells. Therefore, interaction between CaM and CDH23 was investigated in mammalian cells.

Plasmids encoding CaM and CDH23 were transiently co-transfected into mammalian cells. CaM and CDH23 proteins were found to be co-localized, which indicates possible interaction between CaM and CDH23. Co-immunoprecipitation experiments further demonstrated direct interaction between these two proteins. More

importantly, the interaction between CDH23 and CaM is calcium sensitive. Since Ca^{++} is a crucial modulator of fast and slow adaptation and cilia-based amplification, discovery of an interaction between CaM and CDH23 may lead to further steps in understanding the molecular basis of MET function and amplification. (Work supported by NIH Grants DC00089, DC006412 and The Hugh Knowles Center Leadership Fund).

80 Regulated Expression of Surface AMPA Receptors Reduces Excitotoxicity in Auditory Neurons

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Dynamic regulation of the expression of surface AMPA receptors (AMPAR) is a key mechanism for regulating synaptic strength and efficacy in the central nervous system, and is known to regulate auditory sensitivity as well. We have previously demonstrated that acoustic stimulation induces a temporary removal of surface AMPAR with an associated temporary elevation in auditory thresholds. We hypothesize that noise-induced removal of surface AMPAR may help prevent excitotoxic damage to the neuron during prolonged acoustic stimulation. Here we address this hypothesis by blocking clathrin-mediated AMPAR endocytosis in auditory neurons during exposure to moderate level noise in vivo and to glutamate agonists in vitro. We inhibited surface AMPAR endocytosis with a membrane-permeable, dynamin-derived, myristoylated peptide (myr-Dyn). Infusion of myr-Dyn into the mouse cochlea blocked noise-induced reduction of cochlear surface AMPARs, and generated an excitotoxic response to a normally non-excitoxic acoustic stimulus. Noise exposure with myr-Dyn infusion produced elevations in auditory thresholds that continued to worsen for at least 6 hr following exposure and cellular swelling was observed in ganglion cells. Myr-Dyn alone did not produce these effects. Myr-Dyn also exacerbated neuronal death in cultured auditory neurons induced by incubation with NMDA or AMPA. This excitotoxic neuronal death was prevented by calpeptin, a calpain specific inhibitor. These results suggest that the reduction of surface AMPAR induced by excitatory stimulation plays an important role in limiting the excitotoxic damage to the auditory neuron.

81 β-Bungarotoxin Application to the Round Window: An in Vivo Model of Deafferentation of the Inner Ear

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Hearing impairment can be caused by ether pathology of the inner ear with hair cells most commonly affected, or as a primary lesion of the spiral ganglions and the auditory nerve with the hair cells intact. Cell replacement therapy is currently under thorough investigation as a way of treating these pathologies. For those studies there is a need for appropriate animal models. Several different methods of inflicting damage to the hair cells, spiral ganglions and the auditory nerve have been used but only a few have a selective effect on the spiral ganglion cells. β-Bungarotoxin is a venom of the Taiwan banded krait. It is a presynaptic toxin which in *in vitro* studies have been shown to induce apoptosis in neurons leaving further cochlear cells intact. In our study we wanted to create an *in vivo* rat model mimicking the selective damage to the auditory nerve leaving the hair cells intact. Under deep anaesthesia rats received β- Bungarotoxin application to the round window niche. After 3 days there was already a significant raise of hearing thresholds which continued until day 7. After day 7 there was no further change. Counting of spiral ganglion cells was performed and showed no reduction until day 14. Between day 14 and 21 severe reduction or total destruction of the spiral ganglion cells occurred. Cochlear surface preparation and phalloidin staining was performed indicating that the hair cells were intact. These data show that β-Bungarotoxin application to the round window niche is a feasible way of deafening rats *in vivo* if intact hair cells are important for the study

82 Systematic Assessment of Voltage-Activated Calcium Currents in Spiral Ganglia Neurons

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Voltage-gated Ca^{2+} currents confer multiple Ca^{2+}-dependent functions in neurons. Although there is circumstantial evidence to suggest that spiral ganglia neurons (SGNs) express several subclasses of Ca^{2+} currents, the identity of the channels and their functions remain unclear. Here, we recorded both whole-cell and single-channel Ca^{2+} currents in developing and adult mouse SGNs under conditions that allowed evaluation of kinetics, permeation and pharmacology of distinct Ca^{2+} currents in SGNs. Ca^{2+} currents were recorded in whole-cell voltage-clamp configuration, using 3-5 MΩ resistance pipettes. To record Ca^{2+} currents, voltage-dependent outward K^+ currents were suppressed using a pipette solution containing NMG^+ and Cs^+ ions. Extracellular solution contained (in mM) NaCl 110, KCl 6, 4AP 5, $CaCl_2$ 5, TEA-Cl 25, D-glucose 10, Hepes 10, pH 7.3. Intracellular solution contained (in mM) NMG 70, CsCl 75, Na_2ATP 5, $MgCl_2$ 2, Hepes 10, EGTA 10, D-glucose 10, pH 7.3. For cell-attached recordings, patch electrodes were filled with a solution containing (mM): 20-65 Ca^{2+}, Ba^{2+}, or Sr^{2+}, 25 TEACl, 5 4-AP, 5 Hepes (pH 7.3 with TEAOH); *N*-methyl-D-glucamine replaced the divalent cation, when the concentration was reduced from 65 to 20 mM. The composition of the bath solution was as follows (mM): 80 KCl, 25 TEACl, 5 4-AP, 5 Hepes (pH 7.3 with KOH).

We will demonstrate the presence of low-(LVA) and high-voltage-activated (HVA) Ca^{2+}channel in the SGNs. To further classify the HVA current component, specific Ca^{2+}-channel blockers were employed. The L-, N-, and P/Q-type channel blockers nifedipine/nimodipine, ω-conotoxin

MVIIA, and ω-agatoxin-IVA, respectively suppressed partially the total Ca^{2+} current. The remaining current after application of the three blockers was ~15% of the total Ca^{2+} current. The detailed biophysical properties and functions of the Ca^{2+} currents in SGNs will be addressed.
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83 The Contribution of Cav1.2 Channel Currents in Spiral Ganglia Neuron; Lessons Learned from Cav1.3 and Cav3.1 Knockout Mice

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Similar to a variety of neurons, $Ca_v1.3$ channel currents have been demonstrated in spiral ganglia neurons (SGNs). It is expected that multiple voltage-gated Ca^{2+} currents produce diverse Ca^{2+}-dependent functions in SGNs. However, because Ca^{2+} channels function in multicomponent systems, it is difficult to determine several things: 1) the precise contribution of each subclass to specific physiological processes in vivo and 2) the compensatory mechanisms that occur in response to perturbations in the activity of one or more Ca^{2+} channels. This information is essential for a detailed understanding of complex Ca^{2+}-dependent processes occurring in vivo and in vitro. We are developing and analyzing functional Ca^{2+} channel knockout mouse models for a number of these subclasses. This approach provides novel insights concerning Ca^{2+} channels and their physiological processes, which would be difficult to obtain by other means.

Here, we recorded Ca^{2+} currents from $Ca_v1.3$, $Ca_v3.1$ and $Ca_v1.3/Ca_v3.1$ double knockout mouse models. Outward K^+ currents were suppressed using a pipette solution containing NMG^+ and Cs^+ ions. Extracellular solution contained (in mM) NaCl 110, KCl 6, 4AP 5, $CaCl_2$ 5, TEA-Cl 25, D-glucose 10, Hepes 10, pH 7.3. Intracellular solution contained (in mM) NMG 70, CsCl 75, Na_2ATP 5, $MgCl_2$ 2, Hepes 10, EGTA 10, D-glucose 10, pH 7.3. For cell-attached recordings, patch electrodes were filled with a solution containing (mM): 20-65 Ca^{2+}, Ba^{2+}, or Sr^{2+}, 25 TEACl, 5 4-AP, 5 Hepes (pH 7.3 with TEAOH); N-methyl-D-glucamine replaced the divalent cation when the concentration was reduced from 65 to 20 mM. The composition of the bath solution was as follows (mM): 80 KCl, 25 TEACl, 5 4-AP, 5 Hepes (pH 7.3 with KOH). Additionally, current-clamp recordings were performed to dertermine the physiological importance of $Ca_v1.3$ and $Ca_v3.1$ channels. The resting membrane potential was more hyperpolarized, and the spike number decreased in the mutants compared with wild type mice. Surprisingly, the data revealed an unexpected expression of $Ca_v1.2$ channel currents in the mutant mice. The potential roles of $Ca_v1.2$ channels in SGNs will be addressed.

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84 Kinetics of CGRP Receptor Expression and Signaling in Efferent Nerves of Mice and Frogs

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Hair cell systems including lateral-line organs, vestibular organs and cochlea contain an efferent innervation. We discovered that the loss of CGRP (an efferent neurotransmitter) decreased sound-evoked activity in the cochlear nerve, yet the loss of CGRP did not influence cochlear thresholds or noise susceptibility. These data suggested that CGRP normally released from cochlear efferent fibers caused increased sound-evoked activity in the cochlear nerve, which then caused an increase in the dynamic range of sound perception. In agreement with this hypothesis, infusion of CGRP into the cochlea increased sound-evoked action potentials. Likewise, CGRP application to frog lateral-line increased spontaneous discharge rate in primary afferent neurons. However, in the cochlea and in the lateral-line organ, the increase in neuronal activity due to CGRP is developmentally delayed, yet this delay is not caused by lack of efferent CGRP. The CGRP receptor consists of three proteins (CLR, RAMP1, RCP) essential for functional signaling. We investigated if the developmental delay in CGRP efficacy was due to a delay in formation of functional CGRP receptors. We used co-immunoprecipitation on tissues from cochlea and lateral-line organ to detect CGRP receptor protein association. We determined that onset of CGRP efficacy in sound-evoked activity (and spontaneous rate) is due to the formation of a functional CGRP receptor complex. In mouse cochlea, CGRP receptor formation was incomplete at 1 month, but complete by 3 months, which corresponded to onset of suprathreshold enhancement. In lateral-line organ. the CGRP receptor was incompletely formed at post-metamorphic (PM) day 4, and was fully-formed by PM day 90. Such a developmental delay in CGRP receptor association could be responsible for differential susceptibility to acoustic overexposure with age.

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85 Auditory Brainstem Response and Associated Morphological and Molecular Changes in a Mouse Model of Auditory Neuropathy

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Auditory neuropathy is commonly seen in age-related and sensorineural hearing loss. Recent studies suggest that stem cell replacement therapy offers a potential strategy for restoring hearing loss. In a previous study, we have established that stem cells were most effeciently restored shortly after injury (Lang et al., 2008). To characterize how the host microenvironment may play a critical role in the survival of transplanted stem cells, we analyzed the

physiological, cellular and molecular changes resulting from acute and chronic exposure to ouabain, which results in significant auditory neuropathy. In the present study, auditory brainstem response (ABR) testing was done on CBA mice 1, 3 , and 7 days after ouabain treatment. The ABR test indirectly estimates the hearing sensitivity of the auditory periphery and brainstem. Control and ouabain-treated cochleas were subjected to real time RT-PCR assay using primers for several trophic factors, and radial sections of treated and non-treated cochleas were made for histology studies. ABR testing 1 day after ouabain treatment revealed increased thresholds at all test frequencies compared with controls. By day 3 ABR responses were absent at some frequencies; whereas by day 7, ABR responses were absent across most frequencies. Examination of ouabain treated cochleas showed differential expression patterns in both mRNA and protein levels as compared to the control cochleas, and the histological sections revealed associated pathological changes as compared to controls. Our results supported the hypothesis that certain cellular and genetic cues in the injured cochlea can play significant roles in the survival and differentiation of transplanted stem cells.

86 Age Related Volume Changes in Superior Olivary Complex Nuclei of F344BN Rats

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Age related changes in ability to locate the source of an auditory stimulus, studied in both humans (Abel et al., 2000) and rats (Brown, 1984), may relate to changes in the superior olivary complex (SOC). Age related neuronal losses within the medial nucleus of the trapezoid body (MNTB) have been previously described in Sprague-Dawley and Fischer 344 (F344) rats (Casey and Feldman, 1982; Casey, 1990). We examined volume changes in two SOC nuclei that are involved in sound localization, the MNTB and lateral superior olive (LSO). Six F344BN rats were grouped according to their ages, 4-6, 20-22, and 31-33 months, with 2 rats per group. After decapitation of the deeply anesthetized rats, the brains were quickly frozen. Coronal frozen sections were alternately stained for Nissl substance or cytochrome oxidase (or acetylcholinesterase for one 4-6 month rat) or freeze dried for chemical analysis. The LSO and MNTB were identified based on both available stains, and their boundaries were traced bilaterally at 60-120 µm intervals, tightly enclosing the somata of their neurons. Neurolucida software was used to compute the area of each tracing, and volumes were calculated by summing the product of the section area and the distance between adjacent sections. The MNTB volumes in cubic mm, as mean ± SEM, for 4 nuclei in each group of 4-6, 20-22, and 31-33 month old rats, were 0.308 ± 0.017, 0.299 ± 0.012, and 0.281 ± 0.010, respectively. The corresponding results for the LSO volumes by age group were 0.306 ± 0.021, 0.255 ± 0.013, and 0.234 ± 0.017. Only the decline in LSO volume from 4-6 to 31-33 months was statistically significant ($P<0.02$ by one-sided t-test following ANOVA). These results contrast with those of an earlier study on CBA mice (Brescia et al., 2004), which found a significant age related volume change in the MNTB but not the LSO. Our results suggest that changes within the LSO may play a part in age related decline in ability to localize sound.

87 Differential Localization of Estrogen Receptors in the Peripheral and Central Auditory Systems of Young Adult and Aged Male and Female CBA Mice

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Estrogens affect many biological functions, exerting their effects mainly via estrogen receptors (ERs) alpha and beta, which act as ligand-activated transcription factors. Estrogen receptors have been found in the mammalian cochlea as well as in the brain, and there is increasing evidence of their interaction with the auditory system. An important step for understanding how estrogen receptors can affect peripheral and central auditory processing is to determine their distribution pattern in the cochlea and central auditory system. Young adult (3 months) and aged (28 months) CBA male and female mice were used. Immunoreactivity against ER alpha and ER beta selective antibodies was assessed in the cochlea, dorsal and ventral cochlear nucleus, superior olivary complex, medial nucleus of the trapezoid body, nuclei of the lateral lemniscus, inferior colliculus, medial geniculate body and auditory temporal cortex. The localization pattern was found to be divergent between the two types of receptors. No major changes in the localization of estrogen receptors in the CNS were found in relation to age or sex. However, an overall decrease in the expression of the receptors was found in aged mice. In the periphery, notable disparities were discovered for the expression levels of the estrogen receptors between the auditory neurons innervating the base and the apex of the cochlea. These results provide a comprehensive anatomic basis for further studies that will promote our understanding of the mechanisms by which estrogens exert their actions at different levels of the auditory pathway.

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88 Amino Acid Concentrations in the Central Auditory System of Young and Old Rats

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Previous studies have shown metabolic changes in brain during aging, including changes in amino acid concentrations (Banay-Schwartz et al., 1989). We measured concentrations within central auditory regions of

12 amino acids in young (6 months), middle-aged (22 months), and old (33 months old) Fischer 344 rats received from the National Institute on Aging. Rats were overdosed with sodium pentobarbital, decapitated, and their brains removed and frozen within 5 min. Frozen coronal sections were cut from the level of the cochlear nucleus through to the auditory cortex and either stained or freeze-dried for chemical analysis. Freeze-dried sections were microdissected into samples of auditory regions for 2 rats of each age. Samples were weighed and assayed for amino acid concentrations by HPLC. Results for middle-aged rats were commonly between those for young and old rats. As for brain regions generally (Banay-Schwartz et al., 1989), most amino acid concentrations were lower in auditory regions of old than in young rats. Decreases of glutamate concentration statistically significant at $P < 0.01$ include 19% in anteroventral cochlear nucleus (AVCN), 22% in posteroventral cochlear nucleus, 27% and 19% in deep and fusiform soma layers, respectively, of dorsal cochlear nucleus (DCN), 21% in lateral superior olive (LSO), 23% in medial nucleus of the trapezoid body (MNTB), 20% in superior paraolivary nucleus (SPO), 13% in inferior colliculus, 22% in medial geniculate, and 10% in layer 5 of primary auditory cortex. Decreases of glycine concentration that were statistically significant at $P < 0.01$ include 20% in AVCN, 19% and 11% in deep and fusiform soma layers, respectively, of DCN, 9% in LSO, 30% in MNTB, 29% in SPO, and 29% in ventral nucleus of the trapezoid body. The results suggest that hearing deficits in older adults may relate to changes in excitatory (e.g., glutamate) as well as inhibitory (e.g., glycine) amino acid functions.

89 Intense Tone Effects on Central Auditory System Amino Acid Concentrations

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Cochlear damage can result from exposure to intense sounds or ototoxic drugs. Damage to the cochlea leads to changes in the auditory nerve fibers that synapse peripherally with cochlear hair cells and centrally with cochlear nucleus neurons. These changes in the auditory nerve fibers, in turn, lead to changes in the cochlear nucleus, which can also affect more central auditory nuclei. We have been measuring changes in central auditory system amino acid concentrations resulting from cochlear damage and, for comparison, changes that result from direct surgical destruction of the cochlea. Measurements of the effects of intense tone exposure have been carried out in hamsters because of evidence that hamsters exposed to such intense tones develop electrophysiological changes in the dorsal cochlear nucleus (DCN) and experience tinnitus. In preliminary results, at 20 weeks after a 4-hour exposure to an intense tone, there were almost 20% decreases in glutamate and GABA concentrations in the posteroventral cochlear nucleus (PVCN), whereas decreases in DCN layers rarely exceeded 10%. The decrease of glutamate concentration in the PVCN was much smaller than the 40% decrease 12 weeks after treatment with the ototoxic drug carboplatin and the 66% decrease 4 weeks after cochlear ablation in chinchilla or the approximately 35% decrease in rat PVCN 2 days through 4 weeks after cochlear ablation. The decrease of GABA concentration in the intense-tone-exposed hamster PVCN was comparable to the 18% decrease in chinchilla PVCN 4 days through 12 weeks after carboplatin treatment, less than the more than 40% decrease in chinchilla PVCN 7 days through 4 weeks after cochlear ablation, but greater than the 8% decrease in rat PVCN 7 days through 4 weeks after cochlear ablation. Experiments are ongoing to measure changes in amino acid concentrations in the PVCN and other central auditory regions of hamsters after intense tone exposure.

90 Vesicular Glutamate Transporters Are Associated with Specific Cell Types in the Rat Cochlear Nucleus: Deafness Related Changes

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We previously showed the expression and localization of vesicular glutamate transporters (vGluTs) in the cochlear nucleus (CN). In the present study, calcium-binding proteins (CaBPs) calretinin (CR), calbindin-D28 (CB) and parvalbumin (PV) were used to identify cell types associated with vGluTs in the rat CN. Earlier reports and our current observations demonstrate PV in the ventral CN (VCN) in globular and spherical bushy cells, CB in octopus cells and CR in globular bushy and octopus cells.

In hearing animals, vGluT1 and vGluT2 terminals were localized to somata and proximal dendrites of globular (CR+ and PV+) and spherical bushy (PV+) cells as well as octopus cells (CB+). Although expression of vGluT1 and vGluT2 appeared largely complementary, we observed glutamatergic terminals coexpressing both isoforms (in the core and granule cell domain of VCN and in the dorsal CN). Somata and proximal dendrites of bushy and octopus cells contained VGluT3, indicating novel modes of glutamatergic signaling. Following deafness complementary distribution of vGluTs was greatly disrupted and all vGluTs were redistributed to somata.

Within the VCN we observed three phases of vGluT1 terminal degeneration following bilateral cochlear ablation: In 3 day and 3 week-deaf animals, glutamatergic terminals were decreased and vGluT1 labeling was in some somata. However, a region of robust terminal labeling was seen in the magnocellular core of VCN. In 2 month deafened animals vGluT1 was localized to somata and a few very small terminals. We have identified these somata as globular and spherical bushy cells, suggesting that the same cells associated with vGluT1 terminals in the hearing group now express vGluT1 in somata. Interestingly, in 1-year deafened animals, vGluT1 labeling was primarily in terminals often around cell bodies. Those synaptic endings were smaller and fewer in comparison with the hearing group suggesting a deafness-related reorganization of synaptic connections within the CN over time.

91 Relationship Between Noise-Induced Hearing Loss, Tinnitus and Central Auditory Plasticity in the Rat

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Inner ear damage can lead to structural changes in the central auditory system that could contribute to centrally mediated tinnitus. To evaluate this hypothesis, we investigated the relationship between synaptic plasticity in the ventral cochlear nucleus (VCN) of the rat and persistent tinnitus induced by acoustic overstimulation. Rats were unilaterally exposed for 2 h to a narrowband noise centered at 12 kHz and presented at 126 dB SPL. The noise exposure greatly reduced distortion product otoacoustic emission amplitudes in the noise-exposed ear. Rats were screened for tinnitus at 6, 12, 16, 20 or 24 kHz using gap pre-pulse inhibition of acoustic startle (GPIAS). After the noise exposure, some rats were unable to detect the gap suggesting that tinnitus had "filled in" the silent interval which normally reduces the startle response. The evidence of tinnitus in these rats was still present at time of sacrifice 10 weeks post-exposure, while other rats showed no evidence of tinnitus.

Brainstems were immunostained for the growth associated protein GAP-43, a marker of synaptic plasticity. All rats showed increased expression of GAP-43 in VCN and the olivocochlear bundle ipsilateral to the noise-damaged ear; however, the intensity and pattern of GAP-43 expression varied among animals. Rats with persistent tinnitus tended to show less GAP-43 expression than rats with no or little tinnitus. The up-regulation of GAP-43 in the ipsilateral VCN may reflect central compensatory plasticity for decreased excitatory input from the noise-damaged cochlea. Increased inputs to the VCN from olivocochlear neurons as reflected by intense GAP-43 immunostaining may help reestablish the normal input balance from the two ears whereas reduced or mismatched synaptic plasticity in VCN may increase the risk of developing tinnitus. Supported by grant from NIH (RS, R01DC00909101)

92 Low Level Pb Exposure Results in Alterations in the Serotonergic System in the Developing Murine Auditory Brainstem and Inferior Colliculus

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Pb exposure during development alters auditory temporal processing in both humans and animals. Serotoninergic (5HT) has been implicated in auditory temporal processing within the brainstem and inferior colliculus, therefore the serotonergic system could be a potential target for Pb. The current study is designed to examine whether developmental Pb exposure modulates the serotonergic system within the superior olivary complex (SOC) and the inferior colliculus. CBA/CaJ mice were chronically exposed to 0 mM (control), 0.01mM (very low), and 0.1mM (low) Pb-acetate from gestation through postnatal day 21. Brain sections containing the SOC and the central nucleus of the inferior colliculus (CIC) from control and Pb-exposed mice (n=4-6 mice/per treatment group) were immunolabeled for serotonin (5HT), the vesicular monoamine transporter 2 (VMAT2), and the serotonin transporter (SERT) in order to elucidate the effect of Pb on the serotonergic system within the SOC and CIC. Additional sections were immunostained for the vesicular transporters for glutamate (VGLTU1), GABA (VGAT) and acetylcholine (VAChT) in order determine whether Pb exposure might also impact the glutaminergic, gaba-ergic, or cholinergic systems. Pb did not significantly change the immunoreactivity for VGLUT1, VGAT, or VAChT within the SOC, indicating that Pb does not alter these neurotransmitter systems at the low levels utilized in the study. In contrast, Pb significantly decreased expression levels of VMAT2 and 5HT in the LSO. Within the CIC, Pb exposure results in increased expression of 5HT and VMAT2 with very-low levels of Pb and low levels of Pb respectively. No significant changes in SERT expression were observed within the Pb-exposed CIC, suggesting that the effect of Pb is targeted to VMAT2. Our results demonstrated that exposure to Pb during development alters the serotonergic system in the auditory brainstem and inferior colliculus. Supported by NIH/NCRR P20 RR17670, NIH P20 RR015583.

93 Bilateral Effects of Unilateral Cochlear Implantation in Congenitally Deaf Cats: A Critical Period for Synaptic Plasticity

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Congenital deafness results in abnormalities of synaptic structures in auditory nerve endings. These abnormalities are most prominent in the auditory nerve terminal called the endbulb of Held. Endbulbs are large, axosomatic synaptic endings whose size and evolutionary conservation emphasize their importance for auditory functioning. Any perturbation at this synaptic junction could corrupt auditory processing by creating transmission jitter, delay or failure.

We sought to determine whether auditory stimulation of congenitally deaf cats by cochlear implantation would restore the endbulb synapses to their normal state. Three- and six-month-old congenitally deaf cats were stimulated with a unilateral cochlear implant for a period of 10-19 weeks using human speech processors. Implanted cats were trained to respond only to a specific acoustic stimulus, confirming the presence of functional hearing. Auditory nerve restoration was evident on the stimulated side of the 3-month but not 6-month implanted cats by recovery of synaptic structure: postsynaptic densities exhibited normal size, shape and distribution. Synapses of the contralateral auditory nerve in young implanted cats also exhibited a trend towards more normal structure. These results demonstrate that auditory stimulation with a cochlear implant can help preserve synapses through direct and indirect pathways and that there is a definite effect of age. The clinical implication of these findings is

that early placement of a unilateral cochlear implant within a critical time window has widespread, bilateral effects that appear to help sustain the central auditory pathways.
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94 Temporary or Permanent Hearing Loss Is Accompanied by Different Neuroplastic Changes within the Central Auditory Pathway

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Noise exposure leads beside cochlear hair cell loss to profound long term changes within the central auditory pathway. A modified spontaneous activity, changes in neuronal cell density and neurotransmitter action were reported for several auditory structures. It is not possible yet to distinguish between the changes based on the reduced input from the noise-damaged organ of corti (deprivation) and neuronal changes directly related to the auditory overstimulation. For the understanding of noise induced functional disabilities, it seems to be highly important to clarify the influence of these two mechanisms. While hair cell loss and therefore deafferentiation appears slowly in the early days after treatment (temporary threshold shift (TTS)), it should be possible to study effects on central auditory structures at different stages after an acoustic overstimulation (permanent threshold shift (PTS)). In this study, normal hearing mice were noise-exposed (3h, 115 dB SPL, white band noise 5-20 kHz) under anaesthesia. Before and after the noise exposure as well as one week later, hearing thresholds were determined by measurements of the frequency specific auditory brainstem response. In all animals with TTS and PTS the neuronal activity were investigated in subcortical auditory structures in-vivo with manganese enhanced MRI (to monitor calcium dependent synaptic activity) and in-vitro with electrophysiological single unit recordings in brain slices (to measure spontaneous firing rates). In addition, cell densities were determined in these areas to identify a modified cytoarchitecture. The results clearly demonstrate that acoustic overstimulation directly influences the physiology and anatomy in the neural network within the central auditory pathway. Whereas acute noise exposure (TTS) only affects the lower auditory pathway, i.e. the cochlear nucleus, long-term effects (PTS) could also be observed in higher auditory structures.

95 Rescuing Temporal Processing with a Novel Augmented Acoustic Environment in an Animal Model of Congenital SNHL

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Each year as many as 3/1000 children are diagnosed with congenital sensorineural hearing loss. Common in these children are delays in grammar comprehension, vocabulary retention and speech development, related to temporal processing abilities. Studies in a mouse model of congenital hearing loss suggest that early exposure to an augmented acoustic environment (AAE) limits outer hair cell death and maintains peripheral auditory thresholds. However few of these studies have examined the central auditory system. The current study uses a novel temporal AAE containing silent gaps embedded in noise bursts and examines temporal processing in the auditory midbrain.
Mice of the DBA strain were exposed to a traditional AAE stimulus, a novel AAE stimulus, or no stimulus from birth to P30. Auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs) were recorded to assess peripheral auditory function. To assess the effects on central auditory processing we recorded neural activity from a 16-channel electrode in the inferior colliculus (IC). Frequency response maps were measured from 2 to 64 kHz and from 0 to 85 dB SPL, from which we derived each units' best frequency, threshold and tuning sharpness. To assess temporal processing we recorded responses to gap-in-noise stimuli and determined the minimum gap threshold (MGT) from the neuronal response.
Peripheral ABR thresholds decreased and DPOAE amplitudes increased following exposure to both types of AAE stimuli. In the IC, exposure to both types of AAE stimuli increased best frequency (15.1 kHz vs 10.7 kHz), decreased threshold (37.5 dB vs 42.1 dB), and increased Q10 of multi-units (4.2 vs 2.8), compared to the control condition. While exposure to traditional AAE had no effect on neural coding of gap detection, exposure to our novel stimulus significantly decreased the mean MGT to 4.2 ms in the treated group from 10.9 ms in the control group. These experiments pave the way for possible therapeutic intervention in children suffering congenital sensorineural hearing loss.
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96 Auditory Brainstem Responses and Acoustic Startle Reflex Measurements in GluR4 AMPA Receptor Subunit Knockout Mice Suggest a Central Auditory Processing Deficit

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Fast-gated AMPA receptors are required for high-frequency transmission in the adult auditory brainstem. During development, AMPA receptor subunit composition switches from GluR1- to GluR3/4-dominant phenotype, resulting in accelerated receptor kinetics and reduced sensitization. Recent work has shown that deletion of the GluR4 receptor subunit results in increased EPSC decay time, reduced EPSC amplitude, increased incidence of spike failure, and exacerbated short-term depression in the calyx of Held (Yang et al., 2007, SFN abstract 876.4/F44). We measured auditory brainstem responses (ABRs) and modification of the acoustic startle response with noise

offsets in adult GluR4 knockout mice to assess the effects of deficient fast-gated AMPA receptors on brainstem neural synchrony and temporal acuity, respectively. ABR thresholds were similar in wild-type and GluR4 knockout animals. Suprathreshold ABRs, particularly the later waves, were abnormal in GluR4 knockout mice, indicating reduced neural synchrony in the brainstem pathway. The baseline acoustic startle response amplitude was severely reduced, and sometimes absent, in GluR4 knockouts, making a meaningful assessment of temporal acuity using reflex modification measures difficult. Knockout animals showed no obvious signs of retarded motor function. The reason for such strikingly reduced startle amplitude is unclear. GluR4 receptors are widely distributed throughout the brain and may be involved in both sensory and motor components of the acoustic startle response. These results show that fast AMPA-mediated synaptic transmission in auditory brainstem nuclei are required to generate normal suprathreshold ABRs and the acoustic startle response.

97 Effects of Acute vs. Chronic Salicylate Dosing on the Acoustic Startle Response.

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Tinnitus, the perception of sound in the absence of acoustic stimulation can occur with or without hearing loss. The cause of tinnitus may be unknown but it typically begins with some inner ear disorder that likely alters the response properties of the periphery and subsequent higher auditory centers. Two common experimental approaches to induce tinnitus in animals are noise exposure and application of salicylate (SA). The mechanism(s) by which SA induced tinnitus occurs are as unclear as the tinnitus caused by noise. The direct effects of SA on the OHC likely explain the hearing loss associated with SA but SA can cause tinnitus in the absence of hearing loss. SA can cause increases in spontaneous firing rate of auditory nerve fibers (Evans et al., 1981) while low doses do not (Muller et al., 2003). Our previous work (Halsey et al., Abst # 826, ARO 2006) showed that application of SA caused acute hearing loss and with repeated dosing, chronic, significant increase in the neural noise recorded from the round window. We compared the effects of SA dosing on pre-pulse (PPI) and gap inhibition (GI) of the acoustic startle response (ASR). Gaps were presented in four backgrounds of narrowband noise centered at 4, 12, 16 and 20 kHz each noise presented at 57dB SPL. Reduction of the GI of the ASR is believed to suggest evidence of tinnitus. Acute systemic application of SA did reduce the GI of the ASR for the 20 kHz noise condition. The acute application of SA also caused an increase in overall startle amplitude. During the chronic systemic application of SA, the overall startle amplitude was reduced which may reflect adaptation of the ASR with time or a systemic effect of SA with time. Chronic application of SA caused a frequency specific increase in GI of the startle amplitude for the 12 kHz noise condition. This latter effect is opposite the prediction for presence of tinnitus.

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98 Increased Synaptic Activity Within Central Auditory and Non-Auditory Structures Upon Systemic Salicylate Application

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Salicylate is well-known to produce reversible tinnitus in animals and humans as well. Previous in-vivo and brain slice studies have been shown that salicylate application affect the neuronal activity in several central auditory and non-auditory structures (Basta et al., 2005, 2008). These changes should be related to a modified synaptic activity within the neuronal network since single neuron activity was decreased or increased during salicylate application. The present study is aimed at investigating the effect of salicylate on synaptic activity within central auditory and non-auditory structures.

The synaptic activity was determined in adult mice by the manganese-enhanced MRI technique. Manganese ions easily cross the blood-brain barrier and agonise the calcium influx into the cell during pre-synaptic activation. The resulting MRI-T1 signal contrast depend largely on the intracellular concentration of manganese-ions which show a slow clearance over time. This allows an activity coding by the Mn-enhanced MRI-technique and thus the imaging of synaptic activity.

The synaptic activity increased upon the salicylate application in the dorsal and ventral cochlear nucleus, the superior olivary complex, the inferior colliculus and the medial geniculate body. The effect was much stronger in lower than in higher structures of the auditory pathway. There was a remarkable effect on non-auditory structures such as the preoptic area of the anterior hypothalamus. This area is important to maintain the body temperature at a constant level. Salicylate act on this structure during the pharmacological treatment of fever.

The present results suggest that unspecific effects on synaptic activity contribute to the salicylate-induced tinnitus generation.

99 Brainstem Auditory Evoked Potentials During Sleep-Like Cloral Hydrate Effect

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Brainstem auditory evoked potentials (BAEP) shifts during sleep are still under discussion. Authors have reported latency shifts related to body temperature (Bastuji et al. 1988; Litscher, 1995), thus excluding sleep effects. Experimental results in guinea pigs -the auditory nerve compound action potential as well as the unitary activity at the cochlear nucleus, lateral superior olive and inferior colliculus- have revealed shifts in amplitude and firing patterns respectively (Velluti, Pedemonte, 2002), being our hypothesis that at least subtle changes must occur in human sleep,

Preliminary results are based on Cloral Hydrate (CH) producing a sleep-like action. Volunteers without any pathology were stimulated with alternating clicks (80 dB SPL, 10/s) and BAEP were recorded together with on-line

sleep polysomnographic control, during waking and after a HC usual dose. The experimental design was to record during the afternoon nap (from 1:00 to 4:00 p.m.) with monitored skin temperature.
1. During afternoon nap, significant wave V latency increase was depicted in sleep-like stage II in comparison with wakefulness while no skin temperature shifts were recorded.
2. Analyzing the BAEP (10 ms) in the frequency domain (Fast Fourier Transform, FFT), significant decrease in the power spectra were obtained during sleep-like stage II in comparison with a waking epoch.
Conclusion. CH sleep-like effect on BAEP determined significant increase in wave V latency not related to body temperature but to sleep stage II. Besides, a decreasing of the BAEP FFT power was associated.

100 Using the ABR to Assess the Coherence Between Spectral Sensitivity, the Acoustic Environment, and Behavioral Selectivity in Frogs

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The gray treefrog (*Hyla chrysoscelis*) has served as a model system for understanding the mechanisms and evolution of sound pattern recognition in the context of acoustic communication, species recognition, and female mate choice. In this study, we asked three questions: (1) How sensitive is the ear to tones of different frequencies? (2) How well is this sensitivity predicted by the spectral profile of sounds in the frogs' natural acoustic environment? And (3), how well does sensitivity to tones of different frequencies predict behavioral responses to communication-like signals? We measured the ear's sensitivity in awake, wild-caught males and females in response to trains of pure tones (350 - 5000 Hz) using the auditory brainstem response (ABR). To characterize the gray treefrog's acoustic environment, we made extensive recordings of both the sounds of natural breeding choruses and the mating calls of individual conspecific males. We assessed behavioral responses using female phonotaxis. Wild-caught females were allowed to respond to a pulsatile signal that mimicked the temporal properties of conspecific mating calls, but that consisted of a single carrier frequency (500 - 4000 Hz). The ABR audiogram was W-shaped with two distinct ranges of best sensitivity, which correspond to the tuning of the amphibian and basilar papillae. These regions of best sensitivity also corresponded reasonably well with the dominant frequencies in conspecific mating calls and with the frequency-dependent behavioral responses to communication-like signals.
This work was supported by DC-00046 to RJD and DC-008396 to MAB.

101 Second-Order Wiener-Volterra Characterization of Temporal Processing in Primary Auditory Cortex of the Ketamine-Anesthetized Cat

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We apply an extension of the Wiener-Volterra theory (a rigorous nonparametric framework for system identification) to Poisson-distributed impulse train input and spike output in order to characterize the temporal response properties of AI (pyramidal) neurons in the ketamine-anesthetized cat. Linear and second-order nonlinear "Poisson-Wiener" kernels are used to predict neuronal temporal modulation transfer functions (tMTFs), i.e., responses to periodic click trains at repetition rates of 2-64 Hz, as well as their responses to conspecific vocalizations, i.e., cat meows. Linear kernels in anesthetized AI typically show a brief, strong excitation followed by inhibition and then often some rebound activity. Second-order kernels typically exhibit strong compressive nonlinearities which depress the impulse response over a stimulus memory of several hundreds of ms. Interestingly, in neurons with lowpass tMTFs, the nonlinear depression decays monotonically with increasing inter-click interval, whereas in neurons with bandpass tMTFs, the decay is dual-peaked, and the local minimum in depression strength is correlated with the neuron's preferred modulation frequency. We have tested the Poisson-Wiener model on its prediction of the tMTF. The superiority of the second-order over the linear fit is pronounced for any measure of prediction success. Moreover, for the majority of neurons, the second-order fit can be considered very good, with predictability in excess of 80%. We also investigated whether or not the impressive predictive power of the second-order model extends to responses to cat vocalizations, stimuli obviously outside of the class used in the estimation of the Poisson-Wiener kernels.

102 Contribution of the Thalamus to Detection of Novel Sounds: Is There Stimulus Specific Adaptation in the Medial Geniculate Body of the Rat?

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A novel or unexpected sound may represent potential danger for an animal, compromising its survival. In order to respond adequately, it is necessary to rapidly distinguish what is novel from what is familiar. However, despite the obvious importance of novelty detection, the underlying brain mechanisms remain unclear. Studies in the cat auditory cortex (A1) (Ulanovsky et al., 2003), and rat inferior colliculus (IC) (Perez-Gonzalez et al., 2005), have demonstrated that neurons in both of these regions show a reduced response to a stimulus if it is repeated (standard), and briefly resume firing if a novel stimulus (deviant) is presented. This phenomenon is called stimulus-specific

adaptation (SSA) and is a possible single neuron correlate of the MMN in humans. Considering that the MGB receives its inputs from both the IC and A1, one would expect MGB neurons to exhibit SSA and enhanced responses to novel stimuli. However, based on recordings from a few MGB neurons in the cat, Ulanovsky et al., (2003) concluded that MGB neurons do not exhibit SSA. To reexamine this question, we used an oddball stimulus paradigm similar to that of Ulanovsky et al., (2003), examining a larger population of neurons throughout the three main subdivisions of the MGB. Our data demonstrate that SSA is present in all subdivisions of the MGB, being more prominent in the dorsal and medial subdivisions. As in the cortex and IC, SSA in the MGB varies with: 1) inter-stimulus interval; 2) frequency contrast between standard and deviant frequency; and 3) probability of occurrence of the deviant stimulus. In conclusion, our results demonstrate that SSA is present at the MGB level and we hypothesize that it may be shaped in a bottom-up process.
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103 Enhanced Physiologic Discriminability of Stop Consonants with Prolonged Formant Transitions in Awake Monkeys Based on the Tonotopic Organization of Primary Auditory Cortex (A1)

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In a highly influential and controversial report, it was observed that many children with developmental language disorders (DLDs) had difficulty in the perception of stop consonant-vowel syllables with rapid formant transitions, but performed similar to controls when the formant transitions were extended in time (see Tallal, 2004). This observation helped in the formulation of the temporal processing hypothesis, which posits that difficulties in perceiving rapidly changing speech stimuli are caused by the aberrant processing of sounds in expanded temporal windows of integration, "chunking" acoustic events together in time periods too large to allow the fine-grained patterns of speech to be readily discriminated. In order to clarify neural mechanisms potentially relevant for the initial, key observation, we examined the temporal processing hypothesis in light of the perceptual hypothesis of Stevens and Blumstein (1978), which posits that onset spectral features are important for discrimination of stop consonants varying in their place of articulation (POA). Multiunit activity evoked by the synthetic syllables /ba/, /ga/ and /da/ was recorded in A1. The syllables differed in the maximal spectral location of onset frication, and direction and duration of formant transitions. Amplitudes of speech-evoked activity were ranked according to predicted ranks based on responses to tones centered at the points of frication. Responses reflecting POA were partly determined by the onset spectral characteristics of the syllables and the tonotopic sensitivity of the recording sites. Longer intervals of analysis led to a decrement in response specificity. Crucially, response specificity was enhanced for longer duration formant transition stimuli. We conclude that A1 responses evoked by onset spectra of stop consonants are important for POA discrimination. Relevance to normal perception and that occurring in DLD will be discussed. Supported by DC00657.

104 Temporally Dynamic Frequency Tuning in Monkey Primary Auditory Cortex

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Frequency tuning of auditory cortical neurons is typically determined by integrating spikes over the duration of a tone stimulus. However, this approach may mask functionally significant variations in tuning over the time course of the response. For instance, frequency tuning may differ across temporal windows which include initial "on", "sustained", or "off" portions of responses. To explore this possibility, frequency response functions (FRFs) based on multiunit activity evoked by 60 dB SPL tones were examined within 4 time windows corresponding to "on", "early sustained", "late sustained", and "off" portions of responses in A1 of 5 awake macaque monkeys. FRFs of "on" and "early sustained" responses displayed a good concordance, with best frequencies (BFs) differing, on average, by less than 0.25 octaves. In contrast, FRFs of "on" and "late sustained" responses differed considerably, with a mean difference in BF exceeding 0.75 octaves. For 36% of sites, tuning of "off" responses was inversely related to that of "on" responses, with FRFs displaying a trough at a frequency corresponding to the BF of "on" responses. Inversely correlated "on" and "off" FRFs were more common at sites with a higher "on" BF, thus suggesting functional differences between sites with low and high "on" BFs. These results indicate that frequency tuning in A1 may vary considerably over the course of the response to a tone, thus revealing a temporal dimension to the representation of sound spectrum in A1. Implications of the findings for the functional organization of auditory cortex are discussed.

105 Functional Recovery in Gap Detection Following Bilateral Lesions of Auditory Cortex in the Rat

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The purpose of this study was to determine the severity of deficits in gap detection that occur after bilateral auditory cortical damage and to track the time course of functional recovery. Young adult male albino rats (302-322g) were tested for their ability to detect temporal gaps in an otherwise continuous stream of white noise (intensity 75dB SPL). Water deprived animals were first trained to obtain their daily water requirements from a spout located in a small test cage inside a large soundproof room. The animals were then conditioned to avoid a shock delivered through the spout by detecting the presence of a gap in the noise. The durations of the gap were reduced to obtain behavioural thresholds for gap detection in each subject. The animals were then retested following cortical ablation.

Bilateral lesions of the auditory cortex were made with rats under isofluorane, an inhalation anaesthetic that allowed rapid recovery times for post-operative testing. Seventy-two hours following surgery, animals were returned to the test cage and reassessed on gap detection. They were capable of performing the task without any further training. However, their responses typically resulted in a large elevation in gap detection thresholds reflecting a deficit in sensory processing. Post-operative performance was further assessed at 3-day intervals over a 21-day period and showed a gradual recovery in the ability to detect gaps. In several cases thresholds returned to near normal levels. The results were similar to those reported previously by Syka et al., (2002).

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References:

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106 The Level and Distribution of the GABAB[B] Receptor in the Rat's Central Auditory System

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Gamma-butyric acid (GABA) is a major inhibitory neurotransmitter in the central auditory system. The $GABA_B$ receptor is one of the major subtypes of GABAergic receptors. A functional $GABA_B$ receptor is a heterodimer consisting of two subunits, $GABA_BR1$ and $GABA_BR2$. It has been found elsewhere in the central nervous system that the $GABA_B$ receptor exists on presynaptic axon terminals and postsynaptic cell membranes. Presynaptic $GABA_B$ receptors are involved in regulating the release of neurotransmitters including glutamate and GABA. Postsynaptic $GABA_B$ receptors contribute to cell membrane hyperpolarization. These facts indicate that the $GABA_B$ receptor is important for the balance between excitation and inhibition in the central nervous system.

To understand the contribution of the $GABA_B$ receptor in auditory processing, we utilized western blotting and immunohistochemical techniques to determine the level and distribution of the receptor in the rat's central auditory structures. Antibodies for the $GABA_BR1$ and $GABA_BR2$ subunits were used to probe for the receptor. Our results revealed that the $GABA_B$ receptor is expressed at the highest level in forebrain auditory structures including the auditory cortex and the medial geniculate nucleus. The midbrain structure, the inferior colliculus, demonstrates an intermediate level of expression, while brainstem structures including the nucleus of the lateral lemniscus, the superior olivary complex, and the cochlear nucleus have the lowest level of expression. The receptor is essentially homogeneously distributed in the auditory cortex as well as in the medial geniculate nucleus. Among the three major subdivisions of the inferior colliculus, the dorsal subdivision has a substantially higher level of $GABA_B$ receptors than the central nucleus and the external subdivision. These results suggest that the $GABA_B$ receptors make different contributions to neural responses in different auditory structures.

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107 Cortical Processing of Sounds from Multiple Spatial Locations

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Sound source segregation requires resolving a mixture of spectro-temporal and spatial information of multiple sounds. One hypothesis is that the auditory system delineates sounds through a series of filters and sound segregation is achieved on the basis of filter outputs. These filters are usually described by auditory neurons whose firing rates are tuned to sound attributes, such as frequency and spatial location. It remains unclear, however, how single neuron-based filters contribute to the processing of competing sounds that occur both within and outside their tuning preferences. We examined in the present study the effects of spatially close and far apart distracter sounds on neural responses to a target sound in the primary auditory cortex (A1) and the adjacent caudal-medial field (CM) of awake marmosets. Fifteen loudspeakers were positioned in the semicircular frontal field (-90° to 90° along the horizontal axis and at 0°, 45°, 90° elevations). We delivered target and distracter sounds either simultaneously or sequentially from two spatial locations and varied the frequency (or intensity) of a distracter sound to examine the changes in neural responses to a target sound. Analyses revealed that the target responses can be suppressed by distracter sounds placed inside or outside the spatial receptive field of a neuron, indicating that the extent of inhibition is broader across the spatial axis than that of excitation. For near-field and far-field distracters, suppression of target responses usually occurs at similar frequencies, albeit with different magnitude. This suggests that for individual neurons the "feature" frequencies involved in spectral processing remain largely unchanged at different sound locations. Together, these results show that inhibition may improve the neural detection of a target sound by suppressing responses to other sounds carrying competing spectral information across a broad spatial range. [Supported by NIH grant DC03180 (X.W.).]

108 The Ventorostral Belt Area of the Guinea Pig Auditory Cortex Is More Involved in Processing Conspecific Communication Calls Than Other Cortical Areas

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The guinea pig auditory cortex (AC) comprises at least eight areas; two core areas surrounded by six belt areas. To explore the functional roles of these eight areas, a combination of simple stimuli and conspecific communication calls were used, while recording the

responses of cells in core and belt regions of the anaesthetised guinea pig (GP) with custom made multielectrodes.
The full range of GP communication calls were used to investigate whether there is a cortical area, or areas, that are more responsive for communication calls, and / or are better at discriminating between them. A unit was considered to be responsive to sound if it responded to at least one of the following stimuli; broad band noise, clicks, pure tones or communication calls. The proportions of auditory responsive units that responded to communication calls were compared across cortical areas. The ventrorostral belt (VRB) was significantly more responsive to communication calls compared with all other cortical areas; this area was also better than the other areas at coding for the differences between calls.
Horikawa et al. (NeuroReport [2001] 12: 3335-3339) proposed that there are three processing streams from core to belt areas of GP auditory cortex; the caudal, the ventral and the dorsal. Hosokawa et al. (NeuroReport [2004] 15: 1093-1097) presented evidence for the increased involvement of the GP caudal belt areas in processing binaural 'where' related information and proposed that the caudal stream is involved in processing the 'where' aspects of sounds. Our findings suggest that the VRB is more involved in processing communication calls than any other cortical area; this area may be part of a ventral processing stream that is more involved in processing the 'what' aspects of sounds.

109 Stream Segregation of Sinusoidally Amplitude Modulated Tones in the Forebrain of the European Starling

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It has been suggested that sequentially presented sounds from different sources that are perceived as segregated streams of signals are represented in the brain by separate populations of neurons. Different features of sounds such as frequency, level or temporal pattern can be exploited for stream segregation.
In the present study, multi-unit recordings in the auditory forebrain of European starlings were carried out to observe the neural representation of sinusoidally amplitude modulated (SAM) tones, i.e., stream segregation by temporal features. When the modulation frequency (fmod) of a SAM tone with the carrier frequency set to the neurons' characteristic frequency was varied, recording sites generally showed 'modulation tuning'. Different types of modulation transfer function (MTF) were observed: band-pass, low-pass, high-pass, band-reject and all-pass. For studying stream segregation by SAM, the neurons' response was tested using the ABA_-stimulus condition (e.g., Bee & Klump 2004; A and B being SAM tones of differing fmod and _ indicating a silent interval). The modulation frequency of A-signals was chosen to elicit a strong response; fmod of B-signals was 0.5 to 4.0 octaves higher.
Spike rate in response to B-signals dropped as the separation in fmod (Δfmod) between A- and B-signals increased. The rate change as a function of Δfmod was generally larger than that observed for isolated B-signals. Synchrony also decreased as Δfmod increased. Each type of recording site classified by the MTF shape evoked different rate and synchrony patterns in response to B-signals. Faster signal repetition resulted in fewer spikes in response to B tones, suggesting the contribution of forward suppression to stream segregation. The results suggest that similarly to the observation in stream segregation of pure tones, mutual suppression of SAM tones leads to a separated neuronal representation of the A- and B-signals in the forebrain.
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110 Stimulus-Specific Adaptation in the Auditory Thalamus of the Mongolian Gerbil

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Stimulus-specific adaptation (SSA) has been demonstrated in the auditory cortex (AC), but not in the auditory thalamus (MGB). However, experiments based on cortical cooling and electrical stimulation as well as anatomical evidence emphasize that both structures are tightly interlinked. Consequently, even if SSA is generated only in the cortex, it should also affect the thalamus.
We investigated SSA in the MGB with an oddball paradigm involving repetitive stimulation (rate 2 Hz) with pure tones of two frequencies (1 oct separation) in which standard stimuli were interspersed with low probability deviants (10%). Neuronal activity was recorded in anesthetized gerbils from up to 12 sites simultaneously using a multi-channel approach. Hence, relative positions of the two stimulation frequencies varied inside neuronal tuning areas. Spike rates from the first 30ms of pure tone responses were analyzed and compared to a control condition (equal probability of standard and deviant).
We found a clear effect of SSA in 26% of the units (n=100) with a standard-deviant difference in spike rate of >22%, while it was absent in the control condition. Over the time course of 1000 trials SSA induces a fast and substantial inhibition of neuronal responses to the standard tone, whereas the responses to the deviant maintain a constant level of activity. Nevertheless, analyzing the deviant spike rates in dependency to the inter-deviant interval uncovers small single-trial effects: larger intervals correlate with higher spike rates. In addition, separate analysis of the responses to the two stimulation frequencies provided further insights: SSA was found in 34% of the responses to higher frequency stimuli and in 29% of the low frequency responses. In contrast, only 12% of the neurons exhibit SSA for both frequencies, indicating that not all frequencies inside the tuning area are equally affected.
In summary, adaptation in the MGB acts on different time scales in a frequency-specific manner.

111 Effects of Electrical Microstimulation of Prefrontal Cortex on Auditory Response Properties in Primary Auditory Cortex.

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A majority of neurons in the primary auditory cortex selectively adapt to the presence of salient cues in auditory tasks by rapidly changing their neural response properties (i.e., spectrotemporal receptive field (STRF) properties; see Fritz et al., 2003). Presumably these changes are the result of signals originating in higher cortical areas. Currently, our knowledge regarding the source of those signals is limited. The prefrontal cortex (PFC) is one such area that is known to play an important role in attentional task performance and therefore, may have a role in modulating auditory cortical responses. We explored whether electrical microstimulation of the PFC can modulate neural responses in the auditory cortex of awake, passively listening ferrets. We measured auditory cortical neural activity in response to a range of auditory stimuli (i.e. either pure tones or temporally orthogonal ripple combinations) in the awake, non-behaving ferret and compared those responses with neural responses measured while electrical microstimulation was applied to a site in the PFC. Electrical microstimulation of the PFC was paired with a pure tone of a single frequency that was within (or outside) the receptive field of the neuron. We recorded single units in primary auditory cortex of 2 ferrets and preliminary results suggest that neural activity in the auditory cortex can be modulated by low-amplitude microstimulation of the PFC. We shall describe the effects of the PFC electrical microstimulation/pairing manipulation on activity of auditory cortical neurons neurons as well as the effects on the frequency tuning and spectrotemporal receptive field properties of the neurons in the auditory cortex.

112 Sensory-Motor Integration in Primate Frontal Cortex Neurons During a Natural Vocal Behavior: Antiphonal Calling

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Through each species' evolutionary history, selection for neural mechanisms that enable each individual to efficiently navigate their social and ecological worlds was critical. Of particular importance are neural processes that integrate incoming sensory information with the resultant behavioral response. While data on sensory-motor interactions in the primate cortex are available, relatively little remains known about this process species' natural behaviors. Here we recorded single-unit activity of frontal cortex neurons in freely-moving common marmoset monkeys (Callithrix jacchus) as subjects engaged in a natural (i.e. untrained) behavior known as antiphonal calling. This natural vocal behavior involves the reciprocal exchange of vocalizations. Importantly, the production of an antiphonal call is dependent upon first hearing a particular vocalization, a phee call, and producing the same call type in response. As such, each antiphonal call involves the integration of sensory information with a vocal-motor response. In our first set of analyses, we compared neural activity during each of the three elements of this behavior: sensory period (vocalization presentation), latency delay, and motor response (antiphonal call). The aim here was to determine whether neurons across the population showed firing rate changes during any of these three periods. In our second set of analyses, we examined the effects of behavioral context of the neural response observed during antiphonal calling. Specifically, during test sessions, we presented subjects with vocalizations that did not elicit antiphonal callings and subjects produced vocalizations spontaneously. As such, we tested whether neurons responded similarly during either the sensory stimulus or motor response alone compared to the same sensory or motor period during antiphonal calls. This study builds on our previous work examining the ethology and functional neuroanatomy of antiphonal calling in common marmosets.

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113 Vocal Control During Acoustic Interference in Common Marmosets

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Vocal communication is an essential part of social behavior in organisms such as primates, amphibians, birds and insects. The natural acoustic environment of vocal species inherently contains noise that can be periodic or unpredictable in nature. The ability of both human and nonhuman species to avoid intermittent acoustic noise during vocal communications is critical for maintaining the communicative efficacy of the signals. Here we tested the ability of a New World primate, the common marmoset (*Callithrix Jacchus*), to control the timing of vocalizations during antiphonal calling interactions in a controlled acoustic environment. We examined the vocal behavior of marmosets in three different controlled noise conditions: white noise pulses with periodic silent gaps, white noise pulses with predictable silent gaps and white noise pulses with unpredictable silent gaps. We observed that the majority of the calls were initiated during silence and that marmosets were able to modify the timing of the calls according to the given noise environment. Moreover, the duration between the call-onset time and the end of the preceding noise pulse decreased with shorter silence gaps. Overall, these findings suggest that marmoset vocal behavior is an attractive model to study the neural basis of vocal control and feedback.

114 Stimulus-Specific Adaptation Occurs in Neurons of the Medial But Not Ventral Auditory Thalamus

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Neurons in the primary auditory cortex respond more strongly to a rarely presented "deviant" tone than to the same tone when it is common, or "standard". This phenomenon, called "stimulus-specific adaptation" (SSA), has been proposed as a possible single-neuron correlate of the mismatch negativity (MMN), a cortical evoked potential associated with stimulus novelty. Previous studies in cat have suggested that SSA is absent from single neurons in the auditory thalamus (medial geniculate body, MGB) (Ulanovsky et al. 2003, Ulanovsky et al. 2004); however, those reports did not differentiate between the MGB subdivisions. To explore the possibility of thalamic SSA more completely, we recorded extracellularly from 30 single units and 22 multiunit clusters in the ventral, medial, and dorsal subdivisions of the mouse MGB, while presenting the anaesthetised animals with sequences of standard and deviant tones. As in the cat studies, standard and deviant tone frequencies were separated by no more than 0.5 octaves, and evoked similar responses in most neurons. Using stimulation rates of 1.25 - 2.5 stimuli/s, we found SSA in neurons in the medial MGB, but not in the ventral or dorsal subdivisions. The median neuronal stimulus-specific adaptation index was significantly greater than zero in the medial subdivision at all stimulation rates tested (sign-rank test, $p<0.05$), but was not significantly different from zero in the ventral or dorsal MGB for any of the tested stimulation rates. Our results demonstrate that SSA occurs in the auditory thalamus, in the medial, but not ventral or dorsal MGB subdivision. Together with previous findings of SSA in neurons of the "belt" regions of the inferior colliculus, the findings suggest that SSA is either a general property of some neurons in the non-lemniscal auditory system, or a cortical phenomenon that influences subcortical auditory processing only within the non-lemniscal pathway.

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115 Neural Representation of Frequency Modulations in the Mouse Auditory Thalamocortical Circuit

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Frequency modulated (FM) components of vocal communication sounds transmit vital phonological and semantic cues in social mammals from mouse to man. The present studies build upon prior electrophysiological and behavioral studies made in other species to investigate the neural and perceptual encoding of FM sounds. We are investigating how basic properties of FM sounds such as their direction (i.e. upward or downward) and speed are differentially encoded across different zones of the tonotopic map, between the primary auditory cortex (A1) and the anterior auditory field (AAF), between cortical hemispheres and between different stages of the central auditory hierarchy (cortex vs. the medial geniculate body of the thalamus). Preliminary data indicate that in A1 and AAF of both hemispheres, low frequency regions of the tonotopic map are preferentially tuned to upward sweeps and high frequency regions to downward sweeps. The relationship is similar to what has been observed in the cat and ferret, that is, there is a correspondence between BF and direction preference, but the relationship is more moderate than that reported in the rat. Paralleling the neural recordings, we are also studying pre-pulse inhibition behavior to explore the perceptual salience of FM direction and speed. These studies suggest that slower and louder sweeps are more effective at attenuating the acoustic startle reflex whereas upward and downward sweep directions were equally effective. Collectively, these studies will help to determine how complex sounds are encoded and translated into behavior.

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116 Evaluating Auditory Network Connectivity with Combined Microstimulation and Functional Imaging in the Monkey

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A high-level auditory-cortical region was recently identified with functional magnetic resonance imaging (fMRI) in rhesus monkeys. This brain region shows a close functional correspondence to the so called human-voice region. Both human and monkey “voice” regions lie anterior and superior on the temporal lobe and appear to be exquisitely sensitive to certain vocal components in species-specific vocalizations that help to identify other conspecific members of the species. To clarify the in-vivo functional connectivity of the rhesus monkey voice region along with its putative auditory cortical network we used microstimulation in combination with high-resolution fMRI. First we functionally localized the voice region with blood-oxygen-level-dependent (BOLD) fMRI, as previously described. Then we microstimulated this region with glass-coated iridium microelectrodes, using biphasic, cathode leading, 250 to 500 µA pulses of 200 µs duration. We used the fMRI BOLD response to evaluate the anterograde targets of the microstimulation site. Microstimulation of the voice region, which lies on the rostral superior-temporal plane (rSTP), elicited a BOLD response from hierarchically earlier auditory areas (feed-back), and the surrounding superior temporal plane (STP), gyrus (STG) and sulcus (STS) of the ipsilateral hemisphere. We next

microstimulated an upper-bank STS region that was the target of the voice region. The STS microstimulation seemed to show more robust medial and orbital prefrontal cortex activity in comparison to microstimulation of the voice region on the STP. We are currently comparing these results to those obtained from microstimulating the earlier stages of the auditory cortical pathway and aim to compare our functional connectivity results to anatomical tractography from the analysis of retrograde and anterograde tracers placed in some of the microstimulated regions.

117 Contextual Effects in Neuronal Responses to Complex Acoustic Stimuli Differ Between Areas AI and AAF

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The primary auditory cortex (AI) and anterior auditory field (AAF) are both thalamorecipient "core" auditory cortical areas. When stimulated with complex dynamic sounds, neurons in mouse AI and AAF exhibit different linear filtering properties as assessed through analysis of spectrotemporal receptive fields (STRFs) (Linden et al., J Neurophysiol 90:2660, 2003). Generally, AI filters are slower, and more broadly tuned, than those in AAF. We have recently proposed a new way to characterise auditory cortical response properties (Ahrens et al., J Neurosci 28:1929, 2008) with models that incorporate the nonlinear effects of short-term acoustic context. These models identify a "contextual re-weighting field" (CRF) modulating the efficacy of spectrotemporal elements within the stimulus before integration by the STRF. Many contextual effects, including phenomena previously probed only with simple stimuli (such as forward suppression and combination sensitivity), can be captured for a complex acoustic stimulus with the CRF. We compared CRFs identified from responses of AI and AAF cells to dynamic random chord stimuli. We found that: (1) CRFs in both AI and AAF were typically inseparable (the predictive power of the inseparable context model was considerably higher than that previously reported for a separable context model of the same data in Ahrens et al., 2008); (2) CRF modulation was greater in AI than AAF; (3) spectral interactions within the CRF were strongly asymmetric in AI, but less so in AAF; (4) contextual effects in AI were slower and longer-lasting than those in AAF. These findings show that the nonlinear effects of acoustic context differ between AI and AAF neurons. In combination with previous results, they suggest that AAF may be specialised for rapid, temporally and spectrally precise processing, while AI neurons integrate more broadly along both spectral and temporal dimensions.

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118 Cyto- And Chemoarchitecture of Macaque STS

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Cyto- and chemoarchtecture of rhesus macaque STS (superior temporal sulcus) was investigated by staining for Cytochrome Oxidase, AchE, Nissle staining, and immunohistochemistry for calcium binding proteins and SMI 31/32. STS contained three banks and the floor part. In anterior STS, both banks showed parallel tendency, which was that the deep part showed higher order association cortex than superficial part. This may suggest that STS contains the rim part of visual ring and auditory ring. (Supported by NIMH//IRP)

119 Surprise - Extremely High Doses of Cisplatin Do Not Destroy Hair Cells in Rat Cochlear Cultures

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Ototoxicity is one of the major dose limiting side-effects of cisplatin. Previous in vivo studies have shown that as the dose of cisplatin increases, hair cell damage begins in the basal high frequency region and spreads towards the apical low frequency region. In addition, outer hair cells (OHC) are more vulnerable than inner hair cells (IHC). In order to establish a detailed cisplatin dose-response damage model for later studies of otoprotection, post-natal cochlear organotypic cultures were treated for 24 h or 48 h with cisplatin doses ranging from 10 μM to 5 mM. After 24 h of cisplatin treatment, none hair cell bodies were missing with any of the 6 cisplatin doses; however obvious signs of stereocilia damage were found only with the lower doses (10, 50, 100 μM) of cisplatin. After 48 h of cisplatin treatment, cochlear hair cell loss increased nonlinearly with dose. Hair cell loss was approximately 30% at 10 mM, 80% at 50 mM, and 50% at 100 mM. The in vitro hair cell lesions were relatively uniform along the length of the cochlea in contrast to in vivo models that show a base-to-apex gradient. Unexpectedly, when the cisplatin concentration exceeded 400 μM most hair cells survived even when the concentration was as high as 5 mM. Preliminary labeling studies suggest that the lack of hair cell loss at very high cisplatin concentrations may be due to reduced uptake of cisplatin into hair cells. Research supported by NIH (5R01DC006630-05)

120 Role of the Copper Transporter Ctr1 in Platinum-Induced Ototoxicity

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The utility of the platinum chemotherapeutic agent, cisplatin, is limited by high incidence of ototoxicity in up to 40% of cisplatin-treated patients. While cisplatin primarily

damages the outer hair cells (OHCs) of the organ of corti,more extensive injury also disrupts inner hair cells (IHCs), stria vascularis (SV) and spiral ganglion (SG) neurons. A high inter-individual variability in sensitivity to this ototoxic effect suggests that genetic and pharmacokinetic factors play a role in this susceptibility.
Although recent literature establishes the dependence of the antitumor efficacy of cisplatin on the expression of an influx copper transporter (Ctr1), its role in cisplatin-induced ototoxicity remains unknown. The goal of this study was to determine the expression of Ctr1 along with several other platinum compound candidate transporters in mouse cochlear tissue by RT-PCR, quantitative RT-PCR (qPCR), Western blot and immunochemistry. The HEI-OC1 cell line, that expresses many molecular markers representative of OHCs was characterized for expression levels of all candidate transporters as well as for cytotoxic effects of cisplatin and two newer analogs, oxaliplatin and carboplatin.
These experiments revealed that Ctr1 is abundant and highly localized in the inner ear; mainly OHC, IHC, SV and SG and surrounding nerves in the mouse cochlea. Cisplatin was found to be 4- and 20- fold more toxic than oxaliplatin and carboplatin, respectively, toward HEI-OC1 cells, which possess a high endogenous level of Ctr1. Further we observed that the cytotoxicity of cisplatin, but not that of oxaliplatin and carboplatin, was potentiated 3-fold in Ctr1-transfected over the empty vector transfected HEK cells.
Collectively, these results provide evidence for a role of Ctr1 in cisplatin-induced ototoxicity. Inter-individual variation in expression levels of Ctr1 in inner ear cells may be a critical determinant of variation in sensitivity to cisplatin-induced ototoxicity.

121 Extracellular Copper Modulates Cisplatin Ototoxicity

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As one of its side effects, ototoxicity of cisplatin has become more noticeable, since the nephrotoxicity of cisplatin can now be mitigated by therapeutic hydratation. Previous studies revealed that the ototoxic mechanisms of cisplatin were involved with many effectors and multiple apoptotic pathways. Consequently, many practical treatments were performed for protection by blocking cell death pathways according to various ototoxic mechanisms of cisplatin. However, very little know about the platinum transporter, and there are no known ways to block the entrance of cisplatin into cells. Considering cisplatin is a platinum reagent that enters the cell through the copper transporter, we assume that if the copper transporter is occupied by copper, this action may competitively block the entrance of cisplatin and induce efflux of intracellular copper and platinum. To test this hypothesis, post-natal cochlear organotypic cultures were treated with copper sulphate with doses ranging from 10μM to 500μM, or cisplatin with doses of 10μM and 50μM respectively, or a combination treatment of both cisplatin and copper sulphate at concentrations ranging from 10-100 μM for 48 hours. In copper sulphate treated cochlear tissue, we found large lesions if the concentration was higher than 200μM. This suggested that a high dose of copper can damage cochlear hair cells, whereas low dose of copper did not damage the cochlear tissue. 10 μM cisplatin caused about 20% inner and outer hair cell loss. However, most hair cells survived when the cochlear tissue was cultured with both cisplatin and copper sulphate. Unfortunately, copper sulphate did not reduce the high dose cisplatin (50μM) ototoxicity. Western blot examination showed that additional copper sulphate can significantly reduce CTR1 expressions, but greatly increase expression of ATP7A and ATP7B. Considering that CTR1 is responsible for copper and platinum influx, the decreased CTR1 may reflect a cellular feedback reaction that blocks the copper input transporter. The function of ATP7A and ATP7B are of copper export, responsible for copper and platinum effusion. The increased ATP7A and ATP7B suggested that the cells start to pump out the copper or platinum when the copper concentration was high in the extracelluler space. This preliminary data suggests that additional extracellular copper may be able to reduce the influx of platinum while enhancing the output of platinum via a feedback reaction.

122 Nrf2 Activation by Ebselen Suppresses ROS Generation in Cisplatin-Treated Auditory Cells

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Ebselen, an organoselenium compound that acts as a glutathione peroxidase mimetic, has been demonstrated to possess antioxidant and anti-inflammatory activities. However, the molecular mechanism underlying this effect is not fully understood in auditory cells. The purpose of the present study is to investigate the protective effect of ebselen against cisplatin-induced toxicity in HEI-OC1 auditory cells and organotypic cultures of cochlear explants from two-day postnatal rats (P2). Pretreatment with ebselen ameliorated apoptotic death induced by cisplatin in HEI-OC1 cells and organotypic cultures of Corti's organ. Ebselen pretreatment also significantly suppressed cisplatin-induced increases in intracellular reactive oxygen species (ROS), intracellular reactive nitrogen species (RNS) and lipid peroxidation levels. Ebselen dose-dependently increased the expression level of an antioxidant response element (ARE)-luciferase reporter in HEI-OC1 cells through the translocation of Nrf2 into the nucleus. Furthermore, we found that pretreatment with ebselen significantly restored Nrf2 function and ameliorated the cytotoxicity of cisplatin in cells transfectants with either a pcDNA3.1 (control) or a DN-Nrf2 (dominant-negative) plasmid. We also observed that Nrf2 activation by ebselen increased the expression of phase II antioxidant genes, including heme oxygenase

(HO-1), NAD(P)H:quinine oxidoreductase, and ?-glutamylcysteine synthetase ((-GCS). These results suggest that ebselen activates the Nrf2-ARE signaling pathway, which ultimately protects auditory sensory hair cells from free radicals produced by cisplatin.
This work was supported by the Korea Science & Engineering Foundation (KOSEF) through the Vestibulocochlear Research Center (VCRC) at Wonkwang University in 2008.

123 Gingko Biloba Extracts (EGb 761) Prevent Cisplatin-Induced Apoptosis and Down-Regulation of Gap Junctional Intercellular Communication in Auditory Hair Cells

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Purpose: Cisplatin is a well-known anticancer drug showing apoptosis and disturbance of gap junctional intercellular communication (GJIC) in auditory hair cells, which is an important process in organ of Corti (oC) for hearing. Gingko biloba extracts (EGb 761) has been used as an antioxidant or a circulation enhancer. The purpose of this study was to examine the efficiency of EGb 761 in protecting against cisplatin-induced apoptosis and disturbance of GJIC.
Materials and Methods : For evaluation of anti-apoptotic action of EGb, HEI-OC1 cell line was cultured and treated with cisplatin (50uM) with EGb 761 (300ug/ml) for 24 hrs, and then immunocytochemistry (annexin V/propidium iodide) and Western blotting (caspase 3, PARP) were done. And basal turn oC explants from neonatal (p3) rats were exposed to cisplatin (1-10uM) and EGb (50 - 400ug/ml). The number of intact hair cells was counted by co-labeling with phalloidin, MyoVIIa, and DAPI. For evaluation of GJIC, immunocytochemistry and Western blot of connexins (Cxs) and scape loading dye transfer (SLDT) were tested for intracellular location, connexin change, and GJIC on HEI-OC1 cells.
Results : EGb prevented cisplatin-induced apoptosis in immunostaining and decreased caspase 3 and PARP bands, which were increased in cisplatin-treated cells in Western blot. In oC explants, EGb significantly prevented cisplatin (6uM, 10uM)-induced hair cell damage compared with cells treated with cisplatin alone. In this experiment, the number of phalloidin staining was less than that of MyoVIIa in the same situation. In connexin study, EGb prevented abnormal intracellular locations of Cx 26, 30, 31, 43 in cells treated with cisplatin in immunocytochemistry and increased Cx bands, which were decreased by cisplatin in Western blot. EGb also prevented cisplatin-induced disturbnace of GJIC in SLDT study.
Conclusions: EGb 761 prevent cisplatin-induced apoptosis and disturbance of GJIC in auditory hair cells. EGb 761 may be one of preventive medications against cisplatin induced ototoxicity.
This study was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund)(KRF-2006-E00081).

124 Heat Shock Inhibits Cisplatin-Induced Hair Cell Death in the Adult Mouse Utricle in Vitro

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Cisplatin is used to treat a wide variety of cancers; however, a significant proportion of patients receiving cisplatin experience significant permanent hearing loss. The ototoxic side effects of cisplatin result in part from damage to sensory hair cells, leading to the apoptotic death of those cells. We previously showed that upregulation of heat shock proteins (HSPs) inhibits aminoglycoside-induced hair cell death, and that Hsp70 accounts for most of the protective effect of heat shock. We have now examined whether HSP induction can inhibit cisplatin-induced hair cell death. Adult mouse utricles were cultured at 37^{o}C overnight and then were either heat shocked (43^{o}C for 30 mins) or maintained at 37^{o}C (control utricles). Six hours after heat shock, utricles were exposed to either control media or media containing cisplatin at a variety of doses for 24 hours. Our results indicate a protective effect of heat shock against cisplatin-induced hair cell death across the dose-response curve (2-way ANOVA, $F_{1,160} = 40.01$, $p < 0.001$). However, our preliminary data indicate that Hsp70, the most inducible and widely-conserved heat shock protein, does not account for the protective effect of heat shock against cisplatin-induced hair cell death. Thus, we are currently examining the roles of other heat shock proteins in mediating the protective effect of heat shock preconditioning. This work was supported by NIH 5R01 DC007613 from the National Institute on Deafness and Other Communication Disorders, National Institutes of Health.

125 Local Administration of Thiosulfate as Protection Against Cisplatin-Induced Ototoxicity

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Background: Cisplatin is a potent antineoplastic drug that has been used in clinical practice since 1971. The ototoxic side effect has been documented in a number of clinical

and experimental studies. Different approaches have been undertaken to reduce the ototoxic effect of cisplatin. Earlier results from our group show reduced free levels of cisplatin in the blood compartment after concomitant administration of D-methionine. We are developing a novel technique for local administration of drugs to the inner ear to reduce the risk of systemic drug interactions, side effects, and to obtain higher drug levels in the inner ear. The aim of the present study is to reduce the ototoxic effect of cisplatin by intratympanic injection of a gel loaded with the antioxidant thiosulfate. Material and methods: Guinea pigs with normal electrophysiological hearing thresholds were divided into three groups. In group 1, animals were given an intratympanic injection of thiosulfate (0.10 M) in hyaluronic acid gel (0.5%). Group 2 received an intratympanic injection of hyaluronic acid gel (0.5%) without thiosulfate. To assure that thiosulfate is not ototoxic a third group was given an injection of thiosulfate (0.10 M) in hyaluronic acid gel (0.5%) to the middle ear. The animals in group 1 and 2 received an intravenous injection of cisplatin (8 mg/kg) two hours after the intratympanic injection. The animals were sacrificed 96 hours after cisplatin injection and surface preparation was used for quantification of haircell loss. Results: The results will be presented at the meeting.

126 Protection of Cisplatin-Induced Ototoxicity by Transplatin

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Studies from our laboratory provide evidence that the transient receptor potential vanilloid 1 (TRPV1) channels mediate entry of platinum-based drugs into organ of Corti hair cells. We reasoned that inhibition of these channels by transplatin, an inactive isomer of cisplatin, would reduce the entry of cisplatin into hair cells and thereby protect against otoxicity. Using UB/OC-1 cells, a cell line derived from organ of Corti, we shown that transplatin (1-10μM) pre-treatment reduced cisplatin-mediated generation of reactive oxygen species (ROS) and increases in Ca^{2+} influx through TRP channels. Transplatin also abrogated the induction of NOX3 and TRPV1 RNA expression, as determined by quantitative PCR. In addition, transplatin (1μM) reduced cisplatin-induced apoptosis of UB/OC-1 cells, but did not alter its ability to kill different cancer cells lines tested, including rat AT6.1 prostate cancer cells. Intraperitoneal (IP) administration of transplatin (5.5 mg/kg) 30 min prior to cisplatin (13 mg/kg, IP) protected against ototoxicity, as measured by auditory brainstem responses (ABR). Transplatin also reduced damage or loss of rat outer hair cells, as determined by scanning electron microscopy (SEM). Protection against cisplatin ototoxicity was also produced by trans-tympanic (TM) administration of transplatin (100μl, 0.5mg/ml) 10 minutes prior to cisplatin. Transplatin administered alone by either the IP or TM routes did not alter ABR thresholds. This study provides evidence that transplatin interferes with cisplatin-mediated cytotoxicity mechanisms in nonmalignant cells *in vitro* and suggests that this drug may be effective in reducing cisplatin ototoxicity in cancer patients.

127 EDU Protects Cochlear Hair Cells Against Cisplatin Toxicity

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The ototoxic effects of cisplatin are not fully understood, but are believed to be due in part to the generation of reactive oxygen species (ROS) such as the superoxide anion and the hydroxyl radical. EDU (N-[2-(2-oxo-1-imidazolindinyl) ethyl]-N'-phenylurea) is a heterocyclic compound that increases superoxide dismutase and catalase activities in lung, heart and liver tissues without causing obvious signs of toxicity. EDU has also been shown to protect against lung injury; however, it is unclear if it can protect the cisplatin that damages the hair cells and neurons in the inner ear. To determine if EDU can also protect against cisplatin ototoxicity, we treated cochlear cultures from P3 rats with cisplatin alone or in combination with EDU. Cochlear organotypic cultures were treated for 48 h with 10 μM or 50 μM cisplatin alone or in combination with EDU at concentrations ranging from 10-100 μM. EDU was applied at the same time as cisplatin or approximately 12 h prior to cisplatin treatment. When 10 μM of cisplatin was administered alone to cochlear organotypic cultures, it destroyed ~40% of the hair cells. Simultaneous or 12 h pretreatment of cochlear cultures with EDU enhanced hair cell survival by up to 80%. When 50 μM of cisplatin was administered alone, it destroyed ~80% of cochlear hair cells. However, when EDU was administered 12 h before or concurrently with the 50 μM dose of cisplatin, it did not rescue the cochlear hair cells. These results suggest that EDU can protect cochlear hair cells against low-doses of cisplatin, but not against high doses of cisplatin. Research supported by NIH (5R01DC006630-05)

128 Gene Expression Analysis of Mouse Cochlea Exposed to TTS and PTS Noise

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The National Institute for Occupational Safety and Health estimates that there are 30 million American workers exposed to hazardous noise, with 1/3rd of those workers suffering from a detectable noise-induced hearing loss. Noise-induced hearing loss is the most common occupational injury in the U.S. Erway et al. (1996) showed that the C57BL/6J strain of mice were more susceptible to noise damage than the CBA/CaJ strain of mice and this difference was genetic in origin. Davis et al. (1999) developed a noise dose response curve for the two strains

and showed that, not only were C57BL/6J mice about 8 dB more sensitive to noise, their dose-response curve had a different slope than CBA/CaJ. This implied that there were two different mechanisms responsible for cochlear damage. Much is known about how very loud sounds damage hearing in chinchillas. The EPA estimates that 80% of workers are exposed to less than 90 dBA of noise. These levels are assumed to be safe by regulation. The aim of our experiment was to identify markers of the boundary of temporary threshold shift (TTS) and permanent threshold shift (PTS). CBA mice were exposed to one hour noise levels that produced either TTS or PTS. RNA from the exposed mice was harvested immediately after exposure and processed for microarray analysis. In comparing mRNA expression levels between TTS and PTS exposures, we have found 778 genes whose mRNA expression levels up- or down regulated 1.2 fold or greater ($p < 0.05$). First-pass pathway analysis seems to implicate the differential transcription of genes involved in a number of different signaling cascades. Two pathways of note include genes involved in focal adhesion signaling and the TNF-alpha/NF-kappa pathways, whose expression levels are up and down-regulated following TTS and PTS exposure respectively. This work was supported by a grant from NIDCD (R21 DC 7866).

129 Divergence of Noise Vulnerability in CBA/J and CBA/CaJ Mice

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CBA/J and CBA/CaJ (/CaJ) inbred mice frequently serve as 'good hearing' models for functional and genetic studies, and are often assumed to possess the same hearing characteristics. In fact, these strains were separated >70 yrs ago, and differ at many loci. We recently showed that aging characteristics of the CBA/J and /CaJ cochlea diverge after 1 yr of age, such that only /CaJ mice exhibit EP decline (Ohlemiller and Gagnon, ARO 2007). By contrast with aging, CBA/J mice may be more vulnerable than /CaJ to noise (Gagnon et al., HR 2007). We are exploring the latter relation using a dose-response approach.

In keeping with our previous work (Ohlemiller et al., HR 2000), mice of either gender are exposed one time to broadband noise (4-45 kHz, 110 dB SPL) for durations varying from 0.23-240 min in multiples of 2. For each group of up to 8 mice, the proportion of animals demonstrating a criterion permanent threshold shift (PTS, as established by ABR 2 wks post-noise) is calculated, and the duration-vs-proportion relation is fit to a 4-parameter logistic function to determine the 'threshold' exposure for PTS. Because noise vulnerability is different for young (<4 mos) and older mice (K.R. Henry, Audiol. 1983), separate comparisons are made for mice aged 6 wks and 6 mos.

We have completed comparisons for CBA/J and /CaJ mice at 6 wks of age. Separate estimates from 3 cohorts of /CaJ mice place the threshold exposure duration for PTS at >3.45 min. By contrast, the threshold exposure duration in CBA/J is <1.0 min. Having determined that exposures ranging from 1-3 min yield essentially 0% incidence of PTS in CBA/CaJ, but 100% in CBA/J, we will examine the distribution of PTS from 2 min exposures in F1 hybrid and N2 backcross mice formed from CBA/J and /CaJ. In this way, we hope to establish the minimum number of genes that differentiate noise sensitivities in the two strains and lay the groundwork for mapping.

(NIH T35 DC008765 to MRR, P30 DC004665, and R01 DC08321 to KKO)

130 A QTL on Mouse Chromosome 18 Determines How the Cochlear Lateral Wall Responds to Noise

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After a single moderate noise exposure (110 dB SPL, 4-45 kHz, 2 hrs) the endocochlear potential (EP) in CBA/J and CBA/CaJ (CBA) mice transiently declines by 30-50 mV, while that in C57BL/6J (B6) does not (Hirose and Liberman, JARO 2003; Ohlemiller and Gagnon, HR 2007). Differences in how the EP responds acutely to noise correlate well with both transient and permanent changes in stria vascularis and spiral ligament anatomy. Eight wks post-noise, significant reductions are found in Type I and II fibrocytes, strial basal cells, and strial capillary density. EP measures in F1 hybrid and N2 backcross mice to B6 indicated that the noise phenotype in CBA/J could be explained assuming 1-2 dominant-acting QTLs. We have undertaken coarse mapping of this trait, and also have found evidence of recessive inheritance of a noise phenotype similar to CBA in BALB/cJ (BALB) mice.

Whole genome marker analysis of tail DNA from 80 N2 mice revealed a single QTL (LOD 2.98) localized with 95% confidence to the 5-43 cM region of chromosome 18. This interval contains many genes, notably including those encoding aquaporin 4 and the Na-K-Cl co-transporter, which are critical to lateral wall function. We plan to reduce the mapping interval using a larger sample and more closely spaced markers, followed by expression profiling of candidate genes in CBA/J and B6 after noise.

In our initial exploration of the CBA noise EP phenotype, we noted similar changes in BALB mice. However, F1 hybrid mice formed from B6 and BALB showed no EP reduction after noise, indicating recessive inheritance. BALBs may therefore possess a different allele from CBA on Chr. 18, or perhaps produce the same phenotype from a different locus. Our first approach will be to include BALBs in our expression profiling of Chr. 18. We also plan to examine the effects of noise in 13 strains of recombinant inbred (RI) mice formed from B6 and BALB, followed by haplotype analysis.

(NIH P30 DC004665 to R. Chole and R01 DC08321 to KKO)

131 Tumor Necrosis Factor Signaling in Noise-Induced Trauma

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Intense noise causes irreversible damage to the cochlea, resulting in cell death and permanent threshold shift (PTS). To better understand molecular mechanisms underlying noise induced trauma, we performed global gene expression profiling using Affymetrix GeneChips using a rat model. We exposed rats to broad band noise (2-20 kHz) for 90 minutes at 120dB to produce PTS. Total RNA was isolated from rat whole cochleae dissected at 2.5 hr after PTS noise exposure (3 RNA pools per condition), and from non-exposed controls. One significant pathway differentially regulated by intense noise was tumor necrosis factor (TNF) signaling. Expression of Tnfr1 was up-regulated by intense noise. Previous studies have also indicated involvement of TNF signaling in response to noise insults, consistent with our observations. These results indicate that noise quickly activates inflammation response within the cochlea. In the normal cochlea, immunostaining with antibodies specific to TNFR1 was localized to inner and outer hair cells in the organ of Corti. It was also in localized to soma of spiral ganglion cells as well as the marginal and intermediate cells in the stria vascularis. We are currently examining qualitative and quantitative changes in expression pattern in response to noise. We are also conducting studies to determine if inhibition of TNF-mediated inflammation prevents noise induced trauma. (Supported by grants from GM/UAW, NOHR (TWG), UM-COHSE (TWG), and NIH P30 DC05188)

132 WDR1 Expression in the Normal and Noise-Damaged Mammalian Cochlea

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Objects: WDR1 gene has been reported to be involved in actin dynamics and to play an important role in hair cell regeneration in avian cochlea. However, to the best of our knowledge, there has been no report on the role of WDR1 gene in mammalian cochlea. The aim of this study was to investigate expression of WDR1 in the milieu of acoustic trauma in Sprague Dawley (SD) rats.
Methods: Twenty one-day-old SD rats were divided into three groups: the permanent threshold shift (PTS) group, the temporary threshold shift (TTS) group, and the control group. The PTS group was exposed to 120 dB white noise for 5 hours and the TTS group was exposed for 30 minutes after checking auditory brainstem responses (ABRs). Four SD rats from each of the 3 groups were euthanized immediately after the noise exposure, 3 days later, 7 days later, and 28 days later after checking ABRs again. The harvested cochleae were processed and immunohistochemically stained with anti-WD40 repeat protein 1 antibody. Quantitative analyses were done by an immunoreactive density measuring program (Image J, released by NIH) and Western blotting.
Results: We confirmed permanent threshold shift in the PTS group and temporary shift in the TTS group by measuring ABRs. In the cochleae immediately after the noise exposure, both PTS and TTS groups showed elevated expression of WDR1 than the control group. At 3, 7, and 28 days after noise exposure, the PTS group showed relatively higher expression of WDR1 than the TTS group in hair cells, supporting cells, strial marginal cells, and Claudius cells.
Conclusions: We verified the elevated expression of WDR1 as a protective mechanism against noise damage in mammals. In addition, we could corroborate the difference in the intensity and the duration of the WDR1 expression in terms of the intensity of the noise exposure.

133 Time-Dependent Changes in Mcl1 Expression in Noise-Traumatized Cochleae

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Myeloid cell leukemia sequence 1 (Mcl1) is a member of the Bcl-2 family originally isolated from human myeloid leukemia cells. It participates in the regulation of the mitochondrial pathway of apoptosis. The current study was designed to investigate the expression pattern of Mcl1 protein in the normal and the noise-traumatized cochleae of Sprague Dawley rats. In the normal cochlea, immunolabeling showed a wide expression of Mcl1 protein in both hair cells (HCs) and supporting cells, including Pillar cells and Deiters'cells and Hensen's cells. In HCs, Mcl1 immunoreactivity was distributed in the cytoplasm spatially corresponding to the mitochondrial distribution in the cells. Western blot analyses revealed that the expressed Mcl1 was the full length form of Mcl1 (Mcl1-L), which possesses the antiapoptotic property. Following exposure to a broadband noise at 115 dB SPL for 2 hrs, the cochleae exhibited a time-dependent variation in Mcl-1 protein expression. At 4 hrs after the noise exposure, both immunolabeling and western blotting showed the reduction in the expression level of the Mcl1 protein. However, one day after the noise exposure, the Mcl1 protein level was upregulated. The upregulated protein was the short form of Mcl1 (Mcl1-S), which has the pro-apoptotic property. This pattern of the protein expression change was, in general, consistent with that of the mRNA expression change observed in our previous study, which showed an initial downregulation of the mRNA expression level immediately after a noise exposure, and a subsequent upregulation at 4 hrs after the noise exposure. Clearly, the upregulation of the mRNA expression preceded the protein expression. Taken together, the results suggest that Mcl1 is involved in both the maintenance of normal cochlear homeostasis and the regulation of noise-induced cochlear apoptosis. (Supported by New Faculty Startup funds from University at Buffalo and NOHR funds)

134 Decomposition of Mechanical Stresses in Acoustic Trauma

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Acoustic overstimulation generates multiple forms of mechanical stresses, including stretching, shearing, bending, compression/decompression. Interaction of these mechanical stresses can activate acute hair cell (HC) apoptosis. However, it remains unclear how each individual stress induces acute apoptosis. Here, we reported a cochlear model of compression/decompression injury induced by exposure to intense noise. The model was generated by surgical occlusion of the round window of the cochlea of the Sprague Dawley rat. Blocking the round window of the cochlea eliminated the induction of the pressure difference between the scala typmpani and the scala vestibuli, the driving force for basilar membrane vibration during acoustic stimulation. Under this condition, noise stimulation will generate only the pressure fluctuation without inducing the basilar membrane motion, the source of stretching and shearing stresses. Consequently, only the compression/decompression stress was induced. After the surgery, we found 10 to 15 dB threshold shifts across the frequency range between 5 and 40 kHz, which were probably due to the reduction of basilar membrane motion. The animals with the round window closure were exposed to a broadband noise at 120 dB SPL for one hour. After the noise exposure, the cochleae were examined for assessment of HC membrane permeability and apoptotic activity. As compared with the cochleae without the round window closure, the cochleae having the round window closure exhibited a marked reduction in the membrane permeability. The number of apoptotic cells was also reduced. The reduction was more evident in the second cochlear turn, the initial site of HC pathogenesis. The results suggest that removing stretching and shearing stresses reduces acute membrane damage, which in turn prevents the cells from entering the apoptotic pathway.(Supported by NOHR funds and New Faculty Startup funds from University at Buffalo)

135 Changes in E-Cadherin in the Cochlea After Traumatic Noise Exposure

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Leonova and Raphael (1997) reported that the adhesion molecule, E-cadherin, alters distribution patterns resulting from scar tissue formation in the cochlea after administration of kanamycin. They speculated that E-cadherin may play an important role in the transmission of sound and the process of the scar tissue formation in the cochlea after ototoxic drug administration. We investigated changes in E-cadherin in the organ of Corti in rats immediately after exposure to a traumatic noise (10-20 kHz broad band noise at 110 dB SPL for 4 hours) using a confocal microscope. Similar to the previous study (Leonova and Raphael, 1997), E-cadherin was found in the reticular lamina. However, intense E-cadherin staining that outlined the apical part of the outer hair cells (OHCs) was observed in the reticular lamina in the noise-induced damage lesion (basal turn) immediately after the noise exposure. This phenomenon was not observed in the unexposed control animals. The OHCs that showed localization of E-cadherin, were found to be apoptotic or have missing nuclei. This E-cadherin localization may be related to the mechanical stress caused by the intense noise since this phenomenon was not previously observed in the kanamycin-treated cochlea.
This study was supported by NIOSH grant 1R01OH008113-01A1.

136 Noise-Induced Focal Lesions in the Organ of Corti: Distribution and Cell Death Pathways

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Studies have been conducted to identify what death pathways OHCs follow after moderate-severe noise exposures. Identified pathways include oncosis/necrosis, apoptosis & a non-apoptotic, non-oncotic pathway. IHCs, pillar & Deiters cells are also destroyed by noise. In order to develop treatment strategies that will minimize noise-induced hearing loss, it is important to identify death pathways in all cell types in the organ of Corti (OC). Eighteen chinchillas were exposed for 1 h to a 4-kHz OBN at 108 dB SPL. Recovery times were < 1 d to 30 d post-exposure. At termination, animals were anesthetized; their cochleae surgically exposed & fixed in-vivo with 1% buffered OsO_4. The intact cochleae were dehydrated, embedded in plastic & dissected into flat preparations. For each ear, OC length was measured & losses of cells were quantified throughout the OC. Focal hair-cell lesions were identified [≥ 50% loss of OHCs, IHCs or both (i.e., combined) over at least 0.03 mm] and death pathways in dying cells determined. All but one cochlea had one or more focal hair-cell lesions. In different cochleae, some lesions covered a narrow portion of the OC & were close to the 4-kHz OBN location, while some lesions were spread over ~ 50% of the OC. In cochleae with two or more lesions, variable-length areas of reduced damage separated the lesions. Twice as many OHC lesions as combined & IHC lesions were identified. OHC & combined lesions were greater in length than IHC lesions & usually included missing pillars & Deiters cells. IHC lesions rarely involved other cell types. This suggests that IHC lesions are generated by a different mechanism than OHC & combined lesions. Within the OHC & combined focal lesions, dying hair cells were identified that were following the oncotic, apoptotic & non-apoptotic, non-oncotic death pathways. The identification of death pathways followed by IHCs in IHC focal lesions & by supporting cells in OHC & combined focal lesions is in progress.

137 The Effect of Acoustic Trauma on Cochlear Pericytes

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Cochlear blood flow is markedly affected by acoustic trauma, but the concomitant changes in cochlear pericytes are unknown. In this study, we investigated the effect of

noise on cochlear pericytes. Transmission electron microscopy revealed pericytes on the capillaries of the stria vascularis (SV) closely associated with endothelium in the control guinea pigs and mice. Foot processes of the pericytes were tightly positioned adjacent to endothelial cells. Exposure to wide-band noise at the level of 120 dB for 3 hours per day for two consecutive days produced a significant hearing threshold shift and structurally damaged blood vessels in the SV. Serum protein such as IgG leaked from capillaries of the SV. Pericytes lost their tight association with endothelial cells. Expression of desmin, a pericyte structural protein, substantially increased after noise exposure in guinea pigs and mice, with a corresponding increase in pericyte vessel coverage. The increased expression of desmin was associated with the induction of hypoxia-induced factor (HIF-1α) and upregulation of vascular endothelial growth factor (VEGF). Inhibition of HIF-1α could decrease mRNA transcript level for VEGF. Inhibition of VEGF significantly attenuated the expression of desmin in the pericytes. These data are evidence that up-regulation of desmin directly follows activation of HIF-1α, with concurrent increased transcription of VEGF, suggesting that pericytes play an important role in the plasticity of cochlear blood vessels.
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138 The Expression of Proinflammatory Cytokines After Acoustic Overexposure

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Inflammation is originally protective reaction, however the excessive inflammation can result in damage of the tissues. Interleukin-6, Tumor necrosisis factor alpha , Interleukin-1 beta are molecules induced in inflammatory reaction. They are called proinflammatory cytokines. In the present study, we examined the expression of proinflammatory cytokines in the mice cochlea after acoustic overexposure.

The ICR male mice with normal Preyer reflex were used in this study. They were exposed to intense noise (130 dB SPL octave band noise) with a center frequency of 4 kHz for 1 h or 10 h. After acoustic overexposure, bilateral cochleae were carefully removed from the skull base. Total RNA was isolated using Trizol Reagent. RT-PCR were carried out for proinflammtory cytokines(IL-6,TNF alpha,IL-1 beta) and beta-actin.

RT-PCR showed that TNF alpha and IL-1 beta were induced after 1hr noise overexposure. TNF alpha was more induced after 1 h noise overexposure than 10 h. In the mice which have not been exposed to noise , the expression of IL-6 and IL1 beta were not induced.

These results indicate that proinflammatory cytokine may have a various expression pattern each other after acoustic overexposure in the cochlea.

139 Changes of Succinate Dehydrogenase(SDH) Activity After Noise Exposure in Rat Cochlea

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Noise damage to cochlea has been studied in many ways. Altered SDH activity was observed in noise exposed cochlea of Guinea pigs (Fredelius L, et al, 2000). SDH is a part of respiratory chain enzymes in mitochondria, and it represents activity of mitochondria. In this study, rats were exposed to 120dB SPL white band noise. The rats were divided to two groups according to exposed time for 1 hour and 5 hours. The rats were sacrificed 1 hour, 24 hours, 3 days, 7days, and 14 days after the exposure. The hearing level was measured using auditory evoked brainstem response before noise exposure and sacrifice. The SDH staining and immunohistologic study were performed using anti-mitochondrial antibody. The hearing level showed no response to click sound at the immediate after the noise exposure, and it became improved slowly. The activity of SDH was transiently increased after 3 and 7 days after noise exposure. Immunohistologic study showed no significant changes in 1 hour noise group, but in 5 hour group, the expression level of mitochondria was decreased 3 days after the noise exposure. We could observe time difference among hearing level, SDH activity and the immunohistologic findings.

140 Cluster Analysis Reveals Presbycusis Phenotypes That Group Subjects by Degree and Configuration of Hearing Loss

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It has been well-established that auditory thresholds increase with age, with many reports documenting the age-by-frequency progression of mean thresholds in screened as well as unscreened human populations. However, from these reports it is not clear how hearing loss varies by frequency in individuals, or whether there are sub-populations with different patterns of age-related hearing loss, which might be related to different underlying etiology. We examined 'hearing phenotypes' in a database of 962 subjects (552F, 410M, 18 to 92 yrs) having 30 measures of peripheral hearing sensitivity: pure tone audiograms for left and right ears from 250 Hz to 8000 Hz and DPOAE S/N amplitudes for each ear for F_{mean} = 1000 to 6400 Hz. Subjects were recruited from the Rochester community and had a very broad range of hearing abilities. Cluster analysis partitioned the data into seven classes that can be further described by three hearing loss groups: none, flat/gently sloping, and sloping, the latter two groups being comprised of three classes each, differing primarily by degree of hearing loss. DPGrams show a close

correspondence with pure tone configurations. Principal component analysis (PCA) on the same data set reveals that the first two principal directions account for 74 % of the variance in the data and reflect degree (PC1) and configuration (flat vs. sloping; PC2) of loss. Cluster patterns are similar across different age groups and reflect known gender differences including slope and degree of hearing loss. Ongoing characterization includes analyses designed to discover relations among these phenotypes and various measures of auditory perception of speech and non-speech stimuli. Although the verification of presbycusic sub-types based on audiometric configuration alone may not be robust, it is clear that combinations of auditory measures may help to define, characterize, and diagnose hearing loss sub-types as well as provide an index of treatment efficacy.
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141 Structural Integrity of Speech-Related Temporal Lobe Cortex Predicts Age-Related Differences in Word Recognition

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A common complaint of older adults with and without hearing loss is difficulty understanding speech, especially in challenging listening environments. In addition to well known declines in the peripheral auditory system, which contribute to poorer speech recognition, age-related changes in central auditory and attention-related systems are hypothesized to result in speech recognition difficulty. We examined the extent to which functional and structural differences in speech-related and attention-related cortex predicted differences in word recognition between 18 younger adults (19-39 years) and 18 older adults (61-79 years). Each subject performed a word recognition task in an MRI scanner. Words were parametrically low-pass filtered to manipulate word intelligibility across 4 conditions (400 Hz, 1000 Hz, 1600 Hz, 3150 Hz). T1-weighted images were used to examine the extent to which gray matter declines were present within speech-related temporal lobe regions and attention-related frontal lobe regions engaged by the word recognition task. Older adults exhibited significantly poorer word recognition than younger adults in the 1600 Hz low-pass-filter condition, which was associated with age-related differences in gray matter volume of medial Heschl's gyrus (HG. Individual structural variability in the left medial HG also significantly predicted anterior cingulate cortex (ACC) activation. The association between HG gray matter volume, word recognition, and ACC activation was present after controlling for high frequency hearing loss. Competing causal path models exploring relationships among these three variables indicated that individual variability in left HG gray matter volume related directly to word recognition, with indirect influences of ACC activation. These results have clinical implications for rehabilitation and suggest that some of the perceptual difficulties experienced by older adults with hearing loss may be due to age-related structural changes in medial HG.
[Work supported by NIH/NIDCD and the American Federation for Aging Research]

142 Amplitude Modulation Rate Discrimination by Younger and Older Listeners

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Declines in temporal processing are frequently reported among elderly listeners, regardless of peripheral hearing status. However, one aspect of temporal processing that has yet to be quantified among older listeners is temporal pitch coding. Studies reveal that normal-hearing (NH) listeners are often able to perceive a pitch percept through modulations in the temporal envelope, even in the absence of spectral information (Burns & Viemeister, 1976; Miller & Taylor, 1948). Emerging neurophysiological data suggest that the ability to code temporal envelope modulations declines with advancing age, even in the absence of peripheral hearing impairment (Shadduck-Palombi, et al., 2001; Walton et al., 2002). Parallel psychophysical measures among younger and older human listeners have yet to be performed. Modulation rate difference limens were measured in NH listeners. Stimuli were sinusoidally amplitude-modulated broadband noise bursts: reference modulation rates ranged from 50-400 Hz. Preliminary data suggest significant age-related declines in the ability of NH listeners to discriminate between temporal envelope modulation rates. These results are consistent with expectations based on neurophysiological findings, and could have important implications for cochlear-implant listeners, who primarily utilize temporal envelope cues for voice pitch processing.
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143 Temporal Discrimination in Accented Tone Sequences by Young and Elderly Listeners

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The study measured listener sensitivity to increments of a target interval embedded within a tone sequence featuring a single accented component. The reference stimuli consisted of six 1000-Hz tones separated by silent intervals, with equal tonal inter-onset intervals (IOI) of 200ms; tone durations were 50ms, with one tone being elongated in duration (100ms) to produce a perception of accent. Duration DLs for increments of a single sequence IOI were measured, with sequence locations of the target IOI and the accented tonal component varying across discrimination conditions. Listeners included a group of young normal-hearing adults and two groups of older

adults with and without high-frequency hearing loss. Results indicated that discrimination performance of listeners in the two older groups was equivalent, but significantly poorer than that of the younger listeners in each condition. The magnitude of the age-related performance differences was largely independent of both the sequence location of the target IOI and the location of the accented tonal component. Comparative discrimination data collected using unaccented stimulus sequences indicated that the detrimental influence of tonal accent on temporal discrimination performance was much greater for older listeners compared to younger listeners

144 Age Effects in Temporal Envelope Processing: Speech Unmasking and Auditory Steady State Responses

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The aim of this study was to determine whether temporal envelope processing is reduced in older listeners. Two groups of listeners with relatively normal hearing participated: a younger group (mean age = 25.0 yrs) and an older group (mean age = 68.7 yrs). Exp. 1 examined speech unmasking in modulated noise as a function of masker modulation rate (16 Hz and 32 Hz) and target speech rate (normal and 50% time-compressed). Exp. 2 measured ASSR amplitudes as a function of modulation rate (32 Hz and 128 Hz) and carrier frequency (500 Hz and 2000 Hz). Exp. 1 indicated that older listeners show reduced recognition of rapid speech in steady noise and reduced speech unmasking for normal-rate speech. For rapid speech there was no age effect for speech unmasking and no difference in the magnitude of masking release as a function of modulation rate. In general, effects of listener age and masker modulation rate on the magnitude of masking release were observed only for normal-rate speech. Exp. 2 showed that the ASSR amplitudes of older listeners are reduced for a 128-Hz modulation rate but not for a 32-Hz modulation rate, irrespective of carrier frequency. This pattern of results suggests that the reduced speech unmasking seen in older listeners for relatively slow modulation rates is not due to deficits in envelope processing but rather is associated with the more constrained redundancy of the speech material available during the masker minima. Deficits in temporal envelope processing associated with advanced age were evident electrophysiologically, but only at the highest envelope frequencies tested. [Work supported by NIH NIDCD R01-DC01507]

145 Hearing in Persons with Mild Cognitive Impairment (MCI), Alzheimer Disease (AD) and Normal Cognition (NC)

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The general goal is to study the relationship between hearing ability and dementia in persons in late middle- and old age. The specific goal is to study if there is any relationship between an early start of age related hearing impairment and later debut of dementia. Furthermore, this study intends to find out if an early acoustic stimulation/amplification can possibly slow down cognitive deterioration in persons with cognitive impairments.
Persons with memory problems, who have been referred to the Geriatric clinic Karolinska University Hospital, were investigated with neurological, cognitive, neuropsychological and behavioural tests. Depending on the outcome of the investigation, the participants were divided in three groups: 1) a group with normal cognitive functions (NC), N=27; 2) a group with mild cognitive impairment **(**MCI), N=36, and 3) a group with Alzheimer disease (AD), N=28. All participants were tested at the department of Audiology, with the following peripheral and central hearing tests: pure tone audiometry, tympanometry, speech-audiometry in quiet and in background noise, dichotic tests with digits, and mismatch negativity (MMN). The preliminary results showed that the participants of all tree groups had similar age, around 62 years, similar mild high frequency hearing loss, and similar results regarding speech audiometry in quiet. Participants with AD had significantly poorer results regarding speech-audiometry in background noise, and dichotic tests with digits, especially on the left ear, compared to the NC and MCI groups. The outcome of the MMN showed similar results in all tree groups according to the preliminary analysis.

146 Development of a Visual Anticipatory Balance Training System

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Background: Falls can be debilitating in the elderly population. Balance dysfunction in older persons can arise from variable reasons including normal aging process, vestibular and neuromuscular causes. The results of disequilibrium are detrimental and it is crucial to develop tools for identifying fall risk. Objective: To develop an evaluation and training system based on anticipatory balance theory for fall prevention. Method: The system contains two functional modules: the assessment module detects center of force (COF) by a single-axis force plate; the rehabilitation module comprised of a video game for test subjects to accomplish certain tasks requiring eye-head-body coordination so the anticipatory balance training effects can be achieved. These two modules can execute simultaneously by network communication technique (TCP/IP settings). Postural changes of test subjects can be detected, reflected and recorded on the video-game. Average sway velocity, total sway path length and sway range (antero-posterior and lateral) of 3 trials were measured to examine the differences of balance capability between two test

subjects. (one 26 y/o and one 66 y/o female, with no history of fall) The time required to complete the game and the total scores were recorded. Results: The elderly person showed higher COF sway path length (old:young = 1.3:1), lower sway range (1:2) and velocity (1:1.4). The time required to complete the game was higher and the training performance was lower in the old adult. Discussion: On contrary to the conventional thoughts, the elderly person showed decreased body sway range and velocity when confronting fall risks. Increase in sway path length suggests that elderly people might use alternative strategies for fall prevention. Recruitment of more test subjects is required to identify people at higher risks for fall. Conclusion: A visual anticipatory balance training and assessment system was successfully developed. The system produces visual-vestibular conflict situations and can be applied to patients with vestibular and balance disorders for evaluation and training purposes.

147 OtoBase: A Chemical Biology Database for the Inner Ear

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We are implementing a computational strategy to investigate the effects of chemical agents, such as antibiotics and antineoplastic medications, on inner ear organs. As part of this effort we have designed a queriable relational database (*OtoBase*) comprising a wide range of parameters including: the chemical characteristics of drugs that affect inner ear function, drug mechanism of action (structural and molecular targets; reversibility), and therapeutic and ototoxic dose ranges. *OtoBase* links to the Encyclopedia of Molecular Targets database that contains information about the activities of small molecule drugs (EMoT; J. Muhlich; http://emot.mit.edu). EMoT allows the user to view the target ontology or hierarchical clustering of molecular structures and can be queried with a free-text search for drugs and targets. *OtoBase* was constructed using Microsoft® Access database software which promotes easy architectural design, data management, and data mining. *OtoBase* links to the basic and clinical primary research literature specific to drug otic activity that was used to populate field entries with data. *OtoBase* can be mined to analyze trends in ototoxic and protective mechanisms of drugs, for comparisons of ototoxic and therapeutic doses, and to uncover relationships between clinical studies and basic research. For example, a query using “gentamicin” retrieves data that illustrate the wide variation in organisms, experimental design, and inner ear systems (vestibular, auditory) that represent the foundation for knowledge of gentamicin ototoxicity. Analysis of the data retrieved in response to comparative queries with different chemical agents showed that the lack of standardization in pharmacological studies can pose challenges for computational analysis of drug otic activity. Further development of *OtoBase* will allow for integration with genetic and image data as well as open source web publication. Supported by NSF (HRD-0331446) and NIH (GM008136; P50GM068762).

148 Competitive Inhibition of Aminoglycoside Uptake in Vivo

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Aminoglycosides like gentamicin are toxic to both proximal tubule cells in the kidney and sensory hair cells in the inner ear. Previous studies have shown that uptake of a fluorescent aminoglycoside (GTTR) can be inhibited by co-administration of the native drug in the kidney and cochlea in vivo. By determining the intensity of GTTR fluorescence in these tissues, we tested the hypothesis that gentamicin inhibition of GTTR uptake in the kidney and stria vascularis are similar.

Purified GTTR (2 mg/kg in PBS, pH 7.4) was injected i.p. into C57BL6 mice (3-4 weeks). Some mice simultaneously received 2, 20, 200, 600, or 800 mg/kg gentamicin i.p. (n >/= 3). Thirty minutes later, mice were cardiac-perfused, and cochleae and kidneys were excised and examined by confocal microscopy.

Increasing doses of unconjugated gentamicin reduced cytoplasmic GTTR fluorescence in kidney proximal and distal tubule cells. In the cochlear stria vascularis, the baseline intensity of GTTR fluorescence was much reduced compared to kidney tissues. Only high doses of unconjugated gentamicin reduced GTTR fluorescence in the stria vascularis. These data confirm previously published data that show low level aminoglycoside uptake by the cochlea compared to kidney tissues.

The data demonstrate that aminoglycosides readily enter kidney tissues compared to cochlear tissues. This is reflected in (i) the comparative fluorescence of the two tissues during GTTR administration alone, and (ii) the differential inhibition kinetics of gentamicin on GTTR uptake in the two tissues. In the kidney, gentamicin reduced GTTR fluorescence at all molar ratios of gentamicin:GTTR tested, whereas inhibition was not readily apparent at low molar ratios in the stria vascularis. A mass action model of GTTR uptake kinetics gave similar results, with low and high equilibrium dissociation constants fitting the kidney and cochlear tissue data, respectively. These kinetic differences in GTTR uptake likely indicate differences in aminoglycoside uptake mechanisms in the kidney and stria vascularis.

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149 Intracellular Mechanisms of Gentamicin-Induced Hair Cell Damage in Neonatal Rat Organ of Corti

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Purposes: Apoptosis mediated by reactive oxygen species (ROS) and intracellular signaling pathways is thought to play a role in gentamicin (GM)-induced cochlear hair cell loss, however the mechanisms that mediate this have not fully elucidated. The purpose of this study was to assess the significance of intracellular processes, including highly-reactive oxygen species (hROS) formation, JNK pathway activation and apoptosis, in

damage to hair cells in the organ of Corti (oC) after treatment with GM.
Materials and Methods: Basal turn oC explants from postnatal day 3 (p3) or p4 rats were cultured and exposed to GM (35uM) combined with several concentrations of glutathion (GSH, ROS inhibitor), Clostridium difficile toxin B (CDTB, JNK inhibitor), ZVAD-FMK (caspase inhibitor), or combined inhibitors for 48 hours. Hair cells was assessed morphologically by co-labeling with phalloidin, MyoVIIa, and DAPI. The involvement of each pathway was evaluated by staining for hROS, phospho-c-JNK , or activated caspase 3.
Results : GSH, CDTB, or ZVAD-FMK significantly inhibited hair cell damage in a dose-dependent manner, when compared with GM alone. When the explants were exposed to inhibitor combinations, the use three inhibitors simultaneously significantly protected hair cell loss, compared with combinations of two inhibitors. hROS formation was also significantly inhibited in GSH- or CDTB-treated oCs. Caspase 3 activation was decreased most effectively in ZVAD-FMK-treated oCs, followed by GSH, but not in CDTB-treated explants. CDTB inhibited GM-induced JNK activation more effectively than GSH or ZVAD.
Conclusions : hROS formation, activation of the JNK pathway, and caspase activation are each involved in GM-induced damage to oC hair cells. hROS formation appears to be linked to the activation of both the JNK pathway and caspase 3. However, caspase activation may be less dependent on JNK activation, suggesting alternative pathways to hair cell death. The combined use of three inhibitors against ROS formation and the activation of both JNK and caspase 3 showed more effective protection effect against GM-induced hair cell damage that single use of inhibitors, also suggesting that these mechanisms are not completely overlapping.
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150 Gentamicin Uptake in MDCK Cells Via Hemichannels and Possibly Non-Selective Cation Channels

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MDCK cells share many features with strial epithelial cells and uptake of aminoglycoside (AG) antibiotics, and thus have been used as a convenient model for studying the trafficking of the ototoxic drugs. We have shown that Texas Red-tagged gentamicin (GTTR) can rapidly enter MDCK cells independently of endocytosis. However, direct evidence for channels mediating the AG uptake remain scarce. As AGs are typically polyvalent cations, we hypothesize and test that AG uptake is mediated via multiple non-selective cation channels (NSCCs). Using whole-cell recording and GTTR imaging on MDCK culture, we found that: 1) Confluent cells had a low input resistance (~80 MΩ), large input capacitance (~300 pF) and multiple-term exponential capacitive transients, indicating a tight gap-junction-mediated electrical coupling. 18β-glycyrrhetinic acid (18βGA, IC_{50} 27 μM) or 2APB (IC_{50} 76 μM) blocked this coupling. 2) With physiological solutions in the bath and pipette, single MDCK cells showed a resting potential ~-26 mV, and an input resistance ~1.24 GΩ. La^{3+} (100 μM) caused a depolarization of ~4 mV and a net current with a negative slope I/V relation ~-0.67 nS, reversed at ~-20 mV, suggesting existence of NSCCs. A zero Ca^{2+} bath solution reduced the La-sensitive conductance by ~60%, suggesting its high selectivity for Ca^{2+}. 3) Gentamicin (GT, 1 mM) typically caused an outward net current with V-shaped I/V relation: negative slope (~-0.29 nS) between -100 and +30 mV but a positive slope (9.2 nS) between +30 and +80 mV. GT caused no significant change in gap junction coupling. 4) La^{3+} reduced the GT-induced conductance reduction by 60%. 5) The GT-induced conductance increase (30 to 80 mV) was not affected by 1 mM TEA. 6) GTTR uptake by MDCK cells was significantly reduced by 18βGA but not by 2APB. 7) MDCK cells express Cx43, TRPV1,2,4 and TRPA1 transcripts. Our data suggest that GT likely enters MDCK cells via hemichannels and possibly also TRPV channels but not via the La^{3+}-sensitive Ca^{2+}-permeant NSCCs, the latter being blocked by GT. Funded by NIDCD R01 04716 (ZGJ) R01 04555 (PSS) and P30 05983

151 TRPA1: A Novel Perilymphatic Pathway for the Entry of Aminoglycoside Antibiotics Into Cochlear Hair Cells?

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Aminoglycoside ototoxicity is initiated by accumulation of the antibiotics in cochlear hair cells. It is thought that aminoglycosides enter the hair cells through mechanotransduction channels. However, penetration of aminoglycosides to the endolymph surrounding the mechanosensory stereocilia was reported to be a relatively slow process. Here we propose that TRPA1 non-selective cation channels can provide a novel perilymphatic pathway for the entry of aminoglycosides into outer hair cells (OHCs). Perforated whole-cell patch-clamp recordings showed that TRPA1 agonists – allyl isothiocyanate, cinnamaldehyde, and icilin – produced prominent inward current responses in OHCs of wild type mice but not in OHCs of TRPA1-deficient mice. In the absence of reliable antibodies, an electrophysiological approach was utilized to reveal the subcellular localization of TRPA1 in OHCs. A gentle flow of TRPA1 agonist produced a larger response when directed to the basolateral surface of a wild type OHC as compared to a similar application behind the stereocilia. Ca^{2+}-imaging revealed that OHCs respond to TRPA1 agonists only when the tight junction barrier is disrupted to allow agonist access to the basolateral membrane of OHCs. These data suggest that TRPA1 channels are functional and are likely to be localized at the

basolateral surface of OHCs. Therefore, TRPA1 channels might provide a novel pathway for the entry of small organic cations such as aminoglycosides or FM1-43 dye from the perilymph into OHCs. In correspondence with this hypothesis, our preliminary data indicate that TRPA1 activation does allow the entry of FM1-43 into OHCs.
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152 Quantitative Analysis of Partial Lesions Within the Mammalian Crista Ampullares

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Recent data indicating that type I hair cells have higher aminoglycoside antibiotic sensitivity than type II hair cells imply that partial lesions in the vestibular neuroepithelium can be produced from administration of low gentamicin doses. The ability to produce partial lesions provides the opportunity to develop a model for studying pathophysiology of mild ototoxicity and vestibular sensorineural deficit rehabilitation strategies. The aims of this research are to define the extent of lesions in vestibular neuroepithelia caused by low dose gentamicin exposure and characterize the fate of vestibular primary afferents after partial hair cell (HC) loss. In this study, intra-labyrinthine doses of gentamicin (0.1 or 1.0 µg) were acutely administered to *Chinchilla laniger* (aged 6-8 months). Vestibular afferent physiology was recorded (see companion abstract, Hoffman & Sultemeier) and tissues were harvested for immunocytochemical analyses 14-56 days after dosing. Total HC densities (HCs/100µm^2±SD) of cristae central zones were determined using image analysis techniques to directly count HC nuclei in confocal image stacks. Following infusion of 1.0 µg gentamicin, HC densities were reduced at 14 (1.38±0.22), 28 (1.24±0.29) and 56 days (1.18±0.09) post-administration compared to normal specimens (1.68±0.15). No HC density changes were found following infusion of 0.1 µg gentamicin. In damaged specimens, we observed Calretinin-immunopositive (CAL+) parent axons only (i.e. calyces absent) or partial CAL+ calyces (i.e. "hemicalyces"). In most 1.0 µg specimens, we observed primarily CAL+ parent axons. However, CAL+ calyces were not strikingly different than normal in most 0.1 µg specimens. Examples of calyx retraction without loss of contacting HCs were observed in damaged tissues. Data acquired thus far suggest that the affect of low doses of gentamicin on calyx-only afferents appears to precede maximum reduction of HCs.

153 Coding Deficits Associated with Partial Lesions of the Mammalian Crista Ampullares

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Transtympanic applications of gentamicin resulting in putative loss of type I hair cells has been associated with the absence of stimulus evoked modulation in vestibular afferent discharge while spontaneous discharge was preserved (*J. Neurophys.* 93:643, 2005). In view of another recent study demonstrating the variability in gentamicin's effects in vestibular neuroepithelia (*JARO* 8:497, 2007), we sought to establish the direct association between gentamicin-induced lesions and afferent discharge through a model in which the aminoglycoside was introduced directly into the perilymph. Small quantities of gentamicin were delivered through a fine cannula placed in the superior semicircular canal (0.1 – 20 µg gentamicin in 2.5 µl HBSS over one hr.). Afferent recordings were conducted at 14, 28, and 56 days postadministration. Sinusoidal rotational responses were evaluated through traditional Fourier-based methods, but also with multitaper spectral estimation techniques for point processes to evaluate the magnitude and coherence at stimulus frequencies (*Neural Comput.* 13:717, 2001). Following infusion of the highest gentamicin doses (i.e. 5 and 20 µg), evidence of rotational responses were observed in only 2 of 40 neurons exhibiting spontaneous discharge ($p<0.05$). In specimens dosed with 1 µg gentamicin, most afferents exhibited only spontaneous discharge, though clear rotational responses were observed in other afferents at 14 and 56 days post-administration despite dramatic evidence of decreased hair cell density and afferent calyx retraction. While specimens dosed with 0.1 µg exhibited far less morphologic damage, abnormalities in afferent response characteristics were common. These included half-rectification and anomalous associations between spontaneous and evoked discharge. These data provide clear evidence for deleterious consequences in sensory coding that are not accompanied by obvious morphologic changes in the vestibular neuroepithelia.

154 Protective Effect of Pifithrin-Alpha Against Gentamicin-Induced Type I Vestibular Hair Cell Loss

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Aminoglycosides are antibiotics widely used in the treatment of infections in newborns, tuberculosis and cystic fibrosis. However, the clinical use of aminoglycosides is limited by ototoxicity. Gentamicin is one of the aminoglyocides that causes hair cell loss in the inner ear. We have previously reported that pifithrin-alpha (PFT), a p53 inhibitor, protected cochlear and vestibular hair cells against cisplatin-induced apoptosis. In this project, we carried out experiments to determine if PFT has a protective effect against gentamicin-induced vestibular hair cell loss. The vestibular epithelium has two types of hair cells, which are classified by the form of synaptic terminal of afferent nerve fibers. Type I hair cells are innervated by cup-like calyx terminals, and type II hair cells are innervated by bouton terminals. Type I and type II hair cells are distributed in both the striola and extrastriola of rat utricular epithelium. In our study, utricular cultures were obtained from P3 Sprague-Dawley rats. The utricular cultures were randomly assigned to different treatments, gentamicin (500µM or 1000µM) or gentamicin (500µM or 1000µM) plus 100µM PFT. Untreated control cultures were

run in parallel. After 48 hours of treatment, the cultures were fixed in 10% of formalin. Then we carried out double fluorescent immunocytochemistry to label the type I hair cells by staining the calyx with calbindin and all hair cells with phalloidin. The stained tissues were mounted and observed under a fluorescence microscope (Olympus BX51) and a Leica TCS SP2 AOBS spectral confocal microscope. The images were processed with Adobe Photoshop 5.5 software and LCS Lite software. The numbers of vestibular hair cells were counted from three representative regions of each explants and the mean number was determined for each specimen. Data were analyzed by Student t-test. The results showed that PFT significantly protected vestibular hair cells against gentamicin-induced hair cell loss. Particularly, PFT had a protective effect against gentamicin-induced type I vestibular hair cell loss ($p<0.001$).

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155 The Protection of Vestibular Hair Cells with the Oral Administration of Teprenone

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In the previous meeting, we reported that the heat shock proteins play the great role in the protection of the hair cells against the stress. The oral administration of heat shock inducer: teprenone can protect hair cells against the intense noise. In this present study, we studied the effect of teprenone on vestibular hair cell death induced by aminoglycoside.

We used the CBA/N mice (4 w – 6 w). These mice were fed with the food containing teprenone (0.5%) for 4 weeks (teprenone group). The mice fed with the normal food were used for control (control group). 4 animals were used for immunohistochemistry against Hsp70. The utricles of the other animals were cultured in the medium for 24 h. The hair cell death was induced with neomycin. After fixation, the vestibular hair cells were labeled with the immunohistochemistry against calmodulin, and the survival hair cells were evaluated.

In the vestibular hair cells of teprenone group, Hsp-70 was expressed. In addition, after culture, the more vestibular hair cells survived in teprenone group than in control group. The results showed that the oral administration of teprenone can induce the heat shock response in the vestibule and may protect sernsory cells.

156 T-Type Calcium Channel Bloker Flunarizine Induces HO-1 to Protect Organ of Corti Explants from Gentamicin

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Although gentamicin (GM) is an effective antimicrobial agent, it often causes a severe sensorineural hearing loss and balance disturbance due to ototoxic effects on sensory hair cells of inner ear. Recently, we found that flunarizine, a T-type calcium channel blocker, protected the cisplatin-induced damages of sensory hair cells in a calcium-independent manner. However, the effect of flunarizine on the GM-induced death of sensory hair cells is not elucidated. In this study, we examined whether flunarizine prevents the ototoxicity of GM in organotypic culture of cochlear explants from neonatal P2 rats. Treatment with GM obviously caused hair cell damages showing disruption of stereocilia, fragmentation of nucleus of hair cells, disarray of the OHC and IHC row, and loss of FM1-43 staining in sensory hair cells from organ of Corti explants. However, pretreatment with flunarizine significantly suppressed the GM-induced damages in the sensory hair cells of organ of Corti explants. To observe the effect of flunarizine on GM uptake in the sensory hair cells, organ of Corti explants was maintained with fluorescently conjugated GM (GM-Texas Red, GTTR) in the presence or absence of flunarizine. GTTR was observed in all turns of organ of Corti explants. Pretreatment with flunarizine did not attenuate GTTR uptake into sensory hair cells of organ of Corti explants. Interestingly, flunarizine increased the expression of HO-1 in organ of Corti explants increased HO-1. These results suggest that the protective effect of flunarizine on GM-induced ototoxicity is mediated by HO-1 rather than modulation of GTTR uptake into organ of Corti explants.

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157 Rapid Hearing Loss and Hair Cell Degeneration Following Acute Intracochlear Perfusion of Neomycin in Guinea Pigs

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The hair cells in zebrafish lateral line neuromasts are structurally and functionally similar to mammalian inner ear hair cells. Recently, the zebrafish lateral line has been used as a model system for screening of ototoxic and protective drugs. These studies have shown that brief exposure of zebrafish larvae to neomycin in the medium results in rapid and dramatic loss of lateral line hair cells. For example, thirty minute exposure results in selective loss of 80 % of hair cells 1 hr later. To determine if mammalian inner ear hair cells die over a similar time course when directly exposed to neomycin in vivo, we used perilymph perfusion in mature guinea pigs, and followed the kinetics of hearing loss and hair cell death after perfusion of neomycin. Neomycin was perfused for 60 min., animals tested for hearing threshold and the cochlea was fixed for histological analysis. Acute intracochlear perfusion of neomycin induced a dose-dependent hearing loss: 5-25 dB compound action potential threshold shift was noted at lower doses (100 or 330 μM), and 25-65 dB shift was noted at higher doses (660 μM or 1 mM), most marked at high frequencies. When animals were allowed to recover for 1 hour following neomycin perfusion, 5-10 dB of temporary threshold shift

was observed. To determine whether the effects were reversible, neomycin was perfused for 1 hour, followed by a rinse with artificial perilymph (30 min.), and tested at 3 hours. Final CAP threshold shifts remained elevated after this period, suggesting that hearing loss was due to damage rather than a reversible mechanotransduction blockade. . Histological analysis confirmed dose-dependent damage. After treatment with 330 µM neomycin, few outer hair cells in the basal turns showed features of cell death. At 660 µM, most OHCs from the basal turns showed cytoplasmic vacuolization, with no obvious mitochondrial damage. At a dose of 1 mM, hair cells in the basal turn frequently appeared to be undergoing cell death, with mitochondrial swelling. These results suggest that the mammalian cochlea exhibits rapid hearing loss and hair cell degeneration after acute neomycin exposure.

158 Protective Effects of 2-SPBN, Dexamethasone Acetate and D-JNKI on Cochlear Hair Cells from 4-HNE and Neomycin Ototoxicity

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Acute acoustic trauma causes hearing loss through many mechanisms including production of free radicals, cytokines, and gene transcription modulations. Nitrone based spin-trap agents have emerged as promising pharmacologic tools because of their neuroprotective efficacy and large therapeutic window in several models of CNS injury. Glucocorticoid receptors are expressed in most cells and glucocorticoid-mediated protection of cells may result from rapid modulation of calcium channels and calcium mobilization. Other mechanisms are also reported involved in dexamethasone effects, such as gene transcription regulation, inhibition of cytokines. C-Jun N-terminal kinase (JNK) pathway has been studied in the past several years for its involvement in noise induced hearing loss. The D-JNK inhibitor (D-JNKI) has been reported more potent than its L- counterpart.

Cochlear tissues were dissected out from P3 CD-1 mouse pups and cultured in DMEM at 37 °C with 5% CO2. After initial incubation for 24 hours, the cochleae were exposed to 4 hydroxy nonenal (4-HNE, 150 µM) or neomycin (1 mM) for 48 hours with or without the protective reagents: N-tert-Butyl-á-(2-sulfophenyl) nitrone (2-SPBN, 5 mM), Dexamethasone Acetate (1µg/ml) and D/L-JNKIs (2 µM). Hair cells were counted and the results compared among different treatment groups.

The results indicated that 2-SPBN and Dexamethasone Acetate can protect OHCs at cochlear middle turn from 4-HNE damage (survival rate at 50% and 30% respectively), but failed to protect from neomycin exposure. Though L-JNKI did not show protective effect, D-JNKI could conserve 60% of OHCs at the middle turn from both 4-HNE and neomycin induced damage, but not those at the basal turn. Our results suggest that anti-inflammatory steroids, reactive oxygen species scavengers and apoptosis inhibitors are all potential therapeutic candidates for noise induced hearing loss.

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159 Prostaglandin E Recepter Agonists Stimulate Production of Vascular Endothelial Growth Factor in the Mouse Cochlea

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Prostaglandins (PGs) are cyclooxygense metabolites of C20-unsaturated fatty acid such as arachidonic acid. Among PGs, PGE, particularly PGE2, is the most widely produced in the body and exhibits the most versatile physiologic actions. PGE1 and PGE2 are mainly mediated by four difference G-protein-coupled receptors, EP1, EP2, EP3 and EP4. Our prior studies have demonstrated that an EP4 agonist has protective effects on hair cells against noise trauma. Previously, several studies have demonstrated EP4 agonists stimulate production of vascular endothelial growth factor (VEGF) in endothelial cells and cardiac myocytes. In addition, VEGF has been indicated to have otoprotective effects. This study was then designed to examine induction of VEGF in the cochleae by local EP2 or EP4 agonist application. C57BL6 mice were used as experimental animals. An EP2 or EP4 agonist solution was injected into the posterior semicircular canal. Inner ears were collected 24 hours after injection. The expression of VEGF protein and mRNA was measured with ELISA and quantitative RT-PCR respectively. Immunohistochemistry for EP2 and EP4 was performed in frozen sections. The results showed EP2 and EP4 agonists significantly increased both protein and mRNA of VEGF. Immunohistochemistry demonstrated EP2 and EP4 were present in the stria vascularis, the spiral ganglion and the organ of Corti. These findings indicate that EP2 and EP4 agonists have effects on induction of VEGF in the cochlea.

160 Delayed Treatment of Tumor Necrosis Factor Alpha Challenged Organ of Corti Explants with C-Jun N-Terminal Kinase Inhibitor II (SP600125) Protects Auditory Hair Cells

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HYPOTHESIS: Inhibition of c-Jun N-Terminal Kinase either immediate or delayed will protect auditory hair cells in organ of Corti explants challenged with TNF alpha.

BACKGROUND: Previous studies have shown protection of hearing in a guinea pig model of EIT-induced hearing loss in response to treatment with a c-Jun N-Terminal Kinase inhibitor.

METHODS: Organ of Corti explants were cultured for 96 hrs. TNF alpha was used at 2ug/ml, and SP600125 (c-Jun

N-Terminal Kinase Inhibitor II) was used at 10uM. There were 4 experimental groups: 1) untreated control; 2) TNF alpha; 3) TNF alpha + SP600125; and 4) TNF alpha with 16 hr. delay then treatment with SP600125. Explants were stained with fluorscein isothiocyanate-labeled phalloidin, and then inner hair cells (IHC) and outer hair cells (OHC) were counted per 415um section of explant basilar membrane with total hair cell (HC) counts derived from this data.
RESULTS: Control group total HC count averaged 317.8+/-4.4/415um of explant basilar membrane. The TNF-alpha group total HC count averaged 198.3+/-9.8. The TNF-alpha + SP600125 group total HC count averaged 316.3+/-4.9. The TNF-alpha group that received SP600125 after 16 hours of culture had a total HC count that averaged 315.3+/-4.3. There were no statistical differences (p>.05) between control HC counts and the HC counts of both SP600125 treatment groups. The differences between the TNF alpha challenged cultures and both the control and the two SP600125 treatment groups achieved a high level of significance (p<.001). The differences in the HC counts between the two SP600125 treatment groups were not significant (p>.05).
CONCLUSION: Treatment with c-Jun N-Terminal Kinase Inhibitor II (SP600125) prevented hair cell death in organ of Corti explants challenged with an ototoxic level of TNF alpha. The protective effect of this inhibitor on HC survival was also achieved when SP600125 was added to the cultures at 16 hrs. post-TNF alpha exposure.

161 Cytoprotective Effect of Geranylgeranylacetone by HSP70 Expression in Cochlear Damage Induced by Mitochondrial Toxin

Withdrawn

162 Outer and Middle Ear Malformations: A New Solution for an Old Problem

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The indications for the Vibrant Soundbridge (VSB), presently limited to the patients with sensorineural hearing loss (HL) and normal middle ear function, have been extended to include patients with mixed HL due to congenital ossicular chain defects. This study demonstrates that the floating mass transducer (FMT) of the Vibrant Soundbridge (VSB), placed onto the round window (RW), allows optimal amplification in patients with outer and middle ear malformations. These patients at present do not have good options for adequate functional rehabilitation.
Ten patients, who were unsuitable candidates for air and bone conductive hearing aids (BCHAs and ACHAs) and osseointegrative implants (BAHAs) were treated with RW implant. Patients, four children and six adults, had severe congenital malformations of the auricle combined with atresia of the outer ear canal and malformations of the ossicular chain.
Significant improvements were observed in pure-tone threshold and speech understanding after surgery and at follow-up intervals of 12 and 36 months, with no complications or device extrusion.
The post-operative results suggest that RW implant offers a viable new treatment option for patients with congenital malformation of the outer and middle ear.

163 Congenital – Prelingual - Hearing Loss: The Development of Causality

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The concept, at the beginning of the 21st century, that diseases have biological – rational – causes is now taken for granted but this has not always been so. The history of the how specific etiologies of congenital and prelingual hearing loss were identified and used to provide a nosology, which has had some utility for prevention, shows the evolution of this understanding. The knowledge of and the biological causes of congenital deafness have continually changed from unknown, to predominately infectious, e.g. congenital rubella , to perinatal insult, e.g. Kernicterus and now to genetic, e.g. Connexin 26. Up to now, as the cause became known there were developed effective interventions(s) for prevention of deafness. The next challenge is that of genetic deafness. The prevention or cure of genetic deafness will occur through various forms of molecular and genetic manipulations, Also, at present, there is the ability to ascertain the genotype of parents and/or the fetus with the implication, in most instances , of either making an early diagnosis and instituting effective interventions; avoiding the creation of embryo; or the possible destruction of the embryo. These latter two actions have not only the usual ethical concerns but, as some of the deaf community wish to perpetuate their culture, there is a special concern for the autonomy of the effected. This concern is exacerbated by the recent history of extermination and sterilization of the deaf German population. Medicine must respect the whishes of each and every person.

164 Motoneuron Firing Characteristics in Adductor Spasmodic Dysphonia

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Adductor spasmodic dysphonia (ADSD) is characterized by strain-strangled voice quality due to involuntary adduction of laryngeal muscles. Although ADSD has been studied for over twenty-five years by many investigators, little is understood about the etiology of ADSD due to the heterogeneity of findings among electromyographic (EMG), kinematic, therapeutic, and brain imaging studies.
Our current approach is to develop a neuromotor model of ADSD by characterizing vocal spasms at the level of the motoneuron. A neuromotor model is expected to reveal candidate etiologic regions of the central nervous system to guide neuroimaging and pharmacological investigations of spasmodic dysphonia. A microphone signal and

thyroarytenoid vector EMG signals provided by a quadrifilar intramuscular electrode were recorded from eight ADSD subjects during vegetative, phonatory, and linguistic tasks of increasing neuromotor complexity. Decomposition algorithms were applied to vector EMG data to obtain motoneuron firing trains for each task and therefore to obtain specific information about motor unit (MU) recruitment, decruitment, firing rate, and MU coordination during periods of non-spasm, pre-spasm, and spasm. Findings were compared to a corpus of eight normal control (NC) subjects.

During periods of non-spasm, active motoneurons of ADSD subjects exhibited more variability in their firing behaviors than NC subjects, but coordination features common to both groups were largely maintained (e.g., MU "common drive"). Motoneuron firing behaviors of ADSD subjects during pre-spasmodic episodes became increasingly unstable and were characterized by the recruitment of one or two additional motoneurons having firing characteristics dissimilar to the existing pool. Spasmodic periods were characterized by an additional recruitment of motoneurons, with firing behaviors uncoordinated with already-recruited MUs in most ADSD subjects for all vocal tasks.

165 Audiovestibular Dysfunction and Recovery Associated with Adoptive T-Cell Immunotherapy for Metastatic Melanoma

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Adoptive T-cell immunotherapy (ATCI) targeting melanoma/melanocyte-associated antigens (MMAAs) for the treatment of metastatic melanoma has been reported to cause vitiligo and ocular inflammation. This is a result of autoimmune destruction of melanocytes normally present in the skin and eye, respectively. Here we present the frequency, severity, temporal relationship, potential etiologies, and factors facilitating recovery from audiovestibular dysfunction in a series of subjects receiving ATCI therapy for metastatic melanoma.

Thirty-one patients underwent comprehensive neurotologic and audiovestibular assessment before and/or after ATCI. Hearing decline was observed in 55% and detected an average of 10 days following T-cell infusion (range 0–36 days). Eight patients with significant hearing loss were treated with intratympanic (IT) steroid injections. Of 18 patients available for follow-up after treatment (mean interval = 13 weeks), 61% (n=11) had recovery to baseline hearing, 28% (n=5) had partial recovery of hearing, 5.5% (n=1) did not recover due to persistent TM perforation after IT steroids, and 5.5% (n=1) were not tested. Of the eight who received IT steroids, three returned to baseline hearing and five improved but had residual high frequency sensorineural hearing loss. Ten subjects complained of dizziness and imbalance. Seven underwent vestibular tests, which indicated bilateral peripheral vestibulopathy.

While T-cell immunotherapy targeting MMAAs can attack melanoma cells, these genetically altered T-cells can also induce audiovestibular dysfunction, possibly by autoimmune destruction of melanocytes present in the cochlear and vestibular end organs. A majority of the hearing loss was reversible. IT steroid injections may be beneficial in alleviating T-cell mediated inner ear autoimmunity. Close monitoring of audiovestibular function is essential for future MMAA targeting ATCI against metastatic melanoma.

166 Diagnosis of Usher Syndrome and Retinal Degeneration in Infants

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Introduction: Usher Syndrome (USH), an autosomal-recessive disorder affects 15-20% of infants with congenital severe-to-profound sensorineural hearing loss (SNHL). The three USH types vary by degree of SNHL and balance dysfunction and age of onset of retinal degeneration (Retinitis Pigmentosa, RP). The 10 USH gene proteins form a network present in photoreceptors in the eye and hair cells in the inner ear, and mutations result in abnormal hair cell development and photoreceptor function. Historically, late diagnosis of USH occurred because SNHL can result from other causes, requiring identification of RP, often in late adolescence when it was advanced. Photoreceptor function in older USH patients, measured by electroretinography (ERG) and dark adapted thresholds (DAT) is often very abnormal before clinical vision is affected. However, ERG and DAT are difficult-to-perform tests not widely available, so no information exists for photoreceptor function in infants with USH mutations.

Hypotheses: By confirming USH mutations in infants we can: 1) decrease the age of diagnosis; 2) better define early ophthalmologic signs; 3) Provide new knowledge of cellular mechanisms in the photoreceptor disease.

Methods: Starting in January 2008, we offered CMV and GJB2 negative infants aged 0-12 months with moderate-to-profound SNHL USH genetic testing. ERG and DAT were obtained on USH and GJB2 control infants.

Results: 7 infants have been studied so far; 4 USH positive (all MYO7A) and 3 GJB2 positive. One 9 month old had elevated DAT; ERG is pending. One 5 month old had a normal retinal exam and ERG is pending. A third has retinal degeneration at 20 months. The fourth is an older sib of the first patient who walked at 17 months but has abnormal ERG at 3 years. All GJB2 patients had normal retinal function.

Conclusions: ERG/DAT in infants with USH mutations shows retinal degeneration. Correlation with genotype and cellular physiology should provide new therapeutic insight.

167 Peripheral and Central Hearing Function in Turner Syndrome

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Turner syndrome is a chromosomal aberration with a loss of one X- chromosome. It is rather frequently found (1:2000 female births) and the main feature is a female phenotype with a short stature, ovarian dysgenesis with loss of estrogen production and association with ear and hearing problems. Hearing impairment occurs in about 50% of all adult females with Turner syndrome, mainly due to sensorineural impairments. It is a well established fact that the pure tone threshold levels in Turner syndrome, especially in the mid- and high frequency ranges, are exceptionally poor compared to age matched normative data. The pathophysiology of the prevalent sensorineural lesions is, however, not fully understood; it is assumed that cochlear lesions are common. A longitudinal study was performed testing hearing after a 10 year follow up. These women have a decline in hearing over time in the age 30-45 which corresponds to a female in the normal population aged 80-89 years of age. In a group of 30 women, aged 40-67 a battery of peripheral and central auditory tests were performed. Otoaucustic emissions and auditory brainstem recordings were normal implying a cochlear origin of the sensorineural impairment. Phase audiometry- a test for directional hearing showed aberrant results. This indicate that there may be an element of central auditory processing disorder/ mild neurocognitive disability displayed in the abnormal auditory spatial capacity in Turner women, as earlier indicated in the Turner mouse.

168 The Effect of RTMS in the Patients with Intractable Tinnitus

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Background: Low frequency repetitive transcranial magnetic stimulation (rTMS) of the temporal cortex has been tried to manage the patients with chronic intractable tinnitus. rTMS is non-invasive method for altering cortical excitability and low frequency rTMS was reported to reduce the cortical activation.
Objective: The aim of the present study was to report our experience to treat patients with chronic intractable tinnitus and to evaluate the efficacy of rTMS in the treatment of intractable tinnitus.
Materials and Methods: Low frequency rTMS was applied to 12 patients with continual tinnitus for more than 6 months without response to the medications. The neurological examination of these patients was normal. Patients were stimulated by rTMS with 1200 stimuli/day (three sessions of 400 stimuli with an inter-session interval of 30s) at 1Hz and 120% of the motor threshold, for 5 consecutive days. Tinnitus was measured by Visual Analogue Scale (VAS) before and immediately after rTMS.
Results: There were 8 male and 4 female patients with age ranging from 24 to 61 (mean, 48.1 years). Average duration of the tinnitus was 24.1 (6~120) months. After rTMS, VAS score improved to 56.5 ¡¾ 23.5 from 65.7 ¡¾ 22.2 prior to the rTMS. VAS improved dramatically in 5 patients (67.4 ¡¾ 26.4 ¡æ 39.4 ¡¾ 19.7) among 12. There was no specific complication.
Conclusion: Through this study, authors suggest that rTMS may be one of the treatment options in selected patients with chronic intractable tinnitus.

169 Preoperative Cerebral Metabolic Difference Is Related to the Outcome of Cochlear Implantation in Prelingually Deaf Children

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The outcomes of cochlear implant (CI) are various in prelingual children and several factors have been suggested to affect the outcome. Better speech perception after CI is associated with greater extents of presurgical hypometabolism in superior temporal regions, including primary auditory area and according to the speech perception results for prelingually deaf children, children in the age range 5–7 years showed highly variable results. In this study we evaluated the preoperative regional metabolic difference related to the speech outcome after CI in prelingual deaf children.
Fourty-two prelingual deaf children were included in this study. We grouped the patients into younger (n=29, median 3.5 years old, 2~6.8) and older groups (n=13, median 8.7 years old, 7.2~10.3). We carried out F-18FDG brain PET within 1 month before CI in all patients. Speech perceptions test using the institute version of the CID was done for 2 years after CI.
In prelingual deaf children (aged 2-10 years) relative hyper-metabolism of temporo-occipital cortex were related to poor prognosis. In younger prelingual deaf children (under 7 years), relative hyper-metabolism of frontal lobe were related to good prognosis, but not in older children.
Preoperative regional metabolism difference, especially in temporo-occipital cortex in all patients and frontal lobe in younger patients related to speech outcome after CI.

170 Neuro-Imaging of Deaf Children Following Cochlear Implantation: Speech-Evoked Activity Within the Auditory Cortex Detected with Near Infrared Spectroscopy

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Near-Infrared Spectroscopy (NIRS) is a non-invasive technique that uses the transmission of near-infrared light to assess cerebral hemodynamic changes. Like PET and fMRI, it provides information about neuronal activity. However, NIRS is non-ionizing and safe in patients with implanted metallic devices such as cochlear implants(CI). We hypothesize that responses of the auditory cortex measured by NIRS will correlate with responses measured

by behavioral audiometry in children with CIs. We studied pediatric patients with CIs known to have reliable behavioral responses. Stimuli consisted of 20 second speech segments from a children's story alternated with 20 seconds of silence. The intensity of the stimuli ranged from 10-50dB HL in 10dB steps. Laser diodes delivered light at 690 and 830nm to the scalp, and photodiodes detected transmitted light 2.5cm from the source. We developed a custom head-frame to position the source and detector fiber optic bundles against the scalp over the superior temporal gyrus, where the auditory cortex resides. Custom software was designed to analyze the variations in light transmission and calculate changes in deoxygenated and oxygenated hemoglobin. Using our head-frame, we were able to comfortably and reliably position the NIRS probes on children wearing their CIs and make measurements of the auditory cortex response to speech stimuli. There was no NIRS response when the stimulus intensity was below their behavioral threshold. However, there was a NIRS response when the stimulus intensity was above their behavioral threshold. This study shows that NIRS can measure speech-evoked activity in the auditory cortex of children with CIs. NIRS may have a practical application in the objective measurement of speech perception. It could complement CI programming, especially in pediatric patients whose behavioral responses can be variable and difficult to measure.

171 Low-Permeability Microfluidic Components for a Miniaturized Wearable Drug Delivery System

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Precision flow control at low rates and volumes is needed for intracochlear drug infusion. We describe the design, construction and performance of a set of microfabricated low-permeability components comprising a microfluidic drug delivery system used in otology applications. Our adaptable platform for polymer microfluidics readily accommodates integration with silicon-based sensors, printed circuit, and surface-mount electronics technologies. We have used this platform to build flow control components for a wearable, liquid drug delivery system, and demonstrated their effectiveness in animal studies of guinea pigs using infusion of test compounds while monitoring hearing. These components include membrane-based fluidic capacitors and manual screw-valves. The design and performance of a commercial flow sensor integrated into our system additionally demonstrates the ability to integrate silicon-based technologies. We demonstrate fluidic capacitances ranging from 0.015 to 0.15 microliter/kPa, screw valves with on/off flow ratios greater than 38 000, and a 45x reduction in the aqueous fluid loss rate to the ambient environment due to permeation through a silicone diaphragm layer. We also demonstrate an integrated flow sensor with sensitivity better than 0.1 microliter/minute and a sensing range of -50 to 50 microliter/minute. These components are used to modify and control the output flow of a commercial pump, allowing precision control of flow volumes ranging from 0.2 to 1.0 microliter at flow rates ranging from 5 to 40 microliter/minute. Such control is important in ensuring safe flow parameters in intracochlear drug delivery.

172 Intracochlear Drug Delivery with a Miniature Programmable Microfluidic System

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The development and testing of many emerging therapies for diseases of the inner ear requires local drug delivery with controlled, but variable dosage. Long-term studies using animal models further require that the apparatus be miniaturized for placement on the animal. Current devices for chronic drug delivery have limited utility in otology because they either cannot deliver drugs locally to a specific organ or tissue, do not permit changes in delivery rate in situ, or cannot be used in animal trials in an untethered, wearable configuration. Here, we describe a small, self-contained system for liquid-phase drug delivery to small animals. This device enables chronic studies with programmable infusion rates. A commercial miniature medical pump is integrated with microfabricated components to supply ultralow flow rates and stroke volumes for direct intracochlear infusion. Solutions are delivered in pulses as small as 300 nl, with pulses delivered at any interval of 1 min or longer, and the pulse volume and frequency can be altered in situ by remote control. A unique feature of the system is the ability to infuse and immediately withdraw liquid, resulting in zero net volume transfer while compounds are exchanged by mixing with the perilymph. We present in vitro results demonstrating repeatability and precision of the system for nearly 3 months. Furthermore, we present in vivo results of delivery of a model compound in guinea pigs. Using this device, reciprocating perfusion of the guinea pig cochlea with a glutamate receptor antagonist is shown to cause localized, selective, and reversible changes in auditory sensitivity in both acute and chronic studies.

173 Comparison of Systemic and Otic Methods of Drug Administration in a New Animal Model of Otitis Media

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Topical otic antibiotics are extensively used to treat acute otitis media with perforation, chronic suppurative otitis media, and external otitis. Development of topical

medications that can treat otitis media with intact tympanic membrane would also be beneficial, allowing for local delivery of high concentration of medications with minimal systemic exposure. However, little information exists on middle ear accumulation of topically administered antibiotics. Comprehensive comparison between systemic effect of topical and systemic applications is also missing.
To investigate the use of topical otic medications to treat otitis media when the tympanic membrane is intact, we developed a new rat model of aseptic otitis media that allows us to measure accumulation of drugs in fluid within the middle ear. Tissue distribution of the commonly prescribed fluoroquinolone antibiotic, ofloxacin, was studied following external ear canal or systemic administration.
Ofloxacin was detected in middle ear samples within 15 minutes following both types of administration. Systemic application provided slightly greater but more consistent penetration of the drug into the middle ear space throughout the 3 hour period of evaluation. While less pronounced than after systemic application, ototopical application still led to accumulation of ofloxacin in plasma, the large intestine, and the bladder. Our studies indicate that while the external ear canal including an intact tympanic membrane is permeable to ofloxacin, current formulations produce insufficient drug concentrations in middle ear to effectively treat otitis media. We also found that ototopically administered medication is not localized to the ear. Further investigation is needed to ensure that low systemic concentrations of drugs would not create a permissive environment for formation of biofilms and emergence of antibiotic-resistant strains of bacteria.

174 Mini-Invasive Computer Assisted Approach for Cochlear Implantation: A Human Temporal Bone Study

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Objective: The precision of some computer assisted navigation systems (CAS) can potentially guide the surgeon from the mastoid cortex to the cochlea with a trajectory avoiding the facial nerve. The aim of this study was to assess the precision of a CAS with 4 fiducial markers in human temporal bones and evaluate the surgical feasibility of a minimal invasive approach to the cochlea with the aide of this system.
Materials and Methods: Five adult human temporal bone specimens were included. For each temporal bone, 4 titanium screws were placed on the mastoid cortex and a CT scan was performed (helicoidal acquisition, 0.6 mm slices every 0.3 mm). Images were loaded on a an electromagnetic CAS system (Digipointeur, Collin, Bagneux, France). Specimens were registered using all 4 screws. An electrical drill was connected to the CAS emitter in order to monitor the drill progression on CT-scan continuously. A conical approach beginning in the cribriform area, passing through the facial recess and ending in the scala vestibuli through the anterior inferior ridge of the round window was performed with 5, 3 and 2 mm diamond burrs. The trajectory was controlled by a rigid endoscope. A strait 0.5 mm steel wire was inserted into the cochlea. A control CT scan was performed. Temporal bones were dissected in order to detect any injury of the facial nerve canal.
Results: This conical approach to the cochlea was technically feasible in all cases and allowed rectification of the trajectory during the progression. No facial injury was observed. The wire could be positioned in the scala vestibuli in all specimens. The position accuracy of the CAS was < 1 mm on the target in all cases.
Conclusion: Digipointeur CAS system with invasive fiducial markers yield a high precision allowing a mini-invasive approache to the human cochlea. This technique opens insights for robotised surgical procedures.

175 Objective Assessment of Temporal Bone Dissection

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Objective assessment of surgeon competence is recently surfacing as an area of high interest due to changes in patient expectation and a few high-profile surgical cases. Current methods to assess surgical competency typically involve an apprentice-type training where faculty surgeons retrospectively report their expert opinion of a trainee's readiness for the OR. However, these assessments tend to be subject to personal bias and variable standards. Progress has been made by several neurotology training programs to create standardized criteria to rate their trainees' readiness in temporal bone dissection. These include the Welling Scale from the Ohio State University, the Task Based Checklist, the Global Rating Scale, and the Final Product Analysis from the University of Toronto, and a list of metrics specified to assess simulated temporal bone dissections from Stanford. While each scale requires the completion of similar basic components integral to the successful completion of a mastoidectomy, some listed criteria diverge in regard to each institution's preferred approach to temporal bone dissection. The goal of this study is to create a more universal scale for temporal bone dissection assessment that eliminates cross-institutional partialities in terms of the idiosyncratic surgical approaches specific to each training program. To accomplish this, we first compiled the temporal bone grading criteria of each of the three aforementioned institutions into an all-encompassing scale. This new compilation of grading criteria is then sent out as an online survey to the members of the American Neurotology Society with instructions to rate the importance of each criterion. Results are analyzed to identify the specific

criteria that receive the highest consent in terms of their importance and relevance in the objective assessment of temporal bone dissection. These identified criteria will then be used to create the new, cross-institutional scale for the objective assessment of temporal bone dissections. This study will enhance the objectivity of the currently existing grading scales for temporal bone dissections by accounting for differences in surgical traning across institutions.

176 Improvement in Realism of a Temporal Bone Dissection Simulator

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Previous work in the development of a virtual environment for temporal bone dissection has been presented. The purpose of this discussion is to relate recent developments in improved realism within our temporal bone dissection simulator. Simulation training has been shown to be effective within a wide context of realism. Often times, “box simulators” (simple physical models) may be all that is necessary to teach certain skills such as instrument familiarity, instrument navigation, interactivity between instruments and between instruments and the environment. Box simulators provide a cost effective approach in that they are composed of “low tech” components such as using rubber tubing to simulate a vessel to learn vascular anastomosis. Often times however, as one’s skill level improves or as tasks become more complex, more advanced and realistic simulations are needed to continue to engage the trainee as well as offer more complex information required for advance procedures. Early trainees may not appreciate these subtleties or at worst become overwhelmed and therefore learning might not be optimized. As advanced level trainees or “experts” have interacted with our dissection simulator, these observations were made apparent. We present our recent improvements in realism in an attempt to provide the advanced level trainees and experts a more rich and realistic environment. The improvements specifically are the addition of fluid interaction to simulate both irrigation and bleeding and shadowing effects. Additionally, improving the stereo display has been a major advance forward to allow for better depth perception. These additions will allow for a more engaging environment for expert users as well as provide more realistic environment for trainees as they advance in their skill level. Demonstrations of the temporal bone dissection simulator will be presented with emphasis on the aspects of improved realism. This research was supported by the NIDCD RO1- DC06458-01A1.

177 Correlation Between the Depth of Electrode Insertion and Post-Operative Performance in Humans with Cochlear Implants: A Histopathologic Study

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The depth of electrode insertion of a multichannel cochlear implant has been suggested as a clinical variable that may correlate with word recognition. The current study evaluates this relationship using the human temporal bone collection at the Massachusetts Eye and Ear Infirmary.

Thirty-two temporal bones of humans with cochlear implants were reviewed. Temporal bones were harvested at autopsy, fixed and prepared for histological study by standard techniques. Specimens were then serially sectioned, and reconstructed by two-dimensional methods. The depth of insertion of the cochlear implant electrodes, as measured from the round window, was determined

The Inserted electrode Length (IL) was defined as the distance from the cochleostomy to apical electrode tip and the Active Electrode Length (AEL) as the distance between the most apical and most basal electrodes. The ratios of inserted length to the active electrode length (IL/AEL) and to the total length of the cochlear duct (IL/CDL) were calculated. Correlation analyses showed that essentially none of the across-subject variance in NU6 word scores for the 32 subjects could be accounted for by the across-subject variance in either ratio ($r^2=0.02$, $df=30$ and $p=0.48$ for IL/AEL; $r^2=0.02$, $df=30$ and $p=0.39$ for IL/CDL).

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178 Contribution of Acoustic Landmarks to Speech Recognition in Noise by Cochlear Implant Users

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Cochlear implant (CI) user’s performance degrades significantly in noisy environments. The present study examines the hypothesis that when listening to speech in fluctuating maskers (e.g., competing talkers), CI users can not fuse the pieces of the message over temporal gaps because they are not able to perceive reliably the acoustic landmarks introduced by obstruent consonants (e.g., stops). These landmarks, often blurred in noisy conditions, are evident in spectral discontinuities associated with consonant closures and releases and are posited to aid listeners determine word/syllable boundaries. To test this hypothesis, CI users are presented with IEEE sentences containing clean obstruent segments, but corrupted (by steady noise or fluctuating maskers) sonorant segments (e.g., vowels). Preliminary results indicated that cochlear implant users received a substantial gain in intelligibility when they had access to the acoustic landmarks provided by obstruent consonants. Access to the low-frequency (0-1

kHz) region of the clean obstruent consonant spectra was found to be sufficient to realize significant improvements in performance and that was attributed to improvement in transmission of voicing information.

179 Relationship Between Vestibular Function and *GJB2* Gene Mutation in Adult Nonsyndromic Sensorineural Hearing Loss

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The vestibular disturbance recognized in the childhood of nonsyndromic sensorineural healing loss(NSHL) gradually disappears until adulthood. Reports regarding the vestibular function of NSHL in adulthood have been only sporadically seen. Mutation in the *GJB2* gene encoding gap junction(GJ) were identified as the most common cause of NSHL. GJ channels enable neighboring cells to exchange small signalling molecules such as potassium and immunohistochemically exsist not only cochlea but also vestibular organs. NSHL has been thought to be resulted from an altered potassium homeostasis. The aim of this study is to asses vestibular function of NSHL in adults, with special reference to *GJB2* gene mutation.

20 NSHL adults were participated in this study. The median of the pure-tone averages was 92dB for the right ear, and 88dB for the light ear. DNA was extracted from peripheral blood leukocytes of the subjects. The coding region of *GJB2* was amplified by polymerase chain reaction and directly sequenced using BIGDye Terminator sequencing reagents. Vestibular evoked myogenic potencials and caloric test were done as the assessment of vestibular function.

180 Vestibular-Visual-Cognitive Interaction Tests in Patients with Blast Head Trauma

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Traumatic brain injury secondary to blast exposure is a significant operational issue for the military. We have had experience evaluating and treating a large group of patients with this injury pattern. In this abstract we describe the cognitive/visual patterns seen in these patients before and after vestibular physical therapy treatment. Our objectives were to determine the patterns of visual/vestibular and visual/cognitive test results at the onset of vestibular physical therapy treatment for a blast related balance disorder, and to determine which particular tests are valuable prognostic measures for improvement in vestibular function. A set of visual/cognitive and visual/vestibular tests were performed on 82 individuals with blast injury. The dynamic gait index (DGI) was also administered by a physical therapist prior to initiating vestibular physical therapy. Their baseline income results were compared to outcome tests taken 8, 12, and 16 weeks after beginning vestibular physical therapy. Visual testing was performed in a darkened room with an effective viewing distance of 13 feet. The tests included a static visual acuity, perception time, target acquisition, target following, dynamic visual acuity (DVA), and gaze stabilization (GS). Perception time is measured by calculating the time in msec that a randomly presented target must be on the screen before accurate subject recognition. Target acquisition is the time in msec required to make a saccade from the center of the screen to the new optotype position. Target following is the speed in deg/sec at which the subject can accurately track a symbol, and gaze stabilization is the speed in deg/sec at which the subject can move their head and accurately hold a target in view.

There was significant improvement for perception time, target acquisition, target following and DVA after four weeks of vestibular physical therapy. Horizontal GS scores improved significantly after 12 weeks, and vertical GS scores improved significantly between 12 and 16 weeks of vestibular physical therapy. The DGI significantly improved at week 8 and continued to improve at week 12. The patient self report of no dizziness with running coincided with the normalization of vertical GS week between week 12 and 16.

This set of tests seem to be a very sensitive measure of vestibular disorders for patients head injury from blast, and are good measures for improvement in vestibular function following vestibular physical therapy.

181 Vestibular Rehabilitation Using a Virtual Grocery Store in Persons with Unilateral Vestibular Dysfunction

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Little is known about the use of virtual reality to decrease dizziness and improve balance in persons with vestibular disorders. The purpose of this study was to determine if habituation in a virtual grocery store improved patient outcomes in persons with vestibular dysfunction. Subjects: Nine patients participated in the study (mean age: 48, sd 10.6; range 26-60; 9 women). Patients had peripheral vestibular diagnoses including unilateral hypofunction and Meniere's disease. Methods: Patients were treated over 6 weekly sessions in the virtual grocery store (six -4 minute exposures for a total virtual reality exposure of 24 minutes/day). Patients pushed an instrumented grocery cart while walking on a treadmill at their comfortable pace. The speed of movement on the treadmill and through the grocery store scene was based on the amount of pressure applied on the cart handle. The virtual grocery store was displayed on 3 large screens that surrounded the patient, providing them with a sense of walking by the shelves in a grocery store. Over the 6 week period, patients were exposed to more difficult aisles in the store. Difficulty was increased by increasing the product density of the store items (more products per square meter) and greater contrast as they improved. Symptoms were monitored before and after each trial to ensure patient safety. Outcomes were measured before and after the six week intervention by a blinded physical therapist. Outcomes involved both physical performance measures and qualitative measures including gait speed,

the Timed "Up & Go" (TUG), the dynamic gait index (DGI), the functional gait assessment (FGA), computerized dynamic posturography (CDP), the Activities-specific Confidence scale (ABC),the Situational Characteristics Questionnaire (SCQ), and the Dizziness Handicap Inventory (DHI). Results: Data are reported with the number of participants who improved compared to the total number of patients who completed testing. Improvements were noted in all outcome measures including: gait speed (5/8), TUG scores (5/9), FGA (5/8), DGI (6/8), CDP (6/9), SCQ (6/9), ABC (7/9), and the DHI (8/9). Although only the DHI was statistically significant with the Wilcoxon Signed ranks test ($p<.01$), improvements were noted in this case series in the majority of patients for each of the outcomes recorded. Discussion/Conclusion: Virtual reality treatment for persons with vestibular disorders had positive physical and self-reported outcomes. The ongoing trial will compare persons undergoing customized physical therapy intervention to virtual reality intervention to determine if one method demonstrates greater change in physical and self-report measures in persons with vestibular disorders after rehabilitation.
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182 Discriminative Properties of the Situational Characteristic Questionnaire

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Purpose: The Situational Characteristic Questionnaire (SCQ) was designed to discriminate between persons with and without vestibular dysfunction. Scoring of the original instrument can be cumbersome. The purpose of this study was to determine if a new scoring methodology could provide discrimination between persons with and without vestibular disease. Subjects: One-hundred twenty four subjects (48 males, 76 females) participated. Seventy-six persons with vestibular disorders (mean age 57.4 years, SD 17.5 range 22-88) and 48 persons without disease (mean age 49.8 years, SD 19.3, range 22-83) completed the SCQ Parts I and II items. Methods: Subjects were asked to record their perceived symptoms during exposure to various circumstances that might cause discomfort in persons with vestibular disease. Data analysis: The sum of the item scores on SCQ Part I and SCQ Part II as well as overall combined scores were compared between the patient and the control group with the non-parametric Mann-Whitney U test. Receiver operating characteristic (ROC) with area under curve (AUC) analysis was used to describe how SCQ scores (Parts I, II and combined) identified subjects with vestibular disorders. Results: Persons with vestibular disorders demonstrated significantly greater amounts of situational discomfort on the SCQ Part I and SCQ Part II items as well as with the combined score ($p < 0.01$). The sum of the SCQ Part I and II items as well as combined scores identified persons with vestibular disease (AUC range of 0.84 to 0.86, all $p < 0.01$). Optimal sensitivity/specificity for identification of a person with a vestibular disorder was 72%/78% for the SCQ Part I items, 76%/83% for the SCQ Part II items, and 72%/85% for the combined scores. Discussion: Persons with vestibular disorders report significantly greater amounts of situational discomfort as described by items included in Parts I and II of the SCQ. The test items demonstrate the ability to discriminate persons with vestibular disease from control subjects. Future work will be aimed at identifying the optimal number of items needed to describe situational discomfort, to identify if scaling of the items enhances the questionnaire, and to determine the responsiveness of the SCQ items to change in people with vestibular disorders.
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183 Quantitative Analysis of Eye Movement by Image Analysis Technique Using Image J

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Using an infrared CCD camera for recording eye movement is widely accepted and analysis of eye movement is essential for investigating vestibular disturbances. We devised an original eye movement image analysis technique using an infrared CCD camera, a personal computer and public domain software. The analysis was performed using the publish domain soft ware Image J program (developed by the U.S. National Institutes of Health). The video image from an infrared CCD camera was captured at 30 frames per second in 320*240. For analysis of the horizontal and vertical components, the X-Y center of the pupil was automatically calculated using the original macro. For analysis of torsional components, the whole iris pattern, which was rotated each 0.1 degrees, was overlaid with the same area of the next iris pattern, and the angle at which both iris patterns showed the greatest match was calculated. For quantitative analysis, slow phase velocity of each nystagmus, average of slow phase velocity, the visual suppression value, were analyzed automatically.
Using this technique, it is possible to inexpensively perform eye movement analysis, including in quantitative analysis, from video images recorded by many types of infrared CCD cameras.

184 A Small Inexpensive Motor for Head Impulse Testing of Vestibular Function

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The head impulse test (HIT) has become a standard part of the physical exam during clinical evaluation of patients with suspected vestibular disorders. To date, most quantitative HIT testing has been performed using head movements delivered manually by an examiner, with eye movements measured using the scleral coil technique.

Obtaining high quality data using that approach is sufficiently time consuming for both patient and examiner to be impractical for routine clinical use. Manual delivery of head movements is less precise than might be achieved using a motorized system. Motorized chairs suitably powerful for whole-body rotation HIT testing are large and expensive, so interest has grown in development of a smaller unit able to deliver head-on-body rotations.

We have designed and constructed a small, inexpensive HIT testing device comprising a high-torque but fail-safe goniometer coupled to the patient's head via a bite bar, atop which is mounted a video camera that monitors 3D eye rotation responses. We tested the device on 5 healthy volunteers. We recorded head movement using an integrated circuit gyroscope and calculated gain, asymmetry and latency of the horizontal vestibular-ocular reflex (VOR) while subjects viewed a distant target. During horizontal head impulses, peak head velocity was very repeatable at 136±1.6 o/s (mean±SD). Mean acceleration was 2161±272 o/s2. The mean VOR acceleration gain (eye movement acceleration divided head acceleration during the constant acceleration portion of head movement) was 0.92 ± 0.11. Mean VOR latency was 7.6±3.5 ms, and the mean asymmetry of acceleration gain was 4.2± 5%. These values are comparable to those reported by prior HIT studies performed using manual or motorized head impulses, suggesting this small, inexpensive unit may find utility in clinic-based evaluation of vestibular function.

185 Ambulatory Vestibular Monitoring

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Billions of healthcare dollars are expended each year for care of patients with balance disorders. In such patients it is often clinically useful to test vestibular function as an aid to diagnosis and treatment planning. Presently the standard means of testing vestibular function is to utilize specialized equipment in a vestibular testing laboratory to measure eye movements, both voluntary and evoked by vestibulo-ocular reflexes (VOR). The equipment is expensive, big, and heavy. The tests themselves are time consuming and extremely “operator dependent,” requiring a high level of expertise in the technician and a high level of cooperation and concentration in the patient. Patient and physician access to high quality vestibular laboratories is generally limited to tertiary care medical centers. Currently there are no means to record VOR during normal daily activities nor during acute symptoms unless the patient serendipitously has an attack of symptoms while in the vestibular laboratory. Recording and analysis of VOR eye movements would have greater clinical utility if they could be accomplished in a wider variety of times and places than just the vestibular testing laboratory. Herein we present pilot data taken using a protoype Ambulatory Vestibular Monitor (AVM) to enable recording of voluntary and reflexive eye movements outside the vestibular laboratory in ambulatory normal subjects. Our data show that it is feasible to acquire VOR recordings in the ambulatory setting, and that these recordings can have test-retest variability and signal-to-noise ratio comparable to measurements taken with laboratory-based equipment. General design considerations and specifications for a more sophisticated device, capable of simultaneously recording other physiologic parameters, such as EKG, respiratory rate, pulse oximetry, and head movement, will be presented. We believe such a device will open a new field of Ambulatory Vestibular Monitoring that will someday provide clinically and diagnostically useful information about the human vestibuo-ocular reflexes in vestibulopathic patients.

186 Linearity of Stimulus-Response Mapping During Semicircular Canal Stimulation Using a Vestibular Prosthesis

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Selective stimulation of individual branches of the vestibular nerve is difficult to achieve reliably, due to the close spacing of the branches relative to the spatial extent of stimulus current spread. This crosstalk might be effectively nulled through a precompensatory rotational remapping of vestibular prosthesis gyroscope signals. Precompensation would rely on the 3D angular vestibulo-ocular reflex (aVOR) response to prosthetic electrical stimulation exhibiting linearity as described by homogeneity (af(x)=f(ax)) and superposition (f(x)+f(y)=f(x+y)), and sufficient response amplitudes to span the space of normal aVOR-elicited eye movements.

We investigated electrically-evoked aVOR linearity in chinchillas with bilateral loss of peripheral vestibular sensation. Stimuli were delivered via monopolar electrodes to each of 3 semicircular canals of one labyrinth using a multichannel vestibular prosthesis. Animals were first adapted to simultaneous baseline 60 pulse/s asynchronous stimulation of all three stimulus channels. To test homogeneity, a sinusoidal modulation of pulse rate was applied on each canal while keeping the baseline pulse rate stimulation constant on the other two canals. 3D aVOR response velocity and axis were measured for multiple depths of pulse rate modulation. Homogeneity was evident, but responses were modest (<30 deg/s). Stimulating with supernormal baseline pulse rate (200 pulse/s and pulse rate modulation between 0 and 400 pulse/s) elicited larger amplitude responses (>60 deg/s) that diminished 30% over one hour of stimulation.

For single-channel modulation, increasing modulation depth increased the amplitude of aVOR slow phase eye velocity without significantly changing the axis of rotation. Simultaneous presentation of stimuli to different electrode pairs elicited a response approximately equal to the vector summation of individual responses. The accuracy of the rotation angle improved with higher rotation magnitudes.

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187 Functional Reserve as a Balance Performance Indicator

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Functional reserve is a way to describe the range of activity for a specific organ or system, like renal, respiratory or cognition. Usually, when describing a subject balance performance, certain measure is used as a performance indicator. Such as sway velocity, sway trajectory, center of pressure (COP) area, and limits of stability (LOS) among others. In this work, we present a different approach to evaluate balance performance, relating two different measures recorded with a force platform. The COP area with eyes open on a firm surface is used as the basal condition. The largest voluntary displacement of the COP area (LOS) was taken as the maximal capacity of the balance system, before modifying the base of support with a step.

Quantification of the LOS is performed by calculating an ellipse (CE) that will estimate its area. This is achieved by approximating the sway pattern to an ellipse, using the maximum and minimum of the total distance swayed in the X and Y axis:

$$a = \frac{\max(cop_x) - \min(cop_x)}{2}$$

$$b = \frac{\max(cop_y) - \min(cop_y)}{2}$$

$$A\_LOS = \pi * a * b$$

The next step is to compute the area of the CE for the basal condition and determine the ratio of Area of the LOS and Area of the CE. This gives a measurement on how much Area of the LOS is still available for the patient to sway. Area of the CE at 95% is computed as:

$$Area = 2 * \pi * F_{0.05[2,N-2]} * \left(\sigma_x^2 \sigma_y^2 - \sigma_{xy}^2\right)^{\frac{1}{2}}$$

Finally Functional Reserve (FR) is presented as the percentage of the total swaying capacity (LOS) still available to sway. The FR quantifies the percentage of the basal area compared to the whole area of the LOS, computed as:

$$FR = \left(1 - \frac{Area\_Elipse}{Area\_LOS}\right) * 100$$

The FR allows us to quantify how much area is still available for the patient to sway without exiting the limits of stability which will cause instability and fall. The combination of changes in the LOS and COP area (greater or smaller) values can modify balance functional reserve.

We looked at differences in FR between normal healthy subjects (n=45) and patients with balance disorders (n=41), finding a significant difference in the FR values. Also we present the results of balance disorders patients after balance rehabilitation.

188 Dilemmas in Interpreting Results of Vestibular Function Tests in Patients with Unilateral Chronic Otitis Media

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Objects: To evaluate the results of air caloric test in unilateral chronic otitis media (COM) patients with no history of vertigo and to compare the caloric results with those of other vestibular tests and tympanic membrane findings.

Methods: Twenty five patients with unilateral COM were reviewed. Hearing status was assessed preoperatively using pure-tone audiometry and vestibular function was assessed using air caloric, vibration-induced nystagmus (VIN), and subjective visual vertical (SVV) tests.

Results: There was pathologic canal paresis in 8 (32%), pathologic VIN in 7 (28%), pathologic SVV in 5 (20%), and pathologic results in any tests in 15 (60%). Canal paresis was pathologic on the COM side in 6. The other 2 showed pathologic canal paresis on the intact side with inverted nystagmus during warm air stimulation on the COM side. No correlation was observed between the inverted nystagmus and the tympanic membrane findings. Two patients with poorer hearing on the COM side showed vestibular hypofunction on the same side.

Conclusion: Unilateral COM patients showed canal paresis more frequently on the COM side than the intact side. Inverted nystagmus to warm air caloric stimulation on the COM side can mislead to the canal paresis on the intact side. As air caloric test can be influenced by the middle ear pathology, results from various laboratory findings including VIN and SVV tests are helpful in determining the presence and the side of the vestibular imbalance, in assessing a dizzy patient with COM.

189 Vestibular-Evoked Myogenic Potentials and Conductive Hearing Loss as Predictors of Postoperative Symptom Improvement in Superior Canal Dehiscence Syndrome

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Background

Superior Canal Dehiscence Syndrome (SCDS) presents with often bizarre sound and/or pressure induced vestibular and auditory signs and symptoms. Low frequency conductive hearing loss (CHL) and low threshold vestibular evoked myogenic potentials (VEMP) have been reported in SCDS to normalise after surgical plugging repair of the dehiscence.

Objectives

To characterise cervical VEMP thresholds and CHL before and after surgical repair of SCDS and correlate with symptom change postoperatively.

Methods

A review of pre and post operative vestibular and auditory testing was undertaken in six consecutive cases of primary surgical repair of SCDS via a middle fossa approach at tertiary neurotology hospital. cVEMP amplitudes and

thresholds were recorded along with Audiology pre and four months postoperatively and compared with reported symptom change.
Results
Pre operative cVEMP thresholds were significantly lower in all SCDS ears (mean 65 dBnHL range 60 – 70, SE 10) compared with non SCD control ears (mean 90 dBnHL). An average increase in cVEMP threshold of 16 dBnHL (range 10 – 25) followed surgical plugging repair of SCDS. Higher threshold elevations post operatively correlated with better reported symptom improvement.
Conductive hearing loss was most evident at low frequencies with reduction of the air bone gap postoperatively. At 250 Hz mean CHL of 19dB reduced to 3 dB postoperatively; at 500 Hz mean CHL17dB reduced to 2dB; and at 1000Hz mean CHL 13dB reduced to 2 dB. Greater CHL change did not predict greater symptom improvement.
No significant sensorineural hearing loss was recorded with all patients maintaining preoperative class of hearing according to American Academy of Otology Guidelines.
Conclusions
Elevation of cVEMP thresholds following surgical repair was significant and correlated with level of reported symptom improvement postoperatively. Reduction of the conductive hearing deficit was greatest in the low frequencies following surgical plugging of SCDS.

190 Ocular Vestibular-Evoked Myogenic Potentials (OVEMPs)

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The ocular vestibular evoked myogenic potential (OVEMP) is a recently discovered test of labyrinthine function, analogous to the cervical VEMP. Stimulation of the vestibular system by both air- and bone-conduced sound activates vestibulo-ocular pathways, resulting in modulation of tonic extraocular muscle activity. These muscle potentials can be recorded on upgaze by surface electrodes placed inferior to the contralateral eye, since the OVEMP is mediated by a crossed pathway. The typical response consists of an initial negativity caused by extraocular muscle excitation, followed by a positivity. In this study we examine the OVEMP response to various air- and bone-conducted stimuli in normal subjects without vestibular disorders, and in patients with Ménière's disease, superior canal dehiscence (SCD), and unilateral vestibular hypofunction (UVH). OVEMPs were present across all ages and in response to all modes of stimulation: condensation clicks, short tone bursts, and vibration produced by a reflex hammer and a mini-shaker device applied to the forehead. Patients with UVH had absent or markedly decreased OVEMPs on the side contralateral to the diseased ear. Those with SCD had greatly increased OVEMP amplitudes, more so in response to air-conducted than to bone-conducted stimuli. Ménière's patients had reduced OVEMPs from the diseased ear, and their OVEMP asymmetry ratios between the two ears were comparable to those obtained with CVEMPs. In addition to its usefulness in diagnosing these vestibular disorders, OVEMPs offer several advantages to CVEMPs, namely patient comfort and ease of use. The OVEMP is a less strenuous test than the CVEMP because subjects need only look upwards during testing; they do not have to turn or lift their heads up continuously, which can be tiring for older individuals. And finally, since the OVEMP response is an excitatory response instead of an inhibitory response, no threshold muscle activation is required to obtain reliable readings.

191 Vibration VEMPs Suggest That Intratympanic Gentamicin Has a Weaker Effect on the Utricle Than on the Saccule

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Air-conducted, sound-evoked VEMPs have been widely accepted as a test of saccular function. Recently, Curthoys et al. (2006) showed that irregular otolith neurons were selectively activated by bone-conducted, vibrational stimulation in the guinea pig, and labeling of these neurons with neurobiotin demonstrated that they originated in the utricular macula. Previous work in our lab revealed that air-conducted cervical and ocular VEMPs (CVEMPs and OVEMPs, respectively) were absent from the lesioned ear of a patient who had undergone unilateral inferior vestibular nerve section, but that the vibration CVEMPs and OVEMPs were still present, although reduced in amplitude, from that same ear. These data suggest that vibration CVEMPs and OVEMPs originate from either the utricle alone or from both the utricle and saccule. In this study, we examine CVEMPs and OVEMPs in response to sound (clicks and tone bursts) and vibration (tendon hammer and mini-shaker device applied to the forehead) stimulation in patients with unilateral definite Ménière's disease who received intratympanic (IT) gentamicin treatment. Gentamicin was administered following a titration protocol with repeat injections given on an as-needed basis to control vertigo, and most patients required several injections to control vertigo. Sound-evoked VEMP responses were reduced or absent in the majority of patients, but vibration-evoked VEMPs were often preserved. Thus, we suggest that: (1) the absence of sound-evoked VEMPs demonstrates loss of saccular function after IT gentamicin; and that (2) the preservation of vibration VEMPs implies retained utricular function after IT gentamicin treatment. Together, these findings indicate that IT gentamicin has a weaker effect on the utricle than on the saccule.

192 The Reliability and Stability of Dynamic Testing of the Vestibulo-Ocular Reflex in Patients with Vestibular Disease

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Purpose: The purpose of the current study was to investigate the reliability and stability of the Gaze

Stabilization Test (GST) and Dynamic Visual Acuity test (DVA) in patients with vestibular disorders and to determine if subjects' performance on the GST and DVA correlated with dizziness symptoms. Subjects: Twenty subjects were tested (mean age 51.3, sd = 13.4, five males). Method: Using the NeuroCom InVision Tunnel system, patients moved their head at predetermined speeds and ranges in both the pitch and yaw planes while viewing a computer screen 4 m away on which an optotype (the letter E) appeared. Patients indicated the direction of the optotype. The output of the GST is the maximum head velocity (in degrees/sec) at which the subject was still able to identify the orientation of a fixed-size optotype. The output of the DVA is the change in subjects' visual acuity (in logMAR units) when moving the head at a fixed speed. In addition, subjects indicated their level of dizziness on a visual analog scale (VAS) before and after each test. Tests were repeated two times in one session, with a 30 minute rest, and once more 7 – 10 days later. Results: Patients' scores varied considerably among the three repetitions of testing. Intraclass correlation coefficient (ICC) values obtained within session and between sessions ranged between 0.30 – 0.45 for all tests, with better correlations for the within session assessments. Subjects' perceived dizziness did not correlate with their performance on the GST and DVA tests. Discussion/Conclusion: The InVision Tunnel system is a promising new technique to assess the functional status of the VOR in patients with vestibular disorders. The current test protocol requires additional refinement in order to enhance reliability and stability.
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193 The Reliability of Air Caloric Test and Analysis of Middle Ear Status Affecting Air Caloric Test

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Aims: The air caloric test can be used in the presence of tympanic membrane perforation or inflammation of the external ear. It is also better tolerated by pediatric patients than water irrigation. The responses to 24°C and 50 °C air stiumation showed similar to those to water irrigation using 30 °C and 44 °C. But air caloric test has been reported smaller nystagmus intensity and larger intersubject variability compared to water stimulation. In this study, authors hope to clarify that the result of air caloric test may represent vestibular status of patient having chronic middle ear disease.
Materials and methods: Forty-eight subjects who were found to have no evidence of vestibulopathy were investigated. There were 15 subjects (30 ears) of normal hearing with normal eardrum (6 men and 9 women 37 ± 12 years) for control and 33 subjects of unilateral conductive hearing loss due to chronic otitis media with unilateral perforated eardrum (12 men and 21 women, 49 ± 11 years). Both air caloric test and water test were performed in control group. Patient group underwent air caloric test and high resolution temporal bone CT. Maximal eye slow component velocities (MSCV), canal paresis (CP), and patterns of caloric induced nystagmus were analyzed.
Results: Statistically higher total MSCV were obtained for water (131 ± 43 deg/sec) compared to air stimulation (99 ± 35 deg/sec) (p<0.05), but CP did not show significant difference between two medium of caloric stimuli in control group (p>0.05). CP of the patients (24 ± 18%) was significantly higher than that of control (8.4 ± 6.2%)(p<0.05). Inverted nystagmus occurred in 16 disease ear tests (48 %) (15 in warm stimulation, 1 in cold stimulation). Sum of MSCV of diseased ear tests (41± 25 deg/sec) by cold and warm air stimulation was lower than that (52 ± 33 deg/sec) of contralateral healthy ears (p<0.05).
Conclusions: Air caloric test may substitute water caloric test in healthy ear test but consideration of several factors in the diseased ear and seeing the raw data seems to be necessary during the interpretation of the result in the presence of middle ear disease.

194 Subjective Visual Vertical Is Attracted Towards the Side of the Initial Bar Presentation

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Introduction: Measurement of Subjective Visual Vertical (SVV) is a useful approach to understand diseases of peripheral or central otolithic pathways.
It evaluates the cortical integration of gravity. The aim of this study was to evaluate the influence of the initial bar position on the SVV measurements.
Material and Methods: The population comprised 2675 patients (1737 females and 938 males) complaining of disequilibrium or vertigo. Patients were placed in total obscurity and were asked to position a phosphorescent bar in the vertical position using a remote control. The deviation of the bar from the vertical was measured in degrees. The bar was initially presented at 45°, and the side was changed after each measurement. The test comprised 12 consecutive measurements. SVV was calculated as the mean value of the12 measurements. To assess the influence of the initial bar position on the measurement, deviations of the bar to the side of the initial presentation were considered as positive, and those to the opposite side as negative. We defined the visual attraction index (VAI) as the algebric sum of the deviations for the set of 12 measurements.
Results: There was no difference of mean age or SVV between males and females. Mean VAI was different from

0° suggesting the influence of the initial bar position. Visual attraction decreased with the repetition of the measurements during the same test. VAI was lower males, and in patients between 20 to 60 years of age in comparison to those below 20 or above 60. VAI seemed not to be related to the type of the symptoms (otolithic, benign paroxystic positional vertigo, or other).
Conclusion: Visual attraction is probably related to a tilted mental representation of the space. It appears as a new tool to explore the spatial mental representation

195 The Reliability, Stability, and Concurrent Validity of a Test of Gaze Stabilization

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The gaze stabilization test (GST) is a computerized test of the vestibulo-ocular reflex that reports maximum head velocity while maintaining fixed visual acuity. The GST output is more functional than that reported by the Dynamic Visual Acuity (DVA) test. The purpose of this study was to assess the reliability, stability, and validity of the GST in a healthy older population.
Forty subjects (20 older adults with mean (SD) age of 76.3 (5.3) and 20 young controls with mean (SD) age of 25.2 (3.2)) were assessed with GST and DVA testing. The version of the GST used in this study has a tunneled mirror system to ensure a consistent participant distance of 3.96m from the computer screen. All subjects repeated trials within 30 minutes of initial testing. Twenty subjects (10 from each age group) returned within 7-10 days to repeat the GST and DVA testing.
The mean (SD) GST scores for the older group were 124.0 (32.5) deg/s in the yaw plane and 108.6 (30.2) deg/s in the pitch plane. For the young group, mean (SD) GST scores were 159.4 (34.6) deg/s in the yaw plane and 141.3 (26.7) deg/s in the pitch plane. There was a significant between-group difference for GST scores in both yaw and pitch planes ($p<0.0001$). The intraclass correlation coefficient (ICC) for GST scores performed on the same day was 0.71 in the yaw plane and 0.72 in the pitc
h plane. The ICC for the 20 subjects who repeated the GST within 7-10 days was 0.55 in the yaw plane and 0.79 in the pitch plane. Concurrent validity, determined by Pearson Correlation Coefficients between GST and DVA results were 0.41 in the yaw plane and 0.49 in the pitch plane ($p<0.0001$).
These results suggest that the gaze stabilization test (GST) has good same-day test-retest reliability and excellent stability in the pitch plane. The weak correlation between GST and DVA scores suggest the two tests may be measuring different constructs.
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196 Vibration-Induced Nystagmus in Peripheral Vestibular Loss: Comparative Study with Other Vestibule-Ocular Reflex Parameters in the Yaw Plane

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Objectives: Vibration stimulation on the skull or on the neck induces nystagmus in unilateral peripheral vestibulopathy. The intensity has been reported to be correlated with the severity of the unilateral weakness on caloric test. In this study, authors hope to validate the role of vibration induced nystagmus (VIN) as a screening bedside test of unilateral vestibular loss by comparing its horizontal component with several objective parameters reflecting vestibule-ocular reflex (VOR) asymmetry in the yaw plane.
Methods: 74 patients of unilateral vestibular loss of acute onset without history of fluctuating vestibular function were included. Compared parameters of horizontal VOR included the intensities of horizontal component of spontaneous nystagmus, induced nystagmus during mastoid vibration and immediate after head shaking for 15 seconds, canal paresis (CP) on bithermal alternating caloric test and time constants (Tc) on step velocity (100 deg/sec) test. Ipsilesional Tc (Tci) was defined as the average of Tc of per-rotary stimulation to ipsilesional direction and Tc of post-rotary stimulation to contralesional direction. Test parameters were compared with each other by correlation analysis. Receiver operating characteristics (ROC) curves were plotted according to the lower normal value of Tci (10.9 sec) which was acquired from 63 healthy controls, then the area under the ROC curve (AROC) was calculated and compared.
Results: The intensity of VIN showed significant positive correlation with CP and negative correlation with Tci ($p<0.05$). However, the intensity of head shaking nystagmus was not correlated with CP and Tc. The cut off value of VIN was about 7 deg/sec on ROC curve referenced to Tci. AROC of VIN (0.827) was even higher than those of CP (0.805), spontaneous nystagmus (0.646) and head shaking nystagmus (0.498) on the ROC curve referenced to Tci.
Conclusions: VIN is a valid bed side evaluation for the detection of unilateral vestibular loss and the intensity information seems to be as useful as the CP value on bithermal caloric test.

197 Variations on Canalith Repositioning for Treatment of Benign Paroxysmal Positional Vertigo

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Over the past 25 years Epley's canalith repositioning maneuver (CRP) has become the standard treatment for

most cases of benign paroxysmal positional vertigo of the posterior semicircular canal. Our lab has recently shown that this treatment is significantly more effective than sham (1) and can be performed effectively even with a reduced version without rolling the patient onto the contralateral side (2). In this study we pursued the question of relative effectiveness of variations on Epley's basic CRP treatment. We asked if CRP effectiveness would be enhanced by adding a week of the modified Brandt Daroff exercise (B&D), or if a version of CRP based on the recent computational model by Rajguru et al (3) would be more effective than Epley's CRP. We determined the relative effectiveness of self-CRP, recently described in the literature but without supporting data. Finally, we asked if CRP effectiveness was reduced when the patient had more than one semicircular canal involved. Preliminary analyses showed no significant difference among CRP, CRP + B&D, and the Utah CRP. All groups improved significantly from pre- to one week post-test and then maintained that improvement over the 6 month follow-up. The single canal CRP group was slightly but not significantly better than the two-canal CRP group. These data suggest that most treatments which include the basic manipulation of the head as described by Epley are effective. Additional exercise at home does not add to treatment effectiveness. When the patient has involvement of more than one semicircular canal, however, CRP is less effective. Those patients may need treatment with additional maneuver specific to the involved semicircular canals.

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198 Auditory Vestibular Correlation in Meniere's Disease: The Relevance of the Normal Ear

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Objectives: To analyse the correlation between vestibular dysfunction and hearing level of patients diagnosed with Ménière's disease.

Methods: Retrospective study on the correlation between hearing level and unilateral weakness in 60 Ménière's disease patients. Patients were classified according to the AAO-HNS guidelines. Auditory deficiency was assessed by different measures that take into account hearing level form both ears: the one that manifests hearing fluctuations with each vertigo spell and the contralateral. Vestibular function was assessed with the caloric test.

Results. No correlation was found between pure tone average and canal paresis. Nevertheless, when they were grouped by hearing loss AAO-HNS stages, we found a greater canal paresis in those with a higher hearing loss (groups 3 and 4). The different auditory measures used in this study show a better correlation to vestibular fucntion.

Conclusion: As with vestibular function, tha hearing from the so-called normal ear influences the amount of auditory deficit in patients with unilateral Meniere's disease.

199 Extracellular Matrix and Basement Membrane Protein Immunolocalization in Vestibular Endorgans Surgically Obtained from Meniere's Disease Patients.

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The basement membrane (BM) constitutes a distinct compartment of the extracellular matrix (ECM), and form a network of proteins and proteoglycans located at the epithelial and mesenchymal interface of most tissues. ECM molecules have an important structural role and participate in the regulation of extracellular ions homeostasis. We have recently reported the immunolocalization of several ECM proteins in the human vestibule. (Mowry et al. 2008 31st ARO, Abs 958). The identification and localization of BM and ECM proteins becomes relevant given recent reports on the existence of cochlin depositions in stromal tissue in the inner ear of patients diagnosed with DFNA9, an autosomal dominant deafness and vestibular disorder, which in several families presents with clinical disease meeting the criteria for Meniere's disease (Robertson et al.2006 Hum Mol Gen,15,1071-85). The immunohistochemical distribution of collagen IV, nidogen, laminin, a-dystroglycan, tenascin-C, cochlin and vimentin, was investigated in vestibular endorgans surgically obtained from patients diagnosed with Meniere's disease (N=5), and compared with the respective distribution in normal vestibular endorgans obtained from autopsy (N=3). In Meniere's disease cristae and macula utricle, collagen IV and vimentin immunoreactivity was decreased in stromal BMs. In contrast, cochlin immunoreactivity was increased in the stroma. The other ECM proteins immunoreactivity distribution patterns were similar to normative patterns. These results suggest that Meniere's disease is associated with an alteration of the BM and ECM protein expression. Such changes could impair the ionic and fluid homeostasis of the inner ear, with the consequent vestibular or auditory dysfunction.Supported by NIH-NIDCD grant U24DC005028-01 to AI.

200 Inhibition of Trk Signaling and Histone Deacetylase Leads to HSV1 Reactivation in Quiescently Infected Vestibular and Trigeminal Neurons in Cell Culture

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Herpes simplex type 1 (HSV1) is a nearly ubiquitous viral pathogen, with an 80% rate of seropositivity in the adult U.S. population. Following primary viral infection, HSV1 reactivation can occur at variable intervals. Reactivation of latent virus in trigeminal ganglion neurons (TGNs) can lead to painful "cold sores." HSV1 reactivation has been implicated in other syndromes characterized by neuronal death in the head and neck, including vestibular neuritis. Vestibular neuritis is a syndrome of acute peripheral vertigo associated with changes in the vestibular ganglion and nerve that are consistent with a viral etiology. We have developed a murine cell culture system of HSV1 infection and latency. Using HSV1 virus with a green fluorescent protein fusion to the US11 protein, we have infected dissociated VGNs and TGNs, prevented lytic infection with acyclovir, and demonstrated quiescent HSV1 infection. Previous culture studies of herpesvirus reactivation have been performed in neuronal systems that differ from TGNs and vestibular ganglion neurons (VGNs) in several critical ways, including neurotrophin receptor expression. Neurotrophin (NGF) withdrawal in sympathetic neurons results in HSV1 reactivation. VGNs do not express the NGF receptor. Instead they express receptors for neurotrophin-3 (NT3) and brain-derived neurotrophic factor (BDNF). In contrast, TGNs express receptors for NGF, NT3 and BDNF. Using K252a, a panTrk inhibitor, and inhibitors of downstream effectors, we have been able to induce a lytic infection in latently infected TGNs and VGNs. Specific inhibition of BDNF signaling in VGNs also ends the quiescent phase. Additionally, trichostatin A, a histone deacetylase inhibitor, reactivates lytic infection in TGNs and VGNs. These studies should improve understanding of HSV1 lifecycle regulation and lead to novel treatment approaches for people with herpes simplex syndromes, including early acute or recurrent vestibular neuritis and vesicular stomatitis.

201 Behavioral State-Dependent Vestibular Responses in Birds

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The vestibular system contributes to compensatory responses that keep gaze, head orientation and posture stable as animals move around in the world. These responses are often dependent on the animal's behavioral state. Response components may be enhanced or attenuated, turned on or off – depending on the current demands of the behavioral context. Previous studies have shown that simulating gliding flight in pigeons affects head- and body-stabilizing responses to motion stimuli. In the current study, we record eye and head movements from pigeons (*C. livia*) at rest and during simulated gliding flight. We show that the vestibulocollic response (VCR) is enhanced during flight, consistent with a critical role for head-in-space stability specifically during flight. We discuss the ethological significance of this result, as well as the potential neural mechanisms involved. *Supported in part by NIDCD #DC007618 and #DC006913. Stipend support provided by NIDCD #1F31DC009734.*

202 Evaluation of Vestibular Function in Guinea Pigs with Inner Ear Application of Rolipram, an P-CREB Up-Regulator.

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Phosphorylation of the transcription factor cAMP responsive element-binding protein (CREB) is thought to play a key role in neurogenesis. In our previous study, phosphprylated form of CREB (p-CREB) –like immunoreactivities were observed in vestibular ganglion cells after unilateral surgical labyrinthectomy, unilateral TTX infusion, or unilateral lateral semicircular canal transection. But changes of p-CREB-like immunoreactivity in each group were different according to the method of peripheral vestibular lesions. These results indicate that vestibular periphery has a potential of neuronal plasticity and the activation of cAMP-CREB system may facilitate the vestibular plasticity.

Rolipram, a phosphodiesterase (PDE) 4 inhibitor, increases cAMP levels and leads to up-regulate the phosphorylation of CREB. Rolipram was developed as an antidepressant. But now, rolipram disappeared in clinical use due to its severe side effect, vomiting. We thought the possibility that rolipram might be one of the candidates for local application therapy for peripheral vestibular lesion. The aim of this study was to evaluate the influence of rolipram locally applied into unilateral inner ear of the normal guinea pig by osmotic pump using sinusoidal rotation test.

Hartley white guinea pigs with normal tympanic membranes and normal Preyer reflexes were used in this study. A tiny hole was made adjacent to the round window in the right ear, at the end of the 12-h diluted water infusion, the pump then infused 0.2 mg/ml rolipram continuously. During the experimental procedure, we observed static symptoms, such as spontaneous nystagmus and head deviation. To measure the vestibulo-ocular reflexes (VOR), sinusoidal rotation tests were performed before treatment, 12 h, 24 h and 3 days after treatment. The VOR gains were calculated using our analysis system. Rotation test conditions were 0.1 Hz, with a peak angular velocity of 60 degrees/sec. To evaluate vestibular function, we defined the "gain ratio" as follows: VOR gain on the treated side/VOR gain on the untreated side. Differences in gain ratios between the before treatment, 12 h, 24 h and 3 days after treatment were evaluated by Friedman's test with the significance set at $P < 0.05$.

In all animals, we observed neither spontaneous nystagmus nor head deviation in the experimental period. The gain ratio 24 h after treatment decreased, but this change was not statistically significant.

These data indicated that local application of rolipram showed no obvious influence on the peripheral vestibular function in a normal state.

203 Adaptation of the Roll Avor in a Rotating Frame

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Monkeys were oscillated about the naso-occipital axis in darkness while being rotated at a constant velocity around a spatial vertical axis (Roll While Rotating, RWR). The animals were also oscillated about a naso-occipital axis while they received constant velocity rotation of the visual surround (Roll and OKN). RWR for several hrs induced oscillations in pitch velocity and continuous horizontal nystagmus, as well as compensatory oscillations in roll. Afterward, pure oscillation in roll in darkness without vertical axis rotation induced similar roll and pitch oscillations and yaw nystagmus as had occurred during the previous adaptive exposure to RWR. The yaw slow phase velocity in the post-adaptive state started at the onset of roll, rose slowly to a steady state, and fell slowly when the head roll ceased. The rising and falling time constants of the slow phase velocity were characteristic of velocity storage, and the adaptive state only occurred in monkeys with long aVOR time constants. Thus, the adaptation arose in velocity storage. The phase of oscillations in vertical eye velocity relative to head position in the post-adaptive state initially was close to 0°. As this phase reverted toward the normal state (90°), the horizontal eye velocity declined, and only compensatory roll eye velocity was present. Oscillation in roll during horizontal OKN (Roll & OKN) produced a similar adaptive state, also through velocity storage. This was modeled as a shift in the roll orientation vector of velocity storage toward the pitch axis. According to this model, oscillation of the head in roll would activate the roll orientation vector, which has a component along the pitch axis, producing vertical oscillation that are phase-shifted in relation to the time constant of velocity storage. This produced vertical eye velocity that was in phase with roll head position during head roll, thereby generating continuous horizontal nystagmus when the animals were oscillated in roll. Similar adaptive changes in the roll orientation vector could be important in generating motion sickness after exposure to motion environments that induce cross-coupled eye velocities.

204 Specificity and Generalization of Spectacle-Induced Adaptation in the Vestibuloocular Reflex (VOR)

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We recorded the VOR in 5 macaque monkeys during angular rotations in the yaw plane before and after 4 different spectacle-induced adaptation paradigms. Sum-of-sines rotational stimuli were used to induce adaptation while animals wore magnifying (1.7 X) or minimizing lenses (0.59 X) for up to 3 hours. The component frequencies of the sum-of-sines stimuli were either high frequency (2.3, 3.7, 5.9 Hz) or low frequency (0.3, 0.5, 1.4 Hz). The amplitudes of each component frequency were either high velocity (20, 20, 20 °/s) or low velocity (7, 7, 7 °/s) to give us the four different learning paradigms. Following adaptation in the light with these stimuli, the VOR in response to rotations in darkness was recorded at 0.5, 2, 4, 6, and 8 Hz with peak velocities of 20 and 50 °/s before and after each learning paradigm.

Adaptation was greatest over the range of frequencies in the sum-of-sine stimulus, but there was transfer of adapted responses to other frequencies. Similarly, adaptation induced by stimuli with lower and higher peak velocities led to the greatest change in responses to rotations at comparable velocities. The relationship between frequency and velocity was most apparent for adaptation to stimuli at higher frequencies. Velocity specificity of the adapted process was clearly evident. When the adaptation stimuli included high frequencies and velocities, the responses showed greatest changes for responses tested at higher frequencies and velocities. The converse was true for adaptation stimuli at higher frequencies but lower velocities. In contrast, responses following adaptation at lower frequencies showed a less specific relationship to stimulus velocity.

These findings can be understood in terms of a mathematical model of VOR adaptation that includes inputs from tonic and phasic pathways. The phasic component is further characterized by a frequency and velocity dependent nonlinearity. (Supported by NIH R01 DC 02390)

205 Modeling Binocular Fixation During Fore-Aft Translation: Dynamics of Pursuit and the Linear Vestibulo-Ocular Reflex (LVOR)

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During fore-aft translation, the IVOR induces ocular convergence, while pursuit of a target in light induces binocular fixation that also converges the eyes. We studied the dynamics of this convergence and the IVOR-pursuit interaction in monkey and modeled the responses. Monkeys were trained to watch a visual target on a flat computer monitor located 276-mm along the naso-occipital (NO) axis and either the target or monkeys were moved. During pursuit, the target was located centrally, laterally or vertically relative to the NO axis and moved sinusoidally along the screen with an amplitude of 17° and at frequencies of 0.05-1 Hz. Vergence during pursuit modulated ≈1°, and binocular fixation of the target was accurately maintained. When pursuit capability declined at higher frequencies (>1 Hz), the convergence was still maintained over the pursuit trajectory. Even when one eye was covered, convergence was maintained at the extrapolated target position. The amount of variation in yaw, pitch and roll convergence was independent of frequency, of eye position, or of the pursuit profile. Translation in the fore-aft direction (0.05-2 Hz), while

binocularly fixating a central target located 216-316 mm in front along naso-occipital axis or optical axis of the eye, induced a modulation of 0.1-4°, depending on distance to target and frequency of oscillation. For binocular viewing, vergence was maintained over a frequency range of 0 – 4 Hz, regardless of target location in three dimensions. When one eye was covered, both vergence angle and modulation of eye vergence in response to fore-aft translation were inaccurate, with errors of ≈40%. This was modeled by a three dimensional pursuit system, which aligns the visual axis of each eye with the target axis. The model includes accommodation effects on convergence to account for the convergence during monocular stimulation while translating in the fore-aft direction, which was drastically reduced during planar pursuit.

206 Effects of Frequency and Motion Paradigm on Perception of Tilt and Translation During Periodic Linear Acceleration

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Previous studies have demonstrated an effect of frequency on the gain of tilt and translation perception. Results from different motion paradigms are often combined to extend the stimulus frequency range. For example, Off-Vertical Axis Rotation (OVAR) and Variable Radius Centrifugation (VRC) are useful to test low frequencies of linear acceleration at amplitudes that would require impractical sled lengths. The purpose of this study was to compare roll-tilt and lateral translation motion perception in 12 healthy subjects across four paradigms: OVAR, VRC, Sled translation and rotation about an earth-horizontal axis. Subjects were exposed to sinusoidally varying accelerations in darkness at six frequencies from 0.01875 to 0.6 Hz (peak acceleration equivalent to 10 deg, less for sled motion below 0.15 Hz). Subjects used a joystick to indicate the direction of motion and verbally estimated the amplitude of perceived tilt and translation. Consistent with previous reports, tilt perception gain decreased as a function of stimulus frequency in the motion paradigms without concordant canal tilt cues (OVAR, VRC and Sled). Translation perception gain was negligible at low stimulus frequencies and increased at higher frequencies. There were no significant differences between the phase of perceived tilt and translation, nor did the phase significantly vary across stimulus frequency. There were differences in perception gain across the different paradigms. Paradigms that included actual tilt stimuli had the larger tilt gains, and paradigms that included actual translation stimuli had larger translation gains. In addition, the frequency at which there was a crossover of perceived tilt and translation gains appeared to vary across motion paradigm between 0.15 and 0.3 Hz. Since the linear acceleration in the head lateral plane was equivalent across paradigms, differences in gains may be attributable to the presence of linear accelerations in orthogonal directions and/or cognitive aspects based on the expected motion paths.

207 Electrical and Mechanical Correlates of the Sound-Evoked VOR

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Vestibulo-ocular reflexes stabilize the visible image on the retina during head movement. Sound and vibration, by exciting vestibular afferents, produce a short-latency eye movement or sound-evoked vestibulo-ocular reflex (VOR). The same stimuli evoke excitatory muscle potentials recorded beneath the eye (ocular vestibular-evoked myogenic potential: or "OVEMP"). We measured these two responses simultaneously for the first time. The VOR was measured in 3 dimensions using scleral search coils. The OVEMP was measured using infra orbital surface electromyography. Six healthy adults and seven subjects with sound hypersensitivity due to superior canal dehiscence (SCD) were studied. In both groups, an intense 500 Hz pure tone of 120 dB evoked OVEMP responses that began at 8 ms and peaked at 12 ms. The onset of the eye movement coincided with the excitatory peak of the muscle potential. In controls, an inconsistent upward eye movement was followed by a sustained downward movement. In SCD, the eyes moved upwards and torted away from the stimulated ear, in the plane of the affected semicircular canal. OVEMP amplitudes were powerfully modulated by gaze: being maximal on up-gaze and abolished on down-gaze; yet VOR magnitudes were unaffected. When stimulus type was changed from air-conducted sound to bone-conducted vibration, both responses changed concordantly: doubling in magnitude in controls and decreasing in SCDS. Both VOR and OVEMP displayed frequency tuning, and were maximal at the same stimulus frequency. OVEMP amplitudes and VOR magnitudes were significantly correlated.

Selective decrease of the OVEMP upon down-gaze is consistent with relaxation or retraction of the inferior oblique muscles. The temporal relationship of OVEMP and VOR and their identical modulation by stimulus type and frequency indicate that they represent the electrical and mechanical correlates of the same short-latency vestibulo-ocular response.

208 Variability in the AVOR to Head Impulse Testing

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The angular vestibulo-ocular reflex (aVOR) is known to be influenced by factors such as arousal and cognition. However, the inherent variability of the aVOR has not been explicitly examined. The purpose of this study was to determine the variability of the aVOR to active and passive head impulses. Seven subjects with UVH based on history of vertigo and imbalance, physical exam revealing corrective saccade following head impulse testing, abnormal electronystagmography exam (>20% asymmetry), and absence of a mass-enhancing lesion within the internal auditory canals or cerebellopontine angle were studied. The subjects with UVH had

completed vestibular rehabilitation prior to involvement in this study. Dizziness handicap inventory was also completed at the time of each ocular measurement.We also studied eleven control subjects with no history of vertigo or dizziness. Monocular search coil was used to measure aVOR gain during active and passive head impulses at two occasions, separated by 14 days in the UVH and 3-210 days in the normal subjects. Dependent t-tests were used to investigate differences between testing session. As a group, subjects with UVH had no difference in aVOR gain for passive head impulses between the first (T1, 0.40 ± 0.26) and second (T2, 0.49 ± .34) testing sessions ($p = 0.13$); for active head impulses the difference approached significance (T1 0.66 ± 0.23 vs. T2 0.74 ± 0.22, $p = 0.051$). However, difference in aVOR gain within individuals was apparent. Between testing sessions, aVOR gain for passive head impulses improved by 10% or more in 4 of 7 UVH subjects; and in 5 of 7 during passive head impulses. The range of aVOR gain change for passive head impulses was -32 to 87%; active was -5 to 33%. In the controls, there was no difference in aVOR gain for right or leftward head impulses, nor was there any difference between T1 and T2 for passive or active head rotations ($p>0.55$). The range of difference between T1 and T2 was -15 to 32% for passive head impulses and -23 to 21% for active impulses. Interestingly, the DHI score significantly improved by a mean 42 ± 27% for the UVH subjects ($p= 0.02$). Though group differences were less affected, our data suggest the aVOR gain does have an inherent variability to head impulse testing, which may be useful to know for determining efficacy of treatment strategies.

209 Effects of Constant and Incremental Position Error Signals on AVOR Gain Adaptation

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Retinal slip (velocity error signal) is considered to be the most potent strategy for angular vestibular-ocular reflex (aVOR) adaptation. Few studies have investigated position as an adaptive strategy for aVOR gain modification. We sought to characterize aVOR gain adaptation using a position error signal in normal subjects. To date, three subjects have been exposed to 2 separate position error paradigms: constant and incremental. The constant paradigm consisted of 9 trials of adaptation using an error signal that was always 5% greater than the head amplitude; the incremental paradigm consisted of 3 sets of 3 trials of adaptation progressing by 5% up to 15% head amplitude. Monocular search coil recordings were used to quantify the adaptation during, and subsequent to the two paradigms. Two subjects demonstrated significant aVOR gain adaptation to active head rotation. The constant position error signal induced adaptation ranging from 3% to 14% ($p<0.05$) while exposure to an incremental error signal resulted in gain increases ranging from 4% to 9% ($p<0.05$). Passive head impulses revealed limited retention of adaptive effects with significant improvement occurring in only one subject (7%, $p<0.0004$). The third subject did not demonstrate any adaptation. No subject showed recruitment of compensatory saccades during head rotations. Our data suggest use of a position error signal can lead to aVOR gain adaptation. Preliminary analysis suggests that smaller magnitude position error signal may be more effective in up-adapting aVOR gain than paradigms using larger magnitude error signal.

210 Weber's Law and Sensation of Angular Velocity

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Weber's Law states that the discrimination thresholds of a sensory system are proportional to stimulus intensity. Human perception obeys Weber's Law in multiple sensory systems including vision, proprioception and audition. Reflexive responses of the vestibular system to rotations are well described but little is known about the psychophysical ability of the vestibular system to discriminate differences in rotational stimulus intensities. Based on the need for the vestibular system to prevent retinal slip over a wide range of head movements, one might suspect that the vestibular system does not obey Weber's Law. Here, we applied a two-alternative forced-choice paradigm to evaluate Weber's Law for earth-vertical rotations. Healthy young volunteers with no history of vestibular dysfunction were presented with an earth vertical sinusoidal control stimulus of 20 deg/sec, 40 deg/sec or 100 deg/second and a comparison stimulus at varying angular velocities slightly above or below the control stimulus. The two stimuli were presented in immediate succession, randomizing the order of stimuli presentation. A novel stimulus profile was used to minimize the effect of velocity storage. Data were collected using a two-alternative forced-choice protocol where the subject reported if the first or second stimulus was larger. Psychophysical curves were constructed from the responses of the subjects. The threshold, defined as the 80% correct level on the psychophysical curve, was approximately 5 deg/sec at a peak sinusoidal velocity of 40 deg/sec but only 8 deg/sec at a peak velocity of 100 deg/sec. This indicates that that human perception of earth vertical rotations may not behave in accordance with Weber's Law. This result may be related to the requirement to maintain stable gaze across a wide range of head velocities.

211 Vestibular Prosthesis Tested in a Vestibulopathic Model

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We have characterized postural control, tilt psychophysics, and the vestibulo-ocular reflex (VOR) in one rhesus monkey in the normal state, after bilateral vestibular hypofunction was produced with intratympanic

gentamicin, and when the vestibulopathic monkey chronically utilized a one-dimensional canal prosthesis aligned with and stimulating the right posterior canal. Posture was assessed by measuring spontaneous sway of the trunk and the forces applied to the support surface during quadrupedal stance when stance-width and the visual and proprioceptive cues were modified, and by measuring postural responses during stereotyped oblique head turns. Tilt perception in the roll plane was measured with a task derived from the subjective visual vertical (SVV) test commonly employed in humans. The VOR was measured during roll and LARP rotation about the earth-horizontal axis.

The normal monkey demonstrated increasing postural instability when the visual and proprioceptive cues were rendered unreliable, accurate percepts of head orientation during roll tilt, and normal eye movement responses during roll and LARP rotation. When vestibular function was ablated with gentamicin, postural sway increased, particularly in the more challenging conditions, and large abnormal postural responses were evoked by head turns; percepts of head tilt became less accurate, underestimating the true tilt angle by about 50%; and the VOR gain was reduced by 50-60%. After the prosthetic canal was activated, the VOR gain increased substantially, percepts of head tilt improved modestly, and the abnormal postural responses induced by head turns decreased slightly.

These results demonstrate that we can effectively measure postural control, tilt perception, and vestibular-mediated eye movements in normal rhesus monkeys, and can quantify the deficits in these behavioral responses produced by bilateral vestibular ablation. The preliminary prosthesis data indicate that a one-dimensional canal prosthesis can significantly increase VOR responses in vestibulopathic rhesus monkeys, and suggest that it may also improve postural and perceptual function in these animals. We intend to use this approach to determine the effectiveness of a three-dimensional canal prosthesis to alleviate the oculomotor, perceptual, and postural abnormalities associated with bilateral vestibular hypofunction in rhesus monkeys.

212 A MEMS-Based Angular Rotation Sensor for a Vestibular Prosthesis

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Similar to cochlear prostheses providing auditory cues to the central auditory system by targeted electrical stimulation of cochlear nerve elements, implantable vestibular prostheses can encode 3D head movement as electrical current pulses applied to appropriate branches of the vestibular nerve and convey head rotation cues to the brain. Currently, the advancement of vestibular prostheses is impeded by the sensors detecting head rotations—linear accelerometers and gyroscopes. These sensors suffer from excessive size and power consumption that prevents the realization of a fully-implantable system. As an alternative, we propose sensors customized for the application. We are pursuing a biomechanical analogue to the natural human sensor, the semicircular canal (SCC) and ampullary organ. Using a micro-electromechanical systems (MEMS) based approach, we are attempting to capture the essential features from the natural system important during volitional head movements—the velocity meter behavior of the SCC-cupula system, and realize a functionally equivalent microfabricated structure. Such a strategy presents a scalable, biocompatible and low power sensor that enables the eventual realization of fully-implantable vestibular prosthesis.

We model the cupula as a diaphragm and relate its displacement to head angular velocity. Through the MEMS-based approach, a layered stack of thin-film materials serves as the diaphragm. Displacement sensing is achieved with an array of polysilicon strain gauges spanning the diaphragm that translate the diaphragm's deflection into electrical signals. This sensing strategy is dramatically different from using active sensors (accelerometers and gyroscopes) and 2/3 savings in power is projected. Finite element and electrical simulations are being employed to evaluate the necessary range of motion, sensor sensitivity and bias voltage requirements of the cupula sensor. In addition, scaling limits are being assessed to determine the minimum device size for implantation. To functionally mimic the SCC, a rigid polymeric lumen filled with water (viscosity similar to endolymph) will house the MEMS cupula sensor that is placed transversely across the lumen. By assessing the SCC-cupula system in a rotational test system, parameters such as frequency response, linearity, and sensitivity will be determined.

213 Enhancements to the Johns Hopkins Multi-Channel Vestibular Prosthesis Yield Reduced Size, Extended Battery Life, Current Steering and Wireless Control

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The first-generation Johns Hopkins multi-channel vestibular prosthesis (MVP) employed three mutually orthogonal gyro sensors to emulate the transduction of 3D head rotational velocity by the semicircular canals (SCC). The space and power required for that device's core circuitry (~30 x 30 x 11 mm^3 and ~140 mW in typical use) have proved adequate for experimental applications in which the sensors are fixed to the skull and outside the head, connecting to intralabyrinthine electrodes via a percutaneous connector. Long-term chronic stimulation paradigms and transition from percutaneous electrodes to an inductively powered, subperiosteally implanted device comparable to existing cochlear implants will require a significant reduction in device thickness and power consumption.

We describe the design, fabrication and performance of the second-generation MVP. The new device's core circuitry is small and thin enough (~28 x 28 x 5 mm^3) to fit

within a container of size similar to the housings of cochlear implants currently in use. Power consumption has been reduced 50% (to ~70 mW for typical use) and supply voltage has been lowered to 3.7 V, extending battery life while reducing battery size. Other enhancements include: an increased number of electrodes (12) and current sources (4), ability to perform "current steering" through proportional distribution of current delivered simultaneously to neighboring electrodes, a 3-axis linear accelerometer, wireless control and *in situ* reprogramming via a Bluetooth interface, and circuitry for measurement of electrically-evoked compound action potentials.

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214 Implementation of Extended Kalman Filter for Non Invasive Balance Prosthesis

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Balance prostheses can potentially: 1) improve postural stability for balance impaired, 2) reduce imbalance due to vestibular disorders, 3) provide temporary aid during recovery from ablative inner-ear surgery, 4) reduce risk of falling for elderly patients. A noninvasive prosthesis, which utilizes tactile vibrators, has been developed to provide vibrotactile feedback (VTTF) information about a subject's body motion to the torso. Motions are detected by an instrument sensor assembly (ISA) comprised of 3 gyros and 3 accelerometers. A quarternion complementary filter (QCF) was formulated for multi-axis tilt estimation, e.g. estimating changing spatial orientation of the patient for leans or tilts. The QCF provides accurate tilt estimates while standing or during locomotion along a more or less straight path. However, a significant shortcoming is that a pure yaw (rostra-caudal axis) rotation produces tilt error at high yaw rates.

The purpose of this project was to implement an extended Kalman filter (EKF) that provided a more accurate estimate for complex motions, such as yaw motion. Matlab simulation indicated that the EKF did not produce the unwanted tilt artifact, thus the EKF was implemented in C and downloaded to the VTTF device for evaluation. Preliminary testing was conducted on one subject who made three full rotations about the yaw axis at a moderate and realistic speed of rotation. The preliminary results showed that the QCF closely matched the EKF tilt for the first turn, however the QCF had a build up of error for each subsequent turn. When the subject ceased turning a significant tilt error was still present, however the EKF did not show this error. In order to test the filters in a controlled manner, a rate table will be used to provide precise motion inputs. It is hypothesized that the EKF will be more robust than the QCF, and thus possibly prove a more accurate algorithm for the noninvasive balance prosthesis.

215 Human Auditory Memory for Intensity

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Detection thresholds for intensity increments were measured, using listeners varying both in their degree of hearing loss and in age. Stimuli were three 50-ms narrowband noise bursts with widely-spaced center frequencies. The stimuli were presented alone or with a delay of 150 ms between offset and onset. Both factors greatly reduced the energetic overlap of the stimuli at the level of the cochlear filters. Despite this control for energetic masking, detection thresholds were higher when three bursts were presented sequentially than when only a single noise burst was presented. This effect occurred regardless of whether or not the temporal position of the target burst was varied across trials. Despite the hypothesis that age and hearing loss would impact the memory processes required to perform this task, no reliable effects of age or basic hearing sensitivity were found. Decreasing the time between bursts (0 or 50 ms delay versus 150 ms) had no reliable effects on performance. These results are inconsistent with a mechanism that is based on masking at the level of the cochlea, since delay had no effect and the stimuli were well separated in frequency. Nor can these data be related to failures of the strategic focusing of attention, since target uncertainty had no effect. Although there are still other possible explanations, these data are consistent with a memory-based explanation (proposed for similar visual results) in which subsequent stimuli interfere with the encoding and subsequent retrieval of information, but only when each stimulus must be encoded. In order to further constrain the potential explanations, electrophysiological data were collected on a related task with similar stimuli. Together, both types of data can be used to evaluate and refine a preliminary model of how the storage and retrieval of information in auditory memory can impact the ability to make multiple rapid intensity discriminations.

216 The Development of Auditory Frequency Discrimination May Depend on the Assessment Procedure

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Developmental gains in the ability to discriminate sounds based on their frequency (frequency discrimination) are typically attributed to either sensory factors or to the ability to attend to the relevant auditory cue for the duration of the assessment. Here we show that depending on the task listeners are required to perform, discrimination thresholds are either adult- like by 8 years of age (when measured with an oddball procedure in which listeners are asked to select the 'odd-one-out' among three alternatives) or still not adult- like by 14 years of age (when measured with a 2-interval-2-alternative-forced-choice identification procedure in which listeners are asked to determine which of 2 tones is higher). Because the stimuli used in both assessments were similar, sensory maturation seems an

unlikely account for these findings. Among the 14 year olds, only three could be characterized as inattentive based on their psychophysical data. Even when their data were excluded, highly significant group differences between teenagers and adults remained suggesting that attentional factors alone were also not likely to contribute to the group differences. Therefore cognitive functions other than sensation and attention may be responsible for the development of auditory discrimination. Furthermore, these functions continue to develop during adolescence.

217 Psychophysical Models of Auditory Change Perception

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Numerous psychoacoustical studies have measured listeners' ability to detect a small change in a sound attribute or to identify the direction of that change. However, few studies have examined in detail the relationship between performance in these two tasks. Different psychophysical models make different predictions regarding this relationship. Here, we analyzed detailed measurements of detection (D) and direction identification (I) thresholds for changes in pure-tone intensity, frequency, or amplitude-modulation (AM) rate obtained under identical stimulus conditions in the same listeners. We found that neither of the two most commonly adopted models in the psychophysical literature – the constant-variance Gaussian (CVG) model and the dual-state high-threshold (HT) model – could account for the ratios between the measured D and I thresholds. Instead, the measured ratios fell consistently between the predictions of these two models. After eliminating other possible explanations for this puzzling finding, we were led to the hypothesis that human observers have access to more detailed sensory representations of simple auditory stimuli than assumed by the discrete dual-state HT model, but fewer than assumed by the "continuous-state" Gaussian model. Two models based on the assumption of discrete internal representations with more than two states – a quantized-Gaussian model and a Poisson model – were found to account for the measured relationship between D and I thresholds. The Poisson model was further found to correctly predict asymmetric receiver operating characteristics (ROCs) for the detection of changes in intensity. We suggest that the latter model may provide a more faithful representation of the neural and perceptual processes responsible for the detection of changes in basic sound attributes by human listeners than the currently more popular CVG and HT models. [Work supported by NIH R01DC05216]

218 Relations Between Psychoacoustic and Speech Perception Measures in Cochlear Implant Users

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In this presentation we summarize a series of studies that explore the relations between psychoacoustic and speech reception measures in cochlear implant users. The psychoacoustic measures include: pure tone intensity discrimination, pure tone and tone complex frequency discrimination, forward and backward masking of a 300 ms tone burst upon a 10 ms tone burst, gap detection within noise bands of various bandwidths, tone on tone and synthetic formant on formant masking, tone in noise detection, and amplitude modulation detection. Psychoacoustic measures were determined using either using two or three interval alternative forced choice (AFC) paradigms. The speech reception measures include 12 vowel and 20 consonant recognition in quiet, in speech-shaped noise, and in gated speech-shaped noise. Noise levels were adjusted adaptively to estimate the speech reception threshold (SRT). The results for these measures are compared with normal hearing performance towards understanding differences between cochlear implantees and normal hearing listeners. The relative performance of implantees and normal hearing listeners indicate that implantees show average deficits in tone-on-tone masking (20-40 dB), forward and backward masking (10 dB), and tone in noise detection (10-15 dB). Almost all psychoacoustic measures were significantly correlated with measures of speech reception. These findings argue for considering psychoacoustic measures when improving the design of sound processors.
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219 Mouse Ultrasonic Vocal Structure Varies as a Function of Sex, Social Context and Inbred Strain

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Many animals use complex acoustic signals for communication. These sounds are important for facilitating species-specific behaviors such as courtship and mating, establishing and maintaining territories, identifying individuals, and mother-pup interactions. For example, it is well known that male mice produce complex, stereotyped ultrasonic vocalizations during courtship. Until recently there has been little attention paid to the vocal behavior of female mice or to vocalizations emitted in other social contexts. We investigated vocal behavior in male and female mice in two inbred strains: SWR/J and C57BL/6. Animals were pair-housed with same-sex siblings from 3 weeks of age. Recordings were made from pairs of mice in a 2'x2' arena in three social contexts: same-sex social partner, opposite-sex social partner, same-sex social partner reunion (after opposite-sex social partner experience). Female mice produced ultrasonic vocalizations in all three social contexts, and the structure

of these vocalizations was similar in complexity to vocalizations emitted by same strain male mice. SWR/J and C57BL/6 females produced ultrasonic vocalizations with the abrupt frequency discontinuities characteristic of those emitted by male mice during courtship. Moreover, SWR/J females produced ultrasonic vocalizations during courtship, a finding not previously reported. Although the structure of syllables between males and females was similar, particular syllables were more frequent in specific social situations. Finally, the acoustic structure (frequency and duration) of individual syllables emitted by males of the two strains varied. Our findings show that there are consistent differences in vocal structure between males and females, between social contexts and between inbred lines. This variation represents a powerful tool for geneticists, behavioral biologists and electrophysiologists to exploit in unraveling the neural basis of complex social and motor behaviors.

220 Psychoacoustic Characteristics of Residual Inhibition After Loud Noise Stimulation

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Residual inhibition (RI), the phenomenon that tinnitus can temporarily reduce or even disappear after the switch-off of a masker sound, was measured in 84 patients. In 36 of these patients the tinnitus sensation was unilateral, 35 patients experienced their tinnitus bilateral and 13 patients heard the tinnitus centrally in the head.

Three stimuli were used to elicit RI: band-pass noises with center frequencies of 2 and 4 kHz and white noise (BPN2kHz, BPN4kHz and WN resp.). Patients categorized these stimuli in loudness categories ranging from 'inaudible' to 'very loud' or 'too loud' (ACALOS method; Brand and Hohmann (2002)). In the RI-experiment, sounds were presented monaurally for 30 s at the intensity level, which was categorized as 'loud'. After sound stimulation, the patient was first asked to judge the loudness of its tinnitus using a scale ranging from 'completely disappeared' (-50) to 'much louder' (+50). Secondly, patients were asked to indicate when their tinnitus was recovered to its original level (Roberts et al. (2006)).

Pronounced RI could be elicited in 27 of the patients. No differences in RI were found between men and women and no correlation with age or tinnitus duration was found. Also, no significant differences were found in hearing thresholds between RI-patients and non-RI-patients. In most patients, no significant difference was found between using WN, BPN2kHz or BPN4kHz to elicit RI.

Recovery times ranged from tens of seconds to more than six minutes. In those cases in which the tinnitus fully disappeared after stimulation, the recovery time lasted at least 40 s.

There was a clear correlation between the lateralization of tinnitus and RI. In 24 of the 27 patients that clearly experienced RI, tinnitus was inhibited only in the ear ipsilateral to the external stimulus. This suggests that the inhibitory neural circuit that is thought to induce reduction of tinnitus during RI has no or only weak afferent connections with the contralateral ear.

221 A Qualitative Model Relating Loudness to Detection "Near Threshold" and Above, Giving Zero Loudness at a "Hard Threshold" and Nonzero Loudness at a "Soft (Psychometric) Threshold"

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A "near threshold" intensity is one for which the stimulus will be perceived only some percentage of the times that it is presented. Loudness traditionally has no explicit role in defining threshold, but was usually assumed to approach zero as intensity approaches threshold. Empirically, however, "threshold" loudness is nonzero (Buus, Musch, & Florentine, *JASA* **104**, 1998; Buus & Florentine, *JARO* **3**, 2001). Further, loudness is absent when stimulus is absent, such that loudness approaches zero as stimulus intensity approaches zero. And indeed the use of psychometric functions in determining threshold allowed the notion, promoted by Swets (*Science* **134**, 1961), that there is no intensity too weak to produce a sensation some percentage of the time. Altogether, there exist two intensity limits for zero loudness. But any attempt to assign a unique loudness to a stimulus intensity in a loudness equation must assign to zero loudness a single intensity. Rereading of Swets (1961) shows his arguments to be equivocal, and Bialek & Schweitzer (*Phys Rev* **56**, 1986) and Denk & Webb (*Phys Rev Lett* **63**, 1989) note limits on sensitivity that imply an ultimate finite hard threshold. How can we incorporate a hard threshold, resolve the limits problem, and have nonzero threshold loudness? One solution is to assume that acuity is limited by an internal Gaussian distribution of (unheard) loudnesses that acts as Noise. Assume also that a stimulus produces a Gaussian distribution of loudnesses (Signal) whose mean is monotonic with intensity. When Signal mean falls to Noise mean, we set loudness to zero. Overlap of Noise and Signal distributions allows the loudness, *and* the probability of its appearance, to increase with increase in intensity. Variances can be set so that absence of loudness is negligible "well above threshold". Thus loudness is zero in one single limit, i.e. a hard threshold, and loudness is nonzero at a soft (psychometric) threshold.

222 A Behavioral Detection Task in Guinea Pigs Using Air Puffs as Aversive Unconditioned Stimulus

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Compared to other species, guinea pigs are hard to train on a behavioral task because of their erratic behavior. Since guinea pigs are commonly used for cochlear

histology and physiology, there is a need for psychoacoustical tests for guinea pigs. We used a conditioned avoidance procedure, in which, instead of a conventional electric shock, an air puff was applied as aversive unconditioned stimulus (Philippens et al. 1992).
Ten albino female guinea pigs were trained to cross from one to another compartment in response to a narrow-band noise (center frequency 10 kHz, width at -6 dB: 1 octave) in order to avoid a strong narrow stream of air (80 m/s; 0.8 cm^2). The response window was 15 s, and the stimulus lasted until the animal responded. During the initial training sessions a sound level of 78 dB SPL was used. From the 6th session onward 4 levels from 58 to 88 dB SPL were presented. After the 10th session the animals were implanted with an 8-electrode array designed for guinea-pig cochleas (Cochlear®). After the 12th session the animals were ototoxically deafened and subsequently trained to respond to electrical pulse trains (111 Hz, 20 µs monophasic alternating pulses) at four current levels from 142 to 400 µA. Sessions consisted of 20 trials.
The animals learned to respond to the 78-dB SPL noise within 4 sessions (score: ~80%). When the other sound levels were introduced the animals did not immediately respond to the lower levels, but their scores improved in following sessions. Scores increased with sound level (50 to 100%), and response latency decreased with level (8 to 2.5 s). Already on the first session in which electrical stimuli were presented the animals responded well to 400 µA. Scores on lower current levels improved during 5 sessions. Response latencies to electric and acoustic stimuli were similar. We conclude that guinea pigs can be trained fast to detect suprathreshold acoustic or electric stimuli using air puffs as an aversive unconditioned stimulus.
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223 Psychophysical Measurement of the Audiogram of the Common Marmoset Using Saccadic Eye Movements

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In recent years the common marmoset has been used in a number of auditory neurophysiology studies. The complex vocal repertoire of this non-human primate species and successful breeding in captivity make the marmoset an attractive and sustainable model of primate auditory processing. However, to date, little is known regarding the perceptual experience and limits of hearing for the marmoset. Here, we report a psychophysically measured audiogram of the common marmoset using saccadic eye movements. All measurements were gathered from the animal while in a semi-restrained setting that is directly transferable to the constraints of electrophysiological recording. In a 2-alternative-forced-choice (2AFC) computer-controlled task the animal was trained to first fixate and then to perform saccadic eye movements in the direction of an auditory stimulus presented to either the left or right side while simultaneously paired with visual targets presented to both sides. Continuous real-time feedback was provided to the animal in the form of a gaze-controlled visual stimulus. Using this method, psychometric curves were gathered for multiple frequencies as a function of sound level that enabled construction of the audiogram. Advantages of saccadic eye movements as opposed to limb movement are that it allows for testing animals in multiple-choice experiments, provides a continuous response measurement, and generates fewer body movements that are detrimental to electrophysiological recordings gathered during behavior. This research was supported by NIH grants DC03180 (X.W.) and DC009688-01 (P.C.).

224 Self-Motion Direction-Detection Thresholds for Whole Body Roll Tilts About an Earth-Horizontal Axis

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Unlike perceptions of linear translation or rotation about an earth-vertical axis that are evoked by a single sensory modality, processing of tilt perception requires multi-sensory convergence. A number of studies of vestibular perception thresholds for single sensory modalities suggest high-pass dynamic characteristics for rotation and linear translation. However, other than static tilt perception threshold measured during slow tilt experiments, little is known about the dynamic characteristics of tilt perception thresholds. To investigate dynamic tilt direction-detection perception thresholds, nine subjects were tested for roll tilt at nine different frequencies ranging from 0.01Hz to 5Hz. Each roll tilt consisted of a single cycle of sinusoidal acceleration. To investigate the importance of rotation axis location, measurements were taken for two different rotation axes, foot-centered and ear-centered at each frequency, using an adaptive two alternative forced-choice staircase procedure. This procedure found a tilt direction threshold that represented the level at which each subject correctly detected tilt direction 79.4% of the time. The resulting mean thresholds for ear-centerred roll tilt are 1.41, 2.48, 1.48, 1.30, 0.80, 0.45, 0.29, 0.13, and 0.04° for 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, and 5Hz, respectively. Statistical analysis did not indicate a significant difference between ear-centered and foot-centered thresholds even at higher frequencies where we expected inter-aural linear acceleration for foot-centered roll tilt to be suprathreshold and, hence, possibly reduce the measured thresholds. Our data suggest that thresholds at higher frequencies are predominantly determined by canal cues. On the other hand, thresholds at lower frequencies were significantly reduced relative to pure rotation thresholds, indicating that these thresholds are mainly determined by graviceptors, probably primarily otolithic in nature but proprioceptors may also contribute.

225 Three NIDCD Workshops: Clinical Trials, Training and Career Development, New Investigators

[1]*NIDCD*

This year's NIDCD workshop will have three concurrent sessions, each targeted to a specific audience. Session 1 is specifically targeted to individuals interested in Training and Career Development, Session 2 is targeted to New Investigators and Session 3 is targeted to both new and seasoned investigators with interests in Clinical Trials. All sessions will allow for ample question and answer time with the attendees.

"Training and Career Development" will include a profile of research training and career development mechanisms appropriate for graduate students, postdoctoral fellows and new and budding clinician-investigators, including mechanisms for mentored research career development and transition (K08, K23 & K99/R00) and the individual fellowship awards (F30, F31 & F32). In addition, the NIH Loan Repayment Program will be presented. Drs. Dan Sklare and Melissa Stick will lead the presentation.

"New Investigators" will provide practical information on how the NIH/NIDCD works (e.g., institute and study section assignments, scientific review groups, council activities, funding paylines and the role of program and review staff). Specific guidance will be given on the appropriate grant mechanisms for early career stages and transitioning to independence, including the NIDCD Small Grant Award (R03). The ultimate aim of the workshop is to provide information for a successful transition from new investigator to independent investigator (R01) status. Drs. Amy Donahue and Christine Livingston will lead the presentation.

"Clinical Trials" will provide information on the NIDCD clinical trial program. The NIDCD has recently published three funding initiatives for clinical trial development and funding. This session will provide an overview of the new CT process and will provide information on issues pertaining to the design and conduct of clinical trial research. Drs. Gordon Hughes and Richard Mowery will lead the presentation.

226 Introduction to Speech, Language and Communication Disorders

Charles Limb[1]

[1]*Johns Hopkins Hospital*

Despite the abundance of research on auditory mechanisms, only a small portion of this research deals directly with communication disorders. This discrepancy is striking in light of the motto of the National Institute on Deafness and Other Communication Disorders, which is "improving the lives of people who have communication disorders." This symposium will review recent findings as well as historically significant advances in our understanding of speech and language disorders. Four leaders in these fields will present their work in these areas, in an effort to unify progress in both basic and clinical research. In addition, a striking case of spoken aphasia in an opera singer with preservation of singing ability will be presented. The overall goal of this symposium is to encourage auditory researchers to approach their work from the perspective of communication, the most clinically relevant application of the ability to hear.

227 Brain Networks for Language Production and Comprehension

Allen Braun[1]

[1]*NIDCD*

This talk will demonstrate the ways in which functional neuroimaging methods are used to characterize brain networks that subserve language production and comprehension. A multimodal approach – capitalizing on the combined strengths of electrophysiological (EEG, MEG) and hemodynamic (fMRI, PET) techniques, each possessing unique temporal and spatial characteristics – yields the most comprehensive view of the language networks involved. Language can be viewed from multiple perspectives, from the single word to the discourse level. We will present results from studies of picture naming and narrative production – at the opposite ends of this spectrum – to illustrate the use of these methods in traditional clinical and more ecologically valid contexts. Data will be presented showing how language networks are distributed in the normal brain and how they are altered by neurological disorders in which language is impaired. Application of these methods in specific disorders, and the ways in they can provide information about the pathogenesis of these disorders, will be discussed.

228 A Case Study of Expressive Aphasia in an Opera Singer

Jan Curtis[1]

[1]*New England Conservatory of Music*

In September1995, mezzo-soprano opera singer Jan Curtis suffered a severe stroke which left her with significant expressive aphasia. Prior to her stroke, the New England Conservatory of Music trained musician performed in several operas nationally, including the Houston Grand Opera, Kennedy Center Great Performances on PBS, St. Louis Opera, Atlanta Opera, and Opera Company of Boston, among others. Despite her spoken language aphasia, Ms. Curtis remains able to sing. In June 2000, she performed at the National Aphasia Association conference. Later that year, she received the Annie Glenn Award, given in recognition of outstanding achievement by an individual with a communication disorder. In 2003, she was featured in "Afterwords", a documentary film on aphasia that highlights her remarkable recovery, one that she personally attributes to deep personal faith. During this presentation, Ms. Curtis will describe her experience with aphasia and take questions from the audience. Most notably, she will also perform several music selections. Ms. Curtis provides living evidence of the importance of research in communication disorders and reminds us of how much we have yet to learn.

229 The Past and Present of Human Speech Perception Research

Susan Nittrouer[1]
[1]*Ohio State University Medical Center*

The ways in which we frame our questions in research and approach our clinical practice are shaped to at least some extent by a collective history and biases concerning the nature of human speech communication. This presentation will review that collective history of research into human speech communication, and describe how it influences our current clinical practices. Data from children with hearing loss will be presented that indicate how we must shift our current theoretical perspectives. Finally, ways in which these new findings might impact clinical intervention for children with hearing loss will be discussed.

230 Common Information for Auditory and Visual Speech Perception

Lawrence Rosenblum[1]
[1]*University of California, Riverside*

The clinical approach to audiovisual speech perception is largely based on the idea that the speech information available to hearing is different from the information available to seeing (lipreading). This approach relies on low-level featural descriptions of phonetic structure. We will present evidence showing that speech perceivers extract the same informational form from both light and sound. These results are consistent with the theoretical perspective that speech perception is a sensorimotor activity that recovers amodal speech articulation.

231 The "Motor Theory" of Speech Perception

Carol Fowler[1,2]
[1]*Haskins Laboratories,* [2]*University of Connecticut*

The highly controversial "motor theory" of speech perception has been proposed toexplain findings that listeners' perceptions of speech appear to track articulation moreclosely than they track the acoustic signal. There are two major claims associated withthe motor theory: that listeners perceive speech gestures, not acoustic speech signals, andthat they do so by involving their own speech motor systems during speech perception.

Evidence will be presented in support of the first claim, counterintuitive though it maybe. Although there are ways that listeners might achieve gestural speech percepts otherthan through speech motor systems, remarkable evidence in support of the second claim will be presented as well. Furthermore, we will review some of this evidence in the context of much more general findings that perception (not only for speech but arguably all forms of complex sensory perception) generally involves "embodiment."

232 Impaired Neuron-Glia Cross-Talk in a Transgenic (SOD1) Mouse Model for Amyotrophic Lateral Sclerosis (ALS) - Role of Glial Ion Channels and Glia Driven Inflammation

Clemens Neusch[1], Frank Kirchhoff[2], Heinz Steffens[1], Payam Dibaj[1]
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ALS is a devastating late-onset neurological disease with progressive upper and lower motor neuron degeneration. The underlying pathogenesis is complex and involves loss of crucial glial and neuronal membrane proteins that are needed for perineural homeostasis. Futhermore, increased CNS inflammation mainly driven by microglial cells contributes to motor neuron death and disease progression. Here, we present an overview that focus on impaired cross-talk between motor neurons and glial cells in the spinal cord of ALS mice. First, we show data that is derived (a) from a potassium channel (Kir4.1) knock-out mouse to understand the multiple physiological functions of Kir4.1 channels in regulating e. g. K+ homoeostasis, cell swelling and neuronal cell survival in the spinal cord. We, then (b) used transgenic mice expressing a superoxide dismutase mutation as model for ALS to investigate potential implications of Kir4.1 channels in disease progression. Progressive loss of perineural Kir4.1 channels indicate a role in the pathogenesis in this mouse model by inducing K+ excitotoxicity. In a second part we address the interaction of microglial cells with motor neurons in a time-lapse imaging approach using 2-P-LSM in vivo. Taken together, we will discuss (a) the impact of impaired perineural K+ homeostasis and its contribution to motor neuron degeneration by K+ excitotoxicity and (b) the role of inflammation in a mouse model for a classical neurodegenerative disease.

233 Exploring RNAi to Treat Deafness Using Mouse Models of Hearing Loss

Richard Smith[1]
[1]*University of Iowa*

Allele-specific gene suppression by RNA interference (RNAi) is an attractive strategy to prevent hearing loss that results as the dominant-negative consequence of expression of a mutant protein. In a proof-of-principle study, we have shown that potent *GJB2*-targeting short interfering RNA (siRNA) can be used to silence expression of an R75W allele variant of *GJB2* that has been introduced into the murine cochlea through the round window membrane. Selective suppression of $GJB2_{R75W}$ expression by more than 70% of control levels prevents hearing loss in these mice without affecting endogenous murine *Gjb2* expression.

Building on this work, we have studied the delivery of therapeutic agents directly into the otocyst of the E12.5 mouse embryo via trans-uterine microinjection. Highly efficient adenoviral and adeno-associated viral transduction of progenitor supporting and hairs cells, respectively, is observed. The delivery of these viral

vectors during inner ear development does not lead to ototoxic effects during adulthood. These vectors provide the potential to deliver siRNAs or micro RNAs to the developing inner ear to prevent progressive hearing loss. We are exploring these possibilities using mouse models of DFNA2 and DFNA3, and intend to expand their application to gene delivery in dosage models of recessive deafness.
(Supported in part by NIDCD grants DC02842 and DC03544 to RS)

234 Mechanisms of Deafness in Pendred Syndrome

Philine Wangemann[1]
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Pendred syndrome is the most frequent syndromic form of hereditary hearing loss in children. The syndrome was first described by Vaughn Pendred in 1896. Nearly 100 years later the underlying genetic defect was discovered and the first murine model was published in 2001. Pendrin, the protein encoded by the defective gene (*Slc26a4*) is expressed in non-sensory cells of the cochlear lateral wall and contributes indirectly but profoundly to the sensory function of the cochlea. Work performed in the murine model has revealed three synergistic pathways that contribute to the failure to develop hearing. First, loss of pendrin leads to a prenatal enlargement of scala media, which persists postnatally and requires stria vascularis to secrete K^+ at an elevated rate in order to maintain a normal endolymphatic K^+ concentration. Enhanced rates of K^+ secretion cause oxidative and nitrative stress, which lead to the loss of protein expression of the K^+ channel *Kcnj10*, which generates the endocochlear potential. Loss of the endocochlear potential eliminates the driving force necessary for sensory transduction and hearing. Oxidative stress in stria vascularis leads to degeneration followed by an invasion of macrosphages. Second, loss of pendrin leads to a loss of HCO_3^- secretion and an acidification of endolymph, which, in conjunction with the lacking endocochlear potential, inhibits transepithelial Ca^{2+} absorption via cellular and paracellular pathways. Elevated Ca^{2+} concentrations in endolymph block sensory transduction and may contribute to Ca^{2+} overload and the observed loss of stereocilia in the sensory cells. Third, there are several lines of evidence that point to cochlear hypothyroidism, which leads to a failure of the sensory cells to mature postnatally. Taken together, the Pendred syndrome mouse model has played a tremendous role in delineating the multifaceted mechanisms that contribute to hearing loss in Pendred syndrome. Supported by NIH-R01-DC01098.

235 From Muscle to the Inner Ear: Cytoplasmic Actins in Cellular Function and Disease

James Ervasti[1]
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At first glance, skeletal muscle fibers and cochlear hair cells couldn't be more different in structure and function. On the other hand, both cell types generate force and experience mechanical stress during cycles of shortening and lengthening. We are exploring the role of cytoplasmic actins in muscle and the inner ear by generating actin-isoform specific knockout mice. In muscle, cytoplasmic γ-actin comprises just 1/4000th of the total cellular actin and localizes specifically to costameres, structural links which connect the contractile apparatus to the plasma membrane. In contrast, cytoplasmic γ-actin is the predominant actin isoform and more widely distributed in hair cells, localizing to the lateral wall, cuticular plate and to stereocilia. Muscle-specific ablation of γ-actin results in a progressive myopathy characterized by age-dependent decrease in force generation and increased cell death. Consistent with this age-dependent phenotype, loss of γ-actin in the inner ear results in progressive hearing loss with a concomitant deterioration of outer hair cell stereocilia. Taken together, our data suggest that γ-actin plays an important role in the long-term stability of two different mechanically-challenged cell types.

236 *LRTOMT* Is a Fusion Gene with Two Alternative Reading Frames That Is Essential for Auditory Function

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Many proteins necessary for sound transduction have been discovered through positional cloning of genes that cause deafness. We mapped *DFNB63*, a novel locus for nonsyndromic deafness, to a 4.8 cM interval on human chromosome 11q13.3-q13.4 in families from Pakistan, Turkey and Tunisia segregating deafness as an autosomal recessive trait (Khan et al., 2007; Kalay et al., 2007; Tlili et al., 2007). In this study, we report that mutations of *LRTOMT* are associated with profound non-syndromic hearing loss at the *DFNB63* locus. *LRTOMT* has two alternative reading frames and encodes two entirely different proteins, LRTOMT1 and LRTOMT2, that we detected by Western blot analyses. LRTOMT2 has a predicted methyltransferase domain. During evolution, novel transcripts can arise through partial or complete coalescence of genes. We provide evidence that in the primate lineage LRTOMT evolved from the fusion of two neighboring ancestral genes, which exist as separate genes (*Lrrc51* and *Tomt*) in rodents. RT-PCR analysis of 17 different human and mouse tissues revealed a wide pattern of expression of *LRTOMT*, *Lrrc51* and *Tomt*. *In situ* analysis using a probe designed from the unique 3'UTR of *Tomt* showed expression specifically in the

region of the sensory cells of the cochlea, utricle, saccule and crista ampullaris. To determine the cellular and subcellular localization of LRRC51 and TOMT, we performed immunofluorescence confocal microscopy on mouse inner ear. LRRC51 and TOMT immuno-reactivities were detected in the cytoplasm of vestibular hair cells and supporting cells as well as in inner (IHC) and outer hair cells (OHCs) of the organ of Corti. We have generated a knockout mouse model of Tomt and functional analyses of the inner ear of this mouse will be presented.

237 Mechanisms of Deafness Caused by Connexin26 and Connexinn30 Mutations Studied in Mouse Models

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Connexins (Cxs) are protein subunits of gap junctions (GJs), which facilitate intercellular communication. Mutations in Cx26 and Cx30 cause a significant portion (20-50%) of prelingual non-syndromic deafness cases in humans. Molecular mechanisms of deafness caused by Cx mutations, however, are currently unclear.

To study deafness mechanisms caused by Cx26 mutations and to circumvent the embryonic lethality in Gjb2-/- mice, we generated conditional Cx26 (cCx26) null mice by significantly reducing Cx26 expression in either a spatially-specific or time-specific manner through genetic approaches. The cCx26 null mouse models were validated by a multidisciplinary approach and confirmed by profound non-syndromic deafness shown by the animals. Although the gross histological features of the cochlea appeared to be normal, the development of the organ of Corti apparently stopped at around postnatal day 3 (P3) as the tunnel of Corti was nerve opened in cCx26 null mice. Cell degeneration was initially observed at around P14, and all types of cells in the middle turn of the cochlea were completely lost by P30. The cell death gradually spread to the basal turn, while cells in the apical turn were largely spared. Correspondingly, spiral ganglion neurons in the middle and basal turns were substantially degenerated. These findings are in sharp contrast to the much slower time course and milder form of cell degeneration observed in the cochlea of Cx30 null mice. Using an inducible Cx26 null mouse model, we observed that the deafness phenotype was readily induced if the Cx26 expression in the organ of Corti was substantially reduced before P4. Reducing Cx26 expression after P4, however, generated a phenotype more consistent with an early-onset age-dependent hearing loss. These results indicated that Cx26 play essential roles in the development of the organ of Corti, and revealed that the deafness mechanisms caused by null mutations of Cx26 and Cx30 are likely to be different.

238 Many Tales from the Mouse: The Heterogeneity of Phenotypes in Mouse Models and Implications for Human Deafness

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Mutant mice have been instrumental in elucidating the function and mechanisms of the inner ear. The early 1900s brought recognition of the mouse as a model for human disease, and interest in these animals shifted from mouse fanciers to the laboratory. As early as 1928, spontaneous and radiation-induced mutants were discovered to have hearing and vestibular defects. Most remarkable, there is a wide variety of chemical, radiation and spontaneous mouse mutants, supplemented in the last two decades by transgenic, knock-out and conditional mutants, that represent the many forms of human deafness. Examples from our laboratory will demonstrate mouse models for non-syndromic and syndromic hearing loss, as well mice with loss of microRNAs. Each one of these mouse mutants enables us to learn more about the mechanisms of specific mutations, providing key reference points for auditory and vestibular pathology in humans.

239 Multigenic Inheritance of Progressive Early- And Late-Onset Hearing Loss in Mice and Its Relevance to Human Presbycusis

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Progressive hearing loss associated with aging is a common condition afflicting approximately one third of the elderly population in the US. The late onset and confounding environmental influences make genetic studies of human presbycusis difficult. However, many common inbred strains and heterogeneous stocks present with considerable genetic and phenotypic diversity with respect to onset, progression and degree of hearing loss and exhibit cochlear pathologies similar to those described for human presbycusis. In fact, forty out of eighty-five strains of mice, representing the phylogenetic spectrum of laboratory mice, develop early- or late-onset hearing impairment. Initial genetic studies showed that a single-nucleotide exchange in cadherin 23 (Cdh23ahl) is a common variant and confers a substantial risk of late-onset hearing loss in the C57BL/6J strain with a second minor-contributing locus being ahl3 on chromosome 17. The Cdh23ahl variant also acts as sensitizing allele and interacts epistatically with rare and strain-specific alleles (ahl2, ahl4, and ahl8) to control early-onset hearing impairment. Other gene-gene interactions (Phl1-Phl2, ahl5-ahl6) underlie progressive hearing loss in the 101H and Black Swiss strains, respectively. From these linkage studies it appears that hearing loss across laboratory strains of mice is highly heterogeneous and that hearing loss within a strain is due to a small number of loci that act in epistatic or additive manner.

The monogenetic cause of hearing loss is well conserved between human and mouse suggesting that the age-

dependent sensorineural hearing impairment in mouse strains may also provide insights into the genetic predisposition underlying human presbycusis. The mouse studies suggest that the genetic risk for presbycusis is carried by a small number of interacting alleles, which may mostly represent hypomorphic variants of the more severe, rare, and highly penetrant deafness alleles.

240 Perception Different of Synthetic Vowels by Formant Change for Cochlear Implanted Children

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The purpose of this study was to examine the acoustic perception different by formants change for profoundly hearing impaired children with cochlear implants. The subjects were 10 children after 15 months of experience with the implant and their mean was 8.4 years and Standard deviation was 2.9 years. The ability of auditory perception was assessed using acoustic-synthetic vowels. The acoustic-synthetic vowel was combined with F1, F2, and F3 into a vowel and produced 42 synthetic sound, using Speech GUI(Graphic User Interface) program. The data was deal with clustering analysis and on-line analytical processing for perception ability of acoustic synthetic vowel. The results showed that auditory perception scores of acoustic-synthetic vowels for cochlear implanted children was increased more F2 synthetic vowels than these of F1. And it was found that they were perceptive of different vowel in terms of distance rates between F1 and F2.

241 Why Do Hearing-Impaired Listeners Fail to Benefit from Masker Fluctuations?

Joshua Bernstein[1]
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Hearing-impaired (HI) listeners do not receive as much benefit to speech intelligibility from fluctuating maskers, relative to stationary noise, as normal-hearing (NH) listeners. In an attempt to explain this lack of fluctuating masker benefit (FMB), investigators have focused on reduced audibility; deficits in spectral or temporal resolution; or limited cues for target-source separation as possible underlying causes. We present perceptual data suggesting that differences in the signal-to-noise ratio (SNR) at which HI and NH listeners are tested may account for FMB differences, and that the magnitude of the FMB is similar between the two groups when these differences are controlled for. The relationship between SNR and the FMB is predicted by an existing model of speech intelligibility in fluctuating noise, modified by adjusting the assumed shape of the distribution of speech information across the dynamic range. The predicted magnitude of the FMB results from an interaction between this distribution and offsetting effects of masking and unmasking by peaks and valleys in a fluctuating masker. The model is applied to a variety of situations described in the literature where reduced FMB has been reported, including HI listeners and NH listeners presented with speech processed to simulate aspects of hearing loss or cochlear implantation. To the extent that SNR differences can explain these results, the impact of suprathreshold distortions on the ability to "listen in the gaps" of a fluctuating masker may be much less significant than previously thought. [Supported by the Oticon Foundation].

242 The Effects of Pitch and Spatial Cues on Diotic Listening

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The cocktail party effect describes the ability to focus one's attention on one talker when there are competing conversations. In this situation, depending on the position of the target and masking talkers, the head can cast an acoustic shadow giving one ear a higher target-to-masker energy ratio than the other ear. The current study investigates the relative contributions of binaural cues versus "better-ear" acoustic cues on understanding the target. Subjects identified a monotonized five-digit number string in the presence of a monotonized masking sentence. The target and masker either shared the same pitch of 100 Hz, or the masker differed in pitch by ± three semitones (84 Hz or 119 Hz). Anechoic and reverberant settings were simulated using KEMAR-derived binaural room impulse responses. The target was simulated at 0° azimuth, while the masker was from either 0° or +90°. In one condition, the signal at the better ear was presented diotically to isolate the contribution of better-ear listening. Results suggest that the better-ear target-to-masker energy ratio explains performance when segregation and selection of the target is easy. This result contrasts directly with results from many previous anechoic studies in which target and masker were more confusable, and spatial differences in target and masker improved performance beyond better-ear acoustic effects. When segregation of the target was more challenging due to reverberation, performance was once again better for spatial simulations than for the corresponding better-ear diotic presentations. These findings suggest that 1) when segregation and selection of the target are relatively easy, spatial cues contribute little to performance; and 2) in the presence of reverberation, segregation of the target becomes difficult, and spatial cues improve target speech intelligibility.

243 Spectral Integration and Bandwidth Effects on Speech Recognition in School-Aged Children and Adults

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Previous studies have shown that adult listeners are more adept at identifying spectrally-degraded speech than are child listeners. However, the development of the ability to combine speech information from different frequency regions, an issue that may have implications for benefit derived from cochlear implants, has not been well established. The present study had two aims. One was to determine the effect of age on the speech bandwidth

necessary to achieve a relatively low criterion level of speech recognition for bands centered on either 500 or 2500 Hz. The second aim was to determine the improvement in speech recognition that resulted when both speech bands were present simultaneously. Normal-hearing listeners in three age groups (children aged 5-7, children aged 8-11, and adults) were selected for participation. In the first stage of testing, BKB sentences were bandpass-filtered around either 500 or 2500 Hz, and the bandwidth of that filter was varied adaptively to determine the width required for approximately 15-25% correct recognition. In the second stage of testing, these criterion bandwidths were presented in fixed block trials, either separately or together, and percent correct performance was determined. The order of presentation was randomized in both parts of the study to control for possible learning effects. Preliminary results suggest that age is inversely proportional to the bandwidth required to achieve a relatively low criterion level of speech recognition for speech bands centered at either 500 Hz or 2500 Hz. However, both adults and children show a large improvement in performance when both bands are presented simultaneously. These preliminary results imply that, although younger children appear to require more bandwidth to correctly recognize speech filtered around a single frequency, they may be relatively adept at integrating multiple narrowband signals to recognize a composite stimulus.

244 Global Spectra Matching-Based Acoustic Analysis for Understanding and Modeling Perceptual Adaptation to Spectrally-Shifted Vowels

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Human listeners are able to adapt to spectrally-shifted speech with or without explicit training. The degree of spectral mismatch is a critical factor that limits the degree and time course of adaptation. One of hypotheses to explain these observations is that within- and across-vowel acoustic variability in the global spectra might be the limiting factor determining speech performance for spectrally-shifted speech. For example, vowels with relatively low formants may overlap with vowels with relatively high formants. The present study proposes an acoustic analysis framework with which to analyze global spectra matching-based speech recognition performance for unprocessed and spectrally-shifted/-degraded vowels. The framework is a classic template-matching framework, composed of five major modules: feature representation, frequency-warping, metric calculation, template, and post-analysis. The frequency-warping module was added to account for spectral shift and warping invariance. The analysis results from unprocessed vowels indicates that, while the neural system may use global spectra information to identify vowels, invariance to spectral warping and shift may be limited due to within- and across-vowel acoustic variability in the global spectra. The analysis results from spectrally-shifted and/or -degraded vowels (five spectrally-shifted/-degraded conditions and one spectrally-matched/-degraded condition) suggests that there is a limited tolerance range for spectral shifting (450 Hz) that might explain and model speech performance with spectrally-shifted/-degraded vowels. Interestingly, this range is close to the higher-level spectral integration range (~3.5 Barks). By setting two parameters (spectral shifting range and spectral warping range), the proposed model was able to predict both pre- and post-adaptation vowel performance for both spectrally-matched and –shifted speech.

245 Effect of Local Time Reversals on Consonant Identification

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Speech perception depends on both temporal and spectral cues distributed broadly over many frequency channels. The relative importance of spectral and temporal domains for speech perception can be estimated by measuring speech recognition while disrupting cues in one domain and leaving the other mostly intact. One such method is to locally time reverse the speech waveform, which disrupts the temporal information in speech without altering spectral information.

Past studies have shown that word recognition scores are reduced when the speech signal is locally time-reversed (Saberi and Perrott, 1999; Greenberg and Arai, 2001). In this study we measured the effect of local time reversals on consonant identification. Eighteen /aCa/ syllables, spoken by a female talker, were presented to the listeners with time reversals of durations T = 0 (original), 20, 40, 60, 80, 100, 120, 140 and 160 ms. Both auditory and auditory-visual test conditions were included. Preliminary results show that consonant recognition, with scores above 70% at T = 160 ms, is more robust to time-reversals than word recognition. Average consonant scores correlate qualitatively with the primary peak of the complex modulation spectrum (Greenberg and Arai, 2001). However, not all consonants were equally affected by time-reversals. Sibilance and Nasality were not affected by time-reversals, suggesting a primarily spectral nature of these cues. Voice-onset time, which is a primary cue for voicing and which critically depends on timing of the articulatory release and the start of formant transition, was severely affected by time-reversals, resulting in large voicing errors. As expected, visual input increased the received place-of-articulation and manner-of-articulation information, but did not improve voicing information.

246 Phase and the Human Subcortical Representation of Stop Consonants

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The formant frequencies that differentiate stop consonant syllables (e.g. /ga/, /da/, /ba/) are above the phase locking limits of the brainstem (>1000 Hz). In the human auditory brainstem response (ABR), these frequency differences are represented by latency differences with /ga/ responses occurring first, followed by /da/ and then /ba/ (i.e. higher frequencies yield earlier latencies than lower frequencies)

(Johnson et al., Clin Neurophysiol, 2008). In the speech-ABR, this latency pattern (/ga/, /da/, /ba/) is most apparent at four discrete response peaks. Here, we analyzed the speech-ABR to stop consonants using a short-time phase coherence technique. The goal was to develop a new ABR analysis technique (the 'phaseogram'), which would allow us to establish (1) whether time-varying frequency differences manifest as time-varying phase differences and (2) the relationship between response timing and phase. Response phaseograms from 22 typically developing children revealed stark phase differences for frequencies below 1000 Hz. This result is intriguing given that stimulus phase differences are not apparent in this range, and occur only above the phase-locking limits of the brainstem. The mismatch between input and output hints at the existence of distortion products and suggest that subcortical structures transpose stimulus features (phase and/or magnitude) to lower frequency regions that are within the phase-locking limits of the system. This idea is grounded by previous work in the cat inferior colliculus showing that lower frequency neurons are sensitive to the phase-relationships of higher frequencies (McAnnally et al., Hearing Research, 1990). Discussion of our results will include an overview of phase and its role in speech perception, as well as the possible clinical application of our outcomes.

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247 Low-Frequency Phase Coherence in Near- And Far-Field Midbrain Responses Reveals High-Frequency Formant Structure in Consonant-Vowel Syllables

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Stimuli differing in frequencies above the phaselocking capabilities of the brainstem elicit timing differences in human brainstem evoked responses (Johnson et al. *Clin Neurophysiol.* 2008). Specifically, 'ga,' which has a relatively higher second formant (F_2) frequency, evokes earlier peak latencies than 'da' and 'ba' which have successively lower F_2 frequencies. To better understand the mechanisms underlying this phenomenon, we measured near- and far-field recordings to the same stimuli from guinea pig inferior colliculus (IC) and epidural midline (vertex). Results from the vertex of the animal model showed the same pattern seen in humans in response to the consonant-vowel stimuli. To more closely determine the composition of the response that led to the latency shifts, a short-time phase-coherence technique was applied. It was found that the stimuli, which are nearly identical in their phase response below 1000 Hz, evoked far-field responses that were drastically different in their phases at frequencies as low as 100 Hz. Near-field responses from IC were analyzed to explore the relationship between activity from localized neuronal ensembles and the population responses measured at the vertex. The implications of these results on central auditory processing of distortion products will be discussed. Supported by NIH.

248 Subcortical Differentiation of Speech Sounds Is Related to Reading and Perception of Speech in Noise

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The auditory brainstem response to speech has been shown to differ between children with learning impairments and those who are normal learning. Differences are found for transient and harmonic elements of speech, but not pitch (fundamental frequency)[1,2]. The encoding of transients and harmonics, which are used to differentiate phonemes, has been found to be correlated with reading ability[3,4]. In the current study, /ba/, /da/, and /ga/ were presented to children with a wide range of reading ability. The formant frequencies that differentiate these stop consonant syllables are above the phase locking limits of the brainstem (i.e. >1000 Hz). In normal learning children, these frequency differences are represented in the brainstem response as latency differences with /ga/ responses occurring first, followed by /da/ and then /ba/ (i.e. highest formant frequencies to lowest)[5]. In the present study, the presence of this latency pattern (/ga/ < /da/ < /ba/) was found to be positively correlated with phonological awareness and reading ability. Pattern presence was also positively correlated with perception of speech in noise. Moreover, the average magnitude of latency differences was also positively correlated with phonological awareness, silent word reading, speech in noise perception, as well as oral word reading. Thus, it appears that phonological awareness skill, thought to be the most pervasive deficit in children with reading disorders[6,7], is related to subcortical differentiation of speech sounds.

1. Johnson et al., Ear and Hearing, 2005
2. Wible et al., Biological Psychology, 2004
3. Banai et al., J Neurosci, 2005
4. Banai et al., International Conference on Cognitive Neuroscience, 2008
5. Johnson et al., Clin Neurophysiol, in press
6. Szenkovits & Ramus, Dyslexia, 2005
7. Frost et al., Dyslexia, 2005

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249 Stimulus Context Modulates Brainstem Response to Speech in Humans

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Neuronal responses in the auditory cortex are known to be sensitive to the context in which the stimulus occurs. Here, we demonstrate that the human auditory brainstem is also modulated by stimulus context. We examined scalp-recorded auditory brainstem responses to the speech sound /da/ recorded from sixteen normal hearing children under two conditions. In one condition /da/ occurred with a 100% probability (repetitive context); in

another condition (variable context) /da/ was randomly presented in the context of seven other stimuli varying in a number of acoustic features. Spectral analyses of event-matched responses from the two conditions revealed enhanced harmonic and formant representation of /da/ in the repetitive context relative to the variable context. The dynamic nature of the brainstem, as revealed by the difference between responses from the two conditions, positively correlates with speech perception in noise. Thus, repetition appears to induce functionally relevant enhancement in the brainstem responses. Consistent with cortical models that predict sharpening of neuronal responses as a consequence of stimulus repetition, these results are the first to document context-dependent modulation in the human auditory brainstem.

250 FMRI Evidence That Illusory Continuity of Vowels Does Not Depend on Attentional State

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We exploited auditory perceptual organization and the continuity illusion to create illusory vowel sounds. We use fMRI to examine a) the neural correlates of the perception of such illusory vowels, and b) whether the neural correlates are modulated by attentional state. As we have shown previously, when two formants of a synthetic vowel are presented in an alternating pattern, and the gaps in each formant are filled with bursts of noise, the formants are heard as continuous and more vowel-like. When the stimuli of this "Illusion" condition are modified by increasing the formant-to-noise ratio so that the noise no longer plausibly masks the formants, the formants are heard as interrupted ("Illusion Break" condition) and less vowel-like. Replicating our published study, BOLD signal in a region of the left middle temporal gyrus (MTG) was greater for Illusion than for Illusion-Break stimuli. Since this region was also more active for full synthetic vowels than for Illusion-Break stimuli, these differences probably reflect differences in speechlikeness. We manipulated attentional state factorially, by examining BOLD signal for our different sound types under three attentional conditions; full attention to these sounds; attention to a demanding visual distracter task; or attention to a demanding auditory distracter task (on stimuli in a different frequency region to that occupied by the vowel stimuli). Crucially, although a robust main effect of attentional state was observed in many regions, this factor did not modulate signal change in the left MTG illusory-vowel-sensitive region for illusory vowels compared to illusion-break stimuli. This result suggests that illusory continuity of vowels is an obligatory perceptual process, and operates independently of attentional state.

251 Medicine and Science Are Filled with Serious Topics

Joe Palca[1]
[1]NPR

Medicine and science are filled with serious topics. Molecular biology, physics, impacted cerumen. These are nothing to giggle about. And yet... To communicate with the public, it sometimes helps to inject a note of humor into a difficult subject. I will review the use of humor in making a complicated scientific or medical story go from spinach journalism (you should listen because it's good for you) to something that can be both informative and fun

252 What You Need to Know About How Journalists Decide What Stories to Cover

Sandra Blakeslee[1]
[1]New York Times

What you need to know about how journalists decide what stories to cover. How can you make findings on otolaryngology interesting? Hint: think about how much people want to hear clearly, keep their balance and swallow.

253 Sound-Evoked Vibrations in the Basilar Membrane and Reticular Lamina of Living Guinea Pigs Using Optical Coherence Tomography

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[1]Oregon Health & Science University, [2]University of Michigan

In mammals, the cochlear sensitivity depends on the function of an active mechanism termed "cochlear amplification" (CA) in the organ of Corti, in which the outer hair cells (OHCs) generate mechanical forces to enhance the basilar membrane (BM) vibration.CA causes the sharp tuning and exquisite sensitivity of hearing. In such a unique mechanically- physiologically coupled system with active mechanical force generation, knowledge of the differential motion of the key components within the organ of Corti is of crucial importance in understanding cochlear amplification. Using a newly developed optical coherence tomography (OCT) system and homodyne interferometry, we observed sound-induced differential motions of two important surfaces, the BM and reticular laminar (RL), in the sensitive cochlea of living guinea pigs. We found that at the same radial location, the vibrations of BM and RL exhibit similar sharp tuning and sound-level dependent magnitude compression, which are features of mechanical responses in the sensitive cochlea. Differential motion between the BM and RL was observed, as shown by differences in magnitude and phase. We observed that the RL has both a higher level of vibration and a relative phase lead compared to the BM. These data represent the first *in vivo* measurements of the micromechanical motions of the organ of Corti. The data that support the CA mechanism involves a phase shift for the production of outer hair cell force such that vibration generated viscous energy losses are compensated.

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254 Lateral Micromanipulation of the Gerbil Basilar Membrane

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The stiffness and longitudinal coupling within the basilar membrane help shape the frequency tuning of the cochlea. For example, Naidu and Mountain (2001) proposed that longitudinal coupling in the basilar membrane could act to broaden the peak of the cochlear frequency response. In order to better characterize these mechanical properties, we made measurements of lateral point stiffness and longitudinal coupling in excised gerbil cochleae. An integrated optical imaging system and basilar membrane manipulator was devised in which a calibrated glass micropipette is used to displace the basilar membrane laterally. Because the tissue deformations occur in the optical plane of the microscope objective, detailed images of the movement of the micropipette tip and the basilar membrane microstructure were obtained. The tip motion was measured between sequential image pairs by two-dimensional cross-correlation, and these data were used to determine the force applied to the tissue. The lateral point stiffness in the arcuate zone is 0.2–0.5 N/m, in the pectinate zone is 2–3 N/m, and increases to over 10 N/m near the spiral ligament. These results suggest that the arcuate and pectiante zones have distinct material properties, which correspond to histologically observed differences in tissue morphology.

To compute the longitudinal coupling, the displacement field in the tissue surrounding the probe was measured quantitatively using image registration. The tissue in compression has a space constant of 7.6 μm, and the tissue in tension has a longer space constant of 10.5 μm. These space constant values are sufficiently large that they may impact cochlear tuning.

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255 Effects of Inner Hair Cell Bundle Structure on Mechanotransduction

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Recent experiments involving deflection of outer hair cell (OHC) bundles in shaker 2 mice with a mutation that produces abnormally short stereocilia show similar mechanotransduction responses as the control group with normal bundle structure [1]. Although inner hair cells (IHCs) of the shaker 2 mutants have intact stereocilia links they run perpendicular to stereocilia axes in the short bundle rather than obliquely as seen in tip links of normal hair bundles. Electrical recordings and optical measurements indicate that this peculiar structure of the mutant hair bundle does not influence the amplitude and the speed of mechanotransduction responses in shaker 2 IHCs [2].

IHC stereocilia deflection is traditionally thought to be the result of its interaction with the surrounding oscillating, endolymphatic flow. Using our hydrodynamic interaction model, we study an IHC bundle that has the short, uniform structure seen in the mutant shaker 2 mice and compare its deflection and tip-link stretching to that of an IHC bundle with a normal structure. We present results from the model that show the effect of bundle structure and tip link organization on the deflection and subsequent mechanotransduction of a normal and shaker 2 IHC stereocilia bundle. Specifically, we use the model-calculated deflection for each bundle in response to an imposed stimulus to deduce the effect of bundle structure on its stiffness.

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256 OHC Somatic Electromechanical Force Coupled Directly to the IHC Stereocilia

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Nowotny and Gummer (2006) have recently shown that for stimulus frequencies less than about 3 kHz, the somatic electromotility of the outer hair cells (OHCs) leads to anti-phasic motion of the reticular lamina (RL) and tectorial membrane (TM) in the region of the inner hair cells (IHCs). This motion is predicted to cause displacement of the fluid in the subtectorial space, the radial component of which is believed to deflect the IHC stereocilia. Here, the aim is to test this prediction experimentally.

We measured vibration in response to intracochlear electrical stimulation in a two-chamber in-vitro preparation of the guinea-pig cochlea in the 800-Hz region. The upper and lower chambers contained artificial endolymph and perilymph, respectively, with calcium concentrations of 30 μM and 1.3 mM. Vibration of the IHC stereocilia and RL were made in the radial and transversal directions and of the lower surface of the TM in the transversal direction. Transversal and radial motions were measured, respectively, with a laser Doppler vibrometer (Nowotny and Gummer, 2006) and fast line camera (12 frequency multi-tone stimulus from 30 Hz to 4 kHz, background noise less than 1 nm, 10-s averaging time). A complete set of measurements required typically 20 min.

The present results show that i) radial displacement of the IHC stereocilia relative to transversal displacement of the OHC RL is first-order high-pass with cut-off frequency near 800 Hz and gain of up to 5 dB, and ii) OHC contraction causes IHC stereociliary deflection in the excitatory direction. Responses were attenuated by up to 20 dB by 9-AC (1 mM), a somatic electromotility blocker, but not affected by DHSM (500 μM), a blocker of open mechanoelectrical channels.

In summary, in addition to high-frequency tuned amplification, there exists a second amplifying process in the cochlea, operating below about 3 kHz, which positively couples somatic electromechanical force from the OHC directly to the IHC stereocilia.

257 Results of as Study of Differences in Responses Depending on Cochlear Nonlinearities

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A time-domain cochlea model appears to be a good tool to analyze predictions of cochlea models using different assumptions about the nonlinear behavior(s). We have been working with time-domain cochlea models for more than 20 years now, and with a simple graphical user interface it is a reliable tool to predict the dependency of responses on different assumptions about (so far global) parameters of the cochlear partition. For the time being we focus on onset response behavior of evoked OAEs, using the PTPV (primary tone phase variation) method proposed by Whitehead et al. (2000). Some results for a single set of NL-parameters and a 2-tone stimulus with frequencies 1 kHz and 1.2 kHz at different levels have been shown before (Duifhuis, 2006). These data were obtained for a sinh shaped damping nonlinearity, which provides a logarithmic output-input relation over a wide dynamic range.

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258 Joining the TWAMP and Sandwich Models of the Cochlea

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A hybrid cochlear model is formed by joining the Sandwich [E. deBoer "Wave-propagation modes and boundary conditions for the Ulfendahl-Flock-Khanna preparation", *The Mechanics and Biophysics of Hearing Proceedings*. P. Dallos, ed. Berlin, Springer-Verlag: 333-339 (1990)] and the TWAMP [A. Hubbard "A traveling-wave amplifier model of the cochlea ", *Science*, V259, pp.68-71 (1993)]. The TWAMP model postulated the existence of a *lossy, slow* transmission line, actively coupled to a "classical" basilar membrane (BM) model. But nothing in the anatomy seemed to be another line that was actively coupled to the BM. Thus, the TWAMP was abandoned. Recently, however, Ghaffari *et al*. showed that the tectorial membrane (TM) has characteristics of a lossy, slow transmission line [R. Ghaffari, A. Arayosi, and D. Freeman "Longitudinally propagating traveling waves of the mammalian tectorial membrane", *PNAS*, V104, No42, pp. 16510-16515 (2007)]. Its velocities are close to those used in the TWAMP. It is now proposed that the classical line in the TWAMP is the reticular lamina (RL), not the BM. Indeed, the traveling wave on the RL slows down at its tuned location to approximately match the TM wave propagation velocity that has been reported experimentally; and the TWAMP's mechanism amplifies over any region where the two lines' velocities are approximately equal. Therefore, by topology, the coupling between the two transmission lines is via stereocilia forces. Providing additional amplification to the RL motion will subsequently amplify the motion of the BM in the hybrid model's Sandwich portion, whose functional mechanism is based on somatic forces produced via stereocilia-actuated transduction currents that are assumed proportional to RL motion. This joining of the Sandwich and the TWAMP models may resolve issues of somatic *versus* stereocilia forces, by explaining how two distinctly different mechanisms can join together to form the cochlear amplifier.

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259 Outer Hair Cell Electromechanical Properties in a Nonlinear Piezoelectric Model

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Electro-mechanical properties of the outer hair cell (OHC) lateral wall are crucial in determining whether a cochlear traveling-wave can be amplified by OHC feedback. One of these properties, the voltage dependence of OHC axial stiffness, has recently been disputed (Hallworth, 2007). In the present study, the voltage dependence of OHC axial stiffness is investigated using a nonlinear piezoelectric circuit model. This model predicts (a) that the axial compliance (inverse of stiffness) and nonlinear capacitance reach a maximum at the same membrane potential, (b) that the fractional compliance change equals the product of the static stiffness, the nonlinear capacitance, and square of the piezoelectric transformer ratio, and (c) that this product is approximately 0.2 in isolated OHCs. The proposed circuit model also was designed to be integrated into macroscopic models that simulate cochlear wave propagation. Small-signal analyses show that the piezoelectric motor is driven by current at low frequency, therefore circumventing the low-pass filtering problem posed by the membrane conductance and capacitance. However, to achieve cochlear amplification, the OHC receptor current must be assumed to be sensitive to velocity of hair-bundle deflection. (Research supported by NIH-NIDCD R01-DC8318).

260 Mechanical Modeling of Stereocilia Array Inspired by Gating Spring Hypothesis

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A mechanical system inspired by negative stiffness mechanism of inner ear hair bundle was simulated and fabricated in macro-scale. The system consists of an array of rigid inverted pendulums with tip-links interconnecting bistable gates and adjacent pendulums, supported by pivotal springs. Both the open probability and sensitivity simulations displayed greater sensitivity to miniscule stimuli. Parametric study on normalized physical parameters of the model revealed that the greater ratio of tip-link stiffness to pivotal spring stiffness begets greater selective amplification of smaller inputs. This result provides direct evidence that compressive nonlinear sensitivity is induced structurally as in accordance with gating spring hypothesis. A prototype was developed based on the simulated model, and an experiment similar to stiffness measurement experiment of inner ear stereocilia bundle was performed. Even though the number of pendulums and gates were far smaller than actual stereocilia bundle or the simulation model, resulting open probability and sensitivity curves were consistent with the simulation results, showing the compressive nonlinearity. In short, in this study the key physical parameters contributing to selective amplification of stereocilia bundle were identified. Also it was shown that stereocilia bundle employs extremely efficient and frugal method to amplify miniscule stimuli while ensuring robustness of the system by passively and structurally inducing amplification.

261 Introduction to the Vestibular Compensation Symposium on New Clinical and Basic Science Perspectives

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In humans and experimental animals, behavioral recovery usually occurs after one-sided peripheral vestibular lesions, known as “vestibular compensation”. The purpose of the symposium is to discuss new perspectives on patient treatment and the mechanisms underlying vestibular compensation. Although most patients compensated, some patients remain poorly compensated with persistent vestibular symptoms, including disequilibrium, vertigo, and ataxia. In particular, the vestibulocular reflex (VOR) does not produce normal responses to high acceleration stimuli. Thus, patients may experience the perceptual consequences of ongoing VOR deficits, and other problems in cognition and spatial memory due to interactions of the vestibular system with higher brain centers. In the clinic, various surgical approaches are implemented to treat chronic peripheral vestibular disorders, and these approaches have been adapted to animal models. For example, after one-sided labyrinthectomy (UL), peripheral vestibular receptors do not regenerate, nor does the resting spike discharge recover in primary vestibular fibers. Moreover, vestibular nuclei neurons must be intact for compensation to occur. Both whole animal and in vitro studies indicate that recovery of function tends to parallel the reacquisition bilaterally of symmetric resting spike discharge in vestibular nuclei neurons. Recent intracellular recordings from brain slices after UL indicate that changes in firing rate occur only in certain subsets of vestibular nuclei neuron. In addition, vestibular nuclei neurons in compensated and uncompensated animals undergo differential changes in their Na^+ and K^+ conductances. Finally, studies of commissural inhibitory inputs and nonlabyrinthine proprioceptive inputs support their roles in modulating activity of vestibular nuclei neuron during compensation.

262 Vestibular Compensation: Animal Research to Bedside

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Selective lesions of the vestibular apparatus in quadrupeds led us to the conclusion that the structural segmentation of the skeletal system corresponds to a differential distribution of vestibular afferents. For instance unilateral lesion of the utricule causes a tilt of the head neck in the frontal plane, whereas a lesion of the horizontal semicircular nerve induces its rotation in the horizontal plane. These findings have opened new way to bridge the gap between animal research and the bedside. Here are two examples:

At the compensated stage of a unilateral vestibular lesion in quadrupeds, while the rotation of the head in the horizontal plane recovers, a residual head tilt is observed. This prompted us to investigate whether such an enduring asymmetry is also present in Humans following vestibular lesions. Our study has confirmed that there is an enduring vulnerability of postural control in vestibular patients in the frontal plane. This had consequences for rehabilitation and showed that dynamic posturography on a seesaw platform was a valuable tool for clinical diagnosis in vestibular patients up to 1 year after the lesion.

Scoliosis induces structural deformations of the skeleton. While vestibular dysfunctions are often associated with this syndrome, no causal link between vestibular deficits and the development of scoliosis has been made so far. We now show that unilateral vestibular lesions in the larval *Xenopus* lead to structural deformations of the skeleton in adult frogs similar to human idiopathic scoliosis. These deformations are likely to be produced by asymmetric activities of several descending pathways, which activate spinal motoneurons. These asymmetric tonic neuronal discharges persist since vestibular compensation does not take place. Their persistence is due to the lack of proprioceptive inputs in an aquatic environment, which could substitute for the biased gravito-inertial reference frame, as it is likely the case for intra-utero in human.

263 Influence of Unilateral and Bilateral Vestibular Loss on Spatial Memory, Hippocampal Volume and Cortical Visual Motion Processing in Humans

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Input from the vestibular system is important for navigation and spatial memory in animals; however, controversy surrounds the role of the hippocampus. We therefore investigated the vestibulo-hippocampal connections in two studies in humans with chronic bilateral and unilateral vestibular failure. Bilateral, but not unilateral, vestibular deafferentation leads to hippocampal atrophy (-16.9% compared to controls). In a spatial memory and navigation task (the virtual Morris water task) deficits were detected in patients with bilateral vestibular deafferentation.[1] Subtle impairment was also found in patients with right unilateral vestibular deficit.[2] Patients with unilateral vestibular failure experience oscillopsia during rapid head movements due to increased retinal slip caused by vestibulo-ocular reflex impairment. Oscillopsia is always smaller than the net retinal slip and decreases over time in patients with acquired vestibular loss; this correlates with increased thresholds for visual motion detection and increased tolerance to retinal slip. We therefore investigated the underlying cortical adaptive processes using visual motion stimulation in fMRI. We found that a chronic unilateral vestibular deficit suppresses cortical visual motion processing. This is an adaptive mechanism that suppresses distressing oscillopsia in these patients.[3]

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264 Compensation of the Vestibuloocular Reflex (VOR) to Loss of Function in One Labyrinth

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Processes of vestibular compensation are responsible for improvement in the performance of the VOR evoked by horizontal head rotations after inputs from one labyrinth are eliminated. The symmetry of the VOR is restored for responses to low-frequency, low-velocity head movements. Asymmetries in the VOR are noted for responses to high-frequency, high-acceleration rotations. The VOR evoked by these stimuli returns to relatively normal levels for rotations that are excitatory with respect to semicircular canals on the intact side but responses remain markedly diminished for rotations in the opposite direction. These changes in the VOR are dependent upon retinal slip error signals (elicited by head movements in light) that serve as a stimulus for vestibular compensation.
Studies of the VOR in monkeys have shown that responses in normal animals, after unilateral labyrinthectomy, and following spectacle-induced adaptation can be mathematically modeled based upon inputs from tonic and phasic pathways. The improvement in the performance of the horizontal VOR elicited by rotations towards the intact side following unilateral labyrinthectomy can be attributed to selective adaptation of phasic inputs from the intact side to reflex pathways. This representation of pathways mediating the VOR has close resemblance to the properties of the two classes of central vestibular neurons studied in the medial vestibular nucleus of rodents and other species (Straka et al. 2005). Type A neurons have mostly tonic response dynamics and a large linear range. Type B neurons have phasic-tonic dynamics and a smaller linear range. Supporting the modeling results, an increased proportion of Type B relative to Type A neurons has been found in the contralesional MVN after unilateral labyrinthectomy (Beraneck et al. 2004). Further in vivo experiments are required in order to relate these two types of neurons with neurons that mediate VOR responses. (Supported by NIH R01 DC2390)

265 Compensation of Central Vestibular Pathways: Changes in the Encoding of Vestibular and Extravestibular Inputs

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Unilateral vestibular loss results in acute symptoms including spontaneous nystagmus and head tilt as well as long-term deficits in the horizontal vestibuloocular reflex (VOR). Notably, compensation of VOR response has provided a useful model for studying plasticity and its neural correlates, since the direct pathway mediating the reflex is relatively simple (i.e. a 3 neuron arc). Prior behavioral work in humans and monkeys has suggested that neck afferent input could play a role in compensation and supplement changes in vestibular signals following vestibular loss; the substitution of cervical afferent/motor efference copy information would provide central pathways with an improved estimate of head motion. However, prior to our studies, a neural correlate for this proposal had not been found.
In this study, we investigated whether neurons in the vestibular nuclei encode signals that are compatible with sensory-substitution hypothesis. We systematically recorded the activity of single neurons during experiments in which we applied vestibular, non-vestibular (neck afferent and motor), and combined stimulation. First, we found that the time course of changes in the vestibular sensitivity corresponded with the behavioral recovery of the VOR. However, we also found that extravestibular

inputs made a significant contributed to the compensation process immediately following loss. The vast majority of neurons in the contralesional nucleus (> 80%) were modulated in response to passive activation of neck proprioceptors (as compared to 0% in normal animals). Moreover, this neck sensitivity decreased over time (~50% of neurons after 2 months). In addition, we found that the sensitivity of neurons during active head rotations (i.e., when a neck motor command is produced) were higher than for passive head rotations.

Taken together our findings provide evidence that extravestibular signals contribute to vestibular compensation, and that this sensory substitution can take place at the level of the direct 3 neuron arc that mediates the VOR. Interestingly, compensation was dynamic: improvement in vestibular responses was accompanied by decreased and increased weighting of neck afferent and efference copy information. Clinical interventions aimed at exploiting this time window could potentially have important implications for designing rehabilitation programs for patients.

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266 Integration of Nonlabyrinthine Inputs by the Vestibular System: Role in Compensation

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In addition to inputs from the inner ear, the caudal regions of the medial and inferior vestibular nuclei as well as the rostral fastigial nucleus of the cerebellum receive signals from neck, trunk, and limb muscles, the skin, and even the viscera. The functional significance of these inputs has largely been ignored. However, recordings from decerebrate cats that had recovered from a bilateral labyrinthectomy revealed that a fraction of caudal vestibular nucleus neurons respond to whole-body tilts in the absence of vestibular inputs. An upper cervical rhizotomy did not abolish these responses, indicating that the nonlabyrinthine inputs responsible for the modulation of neuronal activity were not exclusively from the neck. Anatomical studies have shown that the caudal vestibular nuclei receive direct or disynaptic signals from a number of central nervous system regions that process sensory signals, including nucleus tractus solitarius, the spinal gray matter, and the dorsal column nuclei. Recent studies in conscious cats confirmed that caudal vestibular nucleus neurons as well as neurons in the rostral fastigial nucleus regain a normal level of spontaneous activity within 24 hours of a bilateral labyrinthectomy, and that a small fraction of cells still respond to whole-body tilts in these animals. Cumulatively, these data support the notion that nonlabyrinthine inputs may play an important role in functional recovery following damage to the inner ear.

267 Na+ and K+ Channel Expression Differs in Vestibular Nuclei Neurons from Compensated and Uncompensated Chicks After Unilateral Vestibular Ganglionectomy

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Vestibular compensation refers to the recovery of function following unilateral vestibular deafferentation. However, some patients remain uncompensated. After unilateral vestibular ganglionectomy (UVG) performed on hatchling chicks (H4), 40% of the chicks remained uncompensated three days after UVG. Principal cells (PCs) of the chick tangential nucleus are vestibular nuclei projection neurons in the three-neuron vestibular reflex pathways controlling posture, balance, and eye movements. Using patch-clamp technique, spontaneous spike firing (SSF), ionic conductances, and spontaneous excitatory postsynaptic events (sEPSCs) were recorded in identified PCs before and after UVG. Three major differences were detected: (1) In compensated chicks, the percentage of SSF cells, firing regularity, and firing rate were symmetric bilaterally, with rates increased over controls. In uncompensated chicks, firing rate increased on the lesion side, but on the intact side only silent cells were recorded. (2) In compensated chicks, sEPSC frequency was bilaterally symmetric. However, in uncompensated chicks, sEPSC frequency on the lesion side increased considerably over controls and the intact side. (3) In compensated chicks, SSF and silent PCs differed in their Na^+ conductances, whereas in uncompensated chicks SSF and silent PCs differed in both Na^+ and K^+ conductances. Finally, using immunolabeling and confocal imaging to measure the surface/cytoplasmic ratio for Kv1.1 in uncompensated chicks, we found an increased ratio for PCs on the intact side, consistent with recorded decreased excitability. Also, decreased Kv1.2 expression was measured in synaptotagmin-labeled profiles contacting PC bodies on the lesion side, consistent with the increased EPSC frequency for PCs on the lesion side. Altogether, the misexpression of Na^+ and K^+ channels underlie differences in the excitability of vestibular nuclei neurons from compensated and uncompensated chicks.

268 Balancing the Vestibular Commissural Inhibitory System: Mechanisms of Vestibular Compensation

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Most theories of vestibular compensation assume that the initial asymmetry in the activity of medial vestibular nucleus (MVN) neurons after unilateral labyrinthectomy (UL) is due to a large imbalance in the vestibular commissural inhibitory system. Thus ipsi-lesional MVN neurons fall silent not only because of disfacilitation after the loss of vestibular afferent inputs, but also because of elevated commissural inhibition from contra-lesional MVN neurons. Indeed this inhibition may be crucial since bilateral labyrinthectomy, which equally disfacilitates the

bilateral MVN, does not silence MVN neurons on either side. It has been proposed that the recovery of activity in ipsi-lesional MVN neurons during VC involves, at least partly, mechanisms that counteract the increased commissural inhibition. However, there have been no direct studies of the imbalance in commissural inhibition after UL, and there is no direct evidence implicating the commissural system in VC.
We used microdialysis in alert animals to directly measure GABA release in the bilateral MVN during VC. Immediately post-UL, in line with the appearance of oculomotor and postural symptoms, there is a marked increase in GABA levels in the ipsi-lesional MVN. This is not prevented by bilateral flocculectomy indicating that it involves hyperactivity of commissural inhibitory neurons. With the development of VC over 48–96 h and the amelioration of behavioural symptoms, the ipsi-lesional GABA levels return to near-normal. Contra-lesional MVN GABA levels do not change significantly in the initial stages of VC. When Bechterew's phenomenon is induced in the compensating animals by reversible inactivation of the intact labyrinth, the symptoms are however not accompanied by changes in GABA levels in either MVN. Thus the commissural inhibitory system is intimately involved both in the initial drastic symptoms of UL and the recovery during VC, while the mechanisms of Bechterew's phenomenon are distinct.

269 Middle Ear Transfer Function Measured in Acute Otitis Media Model of Guinea Pigs

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In our previous study on otitis media with effusion (OME), we measured middle ear transfer function in guinea pig OME model by injection of lipopolysaccharide (LPS) into the middle ear. The change of tympanic membrane and round window membrane vibrations induced by middle ear effusion was measured by a laser vibrometer and reported in a recent paper (Dai and Gan, Hear. Res., 2008). Currently, we have extended the study into acute otitis media (AOM) model of guinea pigs and attempted to measure the change of middle ear mechanics induced by bacterial infection in middle ear tissue and cavity. The aim of this current study is to identify the middle ear mechanical response of these two otitis media models in guinea pigs. The AOM was created by injection of Streptococcus pneumoniae serotype 3 (Sp3, ATCC 6303) into the left middle ear of guinea pig. After 3 days of Sp3 injection, the evidence of AOM was assessed by otoscopy, tympanometry, histology, and final check of the middle ear cavity after the experiment. Vibration of the umbo in response to 80 dB SPL sound in the ear canal was measured with a laser Doppler vibrometer at frequency range of 200~40k Hz. Results indicate that AOM resulted in a deeper reduction of the umbo movement in comparison with the control and OME ears. All the measurements and histology observations from AOM ears provide new insight into differentiation of AOM and OME. (Work supported by OCAST HR06-036 and NIH/NIDCD R01DC006632)

270 Angular Orientation of Human Petrous Ridges

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Background: Otologists are taught that petrous ridges are 45 degrees from the midline. Craniofacial surgeons and physical anthropologists, however, report various angles. From clinical and theoretical vantage points, we suspect that petrous ridges are angled slightly differently in those suffering otitis media, at least in childhood.
Hypothesis: Petrous ridges in crania having small mastoid pneumatization, a correlate of childhood otitis media, are set comparatively toward the coronal. Conversely, petrous ridges of crania with large mastoids are set comparatively away from the coronal.
Study Design: Post-mortem study of 40 bequeathed adult crania. None had clinical otitis.
Methods: In images onto the intra-cranial aspect of the skull base, positioned in the Frankfort plane, we measured angles from the petrous ridges to the anterior cranial base midline, and the inter-petrosal ridge angle. Mastoid areas were from plain Law lateral radiographs.
Results: Relative to the anterior midline, petrous ridge angles ranged 45 to 61 degrees, median 53, right ears; 33-57, 48, respectively, left ears. Although without statistical significance, petrous ridges trended to be more coronally oriented in specimens with the small mastoid indicator of childhood otitis media.
Conclusion: Small mastoids trended to correlate with petrous ridges that are set comparatively toward the coronal.

271 MicroRNA and Cholesteatoma: A Model of Proliferation and Development

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Cholesteatoma is a hyperproliferative disorder of skin typically arising from the external auditory canal and tympanic membrane. These benign tumors cause temporal bone destruction, hearing loss, and chronic infection. Other less common but serious sequela includes meningitis, brain abscess and facial nerve paralysis. Current treatment for cholesteatoma is surgical and repeated resections are common. Surgery for complete removal and control is often destructive and may lead to significant problems with hearing and balance.
Tumorigenesis, both for benign and malignant neoplasms, requires inhibition of apoptotic pathways and activation of growth and proliferation pathways. Recent studies have shown that microRNAs can serve as powerful post-transcriptional regulators of such divergent pathways. MicroRNAs are small (~22nt) non-coding RNAs that negatively regulate gene expression by causing target mRNA degradation or translation inhibition. MicroRNAs may be successfully targeted in anti-neoplastic pharmacotherapies.
We present empirical evidence supporting a model of cholesteatoma tumorigenesis and the role of human microRNA-21 (hsa-mir-21) in this disease process. We

have demonstrated that hsa-mir-21 is up-regulated in cholesteatoma as compared to normal skin. The downstream tumor-suppressor proteins PTEN and PDCD4 are conversely down-regulated in cholesteatoma. The messenger RNAs encoding these two proteins are confirmed targets of hsa-mir-21. This evidence supports a proposed model of cholesteatoma formation in which lipopolysaccharide receptor activation leads to IL-6 production and activation of STAT3. STAT3 is a known activator of hsa-mir-21 production which would subsequently inhibit tumor suppressor protein formation leading to proliferation and invasion. RNA-based therapies targeting hsa-mir-21 and other potentially associated microRNAs in aural cholesteatoma may provide pharmacological therapies serving to reduce recurrence or prevent disease progression.

272 Both Positive and Negative Regulators of Inflammation Are Activated During Acute Otitis Media

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Acute otitis media (OM) is characterized by extensive inflammation, mediated primarily by innate immune responses to bacteria in the middle ear (ME) cavity. Inflammation is a well-characterized response to the activation of innate immune receptors, and the resolution of OM includes reduced innate immune signaling leading to decreased inflammation. However, recovery from inflammation can also be mediated actively, by anti-inflammatory and pro-recovery regulators. We evaluated both pro- and anti-inflammatory gene networks after bacterial inoculation of the ME, to test the hypothesis that recovery from OM is actively promoted by anti-inflammatory pathways. Gene expression was evaluated during acute OM via whole-genome transcript profiling. Mouse MEs were inoculated with nontypeable Haemophilus influenzae. Tissue was harvested at intervals ranging from 3 hours to 7 days. Total RNA was extracted and hybridized to Affymetrix gene arrays. Pro- and anti-inflammatory gene networks were evaluated. The expression of selected genes that exhibited significant changes in OM was confirmed at the mRNA level by real-time PCR and at the protein level by immunohistochemistry. During the initial hours after bacterial inoculation, genes related to the innate immune activation of inflammation were strongly up-regulated. Interleukin 6 (IL-6) and and tumor necrosis factor alpha were prominent pro-inflammatory components of this initial response to NTHi, while IL-1 and interferons were somewhat later pro-inflammatory components. However, anti-inflammatory genes including IL-10 and SOCS3 were also induced in the earliest responses to NTHi. Additional pro-recovery compounds such as IL-1 receptor antagonist, IL-18 and IRAK3 were later additions to anti-inflammatory gene expression. Pro-inflammatory signaling networks that are activated by innate immunity during acute OM are opposed by anti-inflammatory pathways from the very onset of ME bacterial infection. This suggests that inflammation is tightly regulated and actively managed during the entire course of OM. Dysregulation of anti-inflammatory and pro-recovery pathways may underlie increased susceptibility of OM. Moreover, these pathways provide attractive targets for intervention that is designed to promote recovery from OM. (Supported by NIH/NIDCD grants DC00129 and DC006279)

273 Molecular Pathogenesis of Otitis Media in a Mouse Model Hypophosphatemia-Duke Mutation (PhexHyp-Duk)

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Although otitis media (OM) is still a common disease in children and adults, the pathogenesis and the underlying genetic pathways are not yet fully understood. We have discovered that mice with the hypophosphatemia-Duke mutation (*Phex*$^{Hyp\text{-}Duk}$) present with a high incidence of otitis media. *Phex*$^{Hyp\text{-}Duk}$/Y hemizygous males are mildly growth retarded in overall body size, show elevated hearing thresholds, and some exhibit circling behavior. The incidence of OM in *Phex*$^{Hyp\text{-}Duk}$/Y mice was 64%. The goblet cells presented metaplasia and hyperplasia in the middle ear epithelia of *Phex*$^{Hyp\text{-}Duk}$/Y males. Increased proliferating nuclear cell antigen (PCNA) expression indicated proliferation of some ciliated cells, nonciliated cells, basilar cells and fibroblasts in *Phex*$^{Hyp\text{-}Duk}$/Y adult males. In addition, increased expression in the ear of *Muc5ac*, *Muc5b* and *Fgf23* was found in *Phex*$^{Hyp\text{-}Duk}$/Y mutant ears compared to X^{+}/Y wildtype littermate control ears. The *Phex*$^{Hyp\text{-}Duk}$ mutation was previously shown to cause elevated levels of fibroblast growth factor 23 (Fgf23), which in turn is known to increase mouse prostaglandin E2 (PGE2) production. PGE2 is considered to be a mediator of inflammation because of its potent vascular permeability increasing activity. High PGE2 expression may be responsible for the increased *Muc5ac* and *Muc5b* gene expression observed in our study, and be a contributor to OM. We hypothesize that upregulation of PGE2 and Mucin genes represent a new signal transduction pathway for OM. supported by NIH NIDCD R01DC007392 (QYZ).

274 Characterization of Secretory Mucin Proteins in Human Mucoid Middle Ear Effusions

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BACKGROUND/OBJECTIVES: Chronic OM with Effusion (COME) with 'glue' ear is characterized by persistant middle ear effusion, conductive hearing loss, and increased likelihood of surgical intervention. Mucous glycoproteins (mucins) are thought to play a significant role in COME with 'glue' ear by contributing to increased

effusion viscosity. MUC5AC and MUC5B, the two major secretory mucins expressed in respiratory tract tissues and secretions, are also expressed in middle ear tissue. Several studies have shown that MUC5B mRNA expression is more predominant than MUC5AC in middle ear tissue of patients with COME. However, no studies have been reported wherein mucoid middle ear effusions have been analyzed with immunoblotting to specifically identify the secreted mucins. We hypothesized that MUC5B, rather than MUC5AC, mucin represents the predominant secretory mucin present in mucoid middle ear effusions from pediatric patients with COME. METHODS: Thick, mucoid effusions from children with COME undergoing myringotomy with tube placement at Children's National Medical Center, Washington DC, were collected. Electrophoresis, immunochemical analysis with mucin specific antibodies, and densitometry were performed to characterize the presence and identity of secretory mucins in effusions. RESULTS: Western blot results revealed strong MUC5B signal intensity in 9/11 effusion samples. MUC5AC demonstrated strong signal intensity in only 1/11 samples. Semi-quantitative densitometry revealed on average a 6.4-fold increased signal in MUC5B compared to MUC5AC (p=0.0009). CONCLUSIONS: Based on our initial pilot patient sample series, it appears that both MUC5AC and MUC5B mucins can be detected in mucoid middle ear effusions but that MUC5B is the predominant middle ear secretory mucin protein present in thick COME secretions.

275 Reduction of Nitric Oxide Concentration by Various Corticosteroids in LPS Induced Otitis Media

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Otitis media with effusion (OME) is a common childhood disease that can lead to pain, discomfort, and hearing loss. Nitric oxide (NO), one of the inflammatory mediators of OME, is a free radical that is known to regulate cell proliferation, cell death, and angiogenesis. Recently, our group has implicated nitric oxide as a causative agent in sensorineural hearing loss (SNHL). In light of this, we set out to determine which of three commonly used corticosteroids, dexamethasone, fluticasone propionate, and rimexolone, is the most effective in reducing the concentration of NO in OME. To accomplish this, we injected 200µl of either 0.1% or 1% solution of each corticosteroid or vehicle control into the bulla of 53 chinchilla at the -2 hour. At the 0 hour, 0.3mg of lipopolysaccharide (LPS) was inoculated into the bullae of each chinchilla. Same doses of corticosteroid were re-administered at 24 and 48 hours post LPS-inoculation. At 96 hours after inoculation, animals were euthanized and the middle ear effusion (MEE) collected. The samples of MEE were analyzed for NO with the Griess reagent. All three corticosteroids significantly ($p<0.05$) reduced the concentration of NO compared to the vehicle. The concentrations of NO among the different corticosteroid groups were not statistically different, except that all corticosteroids reduced the NO concentration significantly better than treatment with the 0.1% concentration of dexamethasone. Dose response between 0.1% and 1% was the greatest in dexamethasone.
Among the 1% concentration groups, reduction of NO was the best in dexamethasone followed by rimexolone and fluticasone. This study suggests that corticosteroid treatment may reduce inflammation in OME and may protect induction of SNHL in OM.

276 Difference in NF-κB Signaling Between *S. Pneumoniae* and NTHi

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Otitis media (OM) is clinically important due to its high incidence and impacts on language development in children. *S. pneumoniae* is the most frequent cause of severe acute OM and suppurative complications. In contrast, nontypeable *H. influenzae* (NTHi) seems to be more associated with chronic OM with effusion. The underlying molecular mechanism of the different clinical course of pneumococcal OM and NTHi OM remains unknown. Differences in bacterial molecules probably lead to the involvement of diverse signaling pathways such as NF-□B signaling, which is required for induction of inflammatory genes. Previously, we showed that Ser536 of p65 NF-□B is phosphorylated by both NTHi and *S. pneumoniae*, but Ser468 is phosphorylated only by *S. pneumoniae*, negatively regulating the basal activity of p65 NF-□B. We aim to study host genes that are regulated by *S. pneumonaie* and NTHi using Affymetrix GeneChip® Human Gene ST 1.0 arrays.
Using a false discovery rate (FDR) of <0.05 and fold change of >2[1], we found that *S. pneumoniae* regulates 7,303 genes (p=0.02) while NTHi regulates 9,809 genes (p=0.03). 4,287 genes were commonly regulated upon exposure to either *S. pneumoniae* or NTHi. When the p value is <0.01, 2,528 (34.6%) out of 7,303 genes were significantly regulated upon exposure to *S. pneumoniae*, including 999 up-regulated (39.5%) and 1,529 down-regulated (60.5%) genes. In contrast, when the p value is <0.01, 3,529 (36.0%) out of 9,809 genes were significantly regulated upon exposure to NTHi, including 1,307 up-regulated (37.1%) and 2,222 down-regulated (63.0%) genes. Pathway analysis showed that NTHi (p=0.01) is more tightly associated with NF-□B signaling than *S. pneumoniae* (P=0.15). Moreover, *S. pneumoniae* down-regulated NF-□B signaling-related genes more than NTHi. Taken together, we suggest that *S. pneumoniae* is less prone in activating NF-□B signaling compared to NTHi, which may explain the different clinical course of OM between *S. pneumoniae* and NTHi.

277 Differential Expression of Toll-Like Receptors 2 and 4 in Rat Middle Ear

Withdrawn

278 Mucin Gene Expression in an *In Vitro* Mouse Model of Middle Ear Epithelium

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Otitis media is the most common diagnosis in pediatric patients who visit physicians for illness in the United States. Mucin production in response to otitis media causes significant sequelae including hearing loss and the need for surgical intervention. Because mucins play an integral role in the mechanisms of otitis media, investigating the expression of mucin genes in this tissue is vital to the understanding of the pathophysiology of otitis media. Increasingly, mouse models for investigation are being utilized and understanding the similarities between mouse, human and other animal models is important in comparative studies and in translating data from mouse models to human pathophysiology.

Our laboratory has characterized the expression of mucin genes in both *in vivo* and *in vitro* human models. In this study we investigated the expression of the complete spectrum of 19 mucin genes in an *in vitro* model of immortalized mouse middle-ear tissue (MMEEC). Cells grown in culture utilizing standard techniques were assessed for muc1, muc2, muc3, muc4, muc5AC, muc5B, muc6, muc7, muc8, muc9, muc10, muc11, muc13, muc15, muc16, muc17, muc18, muc19 and muc20. The expression of mucin genes utilizing this MMEEC was similar to that in both human *in vivo* and *in vitro* models. However, there did exist some differences with lack of identification of transcripts for muc7, muc8, muc11 and muc17.

This study demonstrates the expression of mucin genes in an *in vitro* model of mouse middle ear epithelium. Further studies are ongoing within our laboratory, to examine the implications of this mucin gene expression, the differences between other models and the correlation with *in vivo* mouse models. Additional studies examining the response of MMEEC with respect to inflammatory challenges and in comparison to other models previously utilized are also ongoing.

279 Fungus Infection of the Middle Ear in a Cetacean

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The hearing organ is one of the major senses in cetaceans. Little is known about the exact mechanism and even less about relevant negative impacts. This is the first report of severe mycotic otitis media in a cetacean, a juvenile female harbour porpoise (Phocoena phocoena) coming from British waters that stranded alive. Gross examinations were added by histological and microbiological investigations. The porpoise was in poor nutritional status. Pathological findings on other organ systems were considered to have minor relevance for the health status of the animal and will also be presented. Examinations of the head region showed intact tympanic bullae with copious greenish-yellow purulent and caseous material within both tympanic cavities and periotic sinuses. No nematodes were found within these structures. Microbiological examinations of the middle ears demonstrated fungal infections with Aspergillus terrus in both tympanic cavities but not in any other site in the body. Histological examinations in and around the oval and round windows showed a massive deposition of proteins and infiltration of inflammatory cells surrounded a prominent mycelium reaching far into the tympanic cavity. Stapes and lateral side of the petrous bone near the oval window were affected by osteolysis. Cyst-like structures, lymphocytes and newly formed vessels filled the space beyond the round window membrane. The Organ of Corti showed massive malformation. Sensory cells and supporting cells were missing throughout most parts of the cochlea. We conclude that this together with the massive changes in the Organ of Corti were factors responsible for severe hearing impairment or even deafness leading to the poor nutritional status. Systematic examinations on the middle and inner ear are important to understand the health status of cetaceans and should therefore be included in routine pathological investigations.

280 Hair Cell MicroRNA Misexpression in Supporting Cells Results in Hair Cell Loss and Deafness

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MicroRNA function is essential for the normal development of the vertebrate inner ear (Soukup, ARO 2008). The miRNA-183 family (miR-183fam) is expressed in neurosensory organs of animal species representing several taxonomic phyla (Pierce et al., 2008). Morpholino induced knockdown of the miR-183fam results in diminished hair cell development (Fekete, MBHDC 2007). Human and mouse mutations in miR-96 are associated with hearing loss (Moreno, MBHDC 2007; Steel, ARO 2008). These observations and the temporospatial expression of miR-183fam in differentiated hair and spiral ganglion cells suggest they are critical regulators enforcing neuronal and sensory cell fates and functions. The mutual exclusion hypothesis of miRNA and target gene expression in adjacent cell types attempts to explain the aggregate functions of animal miRNAs (Stark et al., 2005). According to this hypothesis, misexpression of miR-183fam in adjacent and lineage related supporting/glial cells should yield information relevant to the functions of this miRNA family. To test this hypothesis, we generated transgenic (Tg) FVB/N mice to drive ectopic miR-183fam expression using the core human promoter of the glial fibrillary acidic protein (GFAP). One of 3 resulting transgenic lines exhibited supporting cell miR-183fam misexpression. Homozygotes demonstrated a progressive and nearly complete loss of auditory hair cells by postnatal day (P)132. At P37, a basoapical gradient of Myo7a positive inner and outer hair cell loss was observed, with

the greatest loss in the base. The ordered geometry of supporting cell nuclei and β-tubulin bundles was deranged in the organ of Corti of homozygotes and this disorganization was evident as early as P37, even in the absence of significant hair cell loss, suggesting that there are early defects in supporting cell cytoarchitecture. Click and pure tone ABR thresholds were significantly increased by P37, averaging approximately 70 dB SPL or more across the frequency range tested (2-64kHz). By P90 average thresholds were approximately 90 dB SPL or higher and DPOAE findings suggested that the cochlear amplifier fails to operate in homozygotes. This mammalian miRNA misexpression model demonstrates the potency of the miR-183fam and will be used to identify target genes in regulatory, structural and/or metabolic pathways. The elucidation of miRNA-regulated pathways might provide novel avenues for future therapeutic intervention in treating some forms of hearing loss. Supported by NIH/NCRR P20RR018788 (GAS) and NIH/NIDCD F32DC008253 (MDW).

281 ATP8B1 Deficiency Results in Hearing Loss and Degeneration of Cochlear Hair Cells

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ATP8B1 deficiency is caused by autosomal recessive mutations in ATP8B1, which encodes the aminophospholipid flippase ATP8B1 (formerly called FIC1). ATP8B1 deficiency is primarily characterized by cholestasis, either as progressive (progressive familial intrahepatic cholestasis type 1, PFIC1) or intermittent (benign recurrent intrahepatic cholestasis type 1, BRIC1). In addition, extrahepatic symptoms such as pancreatitis and chronic diarrhoea are found. As patients may also complain about a reduced hearing capability we investigated the role of ATP8B1 in auditory function. We shall show in this presentation that hearing loss is an extrahepatic feature of ATP8B1 deficiency, probably secondary to degeneration of the hair cells, which is consistent with a specific localisation of ATP8B1 in the hair bundle stereocilia. These data open the possibility that the bile salt excretory function of hepatocytes and the mechanosensory function of the hair cells share some common molecular mechanisms and are both critically dependent on the aminophospholipid flippase activity of ATP8B1.

282 Celastrol Induces the Heat Shock Response and Inhibits Aminoglycoside-Induced Hair Cell Death

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Aminoglycoside-induced hair cell death is associated with the induction of apoptotic signaling pathways, resulting in JNK phosphorylation, activation of caspases -9 and -3, and cytochrome c release from the mitochondria. In addition, treatment with aminoglycosides has been shown to result in a spike in the formation of reactive oxygen species (ROS). Our previous work has shown that heat shock inhibits aminoglycoside-induced hair cell death in the adult mouse utricle in vitro. Heat shock leads to robust upregulation of several heat shock proteins in mouse hair cells, including HSPs -27 -70, and -90. The mechanism(s) by which HSPs inhibit aminoglycoside-induced hair cell apoptosis are unknown. A recent screen for molecules that induce the heat shock response resulted in identification of celastrol, a triterpene used in Chinese herbal medicine, as a potent inducer of the heat shock response (Westerheide et al. 2004 J Biol Chem. 279 (53):56053-60.). Celastrol has also been found to have antioxidant and anti-inflammatory properties. We examined the effect of celastrol on aminoglycoside-induced hair cell death and found that treatment with celastrol results in robust upregulation of Hsp70 and Hsp32. In addition, celastrol resulted in significant inhibition of aminoglycoside-induced hair-cell death. Utricles were cultured in 1.5 μM celastrol for 3h and allowed to recover in culture media for 5h. Utricles were then exposed to 1 mM, 2 mM, 3 mM, 4 mM, or 5 mM concentrations of neomycin for 24h. Neomycin significantly reduced hair cell viability (t-test, $p<0.05$, $n=20$). Celastrol inhibited neomycin-induced hair cell death across the neomycin dose-response curve (Two-way ANOVA, $F1,39 = 13.12$, $p< .001$, $n=25$). These data suggest that induction of heat shock proteins and/or antioxidants may represent a viable approach to clinical prevention of aminoglycoside-induced hair cell death and hearing loss. In addition, we have used Hsf1-/- mice to examine whether HSP induction is necessary for the protective effect of celastrol. The protective effect of celastrol was partially retained in utricles from Hsf1-/- mice (t-test, $p< .05$, $n=20$). These data indicate that other, possibly antioxidant, mechanisms are involved in celastrol-mediated protection against aminoglycoside- induced hair cell death.
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283 Overexpression of Hath1 in Noise-Deafened Guinea Pigs Generates New Hair Cells and Improves Auditory Function

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Many factors including sound over-stimulation, aging, ototoxic drugs, pathogenic infection and autoimmune diseases can cause the irreversible loss of cochlear hair cells. To restore the hearing loss due to irreversible loss of cochlear hair cells, it is necessary to produce new functional hair cells. In present study, the deafness of healthy adult guinea pigs was established after the continuous exposure to high-intensity pulsed noise. The deaf guinea pig's ABR could not be elicited at the frequencies 4, 8, 16 and 20KHz, or a very high threshold

(≥ 95dB SPL) and the loss rates of cochlear IHC and OHC were 91.4% and 97.2%, respectively. Subsequently, an adenovirus vector carrying the human-homologous *Hath1*-Drosophila atonal gene was transfected into cochlea. After four weeks of over-expression of *Hath1*, considerable IHC but very little OHC was generated; at this time the hearing function had improved with the ABR threshold in *Hath1*-expressed ears reached an average of 85dB SPL, and the CM curve was recorded at 1KHz. Eight weeks after expression of *Hath1*, large numbers of IHC and OHC were produced. Newly-generated hair cells and OHC cells at the second turn reached 52.4% and 37.1% of the normal values in the healthy guinea pig, respectively, while further improvement of hearing function was found with the ABR threshold in *Hath1*-expressed ears reached an average of 70dB SPL. The CM input-output curve had the preliminary non-linear feature in the hearing recovery ears. Our data indicated that the over-expression *Hath1* after noise-induced heaing loss would induce the differentiation of non-sensory cells into new-generated hair cells and thus improve the hearing function.

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284 Analysis and Comparison of the Adult Mouse Inner and Outer Hair Cell Transcriptomes

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Global expression analysis of the unique cell types in the mammalian organ of Corti should lead to an understanding of the operation of hair cells and their associated supporting cells in the adult cochlea. We have, as an initial step, utilized the DNA-microarray technological to identify the transcript repertoires of adult mouse inner hair cell (IHC) and outer hair cell (OHC) populations. Pristine populations of 100 viable cells per sample were harvested from protease dissociated organ of Corti. Replicate assays were performed using a combined superAmp RNA amplification of each sample and hybridization of Agilent whole genome microarrays. Statistical and differential expression analyses were done using Bioconductor, Expander and Rosetta Resolver. Low copy number transcripts, such as Chrna9, Chrna10, and Slc26a5, were identified in both IHC and OHC transcriptomes. The transcript repertoires of IHC showed greater diversity with ~10% more genes being represented. This differential diversity was also reflected in the number of genes uniquely restricted to each cell type with IHCs and OHCs exhibiting ~2200 and ~540 genes, respectively. Differentially expressed genes shared by both cochlear hair cell populations were also identified with ~560 genes being more highly expressed in OHCs and ~1200 genes were more abundantly found in IHCs. These data sets were also examined for genes involved transcriptional regulation, transmembrane ion movement, neuronal signaling, cell-cell communication, formation of stereocilia and deafness-associated genes. These data will be discussed in regards to the restricted and differentially expressed gene that may impact on the unique properties and functions associated with each hair cell type.

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285 Identification of Proteins That Interact with TMC1 Protein in the Inner Ear

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Mutations in transmembrane channel-like gene 1 (*TMC1*) cause hearing loss in humans and mice. Mouse *Tmc1* mRNA is expressed in cochlear hair cells and is required for their functional development and survival. TMC1 is a six-pass transmembrane protein localized to the endoplasmic reticulum in heterologous expression systems. To elucidate its role in hair cells, we sought to identify proteins that interact with TMC1. We used four bait clones encoding different regions of mouse TMC1 to screen a yeast two-hybrid library of prey clones derived from P1 mouse inner ear cDNA. Two genes, *FKBP8* and *VAPA,* had multiple, overlapping, unique prey clones that interacted with the TMC1 C-terminal bait clone. *FKBP8* encodes an FK506-binding protein and *VAP-A* encodes vesicle-associated membrane protein (VAMP)-associated protein A. We used the STP3 system for *in vitro* synthesis of the TMC1 C-terminus, FKBP8 and VAP-A. TMC1 could be co-immunoprecipitated with either FKBP8 or VAP-A in this system. We co-expressed epitope-tagged expression constructs for full length TMC1 and either FKBP8 or VAP-A in COS-7 and HeLa cells. Immunostaining of the expressed proteins revealed nearly completely overlapping expression patterns. Anti-FKBP8 and anti-VAPA antibodies stain mouse cochlear hair cells in an intracellular reticular expression pattern. We conclude that TMC1 may interact with FKBP8 and VAPA *in vivo* and *in vitro*. We created truncation constructs of TMC1, FKBP8, and VAPA to determine the specific regions of interaction between TMC1 and either FKBP8 or VAPA. Preliminary results of co-localization in HeLa cells seem to indicate that the TM domains of both FKBP8 and VAP-A are required for interaction with TMC1. Since FKBP8 is thought to mediate anti-apoptotic signal transduction and negative regulation of sonic hedgehog signaling in neural tissues, a potential interaction with TMC1 may underlie the requirement for TMC1 in hair cell development and survival.

286 TRPA1 in Hair Bundles of an Ancient Metazoan Animal

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The purpose of the present study is to investigate the possibility that TRPA1 is involved in signal transduction of anemone hair bundles. Hair bundle mechanoreceptors on tentacles of sea anemones detect swimming movements

of planktonic prey. Such movements maximize discharge of nematocysts (stinging organelles) into prey that swim into contact with the tentacles. A homolog of TRPA1 was identified in the database for the model anemone, Nematostella vectensis. A reverse BLAST returned TRPA1 proteins suggesting that anemone TRPA1 is related to known TRPA1s. Like TRPA1 in vertebrate animals, anemone TRPA1 consists of a series of ankyrin repeat domains near the N-terminus and an ion channel near the C-terminus. However, anemone TRPA1 has approximately half as many ankyrin repeat domains as in model vertebrate animals. Known inhibitors of vertebrate TRPA1 including streptomycin, ruthenium red, and lanthanum decrease vibration sensitivity in anemones. Trinitrophenol, an activator of vertebrate TRPA1, is stimulatory in the anemone system. Custom antibodies raised to the extracellular C-terminus of anemone TRPA1 label stereocilia of hair bundles. It appears likely that TRPA1 participates in signal transduction in hair bundles of sea anemones. Supported by NSF IOB0542574.

287 Role of Thyroid Hormone Receptors Alpha and Beta for the Postnatal Regulation of Cav1.3 Currents in Mouse Inner Hair Cells

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Thyroid hormone (TH) controls many processes during the critical period of final differentiation of the cochlea, and TH deficiency causes hearing loss due to structural and functional deficits of the organ of Corti. TH acts through the nuclear receptors TRalpha1 and TRbeta1 which regulate transcription. TRbeta1-deficient mice are deaf, whereas TRalpha1-deficient mice have normal hearing.

In rats and mice, the critical period extends from E17 to P12 including a phase of spontaneous action potential activity in inner hair cells (IHC) evoked by regenerative activation of voltage-gated Cav1.3 Ca2+ channels and delayed rectifier K+ channels. In this phase of spiking, the amplitude of IHC Cav1.3 currents is being up-regulated until P7/P11 and then down-regulated to a lower mature level. In IHCs of hypothyroid rats and athyroid Pax8-/- mice, peak IHC Ca2+ currents were doubled and their down-regulation was delayed (Brandt et al. J. Nsci. 2007, Sendin et al. J. Nsci. 2007) which led to the question which of the TH receptors controls Cav1.3 expression. We therefore recorded Ca2+ currents using Ba2+ (IBa) in IHCs of TRbeta1-/- and TRalpha1-/- mice. IHCs of TRbeta1-/- mice showed an increased peak of IBa (120 % of control) at P9-P11, which was much smaller than in hypothyroid or athyroid animals, suggesting a contribution of TRalpha1 in the developmental regulation of Cav1.3. Indeed, TRalpha1-deficient mice showed an accelerated up- and downregulation of peak IBa amplitude. We also analyzed mice with a targeted mutation of TRalpha1, TRaAMI/S, that blocks TH-mediated relieve of gene repression (Quignodon et al., Mol. Endocrinol. 2007). Here, the developmental up-and downregulation of IBa was altered in comparison with the WT, further indicating a role for TRalpha1 in controlling Cav1.3 expression. To conclude, a concerted action of both TRalpha1 and TRbeta1 seems to be necessary for the developmental up- and downregulation of IHC Cav1.3 current that is decisive for the control of Ca2+ action potentials, exocytosis and gene expression.

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288 Expression and Role of Auxiliary Ca2+ Channel Alpha2delta Subunits in the Peripheral Auditory System of Mice

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Voltage-activated Ca^{2+} channels consist of a pore-forming alpha1 subunit (SU), a beta, an alpha2delta (and sometimes also a gamma) SU. Expression of alpha1 SU is cell-specific as is the co-assembly with the auxiliary subunits, giving rise to Ca^{2+} channels with different, tissue-specific properties. Inner (IHC) and outer hair cells (OHC) express predominantly voltage-activated Ca^{2+} channels with the pore-forming subunit Cav1.3 (Platzer et al., Cell 2000; Michna et al., J. Physiol. 2003), whereas spiral ganglion neurons (SG) express Cav1.2, Cav2.1 and Cav2.2 channels (Roehm et al. Mol. Cell. Neurosci. 2008). Alpha2delta (1-4) SU plays a critical role in trafficking alpha1 SU to the plasma membrane and moreover modulate Ca^{2+} channel properties. Only recently, alpha2delta-1&2 were identified as pharmacological targets for treatment of chronic pain e.g. by gabapentin. So far, the expression of alpha2delta SU in inner and outer hair cells and SG neurons is unknown.

Using RT-PCR and in situ hybridization, we detected alpha2delta-1,2,3 in the organ of Corti and the cochlea. A mouse model with a targeted deletion of alpha2delta-3 (Jackson Laboratories) reported to have a reduced startle response showed increased ABR thresholds despite normal DPOAEs. LacZ reporter staining due to excision of CACNA2D3 gene in alpha2delta-$3^{-/-}$ mice indicated expression of alpha2delta3 in spiral and vestibular ganglia whereas it was absent in IHCs. Ba^{2+} currents in IHCs of alpha2delta-$3^{-/-}$ mice were unaltered, confirming that the alpha2delta-3 SU is not crucial for IHC Ca^{2+} currents.

In conclusion, the hearing deficit of alpha2delta-$3^{-/-}$mice is not due to malfunction of IHCs or OHCs but may rather be caused by altered presynaptic Ca^{2+} currents of spiral ganglion neurons.

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289 Developmental Regulation of Spontaneous Cochlear Activity

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We recently reported that experience-independent 'spontaneous' activity in developing auditory nerve fibers arises from the periodic release of ATP from inner supporting cells (ISCs) in the cochlea; this ATP depolarizes nearby inner hair cells (IHCs) and initiates bursts of action potentials in spiral ganglion neurons (SGNs). ATP-mediated cochlear activity disappears after the onset of hearing; however, it is not known when this activity arises, whether it is solely responsible for triggering auditory nerve activity during development, or what causes this activity to cease after the onset of hearing. To address these questions, we examined the physiological properties and patterns of activity exhibited by ISCs, IHCs and SGNs in acutely isolated cochleas from P0-P20 rats. ATP-mediated spontaneous inward currents were observed in ISCs as early as P0 and persisted until shortly after hearing onset. In contrast, ATP-mediated currents were not detected in IHCs until P3. Focal application of ATP failed to produce detectable currents in IHCs at birth, but IHCs became progressively more responsive to ATP between the ages of P2-P5. Surprisingly, ATP receptor expression by IHCs declined shortly after hearing onset. Thus, the decrease in spontaneous ATP release by ISCs and the developmentally-regulated expression of ATP receptors by IHCs give rise to a temporally restricted period (P3 to hearing onset) during which IHCs experience ATP-mediated inward currents. IHCs are competent to release glutamate onto SGNs at birth, and it has been proposed that intrinsically-generated calcium action potentials in IHCs elicit experience-independent electrical activity in auditory neurons during the first postnatal week. To investigate the relative importance of intrinsically-generated and ATP-mediated activity during this period we recorded spontaneous action potentials from SGNs in P0-10 cochleas. ATP-dependent activity in SGNs emerged at P3, consistent with the onset of purinergic receptor expression by IHCs; prior to that time (P0-P2) IHC-dependent activity was not observed. Together, these results suggest that ATP-mediated signaling in IHCs is the primary excitatory stimulus responsible for initiating action potentials in auditory nerve fibers before the onset of hearing. Supported by the NIH (NIDCD grants DC008860 and DC009464).

290 The Cochlear BK Channel Interactome

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The BK channel acts as a sensor for membrane voltage and intracellular Ca^{2+}, linking cell excitability, metabolism, and signaling. In a series of experiments reported previously, BK was used to coimmunoprecipitate BK-associated proteins (BKAPs) from tissues excised from the chicken and mouse cochleae. Here, we report the results from testing interactions using reciprocal coimmunoprecipitation (coIP) and mining the molecular interaction database IntACT (www.ebi.ac.uk) (Kerrien et al., 2007, Nuc Acids Res 35:561-5) with 174 proteins isolated from membrane/cytoskeletal and cytoplasmic fractions to search for secondary interactions. Binary partners were determined using Envision tool, www.ebi.ac.uk/enfin-srv/envision, with the search limited to murine proteins and not extended to orthologues in other species, and excluding cosedimentation data. Interaction networks were visualized, modeled, and analyzed using the program Cytoscape. The analysis for mouse revealed a network composed of 199 nodes (individual proteins) and 234 edges (lines connecting proteins). We found that 87% of the proteins (160 nodes and 188 edges) are linked to form one large network. The remaining 13% were dispersed among 12 smaller networks composed of 5 nodes or less. Among the larger global network, there were 12 major hubs, formed by a central protein connected to six or more partners, some of which connected to the global network. Ten proteins formed the core of these hubs including α-tubulin, ATP synthase β-subunit, calmodulin, calrecticulin, chromobox homolog 1, γ-actin, NMDA receptor, protein kinase ε, protein SET, and ubiquitin. Reciprocal coIPs verified that BK interacts with 14-3-3-γ, annexin V, apolipoprotein-A1, calmodulin, cofilin, GAPDH, Lin 7c, γ-actin, GST, hippocalcin, and MP0. These analyses provide insights into BK function in mouse cochlea, including connections to mitochondrial and deafness-related proteins found within the network. *Supported by NIDCD grant R01DC04295.*

291 BK Interacts with Cochlear Apolipoprotein A1

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Apolipoprotein A1 (ApoA1) binds lipids and is a major component of high density lipids. Previous studies suggest that ApoE regulates delayed rectifier K^+ channels, whereas little is known about the effects of ApoA1. A yeast two-hybrid screening of a chick cochlea cDNA library, with bait consisting of the BK α-subunit, produced the full-length ApoA1 sequence. The same interaction was observed in chick and mouse when using coimmunoprecipitation, 2-D PAGE electrophoresis, and LC-MS/MS. Both experiments were verified using reciprocal coimmunoprecipitation and colocalization in vivo. In light of these data, voltage-clamp recordings were initiated using excised membrane patches from HEK293T cells cotransfected with BK and ApoA1. These studies revealed that ApoA1 has an effect on the biophysical properties of BK. In the presence of ApoA1, the steady-state voltage conductance curve was significantly shifted to more positive values, with $V_{1/2}$ = 59.2 mV in the absence of ApoA1 and $V_{1/2}$ = 72.1 mV in the presence of ApoA1 ($p \leq 0.002$). Secondly, BK transfected alone shows a linearly

decreasing activation time constant in response to increased membrane potential. In the presence of ApoA1, this response is significantly shifted to a higher value by ~3 msec ($p \leq 0.05$), indicating that ApoA1 decreases the activation time of BK. In contrast, ApoA1 had no significant effect on Gmax or on the slope of the G-V curve. Thus, ApoA1 has an inhibitory effect on BK response properties acting mainly at the membrane as opposed to acting as a chaperone, increasing channel density. In mammalian hair cells, BK channels influence membrane time constants and thus the temporal response of receptor currents. In nonmammals, BK channels take on an additional role in electrical tuning. In both systems, ApoA1 inhibition of BK currents may decrease the frequency response of the hair cell membrane potential, impacting temporal coding in mammals and frequency discrimination in nonmammals. Supported by NIDCD grants R01DC07432, R01DC04295.

292 Putative ER Retention Signals in BK Channel Splice Variants Are Upregulated During Chick Cochlear Development

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The maturation of hair cell excitability culminates in the acquisition of large-conductance, calcium-activated potassium (BK) channels just prior to the onset of hearing. The functional appearance of this channel lags behind an upregulation in BK gene transcripts but appears to be coincident with trafficking of BK subunits to the cell surface. Based on this observation, we hypothesized that dominant-negative isoforms containing endoplasmic reticulum (ER) retention signals limit surface expression early in development. Splicing at the C-terminus generates three possible isoforms, ending in QEDRL, MVYR, or VEDEC. The VEDEC isoform has been shown to retain BK channels in intracellular compartments. We performed quantitative RT-PCR on mRNA obtained from chick basilar papilla throughout development (i.e. embryonic day 12 (E12) through posthatch) using Taqman probes to distinguish between these C-terminus variants. Contrary to our original hypothesis, the expression of transcripts incorporating VEDEC increased 10-fold prior to the onset of hearing from E12 to E18. We used patch-clamp electrophysiology to confirm the dominant-negative effect of VEDEC. Heterologous expression of transcripts encoding VEDEC resulted in a lower BK current density than expression with QEDRL. Antibodies specific to VEDEC and QEDRL were applied to isolated chick hair cells. Anti-VEDEC labeled diffusely throughout the hair cell cytoplasm, supporting the notion that these variants are retained in intracellular pools. Anti-QEDRL labeled in a punctuate pattern at the synaptic pole of the hair cell, where BK channels cluster alongside voltage-gated calcium channels at the presynaptic active zone. Interestingly, expression of variants with QEDRL was unchanged during development, whereas expression of MVYR increased by 9-fold from E12 to E18 then decreased 5-fold in posthatch animals. The functional effect of the MVYR variant is currently unknown. [Supported by NIH R01 DC07432 to RKD and P30 DC0578188]

293 Redox Modulation of the Outer Hair Cell K+ Current Ik,n

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Outer hair cells (OHCs) in the organ of Corti are characterized by an outwardly rectifying potassium current termed Ik,n with unusually negative voltages of activation. Recently, KCNQ4 has been shown to be the molecular correlate of Ik,n in outer hair cells. In OHCs KCNQ4 is restricted to the basal membrane where it is thought to be involved in the cycling of K+ ions from the OHCs into the perilymph. KCNQ4 channel function is essential for the survival of outer hair cells, since KCNQ4 dysfunction leads to progressive OHC degeneration in a hereditary hearing loss both in man (DFNA2) and in a mouse model. It is likely that the unusual biophysical properties of Ik,n are essential for its function in the OHCs. Surprisingly though, heterologously expressed KCNQ4 does not show the same negative activation range as native Ik,n. Recently it has been shown that the functional properties of recombinant KCNQ4 channels are sensitive to oxidative modification, but the physiological relevance is not known.
We thus examined the sensitivity of Ik,n in rodent OHCs to redox modifications. Whole cell patch clamp was used to investigate the physiological response of Ik,n to oxidizing, reducing and cystein-modifying agents in acutely isolated rat OHCs. Oxidizing agents (H2O2) induced an approx. 10 mV shift of voltage-dependent activation to hyperpolarized voltages. Similar to previously reported H2O2 effects heterologously expressed KCNQ4 currents were enhanced in CHO cells. In contrast, reducing agents (GSH, DTT) induced a shift of approx. 10 mV towards depolarized voltages.
Our data suggest substantial impact of redox-dependent modifications on the KCNQ conductance in OHCs. It seems possible that oxidative stress may affect OHC survival via the modulation of hair cell potassium currents.

294 Altered Intracellular Calcium Handling in Hair Cells Suppresses Electrical Activity and Synapse Formation

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We have previously shown that during development chicken hair cells have Ca2+ based action potentials (APs) and that voltage-gated Ca2+ currents play a role in this electrical activity. Here, we investigate the functional expression of calcium-dependent potassium (IK(Ca)) currents, as well as their role in spontaneous electrical activity in developing hair cells of the chicken basilar papilla. In addition, we examine the role of intracellular

calcium handling on K+ currents and electrical activity. Potassium currents were recorded using an extracellular solution (in mM), NaCl 125, KCl 6, CaCl2 0-8, D-glucose 10, MgCl2 1, HEPES 10) , and intracellular solution (in mM), KCl 120, Na2ATP 5, MgCl2 2, HEPES 10, EGTA 1-10, or BAPTA 1-10 D-glucose 10.

We examined the sensitivity of K+ currents and spontaneous electrical activity to intracellular and extracellular Ca2+. We further determined that the Ca2+ sensitive K+ current is sensitive to apamin. Thus, the main IK(Ca) in developing chicken hair cells is Isk. Isk was present at all ages examined (E8-P3), with the most dramatic changes occurring at ~ E10-E18. Specifically, there is a developmental down-regulation of Isk. Additionally, apamin reduced the frequency of spontaneous electrical activity in hair cells, suggesting that Isk participates in determining hair cell firing frequency. To further test the involvement of intracellular Ca2+ signaling, we recorded K+ currents and spontaneous electrical activity in hair cells from Crooked Neck Dwarf (CND) chickens, which have a genetic mutation in ryanodine receptor 1 (RyR1). We will present data that demonstrates: 1) the developmental expression of Isk in developing hair cells and their role in spontaneous electrical activity 2) the role of intracellular calcium handling in hair cell electrical activity 3) importance of electrical activity in synaptic formation during hair cell development.

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295 Regulation of Outward Kv Currents by Extracellular Chloride in Outer Hair Cells

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Two major potassium currents, termed IK and IK,n, are found in outer hair cells (OHCs) of the guinea-pig cochlea. Interesting, we now find that the, 4-AP sensitive, outward K conductance of the OHC is also sensitive to chloride, though, in contrast to prestin, extracellularly. At a holding potential of –40 mV, IK is inhibited by changing extracellular Cl^- levels from 150 mM to 5 mM, with a K_d of 50 mM. Other Kv channel conductances in supporting cells, such as Hensen and Deiters' cells, are not affected by reduced extracellular chloride. We also tested heterologously expressed Slick and Slack K channel conductances, but found no extracellular chloride sensitivity. In order to elucidate the mechanism of IK sensitivity to Cl^- in OHCs, activation and inactivation kinetics of IK were examined. Lowering extracellular Cl^- shifted $V_{1/2}$ of IK inactivation from –40 mV to -50 mV, but had no effect on activation. Thus, Cl^- sensitivity of IK might arise in part from a hyperpolarizing shift in inactivation.

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296 Ca2+-Dependence of 4-AP-Sensitive Outward K+ Current in Developing Hair Cells

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Ca2+ sensitive K+ currents, contribute towards the frequency of firing of spontaneously active cells; such as in the developing hair cells of the chicken basilar papilla. In order to better understand the mechanisms of these spontaneously generated action potentials, we set to investigate the functional expression of voltage-gated K+ currents and their possible sensitivity to intracellular Ca2+ handling. We used 20 mM TEA to block other K+ currents, such as delayed rectifier and (IK(Ca)). TEA insensitive potassium currents were recorded using extracellular solution, (in mM) NaCl 125, KCl 6, CaCl2 0-2, 20 TEA, D-glucose 10, MgCl2 1, HEPES 10; and intracellular solution (in mM) KCl 120, Na2ATP 5, MgCl2 2, HEPES 10, EGTA 1-10, or BAPTA 1-10 D-glucose 10.

We examined the sensitivity of K+ currents to holding potentials, 4-AP and intracellular Ca2+, as well as their roles in spontaneous electrical activity. 4-AP-sensitive currents were present at all ages examined (E8-P3), being down-regulated in taller hair cells during maturation. 4-AP-sensitive currents were more prominent at the basal aspects of the developing cochlea. Surprisingly, the kinetics of the current depended on intracellular Ca2+ handling. The 4-AP-sensitive current decayed faster in 1 mM compared to 10 mM pipette-BAPTA. To further test the involvement of intracellular Ca2+ signaling, we recorded TEA-insensitive but 4-AP-sensitive K+ currents in the hair cells from Crooked Neck Dwarf (CND) chickens, which have a genetic mutation in ryanodine receptor 1 (RyR1). These animals displayed reduced 4-AP-sensitive current compared to control animals. We will present data that demonstrates: 1) the pattern of expression of 4-AP sensitive currents in developing hair cells 2) the role of 4-AP-sensitive current in spontaneous electrical activity, and 3) the modulation of 4-AP-sensitive current by intracellular Ca2+.

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297 Effect of Salicylate on Potassium Conductances of Guinea Pig Outer Hair Cells

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Salicylate induces tinnitus and a loss of acoustic sensitivity, which has been attributed to reduction in the electromotility of outer hair cells (OHCs) with extremely high salicylate levels. The effect of salicylate has not been well studied on membrane conductance of OHCs. In this current study with whole cell recordings, we found that salicylate had substantial effects on $I_{K,n}$ and resting potential of isolated OHCs at relatively lower concentration levels, which may contribute to its ototoxic effect. 1) Salicylate caused a reversible and dose-dependent reduction in $I_{K,n}$ at holding potential of -40 mV by 10.5% (10^{-6} M) 19.2% (10^{-5} M), 29.9% (10^{-4} M), 31.4% (10^{-3} M) and 33.5% (10^{-2} M). Salicylate caused a reduction in the

current at all testing voltages in I/V plots. The reversal potential (Vr) of net salicylate current was ~ -80 mV, indicative of a K selective current. A KCNQ specific blocker, linopirdine (10^{-4} M), completely blocked salicylate-evoked current reduction. In current clamp recordings, salicylate (10^{-4} M) caused a depolarization of OHCs by 7.9 mV (from -73.2 to -65.3 mV). Our data suggests that salicylate blocked $I_{K,n}$, most probably KCNQ4, and accordingly depolarized OHCs, which may reduce the driving force for transduction current and electromotility. 2) Interestingly, when $I_{K,n}$ was decaying and OHC became depolarized from a normally polarized status, salicylate (10^{-2} M) produced a complete repolarization or even hyperpolarization by ~65 mV (from – 25 mV to -80 mV), which was completely reversible and repeatable. At this condition, salicylate caused an increase of the currents at all testing voltages in I/V plots, and Vr of the net salicylate-evoked current was ~-80 mV, suggesting an activation of a K conductance. The data may correlate with increased electromotility by long term salicylate use (Huang 2005 J Neurophysiol) and protection of aspirin against gentamicin ototoxicity (Chen 2007 Hearing Res). Supported by NIDCD DC 00105 and DC 00141.

298 Ionic Current Associated with the Expression of Motor Protein Prestin

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Prestin is now established as the outer hair cell motor. Analysis of its amino acid sequence suggests that Prestin belongs to the SLC26 family of anion transporters. However, it is widely believed that mammalian prestin lacks the ability to transport anions, in contrast to non-mammalian prestin orthologs, which have been shown to induce a current when expressed in HEK cells. Instead it has been hypothesized that mammalian prestin has a hemi-movement of anions that traps anions in the membrane. These trapped anions are thought to act as its voltage sensor and also give rise to prestin's signature voltage-evoked gating charge movement detected as a non-linear capacitance. In this work we demonstrate that prestin-expressing cells possess a current that resembles the current in the related family members SLC26A7 and SLC26A9 in HEK cells. Moreover, data collected from our prestin-HEK cell lines indicates that there is a linear correlation between the size of the current and the size of prestin-induced gating charge movement. The maximum amount of current in these cells was approximately 400pA, which compared favorably to currents in cells expressing the non mammalian ortholog of prestin from zebrafish. These data require further rethinking of the extrinsic voltage hypothesis.
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299 Is There an Intrinsic Voltage Sensor in Prestin?

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Prestin, a member of the SLC26 anion transporter family, is responsible for voltage-driven electromotility in the mammalian outer hair cell. It's been hypothesized that intracellular anions serve as extrinsic voltage sensors for this molecule (Oliver et al., 2001). This hypothesis is based on 1. The supposed lack of an intrinsic voltage sensor indicated by the preservation of unitary charge (z) when mutating individual charged residues in prestin that are absent in SLC26A6. SLC26A6 is prestin's closest relative that also lacks charge movement. 2. The supposed lack of anion transport in prestin. To investigate the possibility that prestin possesses an intrinsic voltage sensor, we individually neutralized 22 charged residues in or in close proximity to the predicted transmembrane domains in prestin, which are also conserved in SLC26A6, and evaluated their functional characteristics (nonlinear capacitance) by whole-cell recording. We found that 12 out of 22 charged mutants significantly reduce z, suggesting that prestin possesses an intrinsic voltage sensor. (Supported by NIDCD DC 007894, DC 000273 and DC 008130)

300 Structure-Function Relationship of Prestin: From Evolutionary Insight

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Prestin (SLC26A5) is the motor protein of mammalian cochlear outer hair cells (OHCs) with unique capability to perform direct, rapid, reciprocal electromechanical conversion. During evolution, the ion transport capability, typical of SLC26A members, was replaced by an innovation unique to the mammalian OHCs, the voltage-dependent motility. The minimal essential motif for the electromotility motor (meEM) was identified through the amalgamation of comparative genomic, evolution, and structural diversification approaches. Within the highly conserved meEM motif two regions, which are unique to all therian species including monotremes and marsupials, appear to be the last features derived in the therian SLC26A5 peptides. Previous studies have examined the structure-function relationships using point mutations, N- and C- termini truncations, and chimera constructions with paralogue sequences. In order to test the functional properties of the evolving meEM, chimera proteins were constructed from different portions (N- and C- termini and the putative meEM domain) of gerbil prestin, chicken and zebrafish SLC26A5 orthologous. Additional constructs were prepared with swapping of these regions among the zebrafish, chicken and the gerbil prestins. Their heterogenic expression, membrane-targeting, nonlinear capacitance (NLC) and transporter current on HEK 293 cells were examined and compared.
Our data showed differences on intracellular expression, membrane-targeting and changes on NLC properties within these chimera proteins, which together with the

evolutionary sequence differences have given us some new insights into the structure-function relationship of prestin.
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301 Guinea Pig Prestin Sequencing, Cloning and Functional Testing

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Outer hair cell (OHC) electromotility is an active cochlear amplifier, responsible for active cochlear mechanics in mammals to increase auditory sensitivity and frequency selectivity. Prestin is a motor protein of OHC electromotility. The prestin gene has been cloned from many mammalian species, such as mouse, rat, gerbil, and human. Guinea pig is a common animal model used in hearing study, especially in study of inner ear functions. However, the guinea pig's prestin gene has not been cloned yet. In this study, we cloned guinea pig's prestin and tested its function by patch clamp recording. We used a PCR based method to clone guinea pig prestin gene from the adult guinea pig inner ear cDNA library. We found that the guinea pig prestin sequence is highly conserved, demonstrating high homogeneity to rat prestin gene. The aligned score is greater than 90%. We also transferred guinea pig's prestin into HEK 293 cells to do functional testing by patch clamp recording. Patch clamp recording showed that OHC electromotility associated nonlinear capacitance was recordable, indicating that the cloned guinea pig prestin has functional expression. This study provides a useful tool to investigate OHC electromotility since most OHC electromotility data are obtained from guinea pigs.
* Student in Lafayette High School Pre-engineering Program in University of Kentucky
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302 Prestin-Associated Charge Transfer: Voltage- And Frequency-Dependence

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Prestin is crucial to the amplification and frequency selectivity of the mammalian ear. It plays a central role in the outer hair cell membrane motor complex that generates forces, dimensional changes, and electric charge transfer in response to changes in the cell membrane potential. The protein performance is critically dependent on intracellular chloride ions, and intrinsic protein charges also play a role. We propose an electro-diffusion model to describe the frequency and voltage dependence of prestin-associated charge transfer. The movement of the charge (including anion and protein charges) across the membrane is described with a Fokker-Planck equation in terms of the probability density of charge transfer as a function of the position and time. This equation is coupled to a kinetic equation that describes the binding of chloride ions to prestin. We find a voltage-and frequency-dependent phase shift between the transferred charge and the applied electric field that determines capacitive and resistive components of the transferred charge. The phase shift monotonically decreases from zero to -90 degree as a function of frequency. The capacitive component as a function of voltage is bell-shaped, and decreases with frequency. The resistive component is bell-shaped for both voltage and frequency. The capacitive and resistive components are similar to those in experimental measurements of membrane charge transfer at high frequencies. The revealed nature of the transferred charge can help to better understand the high-frequency electrical and mechanical abilities of the outer hair cell, and is important for further analysis of the structure and function of prestin.
The work is supported by NIDCD grant DC000354.

303 Deprotonation of Docosahexaenoic Acid Is Responsible for a Hyperpolarizing Shift of Prestin-Associated Charge Movement

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Docosahexaenoic acid (DHA) is an ω-3 polyunsaturated fatty acid that modulates the function of a variety of membrane proteins including ion channels and rhodopsin. It is an essential fatty acid that is enriched in fish oils and often credited in improving cardiac health and being protective against several neurological pathologies, but its role in hearing is unknown. We have shown that increasing either cholesterol or DHA in the membrane of prestin-expressing human embryonic kidney (HEK) 293 cells results in a hyperpolarizing shift in the voltage at peak capacitance. Cholesterol is uncharged and is thought to mechanically modulate the function of membrane proteins. At physiological pH, the hydroxyl group in the hydrophilic head region of DHA is deprotonated. The purpose of this study is to investigate whether the DHA induced voltage shift is dependent on its negative charge. Prestin-expressing HEK 293 cells were incubated with either DHA or methylated DHA, an esterified version of DHA wherein the proton of the hydroxyl group is substituted by a methyl group. Measures of prestin-associated charge movement demonstrated a significant hyperpolarizing shift in the voltage at peak capacitance when incubated in DHA (-82 mV), whereas there was no change in the presence of methylated-DHA (-76 mV), as compared to untreated, prestin expressing HEK cells (-75 mV), nor was there any measurable nonlinear capacitance in the absence of prestin, regardless of incubation media.
Our results demonstrate that deprotonation is required for DHA to modulate prestin-membrane interactions, indicating a lipoelectric effect, which contrasts with the lipomechanic effects of cholesterol. This work is supported by Grant T32 DC007367 (WMG), the W. M. Keck Center for Interdisciplinary Bioscience Training (LR), RO1 DC00354 (WEB, BF, FAP), RO1 DC008134 and NSF BES-0522862 (FAP).

304 Quantitative Relationship Between Prestin Levels and the Cochlear Amplification Gain Using a Hypomorphic Prestin Mouse

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The outer hair cells contribute to the cochlear amplifier by a cycle-by-cycle feedback mechanism, which enhances the BM motion. Cochlear modeling predicts a nonlinear relationship between this feedback efficiency and amplification gain. Thus, one would predict a nonlinear correlation between electromotility and the gain. A previous study showed that prestin heterozygous knockout (*prestin+/-*) mice displayed an up-regulation of prestin and 'wildtype-like' amplification. In vivo, intermediate levels of prestin expression and the degree of amplification relative to the electromotility have not been reported. Therefore, it is imperative to establish a quantitative relationship between the amount of prestin, electromotility, and amplification gain.

In order to provide evidence of the extent of which prestin-based electromotility drives amplification, we created a novel hypomorphic allele, *Prestin*^{neo}. We inserted the neomycin selection marker in intron 6 of the *Prestin locus* attempting to uniformly reduce the prestin mRNA in all OHCs. Real time RT-PCR analysis showed a significant reduction of mRNA levels from *Prestin*$^{neo/-}$ cochleae. This amount represents approximately half of the mRNA levels seen in previous *Prestin+/-* studies. Compared to wild-type mice, no significant change in *Prestin*$^{neo/neo}$ OHC length were observed. Whereas, we observed a significant reduction of *Prestin*$^{neo/-}$ OHC length in the basal turns. Isolated OHCs from *Prestin*$^{neo/neo}$ and *Prestin*$^{neo/-}$ cochleae exhibited a reduction of the maximum transfer charge movements to intermediate levels compared to *Prestin-/-* and wild-type mice. ABR thresholds increased to intermediate levels in *Prestin*$^{neo/neo}$ and *Prestin*$^{neo/-}$ mice. The increases appeared more pronounced in the basal high-frequency range over the apical low frequency range. These results provide crucial intermediate data points that allow us to correlate prestin activity, electromotility, and amplification gain in vivo.

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305 Voltage-Dependent Interactions of the Outer Hair Cell Motor Protein Prestin

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Outer hair cells (OHCs) possess the unique ability to undergo somatic length change in response to sound evoked alteration of transmembrane potential through a process termed electromotility. The acute sensitivity and frequency selectivity of mammalian hearing is dependent upon this process. Electromotility is driven by the transmembrane motor protein prestin that undergoes a conformational change in response to transmembrane potential changes. Previous work demonstrates that prestin exists in multimeric states in the OHC and when exogenously expressed in mammalian cells. However, which states predominantly exist in vivo and which are functionally important is currently uncharacterized. Furthermore, a role, if any, for prestin oligomerization in its voltage-dependent motor function has not been defined. Towards this goal, we explore the role of prestin-prestin interactions by measuring fluorescence resonance energy transfer (FRET) as a function of transmembrane voltage in HEK293 cells co-expressing prestin-CFP and prestin-YFP C-terminal fusion proteins. Our data show that prestin-prestin FRET decreases with depolarization over the operating range of voltages relevant to electromotility. Prestin-prestin FRET reaches saturation at depolarized voltages and preliminary data suggest the same at hyperpolarized voltages. Interestingly, when the FRET transition is modeled by a two state Boltzmann function, the valance of the fit closely agrees with the valance obtained from prestin nonlinear capacitance measurements. Our data suggest that voltage-dependent FRET is due to changes in prestin-prestin interactions within or between oligomers. Whether the FRET changes result from voltage-dependent conformational changes or shifts in the distribution among prestin oligomeric states is currently being explored. This work is supported by grants DC008134 (RMR, FAP) and DC00354 (FAP).

306 Mutation of Conserved Cysteines in Mammalian Prestin

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Several prestin knock-in and knock-out mouse lines have demonstrated the importance of the protein prestin in mammalian hearing. Despite this, the structure-function relationship of prestin remains largely unknown, with even some of its gross features in dispute. Several studies have suggested that prestin forms homo-oligomers that may be stabilized by disulfide bonds, making cysteines important residues to examine. There are three cysteines (C124, C196, C415) that are conserved in mammalian prestin but not conserved in non-mammalian prestin or in prestin paralogs. Two of these (C124 and C196) are located near the conserved sulfate motif. Mutation of C196 to alanine in gerbil prestin had no affect on prestin non-linear capacitance, measured in HEK 293 cells, while mutation of C124 to alanine caused a dramatic negative shift of $V_{1/2max}$ without effect on *z*. To test the possibility that this shift was caused by either a loss of hydrogen bonding or changes in steric hindrance, C124 was replaced with serine, which is slightly smaller but more polar (i.e. has stronger hydrogen bonding properties). The C124S mutation did not rescue the electrophysiology of the wild-type gerbil prestin, suggesting that the shift in non-linear capacitance is dominated by the cysteine's nucleophilic properties rather than either hydrogen bonding or steric aspects. Thus we suggest that C124 forms a disulfide bond to stabilize the tertiary or quaternary structure of prestin.

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307 Not All Cysteines Are Created Equal in Prestin

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Prestin is a member of SLC26 anion transporter family responsible for generation of electromotility in the outer hair cell. Mammalian prestin contains nine cysteine residues, seven of which are located in the hydrophobic transmembrane core region. Data from previous studies showed that mutants of individual cysteine residues within the protein did not adversely affect its function. To test if combinations of cysteine mutations affect the functionality of prestin, we generated multiple cysteine mutants and tested NLC. Some mutants, like C52S/C124S/C679S and C124S/C381S resulted in functional proteins others, like C260S/C415S and C192S/C196S/C395S completely abolished NLC. However, when either C192 or C196 were mutated individually, the resulting C192S/C395S and C196S/C395S mutants were functional, suggesting that C192 and C196 could compensate each other. Although individual mutation of C415 resulted in a normally functional protein, mutation of this residue with any other cysteines resulted in a loss or severe reduction in NLC. Thus, C260S/C415S,C381S/C415S,C395S/C415S, and C192S/C196S/C415S all showed low or no NLC, which could be explained by a special role that C415 plays in holding functional prestin molecule together and/ or targeting the protein to the membrane. All these mutants associated with C415 showed marked reduction in surface expression determined by confocal microscopy. The preservation of near normal NLC in C192S/C381S, and separately in C260S/C395S could be interpreted as due to the formation of two separate intra-subunit disulfide bonds by each of these mutant pairs (C260-C395 and C192-C381). In this interpretation, removal of an individual disulfide bond would not be catastrophic although the disruption of both disulfide bonds would be fatal to its function. In addition, we examined whether mutating cysteines, would affect prestin's ability to form dimers. The fact that dimers were detected in western blot experiments from both WT prestin and prestin with all 9 cysteines mutated (All9) even in the presence of harsh reducing and denaturing agents like urea and EDT, suggests that cysteines are not necessary for prestin dimer formation. (Supported by NIDCD DC 007894, DC 000273 and DC 008130)

308 Modulation of Prestin's State by Efferent Neurotransmitter

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Prestin's signature electrical response, nonlinear capacitance (NLC), is sensitive to a number of perturbations including membrane tension, temperature, and membrane holding potential (Santos-Sacchi and Song, 2007 ARO). The phenomenon involves a shift in the state probability of prestin along the voltage axis and a change of susceptibility to salicylate insult. Thus we suggest that prestin's anion binding affinity depends on its state. Also recently, an important role for intracellular Cl has been demonstrated (Oliver et al, 2001; Rybalchenko and Santos-Sacchi, 2003; Song et al., 2005). We have shown that Vpkcm of NLC shifts in response to changes in membrane holding potential, and such shifts depend on the level of intracellular chloride (Song and Santos-Sacchi, 2008 ARO). This interplay between membrane potential and intracellular chloride can be a key modulator of cochlear amplification.

In an effort to simulate the in vivo condition, we monitored changes of OHC's resting potential and NLC shift by switching between whole cell current and voltage clamp. ACh (100 uM) and GABA (100 uM), as well as their antagonists were perfused on isolated OHCs. We find that ACh and GABA perfusions interactively impact on OHC motor function. In the 160 mM Cl bath condition, perfusion of ACh hyperpolarizes the OHC. Simultaneously, a depolarizing shift of Vpkcm is observed. Perfusion of GABA alone does not change membrane potential under whole cell current clamp. However, simultaneous perfusion of ACh and GABA results in an enhanced hyperpolarization over ACh perfusion alone.

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309 Modulation of Prestin's Function by CFTR

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CFTR (cystic fibrosis transmembrane conductance regulator) is known to activate Cl- and OH-/HCO3- transport by members of the SLC26 family, including DRA (SLC26A3), PDS (SLC26A4), and PAT1 (SLC26A6). We confirmed that CFTR protein is present in the lateral membrane of outer hair cells (OHCs) where prestin is expressed. Hence, it is of interest to determine if prestin's function is modulated by CFTR. In our preliminary study using a mammalian cell line transiently expressing both prestin and CFTR, we found that the voltage-dependent nonlinear charge displacement (Qmax) by prestin was higher in cells treated with forskolin. For a closer look at the qualitative/quantitative nature of the CFTR effect, we measured time-dependent Qmax in individual HEK cells expressing prestin and CFTR with a whole cell configuration during the activation of CFTR by cAMP/ATP directly applied through the recording pipette. In each cell, we found an increment in Qmax accompanied by a decrement of the membrane resistance due to the activation of CFTR with a time lag ~10 sec. The time lag suggests that the increment in Qmax was induced by sequential cellular events, i.e., increment of cAMP, PKA activation, phosphorylation of CFTR, and interaction of the activated CFTR with prestin. We did not observe an increment in control cells expressing only prestin. The degree of Qmax enhancement differed from one cell to another, implying an activation mechanism by a stoichiometric direct prestin-CFTR interaction, which was supported by co-immunoprecipitation assays using OHCs and transfected cells. Finally, no CFTR was found in the

lateral membranes of OHCs derived from prestin-KO mice. This result further supports the idea that prestin and CFTR directly interact with each other in the OHC lateral membrane. [Supported by NIH Grants DC00089, DC006412 and The Hugh Knowles Center].

310 SLC26A Transporter Carboxy-Terminal Domains Bind to Calmodulin in the Presence of Calcium

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Bioinformatical and biochemical data demonstrate that the carboxy-terminal domains (CTD's) of SLC26A transporters contain a ~70 residue intrinsically-disordered region (IDR). One of the common roles for IDR's with particular sequence characteristics is binding to the ubiquitous and well-studied calcium-binding protein calmodulin. Using two independent in silico calmodulin binding site predictors, we found that the SLC26A transporter IDR's possess the requisite calmodulin binding sequence properties, and hence are potential substrates for calmodulin binding. We therefore assayed biochemically the interaction between several purified SLC26A transporter CTD's and calmodulin, and found that the CTDs and calmodulin interact, and that the interaction requires calcium ions. This suggests a possible generalizable calcium-based regulatory role for calmodulin in the entire family of SLC26A transporters, and in the case of prestin (SLC26A5), may provide insight into the role of calcium in the regulation of sound amplification in the cochlea.

311 Ionic Amphipaths Affect Prestin Self-Association

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The electromotile activity of outer hair cells (OHCs) is necessary to achieve frequency selectivity and sensitivity in mammalian audition. Electromotility is generated by the transmembrane protein prestin and is sensitive to amphipathic compounds including salicylate, chlorpromazine (CPZ) and trinitrophenol (TNP). Prestin has been shown to oligomerize in human embryonic kidney (HEK) cells under normal conditions, but the effect of amphipaths on prestin self-association is unknown. Ionic amphipaths are also known to induce observable membrane curvature changes in erythrocytes, and recent work in our laboratory has demonstrated their ability to induce nanoscale changes in membrane curvature in OHCs. In this work, we use acceptor photobleach fluorescence resonance energy transer (apFRET) to study the effects of salicylate, CPZ and TNP on prestin self-association in HEK cells. Our results demonstrated that in cells exposed to CPZ, TNP, and salicylate, there was a decrease in prestin self-association corresponding to our previously reported increase in plasma membrane curvature. These observations suggest that membrane bending may reduce prestin self-association in the plasma membrane and thereby affect electromotility in OHCs exposed to ionic amphipaths.

This work is supported by an NSF CAREER Award (RMR) and NIH grant DC008134 (RMR).

312 Prestin Is Mechanically Independent to Form Tetramers in the Outer Hair Cell Lateral Membrane

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Prestin is the motor protein of cochlear outer hair cells (OHC) with a unique capability to perform direct, rapid and reciprocal electromechanical conversion. Prestin consists of 744 amino acids with a molecular mass of ~81.4 kDa. The predicated membrane topology and molecular mass of a single prestin molecule appear inadequate to account for the size of intramembrane particles (IMPs; ~11 nm) expressed in the OHC lateral membrane (LM). Recent biochemical evidence suggests that prestin forms oligomers, most likely as a tetramer in the heterologous system. However, the oligomeric structure of prestin in OHCs remains unclear. We calculated the moving charge density in the LM of adult gerbil OHCs by measuring the cell's nonlinear capacitance (NLC), showing that the average charge density (22608 μm^{-2}) is four times the average density of IMPs (5686 μm^{-2}) obtained from the existing freeze-fracture data (one-sample *t* test: $P = 0.50$). This indicates that each IMP may represent a tetramer of four prestin subunits based upon the assumption of each prestin molecule transferring one elementary charge. To determine whether the prestin tetramer (i.e., a single IMP) functions as a mechanical unit for OHC electromotility, we compared the values of slope factors of the NLC and motility data simultaneously measured from the same OHCs. If each prestin subunit in the tetramer is mechanically independent (i.e., *valence* = 1), the slope factors of the two should be the same. If, however, four prestin subunits constitute an integrated mechanical unit (i.e., *valence* = 4), the value of the slope factor of motility should be 4 times that of NLC. Our results showed that the slope factors between motility and NLC are not statistically different (Student's *t* test: $P = 0.58$), suggesting that prestin subunits in the tetramer are mechanically independent contributing to the electromotility of OHCs.

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313 Immunohistochemical Localization of Prestin in the Paraffin-Embedded Elderly Human Cochlea

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Introduction:

Prestin, a motor protein of the outer hair cells (OHCs), is a member of a distinct family of sulfate/anion transporters, SLC26A5, and presumably responsible for electromotility. Prestin is immuno-localized in OHCs in animal models. However, it is still unknown whether prestin is also localized in the human OHCs. We aim to study immunohistochemical localization of prestin in the human paraffin-embedded temporal bone sections.

Material and Methods:

Four temporal bones were removed at the time of autopsy from 80-year-old male and 87-year-old female elderly patients. Their otological conditions were not available. The bones were fixed in buffered 20% formalin, decalcified for 9 months in EDTA, trimmed to a cube of 15 mm, embedded in paraffin, and cut at a thickness of 6 micro m. Thin sections were stained with hematoxylin and eosin, and immunostained with a commercially available primary antibody against prestin (goat polyclonal antibody, Santa Cruz Biotechnology, Santa Cruz, CA) and a polyclonal anti-prestin antibody kindly offered from Dr. Kubo, National Institute of Physiological Science, Okazaki, Japan.

Result and Conclusion:

The cytoplasm of OHCs was intensely positive by both antibodies. The inner hair cells, supporting cells, stria, spiral ligament, or spiral ganglion cells were all negative. No cells or tissues throughout the body were also negative on tissue array sections. In conclusion, the present study provides the first convincing evidence that prestin is only expressed in the human OHCs and its expression is essentially preserved in the elderly.

314 Mechanical Power Output of Outer Hair Cell Somata

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Mammalian outer hair cells (OHCs) are endowed with an extremely fast motor that compels shortening of soma upon depolarization of the lateral wall membrane. Function of the motor is dependent upon expression of the transmembrane protein prestin. Under physiological conditions the somatic motor is powered by electro-chemical potential energy between the endolymph and the OHC intracellular space. This battery drives transduction current into the cell via the mechano-sensitive transduction (MET) channels in the stereocilia and leads to mechanical power output through somatic length changes driving against the cochlear load. Piezoelectric-like models of the multi-component lateral wall capture salient features of electromotility including the signature voltage-dependent capacitance, as well as the weak frequency dependence of the voltage-driven isometric force and displacement. In the present work we analyze the temporal characteristics of power conversion by OHCs with specific attention to the timing of peak power output, and force output, relative to peak hair bundle displacement. Results are presented in both the time domain for step hair bundle displacements, and it the frequency domain for sinusoidal hair bundle displacements. Adaptation of the MET current to maintained bundle displacements is included. The analysis predicts that the maximum power output of a given OHC is likely to be tuned to a best frequency that maps to the cochlear place principle. Timing of the force predicted by the analysis also matches requirements of the cochlea for amplification. Results provide specific biophysical mechanisms through which single OHCs may achieve appropriate timing and intrinsic tuning to contribute to the sharp frequency selectivity in the living cochlea. [Supported by NIDCD R01 DC004928]

315 Cochlear Outer Hair Cells in a Dominant-Negative Connexin26 Mutant Mouse Preserved Non-Linear Capacitance with Impaired Distortion Product Otoacoustic Emission

Akira Minekawa[1], Yuya Narui[1], Ayako Inoshita[1], Takashi Iizuka[1], Takahisa Abe[2], Seiji Kakehata[2], Hideichi Shinkawa[2], Takuji Koike[3], Katsuhisa Ikeda[1]
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Mutations in the connexin26 gene *(GJB2)* are the most common genetic cause of congenital bilateral non-syndromic sensorineural hearing loss. We previously reported transgenic mice carrying human Cx26 with R75W mutation that was identified in a deaf family with autosomal dominant negative inheritance (Kudo et al., 2003). The auditory brainstem response of R75W transgenic mice revealed severe to profound hearing loss throughout postnatal development, indicating the disturbance of auditory organ development whereas endocochlear potential was remained within a normal range. Electron microscopy showed that the organ of Corti (OC) was collapsed without detection of the tunnel of Corti (TC) and Nuel fs space from the birth (Inoshita et al., 2008). Although the supporting cells, especially pillar and Deiters f cells, were characteristically deformed, the hair cells were confirmed to be present but seemed to be peculiar. Although Cx26 defect in the *Gjb2* transgenic mouse is found to be restricted to the supporting cells, it is difficult to explain the reason why the auditory response is extensively disturbed in spite of the presence of the OHCs. The present study was designed to evaluate developmental changes of the in-vivo and in-vitro function of the OHC together with the fine ultrastructure of the OHC and its adjacent supporting cells in the R75W transgenic mouse, which would contribute to a better and accurate understanding of the functional properties of the supporting cells as well as the molecular and physiological mechanisms of Gjb2-based deafness.

In this study, no detectable response of DPOAE at any frequencies were observed in R75W transgenic mice throughout postnatal days in spite of the presence of OHCs. And we demonstrated in the accompanying paper that development of OHC motility function is not affected R75W transgenic mice.

316 Developmental Expression of the Outer Hair Cell Capacitance in a Dominant-Negative Connexin26 Mutant Mouse

Takahisa Abe[1], Seiji Kakehata[1], Akira Minekawa[2], Rei Kitani[1], Takahiko Nagaki[1], Ayako Inoshita[2], Takashi Iizuka[2], Hiro-Oki Okamura[2], Katsuhisa Ikeda[2], Hideichi Shinkawa[1]
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Mutations in the connexin26 gene (*GJB2*) are the most common genetic cause of congenital bilateral non-syndromic sensorineural hearing loss. We previously reported on transgenic mice carrying the human Cx26

R75W mutation that was identified in a deaf family with autosomal dominant negative inheritance (Kudo et al., 2003). The auditory brainstem response of R75W transgenic mice revealed severe to profound hearing loss throughout postnatal development, indicating the disturbance of auditory organ development whereas endocochlear potential remained within the normal range. Electron microscopy showed that the organ of Corti (OC) was collapsed without detection of the tunnel of Corti (TC) and Nuel's space from birth (Inoshita et al., 2008). Although the supporting cells, especially pillar and Deiters' cells, were characteristically deformed, the hair cells were confirmed to be present but seemed to be peculiar.

We demonstrated in the accompanying paper that detectable responses of DPOAE were not observed in any frequencies in R75W transgenic mice throughout the postnatal days. Here we investigated development of motor protein activity in the lateral membrane of the mouse outer hair cell (OHC) from postnatal day 8 (P8) to P18 under whole-cell voltage clamp.

Voltage-dependent, nonlinear capacitance (Cv), which represents the conformational fluctuations of the motor molecule, progressively increased during development while Clin, which represents the membrane area of the OHC, showed a relatively small increase with development. The time course of Cv and Clin in R75W transgenic mice and non-transgenic mice showed no significant difference, further showing that they were almost identical to that in C57BL/6J mice (Abe et al., 2007), Morphological study using digitally captured phase-contrast images of developing OHCs showed no significant difference between R75W transgenic mice and C57BL/6J mice. Our results indicate that development of OHC motility function is not affected in R75W transgenic mice.

317 Dopaminergic Signaling in Cochlear Function: Insight from Mice with Targeted Deletion of Receptor Subtypes

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Dopamine is one of several neurotransmitters in lateral olivocochlear (OC) efferent neurons, which project primarily to cochlear nerve fibers in the inner hair cell area. Pharmacological, immunohistochemical and RT-PCR studies suggest cochlear expression of dopamine receptors (DRs); however, the cochlear localization and function of DR subtypes remain unclear.

To assess the role of DRs in cochlear function, we studied the cochlear phenotype in mice with targeted deletion of each DR (D1, D2, D3, D4 or D5). All mutant lines are healthy and fertile, except D1 knockouts, which die by 4 wks. Cochlear sensitivity was characterized by measuring 1) amplitude vs. level functions for ABRs and DPOAEs, 2) the magnitude of DPOAE suppression evoked by electrical stimulation of medial (M)OC fibers projecting to OHCs, and 3) temporary threshold shifts following overexposure to noise. Groups of mice homozygous for the deletion were compared with wildtype littermates.

Baseline cochlear responses were normal in D3 and D4 knockouts via ABR and DPOAEs; however, lower thresholds (5-20 dB) were observed in D1-like knockouts (D1 and D5) for frequencies above 11 kHz, and thresholds were elevated by 5-10 dB in D2 mutants across all frequencies. DPOAE threshold shifts were comparable to ABR shifts suggesting unexpected dopaminergic effects on OHCs via D1, D2 and D5. ABR suprathreshold amplitudes (Wave 1) were significantly higher in D1 knockouts for frequencies below 11 kHz where normal DPOAE amplitude vs. level functions were observed, suggesting D1 effects on IHCs, spiral ganglion cells or synaptic transmission between them. The magnitude of MOC-mediated suppression was unaffected by the loss of DRs in all mutant lines.

Acoustic overexposure (8-16 kHz, 97 dB, 15 min) revealed enhanced vulnerability in D2, D4 and D5 knockouts, with mean threshold shifts ~10 dB larger than wildtypes. In D4 mutants, vulnerability was seen only in ABR, consistent with dopaminergic effects in the IHC area. However, in D2 and D5 lines, vulnerability differences were seen in both DPOAEs and ABRs, again suggesting OHC involvement.

Cochlear localization of the 5 DRs by immunohistochemistry and single-cell RT-PCR is underway to document expression patterns in hopes of clarifying the basis for the unexpected effects on OHC function.

318 Reproductive Hormone Receptors in the Inner Ear of Human and Rodents

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Background: Both men and women suffer from age-related hearing loss, but in men the hearing loss appears to be more profound. There are also well-known sex differences in ABR (auditory brainstem response), where women have shorter latencies then men. However, after menopause, the female values approaches the male values. Women with Turner syndrome (45,X), biologically estrogen deficient, have early rapid age-related hearing loss. On the other hand, there are case reports that hormonal replacement therapy and oral contraceptive use can lead to hearing loss. Such contradictory aspect of the estrogen action are commonly found and may spring from the fact that there are 2 estrogen receptors, but also it could be due to the actions of other sex-hormones, such as progesterone and testosterone.

What explains this difference in hearing? So far, there have not been any published studies on weather there are any other sex-hormone receptors present in the inner ear.

Aim: Are there any sex-hormone receptors, progesterone or androgen receptors, in the inner ear of human and rat cochlea? And if so, where in the inner ear could they be localized?

Methods: Immunohistochemical staining and PCR for progesteron (A and B)- and androgen receptors in the inner ear of female human and rats was performed.
Results: Cytoplasmatic staining with PR-B was seen in the spiral ganglion cells. No visible nuclear staining, of neither progesterone nor androgen receptors, could be found in the inner ear of human cochlea. PCR-results will be presented and discussed.

319 Phosphatidylserine-Expressed Apical Membrane Internalization in Inner Hair Cells of Guinea Pig

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This study explores the segregation of vesicular traffic by membrane component. Using fluorescent Annexin V-Alexa Fluor 488 as a specific marker for the apical membrane phosphatidylserine (PS) of inner hair cells (IHCs), we examined apical membrane PS internalization in IHCs and the effects of temperature, cytoskeleton, and bath-applied extracellular ATP. We found that at room temperature internalized fluorescent vesicles were confined along the cuticular plate and accumulated in regions of the vestigial kinocilium in the IHCs after a 40 min incubation. However, at 37°C, the fluorescent vesicles were transported to the cytoplasm of the apical pole at a distance (~5 - 9 µm). A majority of vesicles concentrated at the subapical compartment to a supranuclear location and aligned with microtubules. No detectable fluorescence signals were seen in the basolateral wall. Disassembly of microtubules and inhibition of F-actin assembly inhibited apical membrane uptake. Inhibition of P2X2 channels by pyridoxal-phosphate-6-azophenyl-2', 4'-disulfonate (PPADS) significantly decreased PS internalization, while activation of P2X2 by ATP increased it. Our results indicate that internalized PS vesicles from the apical membrane in the IHCs traffic along microtubules remain confined in the apical supranuclear domain and are dependent on temperature, cellular cytoskeleton, and apical membrane channel P2X2 activity.
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320 Impairment of SLC17A8 Encoding Vesicular Glutamate Transporter-3 (VGLUT3) Underlies Nonsyndromic Deafness DFNA25 and Selective Inner Hair Cell Dysfunction in Null Mice

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We have identified the vesicular glutamate transporter-3 gene, VGLUT3, as the gene responsible for DFNA25, an autosomal dominant form of progressive, high-frequency nonsyndromic deafness. In two unrelated families, a heterozygous missense mutation, c.632C>T (A211V) was found to segregate with DFNA25 deafness and was not present in controls. In the cochlea, VGLUT3 accumulates glutamate in the synaptic vesicles of the sensory inner hair cells (IHCs) before releasing it onto receptors of auditory nerve terminals. Null mice with a targeted deletion of Slc17a8 exon 2 lacked auditory brainstem responses (ABRs) to acoustic stimuli, although ABRs could be elicited by electrical stimuli and robust otoacoustic emissions were recorded. The number of afferent synapses, spiral ganglion neurons and lateral efferent endings below sensory IHCs were progressively reduced. The remaining ribbon synapses had a normal ultrastructural appearance and underwent normal Ca2+-induced membrane turnover as demonstrated by normal Ca2+ current and exocytic membrane fusion in IHCs. We conclude that deafness in Slc17a8-deficient mice is due to a specific defect of vesicular glutamate uptake and release, and that VGLUT3 is essential for auditory coding at the IHC synapse.

321 Development of an Assay to Detect Serum Antibodies to Recombinant Human Choline Transporter Like Protein 2 (CTL2)

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Choline Transporter Like Protein 2 (CTL2) is a multi-transmembrane protein that is highly expressed in the supporting cells of the cochlea and vestibular system. It has been implicated as a possible target antigen in autoimmune hearing loss (AHL). More than 50 % (37/63) of suspected AHL patients had antibodies that recognized CTL2 in Western blots. A small proportion of the sera that recognized CTL2 were tested against glycosylated and deglycosylated forms of recombinant CTL2, these patients had antibodies to the core protein rather than the modifications. In developing a high throughput assay to detect antibodies against CTL2, we are using the Invitrogen Bac to Bac Baculovirus Expression System to express his-tagged recombinant human CTL2 protein (rHuCTL2). The purified protein will be used in a sandwich ELISA, in which the protein will be anchored by polyclonal antibodies directed against three highly antigenic sites of HuCTL2. Two of the three antibodies recognize only the core CTL2 protein. However, in the Sf9 insect cell system rHuCTL2 is expressed as a triplet of 65-70 kDa in which the 65 kDa band is the core protein and the two higher molecular weight isoforms are differentially glycosylated. In vitro enzyme mediated deglycosylation of rHuCTL2 was successful but expensive. Alternatively, we investigated how the treatment of Sf9 cells expressing rHuCTL2 with tunicamycin would affect these cells and the expression of rHuCTL2. We determined that rHuCTL2 was expressed as a single band at 65 kDa at 72 hours after infection of the Sf9 cells with baculovirus containing rHuCTL2. The deglycosylated protein will be purified using Ni Affinity Chromatography and used to develop a high-throughout sandwich ELISA to detect antibodies against CTL2 in sera from patients suspected of AHL.

322 ACh-Induced Cation Current Is Mediated by Channels Formed Essentially by TRPC3 in Small Arteries in Guinea Pig Cochlea and Other Organs

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Evidence suggest that vascular neuromuscular transmission may include a cholinergic component in vascular beds such as cerebral artery and arterioles in choroid and inner ear. In addition to its endothelium-dependent hyperpolarizing action, acetylcholine (ACh) induces depolarization in vascular smooth muscle cells via activation of a TRP-like non-selective cation channel (NSCC) [Ma et al., 2008]. However, the identity of the NSCC remains to be determined. Using conventional intracellular recording from cells in the cochlear spiral modiolar artery (SMA) and RT-PCR methods on four small arteries, we investigated this NSCC and obtained the following results: 1) Flufenamic acid (30 -100 μM) enhanced ACh-depolarization by ~40% ($p<0.05$) in low resting potential (RP, ~-40 mV) cells, while it inhibited the ACh-depolarization in high RP (~-75 mV) cells. 2) La^{3+} and Gd^{3+} inhibited ACh-depolarization with an IC_{50} of 2.64 and 39.3 μM, respectively, in high RP cells. 3) Chelerythrine (1 μM), a PKC inhibitor, enhanced ACh-depolarization by ~30%. 4) TFP (30 μM), a calmodulin antagonist, enhanced ACh-depolarization by ~40%. 5) Ca^{2+}-free solution enhanced ACh-depolarization by 121% in high RP cells and 83% in low RP cells. 6) RT-PCR analysis showed that, although the mRNA transcripts of both TRPC3 and TRPC6 (the only guinea pig TRPC family members with known complete cDNA sequences) were expressed in the SMA and arteriolar branches of brain (BA), mesenteric (MA) and coronary (CA) arteries, TRPC3 expression level was much higher than that of TRPC6 in all vessels tested, and both TRPC levels were lower in the SMA than those in the BA, MA and CA. Based on data of the channel pharmacology and gene expression, we conclude that ACh-induced depolarization in the arteriolar cells is mainly mediated by activation of a NSCC formed primarily by TRPC3 proteins, although heteromeric channel formation with other TRPC isoforms including TRPC6 remains possible. Supported by NIH NIDCD DC 004716 (ZGJ)

323 TRPML3 Subcellular Localization in the Inner Ear

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TRPML3 is an ion channel and a member of the mucolipin subfamily of TRP channels. Varitint-waddler mice, which possess a mutation in TRPML3, are deaf, exhibit hair cell degeneration, and show vestibular impairment. Our previous results demonstrated that hair cells, marginal cells of the stria vascularis and other cells lining the cochlear and vestibular endolymphatic compartments express TRPML3 mRNA. We also showed that TRPML3::GFP fusion proteins localize to lysosomes and espin enlarged microvilli of LLC-PK1-CL4 epithelial cells, a culture model of hair cells (Nagata, et al, 2008). Here, we used two independent techniques to confirm the subcellular localization of TRPML3 in hair cells. We utilized recently generated antibodies to the N-terminus and C-terminus of TRPML3 together with their appropriate antigenic peptide controls. Simultaneously, we used both adenoviral and adeno-associated viral (AAV) vectors to mediate expression of TRPML3::GFP in auditory and vestibular epithelia. In agreement with our in situ analysis, we found TRPML3 protein expressed in cells that line the endolymphatic compartment, including hair cells and the stria vascularis. On sections and in whole mounts stained with TRPML3 antibodies, we saw prominent staining along the apical region of inner and outer hair cells. In whole mounts infected with viral vectors expressing TRPML3::GFP, we saw GFP expression in stereocilia and in vesicles localized under the cuticular plate in the apical region of the hair cell. While adenoviral vectors specifically infected hair cells, AAV vectors surprisingly infected both hair cells and supporting cells. TRPML3::GFP localized to vesicles in both hair cells and supporting cells. We conclude that TRPML3 is present in apical vesicles of hair cells as well as in stereocilia.

324 Maturation of Synaptic Transmission at the Inner Hair Cell Afferent Synapse

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A comparison of synaptic transmission at inner hair cell afferent synapses of immature and hearing animals was carried out with high resolution using whole-cell patch-clamp recordings at afferent terminals. In the rat hearing onset is around postnatal day (P) 12. We compared pre hearing (P7-12) and post hearing onset (P19-21) excitatory post synaptic currents (EPSCs) from afferent terminals contacting rat apical inner hair cells in excised organ of Corti preparations. All recordings were carried out at room temperature with holding potentials between -84 and -94 mV. After the onset of hearing, EPSC kinetics were significantly faster compared to those recorded before hearing onset: 10-90% rise time: pre hearing onset 0.36 ± 0.09 ms (n = 1807 EPSCs from 8 afferents); post hearing onset 0.136 ± 0.04 ms (n = 1575 EPSCs from 5 afferents) $p < 0.0001$; τ decay: pre hearing onset 1.16 ± 0.13 ms (n = 1807 EPSCs from 8 afferents); post hearing onset 0.529 ± 0.1 ms (n = 1575 EPSCs from 5 afferents) $p < 0.0001$. The median amplitude of EPSCs recorded from afferent terminals of hearing animals was significantly larger than that from immature animals: pre hearing onset 88 ± 72 pA (n = 5468 EPSCs from 8 afferents); post hearing onset 279 ± 184 pA (n = 2120 EPSCs from 5 afferents) $p < 0.0001$. Interestingly, the

range of EPSC amplitudes was similar for the two age groups: 7 to 764 pA pre hearing onset versus 18 to 754 pA post hearing onset. The larger average EPSC amplitude combined with the more rapid kinetics could provide more accurate temporal resolution in the mature afferent terminal, essential for accurate encoding of auditory information.

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325 Rodent Inner Hair Cell Synapses Express NMDA Receptors

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To date, the NMDA receptor activity has only been evidenced in pathophysiological conditions such as: salicylate-induced tinnitus (Guitton et al, J Neurosci, 2003, 23, 3944-3952; Ruel et al., J Neurosci 2008, 28, 7313–7323). We have used here Western blots and immunocytochemistry at the confocal and ultrastructural levels to increase our knowledge on the composition and location of cochlear NMDA receptors as a prerequisite to a better understanding of their implication in the cochlear physiology.

Western blot analysis of cochlear homogenates showed bands at 120 kDa (NR1 subunit), 180 kDa (NR2B) and 150 kDa (NR2D). NR2C could barely be detected at 140 kDa while NR2A was not detected.

The IHC basal poles were surrounded by NR1-immunofluorescent dots. 3D reconstructions clearly showed that these dots were apposed to hair cells. A count on 33 IHCs from the basal and medial turns of 5 cochleae gave 459 NR1-positive dots with a mean of 13.91 ± 0.95 NR1-positive dots per IHC.

At the ultrastructural level, the NR1 and NR2A/B immunoreactivities (NR2B according to our Western blot data) appeared as a dense and dark DAB deposit thickening the postsynaptic membrane facing the presynaptic ribbons. This deposit was still present though the ribbons had disappeared on the presynaptic side. The NR1 immunoreactivity was found on all the 47 synapses from 27 IHCs (9 guinea pig cochleae) we observed. The same observation could be made concerning the NR2A/B immunoreactivity on the 11 synapses observed from 7 IHCs (5 guinea pig cochleae). A careful high magnification examination of these synapses also enabled us to observe a thin DAB deposit on the presynaptic membrane beneath the synaptic ribbon, suggesting the existence of presynaptic NMDA autoreceptors on the IHCs.

In conclusion, our data are in line with previous data suggesting that NMDA postsynaptic receptors on radial dendrites and autoreceptors on IHCs may be the targets of salicylate in the process of induction of tinnitus.

326 Glutamate Transport in the Mouse Inner Ear Is Mediated by the Excitatory Amino Acid Transporter, EAAT5

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Synaptic transmission between hair cells and primary afferent fibres in the inner ear is mediated by glutamate. Type II vestibular and auditory hair cells synapse onto afferent fibres via conventional bouton terminals. In contrast, type I vestibular hair cells are enveloped by cup-like or calyx afferent terminals. The unusual geometry of the calyx, and the tonic release of glutamate by type I hair cells at this synapse means mechanisms must exist to clear glutamate from the synaptic cleft and prevent postsynaptic receptor desensitisation. The exact identity of the glutamate transporter responsible for removing glutamate between type I hair cells and calyx afferents is unclear. Methods: Vestibular organs, cochlea and retina (control) were obtained from mice (overdosed with Ketamine 300 mg/kg), sectioned and incubated in primary antibodies against the glial glutamate-aspartate transporter (GLAST) and EAAT5. Results: GLAST labelling was confined to supporting cells of the vestibular epithelium and organ of Corti as shown previously. Until now, the expression of EAAT5 has only been reported in the retina. Significantly, EAAT5 was expressed in the crista, utricle and organ of Corti. EAAT5 was expressed in both type I and II vestibular hair cells, as well as calyx primary afferent terminals and fibres. In contrast, EAAT5 was only expressed in hair cells of the organ of Corti. Conclusions: EAAT5 is highly expressed in the mouse crista, utricle and organ of Corti. Active uptake of glutamate by EAAT5 by both hair cells and primary afferent fibres may limit glutamate concentration in the synaptic cleft, thereby preventing glutamate receptor desensitisation. The expression of EAAT5 at tonically active glutamatergic synapses such as those in the vestibular epithelium, cochlea, and retina suggests highly efficient glutamate uptake mechanisms have developed to maximise receptor sensitivity.

327 Voltage Dependent Currents in Type I and II Hair Cells and Calyx Terminals of Primary Afferents in an Intact in Vitro Mouse Vestibular Crista Preparation.

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Most physiological studies of the mammalian vestibular crista employ isolated hair cell/afferent preparations. Isolation, however, disrupts hair cells and potential interaction with their associated primary afferent terminals. Purpose: Develop an intact preparation of the mouse crista to study whole-cell currents in hair cells and primary afferents, *in situ*. Methods: Bony labyrinths were isolated from euthanized mice (Ketamine 200 mg/kg; i.p.) and an

intact triad of vestibular organs (anterior, horizontal cristae and utricle) was dissected free and placed into a recording chamber. Whole-cell patch-clamp recordings were obtained from type I and type II hair cells and the cup-like (calyx) afferent terminals that surround type I cells. Results: Outward whole-cell currents in type I hair cells (n = 20; Vm -60 mV) were activated at voltages more positive to -90 mV, whereas currents in type II hair cell were activated at potentials more positive than -60 mV (n = 46; Vm = -60 mV). When compared to data from isolated hair cell preparations, outward currents from some type I cells in the intact preparation differ by revealing a collapsing activation curve, and marked inactivation. Whole-cell recordings from calyx terminals (n = 9; Vm -60 mV) were characterized by a fast transient inward current that activated at voltages more positive than -55 mV, together with outward currents that activated positive to -70 mV. Inward currents were also observed in both type I and type II hair cells and activated negative to -70 mV. Conclusions**:** Our intact *in vitro* preparation preserves the relationship between hair cells and afferents within the crista. Although there are similarities with dissociated preparations, there are important differences in some type I hair cells in the intact preparation. We believe our preparation represents a step towards developing a viable crista preparation to examine information processing between hair cells and primary vestibular afferents.

328 Vestibular Effects of Endolymphatic Ionic and Volume Changes in an Isolated Preparation of a Mouse Labyrinth

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We developed an *in vitro* preparation of the mouse inner ear to examine pharmacological and physiological aspects of vestibular transduction that cannot be easily addressed in whole animal or dissociated preparations (Lee et al., J. Neurosci. Meth. 2005). We have used this preparation to examine the effects of high potassium (K^+) and increased endolymphatic volume on afferent discharge, two factors that may be associated with Ménière's disease. Methods: Mice were anaesthetized (Ketamine 200 mg/kg, i.p.), the bony labyrinths surgically isolated, and anchored in a Ringer-perfused recording chamber. Apertures in the bony labyrinth allowed direct access to the underlying anterior and horizontal membranous ducts and ampullary nerves. An injecting pipette (10-15 µm diameter), attached to a nanoliter pump, was inserted into the semicircular canal duct and used to alter endolymphatic ionic concentrations and volumes. Action potential (AP) discharge was recorded from impaled afferent fibers during manipulation of endolymphatic composition and volume. Results: Injections of high K^+ solution (200 mM KCl, 25 mM $KHCO_3$) caused a 4 to 5 fold increase in discharge rate (n = 10). This was accompanied by diminishing AP amplitude as the afferent depolarized. Within 15 sec to 2 min after injection, AP firing rate and amplitude returned to control levels. While the majority (6 of 10) Injections of "normal" artificial endolymph (140 mM KCl, 25 mM $KHCO_3$), to produce volume changes increased discharge rate in 6 of 10 afferent. However, in 4 of 10 afferent recordings a decreased discharge rate was observed. We are currently testing whether the variable results associated with volume increase is determined by the location of the afferent terminal within the neuroepithelium. Conclusions: Our results suggest that: 1) we can affect afferent activity by altering the ionic composition of the endolymphatic compartment; and 2) volume changes play a significant role in modifying afferent activity.

329 Effects of Lead Toxicity on Cochlear Morphology

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Lead, a heavy metal with no known physiological function in the body, is a persistent environmental toxin that affects several organ systems (Gilbert et. al., 2005). Physiological studies suggest auditory loses both in animals and humans following lead intoxication. However, effects of lead on cochlear morphology is not known. The present study was designed to document the effects of lead toxicity on cochlear morphology in rats at 21 and 30 postnatal day (PND). Pups were culled to 10/litter at birth and exposed to 0.2% lead acetate (E group) via their dams' drinking water from PND 1 to 21 or directly via drinking water from weaning until PND 30. Control group (C) was given regular drinking water. Animals were anesthetized with ether and blood samples were drawn prior to transcardial perfusion with 4% paraformaldehyde and 0.25% glutaraldehyde, in 100 mM phosphate buffer. After dissection cochleas were placed in the same fixative for 6 h, decalcified for 7-10 days, dehydrated in graded ethanol and embedded in paraffin. Five µm serial sections were stained with hematoxylin and eosin and examined on a bright field microscope. Lead level in blood measured by atomic absorption spectrophotometer gave the following results: PND 21C = 47 µg/ml; PND 21E = 133 µg/ml; PND 30C=20 µg/ml; PND 30E= 105 µg/ml. No structural changes were observed in cochleas at PND 21 in lead-exposed rats. At PND 30, in lead-exposed rats, inner hair cells (IHCs) in the organ of Corti showed shrinkage of cell bodies. Vacuolization was observed at their bases, area of synaptic contacts with the peripheral processes of spiral ganglion neurons. These results correlate with physiological changes reported by other investigators (Buchanan et. al., 1999; Tuncel et al., 2002).

330 From the Endolymphatic Sac to the Cochlea

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The endolymphatic sac (ES) and endolymphatic duct (ED) have been thought to provide a bidirectional regulation of endolymphatic pressure. Injections in animals have demonstrated bidirectional flow between ES and scala media due to the overpressure created by the injection. The present study confirms flow from ES to scala media in

human patients. Injections of gadolinium (Gd-DTPA) through the oval window diffuse into the cochlea and MRI images show separate scala vestibule and scala tympani. In the present study we demonstrated in human the diffusion of Gd-DTPA from the endolymphatic sac to the structures of the labyrinth and to scala media. An injection of 0.5 ml cortisone plus 1% Gd-DTPA into the ES in patients with Meniere's disease shows diffusion over 4 days through the saccule and into scala media. MRI imaging shows a single enhanced cochlear canal in contrast to the two separable canals observed from oval window injection. Enhancement of scala media is observed 2 weeks or more following injection. This finding suggests that the ES and the ED could act as a drug delivery access point to the scala media. These data could shed a new light on the pathogenesis of endolymphatic hydrops and its potential therapy.

331 Unitary Current Properties of a Large Conductance K+ Current in the Lateral and Medial Walls of the Cochlear Duct

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The acute sensitivity of the auditory system is conferred partly by the base-line spontaneous activity of hair cells. Moreover, to overcome the intrinsic membrane noise, the sensitivity of hair cells is dependent on the endocochlear potential (EP >80 mV). Thus, EP is indispensable for normal sound transduction. EP is generated by the high throughput of outward K^+ flux across cells of the stria vascularis (StV) and Reissner's membrane (RM) into the scala media by K^+ channels. Consequently, mutations of K^+ channels lining the apical membrane of marginal cells (MCs) result in a reduction in EP and hearing loss (e.g. Jervell and Lange-Nielsen syndrome in humans). To understand the cellular mechanisms for the generation of the EP, we examined the biophysical properties of K^+ channels lining the apical membranes of MCs and RMs.

Whole-cell K^+ currents were recorded using an extracellular solution (in mM, NaCl 145, KCl 5, $CaCl_2$ 0-8, D-glucose 10, $MgCl_2$ 1, HEPES 10), and intracellular solution (in mM, KCl 140, K_2ATP 5, $MgCl_2$ 2, HEPES 10, EGTA 1-10, or BAPTA 1-10 D-glucose 10). Single channel currents were examined in the cell-attached configuration using high external K^+ to clamp the membrane voltage to 0 mV. Unitary current amplitude of a prominent K^+ current in MCs was ~ 12 pA at 40 mV step potential. The unitary conductance was ~200 pS. At the apical portion of the RM, we measured a current with unitary conductance of ~190 pS. We will demonstrate the presence of low-conductance and high-conductance K^+ channel currents in MCs and RMs. The kinetic properties of the channels lining the apical membranes of the lateral and medial walls of the cochlear duct will be delineated. The properties of these K^+ channels are in keeping with sustained-currents that are able to confer the high $[K^+]$, ~150 mM in the endolymph.

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332 Endolymphatic Sodium Homeostasis by Saccular Non-Sensory Epithelium

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The saccule is one of the vestibular organs that transduces linear acceleration to nerve impulses and is situated in the endolymphatic system between the base of the cochlea and the rest of the vestibular labyrinth. It is well established that the utricle, ampullae and common crus contain vestibular dark cell (VDC) epithelia that secrete K, while the saccule is devoid of VDC. In fact, the saccular epithelium, outside of the macular area, has been reported to consist of a single, simple cuboidal cell type [Smith CA., 1970]. Based on these and other observations [Sellick PM et al., 1972], it was proposed that saccular endolymph originates from the cochlea and is not produced in this organ. However, [Na] in saccular endolymph (~3mM) is different from other inner ear organs: higher than in the cochlea (~1mM) and lower than in the utricle (~10mM). In the present study, we sought functional evidence of ion transport by non-sensory epithelium of adult mouse saccule by electrophysiologic and pharmacologic means. A short circuit current, *Isc*, was observed that was directed into the apical side, consistent with cation absorption and/or anion secretion. *Isc* was inhibited by amiloride analogs in the potency sequence benzamil>amiloride>EIPA (the IC_{50} values are 0.06, 0.8, and 8μM, respectively) which is consistent with Na absorption via candidate Na-permeable cation channels, such as ENaC and CNG channels, in the apical membrane. No evidence was found for cAMP-controlled Cl secretion. Our preliminary data also showed that *Isc* was inhibited by a Na/K-ATPase blocker, ouabain (~61%) and by the K channel blockers TEA (~60%), XE991 (~14%), apamin (~25%) and clotrimazole (~19%) but not Ba, 4-AP or glibenclamide. An inhibitor of NKCC, bumetanide, had no effect on *Isc*. These results provide the first direct evidence that the saccule is not merely a "parasite" of the cochlea, but rather that the saccular non-sensory epithelium is involved in vestibular endolymphatic ion homeostasis by absorption of Na from endolymph.

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333 Proteomics Analysis of Perilymph and Cerebrospinal Fluid in Mouse

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Intracochlear drug delivery systems are in direct contact with perilymph and must be designed to function properly in this environment. Proteins in perilymph can adsorb to the surfaces of the implant and may lead to biofouling and changes in delivery profile. Knowledge of protein composition will help anticipate interactions with delivered agents. Previous analyses were performed using

electrophoretic techniques and candidate approaches to identification. In this study, protein composition of perilymph and cerebrospinal fluid (CSF) was analyzed using a capillary liquid chromatography-mass spectrometry based iTRAQ quantitative proteomics approach, searching against a mouse subset of the Uniprot FASTA protein database. Proteomics allows the identification of large numbers of proteins and explicit protein isoforms from small quantities of sample. We adapted Salt et al's (J. Neurosci. Methods, 2006) perilymph collection method to the mouse to minimize CSF contamination and identified over 50 proteins with greater than 99% probability of correct identification. iTRAQ reporter ions allowed determination of relative amounts of proteins between perilymph and CSF samples. Protein in perilymph was on average 2.7 times more concentrated than in CSF. The major protein in perilymph was albumin, followed by a family of protease inhibitors, enzymes, apolipoproteins, complement C3, and serotransferrin. Individual proteins with a high perilymph/CSF (P/C) ratio included murinoglobulin and alpha-2-macroglobulin (P/C ratio ~6), and several apolipoproteins (D and AI and AII) (P/C ratio ~5). In relation to drug delivery, proteins such as albumin have been implicated in biofouling through adsorption to device materials. Enzymes in perilymph may alter the structures of the drugs delivered and other proteins may bind to them making them inaccessible to target tissues. This analysis should prove useful in the design of delivery systems and the choice of delivery agents.

334 P2Y4 Receptor-Mediated Regulation of Amiloride-Sensitive Sodium Transport in the Reissner's Membrane of Gerbil Cochlea

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In the inner ear purinergic receptors are widely distributed along the whole cochlear duct. Ionotropic $P2X_2$ receptors are dominant in the epithelial cells bounding the cochlear duct except in the marginal cells of the stria vascularis where metabotropic $P2Y_2$ and $P2Y_4$ receptors are expressed. Activation of metabotropic purinergic receptors triggers multiple events, including activation of PKC and PLC. The epithelial cells of Reissner's membrane (RM) form much of the boundary between endolymphatic and perilymphatic space in the cochlea and are known to be capable of transporting Na^+ out of cochlear endolymph via epithelial Na^+ channels (ENaC). However, much remains to be known as to mechanism of regulation of Na^+ absorption in the cochlea. In this study, we investigated the effect and mechanism of purinergic signaling on ENaC in the RM of gerbils at the age of postnatal day 21. The vibrating probe technique was chosen to measure transepithelial currents under short circuit conditions.
Results showed that the short-circuit current (*Isc*) toward apical to basolateral direction in the physiologic saline significantly decreased in the presence of amiloride (from -12.1±2.0 to 2.8±0.9μA/cm^2,*n*=6). The addition of UTP in the presence of amiloride showed no change of *Isc.* UTP induced moderate inhibition of the amiloride-sensitive *Isc* from -15.0±2.3 to-7.9±1.8μA/cm^2(*n*=7). The response to UTP was inhibited by 100 μM RB-2, but not by suramin or PPADS. These results point to the P2Y receptor in RM as the $P2Y_4$ subtype. The PLC inhibitor U73122, but not U73343, its inactive analog, inhibited the action of UTP on ENaC. In contrast modulating PKC signaling by PMA (PKC activator) or GF109203 (PKC inhibitor) did not affect purinergic regulation of ENaC. These results indicate that early regulation of ENaC by activation of purinergic receptor is mediated via PLC signaling pathway other than PKC activation.

335 Expression of KCNQ1/KCNE Channels in the Lateral Wall of the Inner Ear

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Alterations in K^+ homeostasis in the cochlear duct have profound effects on the transduction of auditory signals within the cochlea. Indeed, the flux of K^+ through cells of the lateral and medial walls of the cochlear duct is the underlying mechanism for the generation of the battery of the inner ear, the endocochlear potential. To better understand mechanisms involved in the regulation of K^+ in the endolymph, we hypothesized that a cadre of K^+ channels may be involved in K^+ regulation in the cochlear duct. In this report, we have cloned the mouse inner ear-specific KCNQ1 channel and its auxiliary subunits KCNE1-4. Whole-cell K^+ currents were recorded using extracellular solution, (in mM) NaCl 125, KCl 6, $CaCl_2$ 0-8, D-glucose 10, $MgCl_2$ 1, HEPES 10; and intracellular solution (in mM) KCl 120, Na_2ATP 5, $MgCl_2$ 2, HEPES 10, EGTA 1-10, D-glucose 10. Single-channel currents were recorded with extracellular solution (in mM), KCl 120, NaCl 6, $CaCl_2$ 0.5, D-glucose 10, HEPES 10, pH 7.3. K^+ Channels were overexpressed in cultured mammalian cells, and expression was confirmed using immunochemical staining method.
Heteromultimeric complexes of KCNQ1/KCNE1-4 channels or triple-subunit complexes of KCNQ1/KCNE_/KCNE_ channels revealed profound and diverse kinetic and permeation properties of the KCNQ1 channels. We investigated the conductance of K^+ channels composed of the KCNQ1 and 5 different KCNE1-4 subunits; including 2 isoforms of KCNE4, which were reverse transcribed from mouse cochlea mRNAs. Finally, we correlated our functional data with the expression of KCNQ1 and KCNE channels in the cochlear duct. Differential expression of the KCNE1-4 subunits in the cochlear duct provided clues to the potential functions of the KCNQ1 and its auxiliary subunits.
Funded by NIDCD

336 Endolymphatic Acid-Base Balance: Acid Flux from Stria Vascularis

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Disturbance of acid-base balance in the inner ear is known to be associated with hearing impairment or loss in a number of conditions including genetic mutations (e.g.,

Pendred syndrome and hereditary distal renal tubular acidosis) and pharmacologic interventions (e.g., carbonic anhydrase inhibition). Several physiologic and immunohistochemical observations have suggested the involvement of acid-base transporters in stria vascularis, including HCO3 secretion by apical pendrin in strial spindle cells, H secretion by apical H-ATPase in strial marginal cells (SMC), H absorption by basolateral H,K-ATPase in SMC, both apical and basolateral H flux by Na/H exchangers, and H secretion by apical monocarboxylic acid transporters in SMC. We directly measured acid flux from the apical side of isolated stria vascularis in vitro with a pH-selective self-referencing probe. Stria vascularis (without spiral ligament) was separated from the lateral wall of adult C57Bl/6 mice, mounted in a bath chamber on an inverted microscope and superperfused with weakly-buffered physiologic saline at 37oC. Acid efflux was observed that depended on metabolism and on ion transport. The acid flux was greatly decreased by removal of the metabolic substrate (glucose), inhibition of the sodium pump by 1 mM ouabain, inhibition of Na/H-exchange by 100 µM amiloride, inhibition of Na,2Cl,K-cotransporter by 50 µM bumetanide, inhibition of HCO3/anion exchange by DIDS. The flux was increased by inhibition of H,K-ATPase by 1 µM SCH28080, but was not affected by the inhibitor of H-ATPase bafilomycin. These observations suggest that stria vascularis may be an important site of control of cochlear acid-base balance and demonstrate a functional role of several acid-base transporters in stria vascularis, including H,K-ATPase and Na/H-exchange but not H-ATPase. Supported by NIH grants R01-DC00212 and R01-DC01098.

337 Persistance of Dexamethasone Drug Levels and Base to Apex Gradients in the Perilymph of the Scala Tympani After Single Shot Injections Through the Round Window Membrane

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Local drug application to the inner ear is becoming more widely used in experimental studies and clinical applications.

A considerable variability of drug levels in the perilymph of the inner ear could be measured after drug application to the round window niche (e.g. Hahn et al., 2005; Plontke et al. 2007; Plontke et al., 2008).

The administration of drugs to the perilymph using single shot injections through the round window membrane has been shown as a promising alternative approach to deliver drugs quantitatively to the perilymph of the scala tympani (Salt et al., 2007).

We analyzed the pharmacokinetics of dexamethasone, a drug in clinical use for the treatment of inner ear disorders following single shot injections through the round window membrane in the guinea pig. After sequential sampling of perilymph from the apex the prodrug dexamethasone-phosphate and the active moiety dexamethasone-base were measured using HPLC.

Using single shot injections dexamethasone could be delivered to the perilymph of the scala tympani and the variability of drug levels was reduced when compared to round window niche delivery. Short term observations 20 min. after the cessation of the drug application revealed a concentration gradient of dexamethasone from the basal to the apical turn. The peak concentrations of total dexamethasone varied from 206 to 1960 µg/ml (mean: 1101.37 µg/ml, standard deviation: +/- 733.35 µg/ml). After an additional observation time of 1 h a gradient was still present with peak levels of total dexamethasone varying from approx. 93 µg/ml to 1616 µg/ml (mean: 633.08 µg/ml, standard deviation: +/- 714.95 µg/ml).

Future goals include long term evaluations of dexamethasone pharmacokinetics in the perilymph after intracochlear injections and comparison of experimental data with predictions from computer simulations calculated with a three-dimensional model of the guinea pig cochlea.

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338 Perilymph Pharmacokinetics in the Guinea Pig Inner Ear Compared for Local Injections at Different Sites

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Local applications of drugs and other substances to the inner ear are increasingly being used in research and for the treatment of clinical disorders. However, many delivery methods have yet to be characterized quantitatively. Furthermore, in order to interpret pharmacokinetic measurements, a basic understanding of the mechanisms underlying drug dispersal in the ear is necessary. In the present study, we applied the marker TMPA into perilymph by direct injections at three different injection sites, the basal turn of scala tympani (ST), the cochlear apex and the lateral semi circular canal (LSCC). Marker concentration time courses were monitored with TMPA-sensitive electrodes in the basal turn of ST and scala vestibuli. Injection and recording electrodes were sealed in place to prevent perilymph leakage. Failure to effectively seal an insertion site resulted in rapid washout of perilymph by CSF. Measured time courses were interpreted using our established cochlear fluids model. Results were consistent with the injected solutions displacing perilymph through the cochlear aqueduct with a resulting flow from the injection site to the aqueduct. Injections in the basal turn of ST thus provide a spatially restricted perfused region, with subsequent redistribution of TMPA from the basal turn to other areas of the ear predominantly by diffusion. In contrast, injection into the LSCC allowed the perilymph space to be almost completely filled with TMPA, markedly reducing redistribution and producing substantially more stable concentrations with time. The studies demonstrate important issues that must be considered in order to interpret pharmacokinetic studies quantitatively, including:

fluid leaks at perforation sites, CSF entry and flow caused by evaporation from the bone of the exposed cochlea, redistribution between compartments of the ear and clearance from inner ear compartments.
This study supported by NIH/NIDCD grant DC01368.

339 Does an Ototoxic Dose of Cisplatin Accumulate in the Guinea Pig Cochlear Fluid?

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Introduction: Cisplatin is a potent anticancer drug but unfortunately hearing loss is a dose limiting side effect. In a previous study using a non-ototoxic dose of cisplatin (5 mg/kg) we observed that the ratio between the perilymphatic and the blood ultrafiltrate concentration increased from 0.05 to 0.8 during the sampling period (5 – 90 min). In the present study we want to see if an ototoxic dose of cisplatin (8 mg/kg) produces a similar accumulation of the drug in the guinea pig cochlea. We have taken samples up to 4 hours after an intravenous administration of the parent drug to determine if the pharmacokinetic profile can constitute an explanation for cisplatin-induced ototoxicity. Materials and methods: 17 female albino guinea pigs were used in the pharmacokinetics experiment. All animals received a single intravenous dose of cisplatin (8 mg/kg, given 1 ml/minute). Two blood samples, one samples of cerebrospinal fluid (CSF) and one sample of scala tympani perilymph from the right and left cochlea, were drawn at the same target time, within a time range of 30 to 240 minutes (30, 60, 90, 120, 180 and 240 minutes) after administration of the drug. A dorsolateral approach was used to collect scala tympani perilymph and a suboccipital puncture was preformed to aspirate clear CSF from cisterna magna. Liquid chromatography with postcolumn derivatization was used for quantitative determination of the parent drug. Results: The results will be presented at the meeting.

340 Functional and Molecular Expression of Epithelial Sodium Channels in Cultured Human Endolymphatic Sac Epithelial Cells

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The endolymphatic sac (ES) is part of the membranous labyrinth in the inner ear that plays an important role in maintaining homeostasis of the endolymphatic fluid system. However, the exact mechanism of fluid volume regulation is not yet known. We analyzed the molecular and functional expression of epithelial sodium channels (ENaCs) in cultured human endolymphatic epithelial (HESE) cells. The ES specimens were harvested during acoustic neuroma surgery (n=13) using the translabyrinthine approach and were subcultured with high-epidermal growth factor (EGF) (25 ng/ml) media. The serial-passaged HESE cells differentiated into a monolayer of confluent cells and some of the cultured cells had features of mitochondria-rich cells. RT-PCR revealed that ENaC subunits are expressed in the cultured HESE cells. We also confirmed the presence of an ENaC-dependent short-circuit current in the cultured HESE cells. Interestingly, ENaC mRNA expression and ENaC-dependent current decreased after treatment with IL-1β (10 nM for 24 h). These findings suggest that ENaC plays an important role in fluid absorption in the human ES, and its function may be altered during inflammatory conditions.

341 Kidney-Specific Chloride Channels (CLCNK) in the *Xenopus* Inner Ear

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We have probed the *Xenopus* inner ear transcriptome with a *Xenopus laevis* Affymetrix GeneChip® and analyzed expression level data for membrane transport protein probe sets. Among the most highly expressed transport proteins in the inner ear were: gap junction protein beta2 (GJB2), sodium channel non-voltage gated 1 beta (SCNN1B), and chloride channel Ka (CLCNKA). RT-PCR analysis confirmed the microarray expression levels for GJB2, SCNN1B, and CLCNKA. We selected the CLCNKA gene for more detailed structure-function studies because it, and its beta subunit BSND (barttin), have been linked to Bartter syndrome, a renal tubular disorder that can be associated with sensorineural hearing loss (Bartter syndrome Type 4). CLCNKA is a member of the voltage-gated chloride channel (CLC) family and is hypothesized to form 12 transmembrane domains with C and N intracellular termini. We have cloned a 2,155 bp *Xenopus* CLCNKA inner ear transcript using RT-PCR RACE protocols. The *Xenopus* sequence shares 60% protein identity with both human and mouse CLCNKA. We have successfully synthesized two fluorescent fusion constructs, GFP-CLCNKA and CLCNKA-GFP. Using transient transfection methods in cultured cells we are expressing our constructs with the preliminary goal of determining whether expression levels are affected by GFP position relative to the channel terminus. Experiments are underway to clone BSND for *in vitro* co-expression physiological studies. Bioinformatics tools and resources such as ClustalW, the DOE *Xenopus tropicalis* genome, and the San Diego Supercomputer Center Biology Workbench are being used to facilitate sequence curation and analysis, and creation of phylogenetic trees correlating to our highly expressed membrane proteins. Supported by NSF (HRD-0331446) and NIH (GM008136; DC003292; P50GM068762).

342 Reconciling Distortion Product Otoacoustic Emission Theory and Data

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It has been commonly assumed that distortion product otoacoustic emissions(DPOAE) are generated near the F2 place and propagate back to the stapes via a transverse wave that is similar to the conventional forward traveling wave (Mahoney and Kemp, 1995, JASA 97:3721; Mountain et al, 2000, Recent Developements in Auditory Mechanics, World Scientific). This assumption has been challenged by Ren and his colleagues (Ren et al, 2006, J Neurophysiol 96:2785; He et al, 2008, PNAS 105:2729) who argue that the reverse traveling wave hypthesis is not consistent with data from their experiment where motion at the distortion product (DP) frequency was measured simultaneously at the stapes and on the basilar membrane. They suggest that their data imply that the DPOAE travels back via a compressive fast wave rather than via a slow transverse wave.
In an attempt to reconcile these two points of view, we have used a simple one-dimensional nonlinear transmission-line model to explore the generation and propagation of DPs within the cochlea. Although this type of model can not support compressional waves, we find that the model produces DP responses on the basilar membrane and at stapes that are similar to the experimental data. By replacing the nonlinear elements that generate the distortion product with linear elements coupled to active sources (generators), we can analyze the individual contribution of these generators to the DPs. We show that a wide range of cochlea locations can contribute to the DPs measured at stapes, and that it is this distributed nature of DPOAE generation that leads to the stapes and BM vibration data that has been used to support the compression-wave hypothesis.
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343 Do Otoacoustic Emissions Travel in the Cochlea Via Slow or Fast Waves?

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While the slow speed of the traveling wave on the basilar membrane is well established for the forward direction (Békésy 1960), still controversial is whether otoacoustic emissions travel to the stapes via a slow or fast wave (Wilson, Physics Today, April 2008). Ren and colleagues suggest a fast wave is involved (Nat Neurosci 2004; PNAS 2008), but Dong and Olson (2008 JASA) suggest a slow wave is involved. To directly measure the propagation speed of such a reverse wave, the basilar membrane was stimulated at a single point (~12 mm from the stapes) in a human cadaver temporal bone preparation, the velocity of that point and the resulting stapes velocity were measured using a laser Doppler vibrometer, and the group delay of the transfer function was computed. A hole was drilled in the first turn of the scala tympani and an SmCo magnet (<0.1 mg) was placed on the basilar membrane. A coil with a ferrite core was glued to a portion of the opening, less than 2 mm above the magnet, and the remainder of the opening was covered with plastic wrap to maintain the fluid level. The average group delay for the 0.3-5kHz range was around 1 ms, (1.6 ms from 0.3-1kHz and 0.7 ms from 1-5kHz). Since a delay of 1 ms corresponds to a wave speed of approximately 12 m/s (125 times slower than the speed of sound in saline), these results support the idea that otoacoustic emissions travel within the cochlea via a slow wave.

344 Long Delays Without Basilar-Membrane Traveling Waves: Correlations Between Tuning Bandwidth and Emission Delay in a Model of the Gecko Inner Ear

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Although lizards lack the basilar-membrane traveling waves evident in mammals, their ears produce stimulus-frequency otoacoustic emissions (SFOAEs) with latencies comparable to those measured in many mammals (1–2 ms or greater). To probe the origin of these relatively long OAE delays, we developed a model of SFOAE generation in the gecko. The model inner ear comprises a collection of gammatone filters (representing the hair bundles and associated tectorium) whose effective damping manifests a small degree of irregularity. The model reproduces the major qualitative features of gecko SFOAEs, including their substantial delays. The SFOAE delays predicted by the model increase with the assumed sharpness of tuning, reflecting the build-up time associated with mechanical resonance.
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345 Comparisons Among Stimulus-Frequency Otoacoustic Emission Phase-Gradient Delays

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Measurements of stimulus-frequency otoacoustic emission (SFOAE) phase-gradient delays have been used to test models of emission generation, determine round-trip propagation times, and estimate the sharpness of cochlear tuning. Phase-gradient delays are defined as the slopes of phase-versus-frequency functions. For SFOAEs, phase-versus-frequency functions can be obtained with at least two different methods. Traditional compression- and suppression-based methods of measuring SFOAEs use the phase of the nonlinear residual as a function of probe frequency (e.g., Shera and Guinan 2003). Phase-gradient delays can also be obtained by using an additional tone (e.g., a bias tone) or multitone complex to modulate the amplitude of the SFOAE and then measuring the phase of the modulation as a function of the modulation frequency (e.g., Meenderink and van der Heijden 2008). Simple

models suggest that these two methods of measuring SFOAE-associated phase-gradient delays differ systematically, depending on quantities such as the relative magnitudes of forward and reverse propagation delay. Here we compare values of these different phase-gradient delays measured at the same frequencies in the same ears.
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346 Measuring the Travel Time of Distortion Products from Their Cochlear Site of Origin to the Ear Canal

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Cochlear nonlinearities can produce distortion products (DPs) when two or more tones are presented together. Part of the DP energy travels back to the ear canal, and can be measured as DP-otoacoustic emissions (DPOAEs) by a sensitive microphone. We developed a method for the precise measurement of the time it takes for the DPs to travel from their intracochlear site of origin to the ear canal. We simultaneously recorded the sound pressure in the ear canal and the cochlear microphonic potentials (CM; measured at the round window using a silver ball electrode). Using Mongolian gerbils, we performed two series of experiments. In a first series of experiments we showed that the DPs in the CM are of the same origin as those recorded as DPOAEs in the ear canal – namely, the region of the cochlea tuned to the stimulus tones. Because CM is an electrical signal, the travel time to the electrode can be neglected, so that the DPs in the CM recordings can be considered as direct measurements of the DPs at their site of origin. In a second series of experiments we determined the backward propagation time of the DPs from their cochlear site of origin to the ear canal by analyzing the phase differences between CM and DPOAE recordings. Our use of multitone stimuli makes a single recording sufficient, circumventing any effects of inter-recording variability. After taking into account the delays associated with reverse propagation through the middle ear and from the tympanum to the microphone, significant delays (>> 100 μs) remain that must be attributed to reverse travel time within the cochlea. The cochlear dimensions and the speed of sound in water rule out compression waves considered by Ren (Nat. Neurosci. 7:333-4, 2004). Instead, the backward propagation of cochlear DPs seems to be dominated by slow traveling waves.

347 Long Lasting Components of Transiently Evoked Otoacoustic Emissions

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Transiently evoked otoacoustic emissions were measured in 80 ms interval between click stimuli. The responses obtained were decomposed into basic waveforms by means of adaptive approximations using a matching pursuit algorithm. The method allows for description of the signal components in terms of frequencies, time occurrences, time spans, and energy. The main advantage of this method over techniques such as filtering and wavelets is that the signal is not divided into frequency bands. For the same frequency several components having different time occurrences can be extracted. These waveforms can also have different duration which can be evaluated by their time span parameter. This approach made possible observation of two types of long-lasting components: ones that were present during the whole measurement period, and exponentially decaying components. The distributions of the frequencies of components of different durations were similar, with most components falling within the 1-2 kHz interval. The incidence of slowly decaying components was higher then stable components.

348 Temporal Adaptation in Click-Evoked Otoacoustic Emissions

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The level of a click-evoked otoacoustic emission (CEOAE) is determined by the point on the internal CEOAE level-curve set by the evoking click. For clicks below 30-40 dB, the CEOAE-level grows linearly while it saturates for higher input-levels. This study investigates how the CEOAE level-curve changes with the time between two click-presentations. It was found that when the time between two clicks was less than 10 ms, the two clicks operated from different CEOAE level-curves. This effect is referred to as temporal adaptation of the CEOAE level-curve and results in CEOAE-levels that are different even though the clicks have the same input-level.
Temporal adaptation in CEOAEs was investigated by means of temporal suppression, which is the level-variation that occurs when presenting a so-called suppressor-click close in time to a test-click. Temporal suppression consists of a phase- and magnitude-component, and it is the magnitude-component only that can be used to quantify the CEOAE-level curve. Unlike historical studies, the phase-component was removed from the temporal suppression measure to quantify temporal adaptation of the CEOAE.
The results for four subjects showed that the compression-threshold of the CEOAE level-curve, i.e. the knee-point between linearity and compression, changed as a function of the time between suppressor- and test-click. The compression-threshold decreased (reflecting positive suppression) or increased (reflecting negative suppression) depending on the subject and the exact point

in time within the interval between suppressor- and test-click. It is claimed here that this temporal compression-threshold variation indicates the existence of a subject-dependent adaptation process in the gain mechanisms described by the CEOAE level-curve. These fast-acting changes in cochlear compression are important for a full understanding of the temporal properties of cochlear amplification.

349 Comparison of Suppression Estimates from Simultaneous Masking, Forward Masking, and DPOAE Measurements

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One reason simultaneous masking (SM) is larger than forward masking (FM) may be that mechanical suppression within the cochlea occurs only when masker and signal are presented simultaneously. This study compared behavioral and acoustical (DPOAE) estimates of suppression. Behavioral suppression was estimated as the SM-FM difference at any given masker level. FM signal delay was 0 ms (after the end of a 200-ms masker) to minimize any recovery from neural adaptation. The SM signal was presented 15 ms before the end the masker. The horizontal shift of DPOAE I/O functions due to the addition of a third (suppressor) tone provided an independent estimate of suppression to which the behavioral data were compared. Behavioral and DPOAE suppression estimates were obtained from 30 normal-hearing subjects for a 4000 Hz signal and two suppressor/masker frequencies: 2141 Hz and 4281 Hz. Signal level, which was L_2 for DPOAE measurements, was held constant at levels ranging 20-45 dB SPL for FM, 20-70 dB SPL for SM and 25-60 dB SPL for DPOAE measurements. Suppressor (L_3) levels varied in 5 dB steps from -20 to 85 dB SPL for DPOAEs, while masker level was adaptively varied, with a maximum limit of 95 dB SPL. The overlap of the resulting physiological and psychophysical estimates of suppression at both masker/suppressor frequencies supports the hypothesis that the difference in amount of masking between FM and SM is largely due to suppression.

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350 Differential Behaviors of Quadratic and Cubic Distortion Product Otoacoustic Emissions in Humans

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Distortion product otoacoustic emissions (DPOAEs), evoked by two pure tones (f1, f2, f1<f2), are used clinically to examine cochlear functions. Quadratic and cubic difference tones (QDT, f2-f1 and CDT, 2f1-f2) are dominant even- and odd-order DPOAEs that reflect different aspects of the nonlinear function of cochlear outer hair cells (OHCs). In this study, behaviors of QDT and CDT were explored by varying the f2/f1 frequency ratio from 1.15 to 1.8. For each frequency ratio, the primary levels (L1 and L2) were swept independently from 54 to 75 dB SPL in 3 dB steps. For a fixed f2/f1 ratio, CDT amplitudes in the L1×L2 primary level space showed a ridge when L1 was greater than L2 and the level difference became progressively larger at lower signal levels. When the frequency ratio increased, this deviation of L1 from L2 also enlarged. In contrast, QDT amplitudes could be maximized by increasing either L1 or L2, regardless of the frequency ratio. The QDT amplitude seemed to prefer a larger sum of primary levels (L1+L2), but not a particular level difference (L1-L2). For a given L1×L2 combination, CDT magnitude reached a maximum at ratios of 1.22 to 1.25 depending on the primary levels and diminished rapidly above these ratios. However, the QDT amplitude showed an opposite change: it produced a notch at these ratios. Large QDT appeared at the ratio of 1.3 and very low f2/f1 values with no influence of the primary levels. The differential behaviors of QDT and CDT may represent the compression and gain characteristics of the OHC transducer. Manipulating the two-tone stimulus could alter the interaction of the traveling waves and shift the cochlear transducer operating point, thus resulting in different changes in QDT and CDT amplitudes. Optimizing both CDT and QDT recordings may provide more accurate and comprehensive information about the inner ear function.

351 Pymetrozine and Procaine Affect the DPOAE-Generation in Tympanal Organs of Insects

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Tympanal organs of insects emit distortion-product otoacoustic emissions (DPOAEs) that are indicative of nonlinear ear mechanics. They possess properties that are similar to those measured in vertebrates despite different receptor morphology. DPOAEs in insects are assumed to be produced by the scolopidia, the type of mechanoreceptor characteristic of all chordotonal organs, including insect ears [Möckel et al.: J Comp Physiol A193, 2007]. To further test the involvement of auditory scolopidia in DPOAE generation, we applied the neuroactive insecticide pymetrozine and the local anaesthetic procaine to the tympanal organ of the bush cricket, Mecopoda elongata (Tettigoniidae).

The insecticide pymetrozine selectively affects proprioceptive chordotonal sensilla in locusts [Ausborn et al.: J Exp Biol 208, 2005]. We assume that it affects the scolopidial mechanoreceptors in bush crickets as selectively as it does in locusts. Application of pymetrozine (in concentrations of 10-3M to 10-10M) to the auditory chordotonal sensilla of the tympanal organ caused progressive decrease of the DPOAE amplitudes, with maximum reduction found 60 min after application. The local anaesthetic procaine can be used to reversibly block neuronal activity in insect neurons, with the primary target being voltage-sensitive Na+-channels [Devaud et al.: Europ J Neurosc 26, 2007]. Application of procaine (in concentrations of 740mM to 74mM) to the tympanal organ

is followed by a reversible effect on the DPOAE-amplitudes. These findings suggest that auditory sensory neurons are involved in creating sensitive nonlinear ear properties. Both pymetrozine and procaine could be useful tools to elucidate the mechanisms of DPOAE generation in insects.

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352 Intracochlear Drug Delivery in the Mouse: Correlation of Simulated Concentration Gradients with Shifts in Measured Auditory Response

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Intracochlear drug delivery in the mouse is challenging due to the extremely small size and volume of the inner ear. Physiological measures can access the impact of delivered compounds on peripheral auditory function, but infusate distribution characteristics must be inferred. Computer simulations offer opportunities to calculate cochlear concentration gradients for different delivery approaches and are an excellent complement to in vivo studies. Fluid modeling software is available to simulate a variety of delivery protocols representing infusions, perfusions, and extracochlear round window drug applications. Diffusion, volume flow, interscala transport, and clearance mechanisms are modeled allowing determination of drug concentration with position in the cochlea. When coupled with a cochlear tonotopic map, simulated concentration profiles can be correlated with shifts in measured auditory responses as a function of sound frequency. Two different drug delivery approaches are considered in the present investigation involving mice: scala tympani infusion through a basal turn cochleostomy, and this approach coupled with a fluidic exit hole in the posterior semicircular canal. Distortion product otoacoustic emission thresholds were measured from 8-44 kHz before and during delivery of artificial perilymph and sodium salicylate. Shifts in threshold were compared to simulated concentration gradients to highlight the relationship between drug concentration and frequency-dependent changes in auditory function. Model parameters were adjusted to match the profile of these characteristics and to provide insight into mechanisms impacting drug distribution in the cochlea. Strengths and limitations of currently available cochlear infusion models are discussed and critiqued for improvements.

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353 Intracochlear Infusions: a Surgical Approach to Reduce Concentration Gradients Within the Cochlea

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Direct delivery of compounds to the inner ear in animals is most commonly achieved by absorption or direct injection through the round window membrane, or infusion through a basal turn cochleostomy. These methods provide direct access to cochlear structures, but with a strong basal-to-apical concentration gradient consistent with a diffusion-driven distribution. This gradient limits the efficacy of therapeutic approaches for apical structures, and puts constraints on practical therapeutic dose ranges. A surgical approach involving both a basal turn cochleostomy and a posterior semicircular canal canalostomy provides opportunities for facilitated perfusion of cochlear structures to reduce concentration gradients. A cannula inserted into scala tympani at the cochleostomy site allows controlled delivery of solutions into perilymph, with the canalostomy in the PSCC serving as a fluidic exit port. The theoretical flow pattern is from base to apex in scala tympani, through the helicotrema, from apex to base in scala vestibuli, and then through the semicircular canals. This contrasts with infusion at the basal turn of scala tympani with no exit hole (cochleostomy only) where presumably excess fluid is pushed through the cochlear aqueduct.

Infusion (1µl/hr) of fixed volumes of artificial perilymph (AP) and sodium salicylate were used to evaluate the two surgical approaches in the mouse. Cochlear function was evaluated via closed-system DPOAE threshold level measurements. AP infusion confirmed no surgical impact to auditory function, while shifts in DPOAE thresholds were measured during infusion of sodium salicylate (10 mM) and AP (washout). Different fluids were separated by 10 nl air bubbles within the infusion tubing to avoid within-tube diffusion. Frequency dependent shifts were compared for the cochleostomy-only and the cochleostomy-plus-canalostomy approaches.

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354 Cochlear Monitoring Using Continuous Loop Deconvolution Averaging for the Evaluation of Otoprotection Against Electrode Insertion Trauma-Induced Hearing Loss by Drug-Eluting Electrodes

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A method to objectively determine auditory thresholds is specifically developed to test guinea pigs exposed to cochlear trauma and the efficacy of protective drug-eluting electrodes. The method uses a novel Continuous Loop Acquisition Deconvolution (CLAD) technique that can be tuned to simultaneously produce Quasi Steady State Responses (QSSR), suitable for objective detection, and a low-noise transient Auditory Brainstem Responses (ABR) as the conventional threshold detection methods. The analysis of signal strength and noise spectral characteristics of different electrode configurations ranging from chronic epidural electrodes to conventional scalp needle electrodes are also studied.

Tone bursts of 16, 4, 1 and 0.5 KHz were used. Eight identical tone pips, triggered by a slightly jittered sequence, were delivered in each sweep providing a mean stimulation rate of 78.13 Hz. The convolved response QSSR was obtained by averaging 20 blocks of 64 sweeps. The QSSR and the raw background activity (EEG, EMG), coming from both channels, were simultaneously acquired using a commercial system (Intelligent Hearing Systems, Miami). The individual 64-sweep QSSR recordings were analyzed in frequency domain to obtain their 78-Hz fundamental component (real and imaginary parts) and the 156-Hz first harmonic. The multivariable Hotelling's T2 test was applied to the data from the 20 blocks to perform the statistical response detection. The average QSSR is deconvolved to produce the ABR that is used for visual verification by the experimenter. For different electrode configurations the background activity was analyzed to assess its spectral characteristics, critical in the objective threshold detection problem.

The method is being successfully tested in animals in which the thresholds are being consistently detected with a resolution of 5 dB SPL. The method provides an objective statistical detection of thresholds with simultaneous acquisition of transient ABR.

355 Mastoid Cavity Dimensions and Shape: Method of Measurement and Virtual Fitting of Implantable Devices

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Temporal bone implants can be used to electrically stimulate the auditory nerve, to amplify sound, to deliver drugs to the inner ear and potentially for other future applications. The implants require storage space and access to the middle or inner ears. The most acceptable space is the cavity created by a canal wall up mastoidectomy and commonly access is gained through the facial recess. Detailed knowledge of the available space for implantation and pathways to access the middle and inner ears is necessary for the design of implants and successful implantation. Based on temporal bone CT scans a method for 3D reconstruction of a virtual canal wall up mastoidectomy space is described. Using Amira® software the area to be removed during such surgery is marked on axial CT slices and a 3D model of that space is created. The average volume of the models is 12.6 cm^3 with standard deviation of 3.69 cm^3, ranging from 7.97 cm^3 to 23.25 cm^3. Critical distances were measured directly from the models and their averages were calculated: height 3.69cm, depth 2.43cm, length above the external auditory canal (EAC) 4.45cm and length posterior to EAC 3.16cm. These linear measurements did not correlate well with the computed volumes. The shape of the models was variable to a significant extent making the prediction of successful implantation for a given design based on linear and volumetric measurements unreliable. Hence, to assure successful implantation, preoperative assessment should include virtual fitting of an implant to the intended storage space. The above mentioned 3D models were exported from Amira® to a Solidworks application where virtual fitting was preformed. Our results are compared to other temporal bone implant virtual fitting studies. Virtual fitting has been suggested for other human applications.

356 Analysis of Alternate Adenovector Serotypes in the Inner Ear

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Gene delivery has been examined as a potential treatment for a variety of inner ear disorders. Adenovectors provide a large carrying capacity and are easy to produce at a high titer. While the common adenovectors based on the human Ad5 serotype offer promise for gene delivery to the inner ear, we have begun looking at adenovectors based on other human serotypes for their ability to deliver genes

to inner ear tissues. The cellular tropism for the alternative serotypes is different so altered cellular distribution patterns within the inner ear are expected. Some of these new adenovectors are based on serotypes that have been shown to have limited preexisting immunity in human populations which could address concerns in some studies which have suggested that Ad 5 serotype vectors have the potential to cause inflammatory reactions and damage to the inner ear. We examined a series of different adenovector serotypes including Ad 5, Ad 35, and Ad 41 using both in vitro and in vivo techniques. Transfection kinetics was examined using macular organ cultures to follow the general ability of the new adenovectors to enter and express genes. All vectors tested showed the ability to transfect adult utricle cultures but demonstrated different transgene expression timing patterns. Vectors were then delivered to the inner ear of adult C57Bl/6 mice via the round window or a canalostomy in the posterior semicircular canal. Expression of the GFP transgene was examined by immunohistochemistry and confirmed by laser capture dissection and PCR in order to begin to determine cell type distribution within the inner ear. Hearing was evaluated before and after vector delivery. Our results show that alternate serotype adenovectors successfully transfected the inner ear of mice and that we can follow intraorgan biodistribution using our techniques to quantify differences in delivery within the inner ear.

357 AAV-Mediated Cochlear Gene Therapy Aiming to CLARIN 1 Gene Replacement in Knockout Mouse Cochlea

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Hearing impairment is one of the most common forms of sensory disorder. Absence of effective therapy for most forms of hearing impairment has prompted the investigation of novel therapeutics including cochlear gene therapy. Usher syndrome (USH) is an autosomal recessive disorder which in general is defined by a bilateral sensorineural deafness and retinitis pigmentosa caused by mutations in at least 10 gene loci. USH is divided into three main clinical types USH1, USH2 and USH3 from which the USH3 is the most common form of Usher syndrome in Finland. USH3 is caused by mutations in the *clarin 1* (*CLRN1*) gene, which encodes the four transmembrane domain protein clarin 1, expressed in the organ of Corti and spiral ganglion cells of the mouse ear.
The aim of the study is to find efficient AAV vector complex which can be used in AAV-mediated cochlear gene therapy aiming to *Clrn1* gene replacement in *Clrn1* knockout mouse cochlea.
We have studied transduction efficiencies of self-complimentary recombinant AAV2/1, AAV2/2, AAV2/5 and AAV2/8 vectors encoding green fluorescent protein (GFP) or hemagglutinin (HA) tagged *CLRN1 in vitro* in human embryonic kidney (HEK) -293 cell cultures and mouse cochlear tissue cultures. Transduction efficiencies of AAV2/1 and AAV2/2 vectors encoding GFP were studied *in vivo* in wild type (wt) c57bl mouse. AAV2/1- and AAV2/2-GFP constructs were delivered to cochlea with either direct microinjection through the round window membrane (RWM) into the scala tympani or through the cochleostomy into the scala media.
Serotypes AAV2/1 and AAV2/2 transduced HEK-293 cells much more efficiently than serotypes AAV2/5 and AAV2/8. Transduction efficiency of self-complementary AAV2/2 was significantly higher than conventional AAV2/2. AAV2/1 and AAV2/2-mediated GFP expression was detected in the organ of Corti of wt c57bl mouse after injection through the RWM and through the endosteum in cochleostomy.

358 Hearing Preservation Following Vector Delivery

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Many studies have focused strategies for prevention or treatment of sensorineural hearing loss. A potential disadvantage of pharmacologic solutions to this problem is that protective agents have to be delivered over long periods of time for many causes of progressive hearing loss. One potential approach to avoid chronic drug delivery is to use gene therapy. Gene delivery to the inner ear has been suggested to cause inner ear trauma through inflammation and the physicial trauma of injection. In order to further develop gene therapy for the inner ear, it is important to prevent surgical trauma. We evaluated the effect of Ad5 capsid adenovectors (Ad.11D, Ad.11D.bcl-2) on hearing function after delivery to the perilymph of adult C57Bl/6 mice. Hearing was checked prior surgery and three days post surgery by ABR and DPOAEs. A second group of mice underwent surgery two times to determine if a preliminary exposure to an Ad vector could induce an immune response leading to further loss. The first adenovector (Ad.LacZ) was delivered to the horizontal semicircular canal or via round window. After the surgery the animals were allowed to recover for 72 hours. In the second surgery a second adenovector (Ad.11D) was delivered to the semicircular posterior canal. The functional outcome was tested prior, three days post first vector delivery and 5 days post second vector delivery via ABR and DPOAE. The histological results demonstrated a successful delivery of the vectors by immuno-histochemistry for LacZ and GFP with minimal loss in function.

359 Cy-3.5 Labeled SiRNA Spreads Into Cochlear Epithelium Via Transtympanic Inoculation

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Small interfering RNA (siRNA) molecules bind to RNA and block transcription. These molecules can be used for protection against cell death and other types of pathologies. In general, siRNAs have minimal side effects and long term stability in the target tissue. Clinical applicability for inner ear therapy will depend on feasible routes of delivery to the target. The middle ear cavity is one potential site which is accessible and therefore feasible for future inner ear treatment. We investigated efficacy of delivery of Cy-3.5 labeled siRNA in the cochlear epithelia via transtympanic injection. We inoculated 100-150 µl of Cy-3.5 siRNA (2 µg/µl) transtympanically into the left middle ear fossa with a 30G needle. The right ear was inoculated similarly with sterile PBS and served as a control. Histology was assessed 2, 7, 13, 20, 30, or 40 days after inoculation by fluorescence stereoscopy of entire temporal bones, followed by epi-fluorescence of whole mounts of the organ of Corti. Alexa 488-phallodin was used to label actin. Stereoscopy revealed fluorescence of Cy-3.5 in the spiral ligament and the auditory epithelium on days 7, 13, and 20. Epi-fluorescence showed intense Cy-3.5 expression in the inner sulcus and Hensen cell areas, especially in the lower turns of the cochlea on days 7, 13, and 20. In contrast, the intensity of fluorescence at earlier and later time points was less consistent. Epi-fluorescence examination of the actin staining revealed little or no side effects of the presence of siRNA on the morphology of the organ of Corti. Control ears were negative for Cy-3.5 fluorescence. The spread of siRNA into the cochlear epithelium via transtympanic inoculation, and the lack of toxicity indicate that this reagent is feasible for experimental and clinical inner ear applications.

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360 Noninvasive in Vivo Delivery of Transgene Via Adeno-Associated Virus Into Supporting Cells of the Neonatal Mouse Cochlea

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There are a number of genetic diseases that affect the cochlea early in life, which require normal gene transfer in the early developmental stage to prevent deafness. The delivery of adenovirus (AdV) and adeno-associated virus (AAV) was investigated to elucidate the efficiency and cellular specificity of transgene expression in the neonatal mouse cochlea. The extent of AdV transfection is comparable to that obtained with adult mice. AAV-directed gene transfer after injection into the scala media through a cochleostomy showed transgene expression to the supporting cells, inner hair cells (IHCs) and lateral wall with a resulting hearing loss. On the other hand, gene expression was observed in the Deiters cells, IHCs and lateral wall without hearing loss following the application of AAV into the scala tympani through the round window. These findings indicate that the injection of AAV into the scala tympani of the neonatal mouse cochlea therefore has the potential to efficiently and noninvasively introduce transgenes to the cochlear supporting cells, and this modality is thus considered to be a promising strategy to prevent hereditary prelingual deafness.

361 Effects of Cidofovir Via Intratympanic Delivery in GPCMV Related Hearing Loss

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Congenital cytomegalovirus (CMV) infection affects 1% of all newborns and is considered to be one of the most common causes of sensorineural hearing loss (SNHL). Approximately 90% of newborns infected with CMV are asymptomatic at birth. Yet, 20% go on to show SNHL. The therapies available to treat CMV infection have proven to be toxic and have severe side effects. Therefore, finding a safe and effective treatment for CMV induced SNHL is needed.

Cidofovir (CDV) and other antivirals have been shown to stabilize and improve hearing loss in infants. CDV given systemically has shown effectiveness on treatment of both symptomatic and asymptomatic CMV infection. The potential toxic side effects of CDV are the major deterrents for its clinical use. Therefore, intratympanic (IT) delivery warrants investigation as a non toxic alternative to systemic treatments. Our proposal is that CMV related hearing loss can be treated via IT drug injection.

Previous work has shown that direct inoculation of guinea pig cytomegalovirus (GPCMV) into the bulla of a guinea pig (GP) is a consistent and reliable model of infection. The similarities in the anatomy and physiology of the GP and human ear make this model extremely relevant. In this study various concentrations of CDV are injected IT into the GP inner ear. Pharmacokinetic data is compiled and the appropriate concentration of CDV is injected 12-24 hours after GPCMV infection. Hearing tests, inner ear histopathology, viral load, toxicity and cochlear drug levels are all monitored in the infected and saline control animals. The results of this study will determine the safety and effectiveness of IT injections for the treatment of GPCMV related hearing loss.

362 In Vitro and in Vivo Toxicity Studies on Block Copolymer Micelles: A Novel Nonviral Vector for Inner Ear Treatment

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Sensory-neural hearing loss is based on cell dysfunction in the inner ear: hair cell loss, degradation of spiral ganglion cells or dysfunction of the stria vascularis can affect hearing adversely. These cell types can be treated effectively using plasmids, genes or neurotrophic factors whereas a novel generation of drug carriers would advance the current application methods by combining targetability, biodegradability, traceability and controlled drug release.

In this study block copolymer micelles (BCM) were examined for their uptake and possible toxicity in vitro and in vivo as first step in developing novel nanosized multifunctional carriers.

Therefore BCM labeled with DiI (diameter around 80 nm) have been incubated in different concentrations for 24 hours with L929 and PC-12 cells. For localization in the cells confocal laser scanning microscopy (CLSM) was performed after staining the actin-filaments with phalloidin and the nuclei with DAPI. Additionally, the cell viability was examined using vital staining with neutral red.

For in vivo examination 5 µl of BCM solution was injected via cochleostomy into the scala tympani of guinea pigs. The hearing function was determined by acoustically-evoked auditory brainstem response measurements before, 48 hours and 14 days after treatment. Finally, the harvested cochleae were stained with phalloidin, before the basilar membrane and the stria vascularis were dissected, stained with DAPI and coverslipped for hair cell counting and CLSM.

BCM were taken up by all cells in vitro and in vivo accumulating in the cytoplasm near the nucleus. No signs of toxicity were observed: neither the neutral red assay nor hair cell or hearing loss showed up in this time period.

In summary, BCM comply with the basic requirements for novel drug carriers for inner ear treatment.

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363 Effect of Disulfiram-Loaded Nanoparticles on Cochlear Morphology and Function in the Rat

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Nanoparticles (NPs) are being investigated as a transporting tool for targeted drug delivery to the inner ear. Since disulfiram (DSF) has been previously shown to induce apoptotic and necrotic cell death, DSF-loaded NPs were used as a toxicity model drug to study the effectiveness of NP transport from the middle ear through the round window (RW) membrane to the cochlea.

Under ketamine (35 mg/kg) and xylazine (6 mg/kg) anaesthesia the acoustic bulla in the adult rat was exposed and a small piece of gel foam was placed on the RW. DSF-loaded NPs (liposomes prepared in the University of Helsinki) were continuously delivered to the middle ears of the rats using an Alzet osmotic pump (100 ƒÝl of DSF-loaded NP suspension for seven days). Cochlear function was evaluated using the compound action potential of the auditory nerve (CAP), auditory brainstem responses (ABRs) and distortion-product otoacoustic emission (DPOAEs) recordings. Paraffin-embedded cochlear sections were stained with hematoxylin-eosin, Nissl, FluoroJade C and antibodies against S100 protein.

Seven-days delivery of DSF-loaded NPs to the middle ear in rats damaged the spiral ganglion neurons and their dendrites (FluoroJade C staining) and significantly diminished the number of Schwann cells (S100 protein staining) as revealed 36-45 days after the start of DSF-loaded NP delivery. The morphological deterioration was reflected in a marked CAP amplitude decrease and a 20-40 dB hearing threshold shift in the frequency range 8-25 kHz. However, DPOAE amplitudes remained unchanged during the whole period of DPOAE monitoring, indicating negligible deterioration of the outer hair cells. The results demonstrate that DSF-loaded nanoparticles spread through the round window to the scala tympani and impose their toxic effect on neuronal-derived cells in the spiral ganglion.

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364 Incorporation and Release of Various Forms of Dexamethasone in Biodegradable Poly(Lactic-Co-Glycolic Acid) Nanoparticles

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About 28 million Americans, 8 percent of the population in the U.S. have impaired hearing. Sensorineural hearing loss, which usually associates with hair cell abnormalities, accounts for 90 percent of all hearing loss. The development of an effective treatment for hearing loss is of great importance. Our overall goal is to develop an efficient inner ear drug delivery systems using nanoparticles. Local, round window membrane (RWM), application of therapeutic drugs has many advantages over systemic drug application including reduced dosage and associated side effects of some drug. Using a drug-nanoparticle complex to assist RWM drug delivery has the additional advantage for controlling dosage and maintaining a sustained drug release thereby improving the effective therapeutic window. Dexamethasone (DEX), an angiostatic corticosteroid, is one of the drugs which have shown potential hair cell protection in our lab. Three forms of DEX are readily available: the base form, the acetate ester form (DEX-Ac) and the basic phosphate salt

form. Encapsulation of the three forms of DEX into biodegradable poly (lactic-co-glycolic acid) (PLGA) particles is investigated and compared in this study. DEX base and DEX-Ac particles are synthesized by an oil-in-water (O/W) emulsion/solvent evaporation method, while DEX phosphate particles are fabricated by water-in-oil-in-water (W/O/W) emulsion/solvent evaporation method. DEX-Ac particles exhibit highest loading compared with DEX base particles and DEX-phosphate particles. Release profiles show that sustained release of DEX acetate is achieved over a week, while burst release occurs for other two types of particles when placed in an aqueous solution. The higher loading and controlled release behavior of DEX-Ac loaded PLGA particles compared to the other forms of DEX suggest the potential of a new therapeutic strategy in hearing loss treatment using a nanoparticle delivery system.

365 The Effect of LOS Mutations of Non-Typeable Haemophilus Influenzae on the Permeability of the Round Window Membrane and Damage of the Inner Ear

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The round window membrane is permeable to bacterial products and inflammatory mediators allowing their passage from the middle ear to the inner ear. An understanding of host and pathogen interactions is important for the rational design of better treatment of bacterial otitis media and inner-ear complications.

Nontypeable *H. influenzae* (NTHi), an opportunistic pathogen lacking capsular polysaccharide, is a leading cause of otitis media in children. Lipooligosacchide (LOS), the major immunogen of *H. influenzae*, has a highly heterogeneous composition. LOS glycoforms mimic host structures to allow the organism to evade innate defenses and manipulate host cell metabolism.

The purpose of this study was to determine the virulence of NTHi 2019 and its two LOS mutant strains, B29 (gene *htrB*) and DK1 (gene *rfaD*), and their ability to penetrate the round window membrane. NTHi 2019 B29 mutant strain was associated with acylation of lipid A, and DK1 expressed a truncated LOS. We inoculated 15 chinchillas with 0.5 ml of 10^2 CFU wild-type or B29, or DK1 mutant strains. Two days after inoculation all animals had otitis media; however, the effusions of animals inoculated with the B29 mutant appeared less thick than the wild-type or DK1-inoculated animals. Histopathological findings using wild-type and mutant strains were different. Although otitis media was seen with B29 and DK1 mutants, we observed reduced passage of bacteria through the round window membranes and less histopathological changes of the round window membranes and inner ears compared to the wild-type strain. These data show a significant effect of the LOS gene on middle ear pathology and the ability of NTHi to penetrate the round window membrane and cause inner ear pathology.

366 Precision and Reliability of the Mouse Calyx of Held Synapse

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The medial nucleus of the trapezoid body (MNTB) is an important component of the sound localization circuit of the auditory brainstem. Each MNTB principal neuron is contacted by a single, giant axosomatic terminal, called the calyx of Held. Traditionally, the calyx of Held synapse is viewed as a highly reliable relay, with every presynaptic action potential triggering a postsynaptic action potential. However, more recently, *in vivo* extracellular recordings showed evidence for frequent spike failures. To investigate the reliability and precision of the calyx of Held synapse, we made both extracellular and whole-cell recordings from MNTB neurons *in vivo* in adult, anesthetized mice. In about half of the intra- and extracellular recordings, we found evidence for action potential failures. We could identify two different causes for these failures, a relatively small EPSP amplitude and a decrease in postsynaptic excitability. The time course of changes in postsynaptic excitability was quite rapid, suggesting that sodium channel availability played an important role. The EPSP amplitude generally did not show a clear dependence on interval, whereas experiments in slices indicated the presence of rapid short-term depression under conditions when release probability was high.

To investigate the importance of these findings for the precision and reliability of the sound localization circuit, we recorded responses to trains of brief tones at the characteristic frequency. MNTB neurons showed a phase-locked response up to 200 Hz. Most of the observed decrease in temporal precision and reliability at high frequencies was already present in the inputs to the MNTB. We therefore conclude that although the calyx of Held synapse can fail, it is generally safe enough.

367 In Vitro Multielectrode Array Recordings at Adult Giant Synapses of Held

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The giant synapses of Held in the anteroventral cochlear nucleus (AVCN) and in the medial nucleus of the trapezoid body (MNTB) are established models for investigation of the properties of excitatory neurotransmission *in vitro* and *in vivo*. However, due to extensive myelinization whole cell recordings from AVCN spherical bushy cells and MNTB principal neurons are rarely done past the third postnatal week. This could be a limiting factor in an attempt to scrutinize physiological properties of adult synapses.

To simultaneously assess presynaptic and postsynaptic activity from adult-like (P45-55) calyceal synapses *in vitro*, we established multielectrode array (MEA) recordings in the gerbil AVCN and in the MNTB. Electrical stimulation of the afferent nerve fibers in a parasagital preparation of the CN or in coronal MNTB slices evoked respective compound field potentials (FP) consisting of presynaptic volleys and postsynaptic responses. The presynaptic

component was sensitive to TTX and the postsynaptic response could be blocked by low Ca^{2+} external solution (0.1 mM), by NBQX (10 µM) or by GYKI52466 (100 µM). The transmission delay (TD) at 32°C, measured as distance between the maxima of the pre- and postsynaptic signals was 0.61±0.01 ms in the AVCN and 0.45±0.01 ms in the MNTB. TD progressively increased at higher stimulation frequencies (50-500 Hz); yet this increase was less pronounced in the AVCN than in the MNTB. Contrary to this, activity dependent depression of FP amplitudes at 32°C was significantly more prominent in the AVCN, particularly for 400 and 500Hz stimulation frequencies. While the input frequencies ≤200Hz induced facilitation in the MNTB (18±5% at 200Hz), the amplitudes in the AVCN showed frequency dependent depression (-27±3% at 200Hz). At 333Hz the MNTB amplitude decreased by only -8±3% and the AVCN amplitude by -44±4%. It is concluded that MEAs provide a useful tool to investigate certain aspects of neurotransmission at adult calyceal synapses.

368 Synaptic Transmission Delay at the Calyx of Held *in Vivo* : Rate-Dependence, Phenomenological Modeling and Relevance for Sound Localization

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Transmission at central synapses exhibits rapid changes in response amplitude under different patterns of stimulation. Whether the synaptic delay is similarly modifiable is important for temporally precise computations. We address this question at the calyx of Held of the medial nucleus of the trapezoid body (MNTB) in Mongolian gerbils, which allows simultaneous recording of pre- and postsynaptic activity *in vivo*.

We find that the synaptic transmission delay (TD) increases as a function of spike rate (10-40 %). The temporal dynamics of the TD increase exhibited exponential shapes with activity-dependent time constants (~15-25 ms). Recovery dynamics of TD were mono- (20-70 ms) or biexponential with fast (3-20 ms) and slow time constants (50-500 ms). Using a phenomenological model to capture TD dynamics, we estimated ΔTD=5-30 µs per transmission event. Since the TD changes cover the behaviorally relevant range of interaural time differences in gerbils, these results constrain models of sound localization.

369 Persistent Na^+ Current in the Calyx of Held

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The medial nucleus of the trapezoid body (MNTB) is a sign-inverting relay for auditory signals used in sound localization. Fast transfer of signals from bushy cell to MNTB principal cell is facilitated by the calyx of Held, a giant glutamatergic nerve terminal. Patch clamp and immunohistochemical studies have revealed diverse voltage- and ligand-gated ion channels on the calyx and associated axon. Few studies have examined how these two classes of channel interact. We have identified in the calyx a large non-inactivating (persistent) Na^+ current with properties well suited to a role in regulation of subthreshold properties of synapses. Recordings were made from calyces in brainstem slices of P8-12 rats, with K^+ and Ca^{2+} channels were blocked. Long, depolarizing voltage-clamp pulses from -100 mV revealed a stable, TTX-sensitive inward current (peak amplitude ~ –200 pA). Surprisingly, this conductance began to activate near -85 mV, suggesting that it could play a role in the resting properties of the calyx. Indeed, application of TTX hyperpolarized the terminal by several mV and decreased resting conductance. Our previous studies showed that calyces express GABAA and glycine receptors, and have an elevated [Cl]i. Thus, activation of the receptors can depolarize the terminal and increase transmitter release probability. We thus asked if the glycine-induced depolarization could activate Na^+ current. Application of TTX reduced the amplitude of slow glycine-induced depolarization when the resting voltage was between -90 mV and -70 mV, indicating that persistent Na^+ current was activated and served to amplify the effects of glycine on voltage. Moreover, glycine-induced increases in the frequency of spontaneous (miniature) EPSCs were significantly reduced by TTX. Our data show that a presynaptic persistent Na^+ current regulates the resting properties of a synapse and contributes to the effectiveness of presynaptic modulators.

370 Modulation by GABA at the Endbulb of Held

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The properties of synapses can change through the actions of many types of neuromodulators. However, the functional importance of neuromodulation is not well understood. To address this issue, we study modulation by GABA receptors at the auditory nerve to bushy cell synapse, the so-called "endbulb of Held", which plays an important role in the processing of auditory information. Application of GABA in voltage-clamp recordings in mouse brain slices indicate that both $GABA_A$ and $GABA_B$ receptors are present at this synapse. This is striking because all spontaneous and evoked IPSCs in bushy cells are blocked by the glycine-receptor antagonist, strychnine, and no rapid $GABA_A$-dependent IPSCs are seen. This suggests that both $GABA_A$ and $GABA_B$ receptors are effectively neuromodulatory. Application of the $GABA_B$-receptor-specific agonist, baclofen, causes strong inhibition of the synapse. In addition, short-term plasticity changes from strongly depressing to facilitating, suggesting that baclofen acts presynaptically. Consistent with this, lowering the external calcium concentration led to similar effects. Baclofen application also significantly decreases mEPSC frequency but not amplitude, indicating changes in the neurotransmitter release machinery.

Current-clamp recordings showed no effects of baclofen on postsynaptic resting potential, action potential threshold, or input resistance, suggesting $GABA_B$ receptors are primarily presynaptic. Recordings at room temperature reveal a tonic level of $GABA_B$-receptor activation, indicating the existence of an endogenous GABA source. Tonic activation could be further elevated by SNAP-5114, a GAT-2/3 blocker, suggesting that GABA spillover from a distant site is driving GABA-receptor activation.

371 Impact of Synaptic Depression on Spike Timing at the Endbulb of Held

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Synaptic strength is subject to a number of activity-dependent processes, such as short-term depression. However, the consequences of these changes on synaptic computation are not well understood. We study this issue at the endbulb of Held synapse, which is formed by auditory nerve fibers onto bushy cells in the anteroventral cochlear nucleus. This synapse plays an important role in refining and relaying temporal information about sounds for sound localization. We used voltage clamp in mouse brain slices to measure short-term plasticity at the endbulb. The amount of depression varied considerably over the population of endbulbs. We used dynamic clamp of bushy cells to study how different levels of endbulb depression would affect the processing of temporal information under a variety of activity paradigms. Low levels of depression enhanced the probability of spiking for most of these paradigms, whereas high depression reduced the likelihood of response. The precision of postsynaptic spiking was enhanced by low depression in some situations, but in others, precision increased for high depression. This may suggest that having different levels of depression at different endbulbs is adaptive for the auditory system, by allowing multiple strategies to operate simultaneously for improving temporal precision.

372 Early Postnatal Development of Spontaneous and Acoustically Evoked Discharge Activity of MNTB Principal Cells: An in Vivo Study in Mice

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The calyx of Held synapse in the medial nucleus of the trapezoid body (MNTB) serves as a model for investigation of excitatory synaptic transmission in the mammalian brain. So far, most studies were based on in vitro recordings. Since no in vivo data of the activity of MNTB principal cells during the early postnatal period is available, characteristics of spontaneous discharge activity before and around the onset of acoustically evoked signal processing (hearing onset) and properties and possible changes in responsiveness to acoustic stimuli are still unknown. Here, we report spontaneous and acoustically evoked discharge activities of MNTB principal cells in vivo in mice from P8-P28 with a specific focus on developmental changes around hearing onset at P12. Data were obtained from two strains commonly used in brain slice recordings: CBA/J and C57Bl/6J. Prominent developmental differences were found for the rates and patterns of spontaneous discharge activity before and after hearing onset. The response patterns to acoustic stimuli were comparable at different ages, but significant developmental changes were observed with respect to response thresholds, latencies and stimulus-evoked discharge rates. Adult-like conditions were reached in the second or third postnatal week. There were no discernible strain-specific differences with exception of the early onset of hearing loss in C57Bl mice at P28. Employing multiunit recordings, we also investigated the tonotopic organization of the MNTB in all three spatial dimensions. The nucleus showed a high-to-low frequency gradient along the mediolateral and dorsoventral axes, but no systematic change in nuclear frequency representation was found in the rostrocaudal dimension. The results might contribute to the interpretation of synaptic transmission acquired in vitro and help to get a better understanding of the functional relevance of some of the developmental changes described earlier.

373 Synaptic Inputs Drive Bursts of Action Potentials in the Rat Auditory Brainstem Before the Onset of Hearing

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The calyx of Held synapse, a giant glutamatergic synapse located in the medial nucleus of the trapezoid body (MNTB), forms before the onset of hearing. It is not known when the neurons in the MNTB become electrically active and whether electrical activity contributes to the formation and maturation of this synapse. We therefore made in vivo recordings from the neonatal rat MNTB and observed both bursting and non-bursting units. The proportion of bursting units increased with age, and after P3, the day at which the calyx of Held synapse typically forms, only bursting units were observed. To determine the cellular mechanisms involved in generating these two modes of activity, we made whole cell recordings of MNTB cells in brain slices. At P0-3, the input resistance of MNTB cells was relatively high, and in some cases spontaneous inputs could already be large enough to trigger spikes. Similar results were obtained during whole cell recordings from identified MNTB neurons in vivo, where we observed that firing was also driven by EPSPs (6/6 recordings between ages P1-6). Further analysis of the complex waveforms observed in extracellular recordings from the MNTB provided evidence that both short-term facilitation and depression contributed to the precision and reliability of transmission in the neonatal MNTB.

374 Developmental Changes in Hyperpolarization-Activated Cation Channels Control the Resolution of Coincidence Detection in MSO Principal Neurons

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The temporal resolution of ITD detection in the medial superior olive (MSO) depends critically on the resting membrane properties of MSO cells, which limit the rise and decay of synaptic events. Our previous work has demonstrated that hyperpolarization-activated cation currents (Ih) provide an unusually strong contribution to the resting potential and input resistance of MSO neurons. Here, we examined how this influence is established near the onset of hearing (~P12) using whole-cell patch recordings from MSO neurons in gerbil brainstem slices. In whole-cell voltage-clamp recordings (35°C) in neurons prior to hearing onset (P10), Ih activation was relatively conventional (boltzmann fit: V1/2 =-84.7±2.1 mV, slope=-10±3 mV, n=4). However, during the first week of hearing, Ih maximal conductance increased by ~8 fold (8.3±1.5 to 62.6±5.2 nS), and the voltage-dependence of activation shifted to more positive potentials by 24 mV (V1/2=-60.9±1.8 mV; slope=-5.9±1.1 mV at P21). Hearing onset was accompanied by a shift in MSO neurons' modulatory profile as well. Between P10 and P13, activators of cAMP shifted the V1/2 of channel activation by 9.8±2.0 mV (n=4). However, from P18 onwards MSO neurons were completely insensitive to both activators and inhibitors of cAMP, suggesting a developmental change in subunit composition or other molecular modification of the channel. The impact of these developmental changes on synaptic integration was drastic near hearing onset: between P10 and P13, Ih contributed extensively to a 74% reduction in input resistance, from 117±11 to 30±4 MΩ, as well as a 7 mV depolarizing change in the resting potential (-64.6±0.5 to -58.2±0.6, n=13 and 5). Together, these results demonstrate that changes in both the density and voltage dependence of Ih are critical for establishing the submillisecond time resolution underlying sound localization.

375 Presynaptic Actions of Metabotropic GABA and Glutamate Receptors on GABAergic Transmission in Nucleus Laminaris Neurons

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GABAergic transmission in nucleus laminaris (NL) neurons, the third-order avian auditory station, is subject to dual modulation by GABAb receptors (GABAbR) and metabotropic glutamate receptors (mGluR) involving groups II and III (Gao and Lu, 2007 ARO). Here, we investigated the action loci of these receptors, using whole-cell recordings in slice preparations obtained from late chicken embryos. We examined the effects of specific agonists for these receptors on miniature inhibitory postsynaptic currents (mIPSC), paired-pulse ratio (PPR, with a pulse interval of 100 ms), and postsynaptic currents (I-mus) elicited by puffing a GABAaR agonist muscimol (10 μM) onto the recorded NL neurons. Neither of the agonists (100 μM baclofen, 2 μM DCG-IV, and 10 μM L-AP4 for GABAbR, group II and III mGluR, respectively) affected I-mus, excluding the possibility of postsynaptic actions. Consistent with this, baclofen reduced the frequency of mIPSC, without affecting the amplitude. However, DCG-IV produced only a marginal inhibitory effect on mIPSC frequency but not the amplitude, whereas L-AP4 had no noticeable effects on either parameter. Surprisingly, PPR remained unchanged even though evoked IPSC was dramatically suppressed by each of these agonists, suggesting that PPR might not be a sensitive indicator at this synapse that can be used to distinguish pre- from postsynaptic action. Combined with our previous findings that each of the agonists increased failure rate of GABA release and had no effects on cellular excitability, the present results suggest that both metabotropic GABA and glutamate receptors mediate modulation of GABA release in NL neurons via presynaptic actions. Supported by NIDCD Grant DC008984 to YL.

376 In Vitro Analysis of Synaptic Inputs to MSO Neurons of Adult Mongolian Gerbils

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In mammals the location of low frequency sounds in the horizontal plane is initially processed in the medial superior olive (MSO) by a neuronal coincidence mechanism. These coincidence detector neurons are highly sensitive to the timing of binaural excitatory and inhibitory inputs. It is known that both types of presynaptic neurons fire temporally precise at high frequency for up to several milliseconds. However, it is unclear how the synaptic short-term dynamics and synaptic receptor kinetics at MSO neurons behave during high frequency firing in adult animals.

To investigate the short-term dynamics of MSO inputs we performed whole-cell voltage-clamp recordings from visually identified MSO neurons of adult Mongolian gerbils (P60 - P130). Recordings were carried out at ~35°C or at room temperature. Afferent fibers were stimulated using a glass electrode.

We found that excitatory (AMPA) and inhibitory (glycine) synaptic currents depress substantially during 10 successive pulses of fiber stimulations given at low frequencies (~65% of initial amplitude at 0.5 Hz). At higher frequencies (200 Hz) the synaptic depression for both currents reached 25% of initial value. Depression of excitatory currents is mediated by presynaptic mechanisms since neither saturation nor desensitizing of AMPA-receptors contributed to depression. AMPA mediated currents are fast with decay time constants between 200 and 400 μs. The decay time constants of glycinergic currents ranged from 1.1 and 2.7 ms. Both,

excitatory and inhibitory inputs have a large quantal content and similar recovery from depression time constants.
Together these data, suggest that short-term-dynamics at the MSO has to be taken into account for understanding temporal integration at the level of the coincidence detectors, and that these dynamics of both the excitatory and inhibitory appeared largely similar.

377 Synaptic Organization of MSO Principal Neurons in Hearing, Deaf, and Cochlear-Implanted Cats

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The medial superior olive (MSO) is a key component of the auditory pathway that has been implicated in the processing of interaural time differences (ITDs) used for sound localization. The deaf white cat is a proven animal model of congenital deafness and was utilized to examine how deafness and cochlear implantation affected the synaptic organization of MSO principal neurons. Synaptic inputs to the MSO were investigated through a novel method of synaptic vesicle (SV) analysis in which synapses are classified as excitatory or inhibitory based on a quantitative assessment of SV size and roundness. Analysis shows that the cell bodies in the MSO of deaf cats have a decreased number of endings containing pleomorphic or flattened vesicles and symmetric postsynaptic densities as compared to hearing cats. To examine the possible restorative effects of cochlear implant stimulation on the MSO, cats aged 3 months received either unilateral or bilateral cochlear implants that had been modified by Advanced Bionics Corporation for use in kittens. Implantation and stimulation strategies were virtually identical to those used for human recipients of cochlear implants at the Listening Center at Johns Hopkins Hospital. The animals were stimulated 5 days a week over a period of 3 months. Preliminary results show that the principal neurons of the unilateral cochlear implant animals have an increased number of inhibitory axosomatic inputs as compared to deaf cats. There is also a return of excitatory inputs to MSO dendrites. These results support the hypothesis that early cochlear implantation can induce synaptic plasticity to restore auditory neurons to a more normal state. Experiments with bilaterally implanted animals are underway, and we predict the resulting changes in synaptic organization to further support the hypothesis for restoration of inhibitory synaptic inputs.

378 Deafferentation-Induced Axonal Sprouting Is Not Correlated with Dendritic Atrophy in the Gerbil VCN-MSO Pathway

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Nuclei of the superior olivary complex integrate bilateral inputs to detect interaural time and intensity differences. The ventral cochlear nucleus (VCN) projects bilaterally to the medial superior olive (MSO). Ipsilateral and contralateral VCN inputs terminate on lateral and medial dendrites of MSO neurons, respectively. When the cochlea or VCN is lesioned unilaterally at early postnatal ages, intact VCN axons sprout and project to denervated regions of MSO. In this study we examined the critical period for this change in gerbils. We found that lesion-induced sprouting to MSO is observed only if the lesion occurs prior to postnatal day 10 (P10). We have begun to explore the mechanisms that limit this sensitive period. One possibility is that dendritic retraction limits the extent of VCN axonal sprouting. To test this possibility, cochlea removal (CR) or cochlear nucleus removal (CNR) was performed on P4 or P7 gerbils. After 7 days, projection patterns were traced in vitro using dextran dyes. MSO dendritic morphology was assessed using MAP2 immunofluorescence and a dendritic length ratio (denervated side/intact side) was obtained for each MSO. Following P4 CR and CNR we observed more dendritic atrophy in the contralesional MSO (ratios of 0.51±0.04, n=3 and 0.54±0.05, n=4, respectively) than in the ipsilesional MSO (0.86±0.02 and 0.92±0.03, n=3 and 4). However, the degree of VCN axon sprouting did not appear to differ between the two sides. Furthermore, P7 CR produced less dendritic atrophy (0.89±0.01 contralesional and 1.0±0.01 ipsilesional, n=6 and 6) and axonal sprouting than the P4 manipulations. These results indicate that VCN axonal sprouting is independent of the extent of MSO dendritic atrophy. Differences between the contralesional and ipsilesional MSO after P4 CR and CNR may be due to differences in the amount of excitatory inputs to MSO following deafferentation, taking into account GABAergic projections that are excitatory at early postnatal ages.

379 Vascular Differences Between the Cat Medial Geniculate Body and Inferior Colliculus

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The capillary density in a brain structure may relate to levels of neural activity. In prior work on the cat inferior colliculus (IC), we found that the central nucleus (CN) has 50% more capillary profiles per cross-sectional area than the non-lemniscal dorsal (DC) and the lateral cortices (LC). Here, we assessed whether similar differences exist in the medial geniculate body (MG) ventral (MGv), dorsal (MGd), and medial (MGm) divisions, which we compared with the IC CN, DC, and LC, respectively, since these structures are connected. IC and MG capillary distributions were studied in plastic-embedded, 1 µm-thick, toluidine blue-stained sections in a caudo-rostral series; architectonic subdivisions were drawn independently. We plotted vascular profiles <8 µm in diameter in three semithin MG sections. Capillaries were counted in 200x200 µm areas; those in the brachium of the IC (BIC) were excluded. There were significant capillary density differences between the MGv and MGm ($p<0.05$, t-test). There were also significant density differences between the MGv and the MGd ($p<0.05$, t-test). A subdivision of the MGd, the caudal MGd, had the same capillary density as MGv

(p>0.05; t-test, df:2; n=80), suggesting a high level of metabolic activity in an extralemniscal part of the MG. This suggests that a different vascular organization exists in the MG than in the IC. While IC vascular organization is consistent along its extent (with the CN most vascular), the MG contains regional vascular differences within its subdivisions. Such differences suggest functional variation within MG subdivisions. The IC is 60% more vascular than the MG. The high MGv and CN capillary density implies that the lemniscal auditory pathway has a vascular substrate for a high level of metabolic activity. Supported by USPHS grant R01DC02319-29.

380 GABAergic Tectothalamic Neurons in Inferior Colliculus Receive a Special Glutamate Synapse

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Cell bodies and proximal dendrites of large neurons in the inferior colliculus (IC) of the rat are totally covered by unique, “calyx-like” glutamate terminals that are immunopositive for vesicular glutamate transporter 2 (VGLUT2). The aim of this study on the rat and transgenic mouse is to identify the neurons that receive this special input. We show that these specialized VGLUT2-positive terminals make axo-somatic asymmetric synapses with immunoelectron microscopy. At the light microscopic level, colocalization analysis of VGLUT1- and VGLUT2-immunofluorescence revealed that there are three populations of terminals in the IC: terminals positive for VGLUT1 but negative for VGLUT2, positive for VGLUT2 but negative for VGLUT1, and positive for both VGLUT1 and 2. The specialized terminals on large IC neurons were positive for VGLUT2 but not VGLUT1, and this suggests that glutamatergic inputs to large IC cells are segregated. Since IC GABAergic cells are large, we immunostained rat IC sections for VGLUT2 and GAD67, a synthetic enzyme for GABA, and found that a subpopulation of GAD67-positive cells received the specialized synapse, and those neurons were significantly larger than GAD67-positive cells lacking it. GABAergic neurons with the specialized synapse were distributed evenly in the all subdivisions of the IC. In GAD67-GFP knock-in transgenic mice, where all GABAergic neurons express GFP, we obtained very similar results: Only the large GFP-expressing cells received dense VGLUT2 inputs to the somata and proximal dendrites. We made whole-cell patch-clamp recording from GFP-positive cells in brain slices of GAD67-GFP mice and identified the specialized VGLUT2 synapses with immunofluorescence in the postfixed slices. The GABAergic neurons with the specialized synapse had regular or pause/build firing patterns and lacked the post-inhibitory rebound found in other GAD67-GFP neurons without special input. Finally, we injected retrograde tracer into the rat medial geniculate body (MGB). Almost all the GAD67-positive retrogradely labeled neurons that project to the MGB were encircled by VGLUT2-positive terminals. These data clearly demonstrated a novel circuitry for GABAergic tectothalamic projections and suggest a unique function for inhibitory inputs to the auditory thalamus. (Supported by NIH grant DC00189 and Uehara Memorial Research Fellowship.)

381 Cortical Projections to Midbrain Cholinergic Neurons That Project to the Inferior Colliculus

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The majority of cells in the inferior colliculus (IC) are affected by acetylcholine. The acetylcholine originates from two midbrain nuclei – the pedunculopontine tegmental nucleus (PPT) and laterodorsal tegmental nucleus (LDT). Several studies have suggested that projections from auditory cortex activate the midbrain cholinergic cells, but the underlying circuitry is unknown.
We injected FluoroRuby (FR) into auditory cortex in pigmented guinea pigs to label cortical projections to PPT and LDT. In the same animals, we injected Fast Blue (FB) into one IC to label PPT and LDT cells that project to the IC. We subsequently processed the brain to identify presumptive cholinergic cells by labeling them with an antibody to choline acetyltransferase (ChAT), which was visualized with a green fluorescent marker distinguishable from both FR and FB. We then examined the PPT and LDT with a fluorescence microscope to determine whether boutons of FR-labeled cortical axons were in close contact with cells that were double-labeled with the retrograde tracer FB and the ChAT immunolabel. We observed numerous such contacts; all these contacts were located ipsilateral to the cortex that was injected with FR. The contacts were more numerous in PPT than in LDT. In both nuclei, cortical axons appeared to contact either cell bodies or dendrites. In some animals, the FB injection was on the same side as the cortical injection; data from these experiments suggest a projection from auditory cortex to ipsilateral PPT and LDT cholinergic cells that project to the ipsilateral IC. In other animals, the FB injection was in the IC contralateral to the injected cortex. Data from these animals suggest a projection from auditory cortex to ipsilateral PPT and LDT cholinergic cells that project to the contralateral IC. We conclude that projections from auditory cortex are likely to project directly to brainstem cholinergic cells that innervate the ipsilateral or contralateral inferior colliculus. This suggests in turn that cortical projections could elicit cholinergic effects on both sides of the auditory midbrain.
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382 Inputs to Neurons Expressing HCN Channels in the Auditory Brainstem

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Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels have been associated with the generation of pacemaker-like firing, regulation of the resting potential and the integration of dendritic inputs. HCN channels are fully activated at potentials greater than -100 mV and this raises questions about the origins of such

hyperpolarization, and the role of inhibitory inputs in the activation of these channels.
The aim of this study was to map the distribution of neurons that express HCN1 in the inferior colliculus (IC) and lateral lemniscus (LL) of the guinea pig, and discover the types of projections these cells receive, using VGLUT and VGAT as markers of glutamatergic and inhibitory terminals, respectively.
Fresh-frozen brain sections were collected from adult guinea pigs, and were fixed by immersion in paraformaldehyde. HCN1, VGLUT-1 and -2 and VGAT were detected using monoclonal and polyclonal antibodies, and were visualized by means of fluorescent secondary antibodies.
HCN1 is present in the somatic and dendritic membranes of neurons in the IC and LL. Within the IC, it is most significantly expressed in the ventral part of the central nucleus, whereas in the other subdivisions the level of expression is considerably lower. HCN1 is not expressed in the dorsal nucleus of the LL, only in the ventral nucleus (VNLL), mainly its dorsal part.
In all the studied nuclei, the main type of projection to HCN1+ cells is axo-dendritic, being denser in the VNLL than in the IC. Axo-somatic contacts are less frequent, being only significant in the IC and only for VGAT+ terminals.
The relatively limited inhibitory projection to HCN1+ somata may indicate that inhibition is not a major contributor to the activation of HCN1 channels in these nuclei, where perhaps the intrinsic process of repolarization is sufficient. On the other hand, since most of the contacts are axo-dendritic, HCN1 may play a role in the integration of dendritic inputs.
Supported by the EPSRC.

383 Coding of Frequency by Neurons in the Inferior Colliculus of the Unanesthetized Rabbit

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Frequency analysis is a key function of the auditory system. Nevertheless, most studies of frequency coding have used anesthetized preparations, even though anesthesia alters neuronal responses. Here we describe tuning curves and frequency-response maps of neurons in the inferior colliculus (IC) of unanesthetized rabbits, which were constructed from responses to tone bursts of varying frequency and intensity. Stimuli were usually presented contralaterally.
About half the neurons studied were inhibited at some frequencies as evinced by suppression of spontaneous activity. However, not all neurons had sufficient spontaneous activity for inhibition to be apparent. Among neurons with higher spontaneous activity, about three-quarters were inhibited, implying that the bulk of neurons in the IC are inhibited across some frequencies.
Few neurons had the "V-shaped" tuning curves of auditory nerve fibers. In the IC, tuning curves were broader near the tip, and could comprise multiple responsive regions. Some tuning curves could also be irregular in shape or even be closed. The broader tuning was confirmed by calculating values of Q-10 and Q-40 that were, on average, smaller in the IC across all characteristic frequencies than the values reported for auditory nerve fibers.
Absolute thresholds were lower by ~20 dB than in the auditory nerve. Average thresholds of neurons near their characteristic frequencies were, however, similar to reported behavioral thresholds at similar frequencies, particularly below ~10 kHz.
The lower thresholds and broader tuning in the IC suggest that each neuron receives the output of many auditory nerve fibers, reducing neural noise and hence thresholds. In the auditory nerve, the presence of a soft tone is encoded by the discharge of the most sensitive fibers tuned to the frequency of the tone. In the IC, it is encoded by the firing of a majority of neurons tuned to that frequency.
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384 Frequency Organization of the Inferior Colliculus Determined with Voltage-Sensitive Dyes

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The inferior colliculus (IC) is tonotopically organized with low frequencies represented dorso- and dorso-laterally and high frequencies ventromedially. We have used transverse brain slices of the IC, which contain anatomically distinct frequency laminae, to examine the physiological and anatomical determinants of frequency coding. Slices were from either the rostral-to-middle or the more caudal regions of the IC. Inputs to the IC were activated by stimulating the lateral lemniscus with trains of current pulses and activity recorded simultaneously across different regions using voltage-sensitive dyes.
Activation of lemniscal inputs resulted in a pattern of excitation and inhibition confined to spatially segregated bands oriented similarly to previously characterized frequency laminae. Adjacent bands exhibited excitation and inhibition, with a periodic shift occurring during the stimulus train. This periodic pattern suggested the presence of a pattern generator in laminar formation. We therefore examined the effects of post-inhibitory rebound activity, a known pattern generator in other systems, on laminar formation in the IC. Responses were measured during rebound generation in control conditions and during block by nickel chloride. Rebound activity was concentrated in the ventro-medial IC in rostral-middle slices and the dorsolateral IC in caudal slices. Response patterns in these areas showed a strong inhibition followed by rebound excitation. The pre-rebound inhibition was temporally correlated with excitation in the dorsolateral IC in rostral slices and ventromedial IC in caudal slices.

Laminar segregation during rebound activity was distinct within spatially confined bands. Blocking rebound activity resulted in weak excitation in these bands, and a wider spatial spread.
Our results confirm earlier studies that frequency laminae in the IC are determined by input segregation, but suggest further that rebound neurons might enhance frequency discrimination.
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385 Spectral and Temporal Resolution Tradeoff Along the Tonotopic Axis of the Inferior Colliculus

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According to the uncertainty principle, the spectral (bandwidth, Δf) and temporal (duration, Δt) resolution of an auditory filter should be inversely related. Results from auditory nerve data reflect this assumption, showing that modulation sensitivity improves with an increase in the characteristic frequency and filter bandwidth (Joris & Yin 1992). Here we examined whether similar trends in the behavior of temporal resolution (Δt) and spectral resolution (Δf) are present in the central nucleus of the inferior colliculus (CNIC). We recorded the electrical activity in the CINC as a response to a Dynamic Moving Ripple (DMR) sound stimulus with a 16 channel acute probe inserted orthogonal to the CNIC isofrequency lamina. The neuronal responses were then used to compute the spectrotemporal receptive field (STRF) and Ripple Transfer Function (RTF) of each unit as a function of frequency. The spectral and temporal resolution and modulation preferences of each unit were then extracted from the calculated STRF and RTF. STRFs were systematically organized along the tonotopic axis of the CNIC. Although spectral resolution decreased with increasing best frequency proportional spectral resolution (in octaves) increased from coarse resolution at low frequencies to high resolution at high frequencies. An opposing trend was observed for the STRF temporal resolution, which decreased systematically with increasing frequency. Surprisingly, the observed trends were inconsistent with the expected pattern imposed by the peripheral auditory filters. This was further supported by the fact that the amount of temporal and lateral inhibition increased systematically with increasing receptive field frequency. The observed tradeoffs between spectral and temporal preferences imply that the modulation and tuning characteristics are systematically reorganized along the tonotopic axis of the CNIC. (Supported by NIDCD R01DC006397).

386 Spectrotemporal Resolution Trade-off Within and Across the Isofrequency Lamina of the Central Nucelus of the Inferior Colliculus

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We examined the organization of spectrotemporal preferences within the Central Nucleus of the Inferior Colliculus (CNIC) of cats. Neural responses to dynamic moving ripple sounds were recorded with 16-channel recording probes. The probe spatial position was referenced to a three-dimensional coordinate system using a stereotaxic frame assembly within the CNIC. Each penetration was separated by ~300μm along the laminar plane. This procedure allowed us to fully sample the three-dimensional volume of the CNIC. Spectrotemporal receptive fields (STRF) were computed from the unit response via reverse correlation procedures. The reconstruction of the spectrotemporal preferences along the isofrequency lamina exhibits a systematic organization for important functional parameters including temporal and spectral modulation frequency (TMF and SMF respectively), receptive field duration, bandwidth, and delay. Within an Isofrequency lamina, TMF showed a progression from low to high modulation frequency along the dorso-medial to ventral-lateral axis of the CNIC, whereas the SMF exhibits an opposing progression along the same axis. Furthermore, spectrotemporal resolution varied systematically along the tonotopic axis (across lamina). Low spectral and high temporal resolution was observed for low BF sites. Progressively higher spectral and coarser temporal resolution was observed with increasing BF. The reconstruction of these preferences within the lamina and across lamina suggests that spectrotemporal preferences are systematically organized within CNIC. Such a distributed organization has implications for how spectrotemporal information in natural sounds is encoded within the CNIC. (Supported by NIDCD R01DC006397).

387 Spectrotemporal Response Properties of Neighboring Neurons in the Central Nucleus of the Inferior Colliculus: Implications for Local Circuitry and Functional Integration

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The central nucleus of the inferior colliculus (CNIC) is organized into anatomically distinct isofrequency lamina that form the basis for its tonotopic organization. However the range of computational possibilities within a lamina and the interactions between them are presently not understood. Its been suggested that afferent inputs from the DCN, LSO, MSO, and DNLL are organized into "synaptic domains" (Oliver 2000). Based on this hypothesis, it is expected that local neuronal populations of the CNIC lamina will be fairly homogenous with functionally distinct properties. A recent study using pure

tone stimuli (Seshagiri and Delgutte 2007) confirmed that the best frequencies (BF) of neighboring neurons are mostly conserved, but other important functional properties are significantly more heterogeneous. In this study we examine micro organization of receptive field preferences by comparing spectrotemporal receptive fields (STRF) between neighboring units in the cat CNIC. STRFs were obtained by presenting dynamic moving ripple stimuli and recording neuronal activity from neighboring neurons with four-tetrode (4x4) acute probe. Spikes are detected and then sorted with assistance of MClust software (Redish et al) before computing STRFs. In order to compare STRFs from adjacent units, we first measured the normalized STRF crosscorrelation between all nearby neuron pairs. Once the STRF delay disparity between neighboring neurons was taken into account, the receptive field of neighboring units were generally highly correlated. We also measured several spectrotemporal parameters to determine which spectrotemporal integration properties were most conserved. We found that the best frequency is the most conserved parameter, which is in line with previous studies. The response latency and temporal duration were also highly correlated. Best modulation frequency, frequency bandwidth, and best ripple density were significantly correlated, however, these high-order parameters generally were less conserved. These results suggest that spectrotemporal preference of locally isolated neurons in the CNIC can be similar, although certain functional properties appear to be more heterogeneous. Such functional properties support the idea that local neuronal populations in the CNIC can inherit some basic receptive field features (e.g., BF and delay) from their inputs although they integrate high-order features (e.g., spectrotemporoal modulations) to construct new functional distinct outputs. (Supported by NIDCD R01DC006397)
Keywords: inferior colliculus, spectrotemporal, receptive field, tetrode

388 How Are Concurrent Stimuli with Different Spatial Locations Encoded in the Inferior Colliculus?

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Most sounds occur in conjunction with others, and the auditory system segregates these concurrent sounds into separate sources, that generally arise from different spatial locations. Sounds such as speech, music and animal communication are often partially composed of harmonic complexes, for which pitch is a powerful cue for segregation. Harmonic complexes are composed of components that are multiples of a fundamental frequency (f0). The pitch of a harmonic complex is determined by the f0; sounds with a pitch difference of greater than >6% can be easily segregated. In order to examine how the auditory system represents concurrent, segregated sounds at different spatial locations, two broadband harmonic complexes with different f0s were presented at different interaural level differences (ILDs) to anaesthetized, pigmented guinea pigs. The responses of inferior colliculus neurons to these stimuli were recorded. Almost all neurons responded to both harmonic complexes, i.e. the different harmonic complexes were not represented in different neural populations. However, the magnitude of the temporal response, but not the mean rate response, to a harmonic complex contained information about the spatial separation between the two complexes . This representation is the result of both the ILD of the stimuli and differences in the levels of the two complexes at the contralateral ear.

389 Binaural Processing in EI Neurons Revealed with In-Vivo Whole Cell Recordings

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Cells that receive excitation from one ear and inhibition from the other (EI cells) process interaural intensity disparities, the cues all animals use for localizing high frequencies. EI cells in the inferior colliculus (IC) are characterized by firing to contralateral stimulation whereas ipsilateral stimulation evokes no response. With binaural stimulation the spike-count evoked by contralateral stimulation is reduced or even completely suppressed. Previous iontophoretic studies have shown that in some IC cells, the ipsilateral inhibition occurs in a lower center (e.g., the LSO) whereas in other cells the ipsilateral inhibition actually occurs in the IC. In those cells in which blocking inhibition at the IC reduces or eliminates the ipsilaterally evoked inhibition, it is inferred that ipsilateral stimulation evokes IPSPs (inhibitory postsynaptic potentials), although the IPSPs could not be viewed directly from extracellular recordings. In order to measure PSPs directly, we made whole-cell patch-clamp recordings from 22 EI neurons in the IC of Mexican free-tailed bats. In all cells contralateral stimulation evoked spikes and/or EPSPs, and the number of spikes or the magnitude of EPSP decreased with binaural stimulation. In 2/22 cells, ipsilateral stimulation evoked IPSPs, showing that the EI property was created in the IC, and in 4/22 cells, ipsilateral stimulation evoked no response, showing that the EI property was inherited. However, in 16 EI cells, sound presented to the ipsilateral ear evoked only EPSPs, even though the ipsilateral stimulation suppressed spikes evoked by contralateral stimulation. Ipsilaterally evoked excitation in EI cells is surprising and shows that the circuitry activated by the ipsilateral ear is both different and more complex in EI neurons than was previously thought. Supported by NIH Grant DC007856.

390 Auditory-Space Coding by Neuronal Populations in the Collicular Pathway

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Previous studies indicated that many neurons in the collicular pathway, namely the central and external nuclei of the inferior colliculus (ICc and ICx, respectively) and the superior colliculus (SC), are sensitive to sound source directions. However, the details of the response properties (e.g., temporal response pattern, and robustness to stimulus level variation) varied among neurons and among nuclei, and the effect on space representation of

combining information across such neurons remains unclear. This study attempted a quantitative comparison of nuclei in terms of the fidelity of space representation at the neuronal population level. The amount of space-related information carried by single neurons or neuronal populations was evaluated in terms of the mutual information (MI; unit: bits) between the stimulus azimuth and the spike responses. Single unit responses were recorded from three nuclei of anesthetized gerbils. The stimuli were 50-ms wide-band noise bursts at various sound levels that varied in terms of the azimuth on the horizontal plane. In all the nuclei, the majority of units exhibited some degree of azimuth tuning for at least one stimulus level. MI was derived from the classification results based on the distance metrics of spike trains [van Rossum (2001) Neural Comput 13:751-763]. For the population analyses, the spike trains of individual neurons in the populations were concatenated to form long "ensemble trains," which underwent MI computation. The MI for single neurons was generally similar among the nuclei. As expected, the MI tended to increase as the number of neurons increased in the ensembles. However, the efficiency of combining neurons varied markedly among nuclei, i.e., MI: ICx > ICc > SC. This result can be interpreted as indicating that individual neurons in the ICx carry information about the space most independently, whereas the SC neurons are more stereotypical in terms of information content.

391 Comparison of Auditory Responses in the Medial Geniculate and Pontine Gray of the Big Brown Bat, Eptesicus Fuscus

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The inferior colliculus (IC) has been studied extensively in its role of transmitting information from the brainstem to the medial geniculate (MG). However, the IC is also the source of a major pathway to the cerebellum, via the pontine gray (PG). As a first step in determining how information is processed in these two pathways, we examined auditory responses of single neurons in both nuclei.

In the PG, neurons responded to either pure tones (PT, 60%) or to simple frequency modulated (FM) sweeps (40%). In the MG, 52% responded to PT, 36% to FM sweeps, 6% each to broadband noise and sinusoidal FM tones. Both MG and PG contained a complete frequency representation, but PG had more neurons with best frequencies > 50 kHz. Mean response latencies were longer in the MG (20 ms ± 12 ms) than in the PG (12 ms ± 8 ms).

Both nuclei contained monaural and binaural cells. Binaural cells were 67% of the population in MGB and 77% in the PG. EI neurons (excited by contralateral, inhibited by ipsilateral) comprised 63% of both binaural MG and PG cells. EE neurons (excited by either ear) comprised 34% of binaural MG cells and 33% of PG cells.

MG and PG contained neurons with both transient and sustained discharge patterns. The most noticeable difference between nuclei was in the number of neurons with prolonged responses that lasted longer that the stimulus duration. In the MG, only 10% of cells had this pattern while the PG had 50%.

Although both the MG and PG receive input from the IC, response properties in these regions differ in many respects. Most notably, neurons in the PG had shorter latencies than those in the MG, but were much more likely to fire for a prolonged period of time beyond the stimulus duration. This suggests that the thalamocortical pathway performs integration over time for cognitive analysis, thus lengthening latency, while the pontine-cerebellar pathway, which focuses on sensory-motor control, requires rapid input that provides a lasting trace of sound.

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392 Chronotopic Organization of the Auditory Cortex in the FM-Bat <Carollia Perspicillata>

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Carollia perspicillata is a frugivorous FM-bat that uses echolocation for general orientation and navigation but presumably not to catch small insects. In insectivorous bats, target range is coded by combination sensitive cortical neurons that respond to specific pulse-echo delays, in certain CF-FM bats the delay-tuned neurons are organized chronotopically with shortest delays represented most rostrally.

In the auditory cortex (AC) of the bat *Carollia perspicillata*, combination-sensitive delay-tuned neurons are embedded in the high-frequency field (HF) field. The neurons responded both to pure tones and to pairs of FM stimuli that mimicked certain echolocation pulse-echo delays. These FM-FM neurons were responsive to the same FM harmonic component in pulse and echo (e.g. FM_2+FM_2, FM_3+FM_3).

In 27 anesthetized bats a total of 212 frequency-tuned neurons throughout the HF field in the AC of *Carollia perspicillata* were recorded and maps of the topographic organisation of delay-tuned neurons were constructed. Their characteristic delay, measured at low echo levels close to threshold, ranged between 1 and 24 ms (mean and SD was 6.84 ± 9.56, *n=212*), the best delay, that evoked maximum response, was between 1 and 30 ms (mean and SD was 6.84 ± 9.22, *n=212*). Neurons located rostrally were tuned to short delays and were overrepresented compared to those located caudally and tuned to long delays. The AC of *Carollia* was chronotopic organized. The HF area has an echo-delay axis representing target-distance information along the rostrocaudal axis of the brain

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393 Non-Acoustic Signals May Affect the Baseline Firing of a Specific Subset of Neurons in the Primary-Like Auditory Cortex, Field A, of Guinea Pig

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@Primary sensory cortices are believed to be involved solely in processing of bottom-up signals from periphery. However, anatomical feedback route from higher cortical fields to the primary cortex suggests the potential influence of certain internal activity of animals on this field.

Guinea pigs have two primary-like fields, field A and field D. Recent imaging techniques have revealed that both fields have tonotopy of a mirror image. We recorded unit responses from field A while animals are freely-moving. This approach allows an animal to respond to stimuli with its initiation of a state-dependent behavior, probably together with some altered internal states, such as emotion, expectation and so on.

Guinea pigs showed a stereotypic behavior following the shift of environmental illumination from light to dark. Here, utilizing this behavioral shift, we explored possible changes in the responsiveness of field A units in association with this illumination change. For a subset of units, we found robust decreases (off-decrease) or increases (off-increase) in baseline firing (BsF) to be initiated, in synchrony with an invoked action or a changed behavior state, possibly reflecting changed internal state of the animal, immediately after room light was silently extinguished. In the dark, preferred acoustic stimuli evoked salient excitatory responses against the reduced BsF level for the off-decrease units or salient inhibitory responses against the increased BsF level for the off-increase units. Besides, the units showing such BsF changes were located predominantly in layer 5 of the field A or its vicinity. Previous studies have suggested that direct application of neurotransmitters known to be in brainstem nuclei into cerebral cortex could modify the spontaneous firing rate and pattern. Our results are discussed in line with modulation effect of the brainstem norepinephrine system known to be activated for the behavioral adaptation to a new environment.

394 Assessment of Functional and Cellular Neurotoxicity of Styrene Using an in Vitro Model of Auditory Cortex Networks

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Styrene, a widely used industrial solvent, is potentially hazardous to the auditory system. Workers exposed to styrene exhibit hearing loss and auditory processing difficulties, suggesting the involvement of the central auditory nervous system. Using an in vitro model of auditory cortex networks (ACN), the functional and cellular neurotoxicity of styrene was evaluated. Functional neurotoxicity refers to the alteration or impairment of normal electrophysiologic function in neural networks without neuronal death, whereas cellular toxicity refers to neuronal death.

Neurons dissociated from auditory cortex of E17 mouse embryos were grown on photoetched microelectrode arrays (MEAs) containing 64 transparent indium-tin oxide electrodes. These networks in culture develop stable spontaneous activity in three weeks and allow extracellular recording of multisite action potential patterns. After 30 minutes of reference activity, ACNs were acutely exposed to styrene oxide dissolved in DMSO at concentrations ranging from 0.1mM to 2 mM, during continuous recording of the network activity. Responses were quantified in terms of percent changes from reference as a function of concentration. Results indicate functional and cellular concentration-dependent neurotoxicity, with functional toxicity occurring at lower concentrations. Average spike and burst activity showed minimal changes at 0.1 mM, an IC50 of 0.5 mM and cessation of activity at 1 mM. Replacement of the bath medium restored the activity in ACNs exposed to styrene concentrations of 1 mM or lower. Morphologic alterations such as somal swelling and deterioration of processes were identified at concentrations above 0.5mM with neuronal death occurring within a few hours at concentrations above 1 mM. The concentration range used in this study is comparable to the levels of occupational exposure in vivo of 12 ppm to 240 ppm, and provides quantitative data on electrophysiologic and morphological progression of neurotoxity.

395 Feed-Forward and Feedback Modulations Between Three Cortical Auditory Areas Specialized for Time-Domain Signal Processing

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The auditory cortex of the mustached bat consists of at least nine areas. Out of these nine, the FF (F means frequency modulation), dorsal fringe (DF) and ventral fringe (VF) areas are composed of neurons tuned to the combination of the emitted biosonar pulse and its echo with a specific echo delay (best delay: BD) and are specialized for processing target distance information carried by echo delays. The DF and VF areas are hierarchically at a higher level than the FF area. Focal electric stimulation of the FF area evokes the BD shifts of DF neurons away from the BD of the stimulated FF neurons. Such a shift in tuning is called a "centrifugal" shift. In contrast, stimulation of the DF area evokes the BD shifts of FF neurons toward the BD of the stimulated DF neurons. Such a shift is called a "centripetal" shift. In our current studies, we found that focal electric stimulation of the FF area evokes centrifugal BD shifts of VF neurons as well as DF neurons and the BD shift-difference curve of VF neurons is very similar to that of DF neurons. Focal electric stimulation of the VF area evokes centripetal BD shifts of FF neurons. The centripetal BD shifts of the FF neurons evoked by the VF stimulation are 2.5 times larger than the

centrifugal BD shifts of the VF neurons evoked by the FF stimulation. Our research indicates that the feedback modulation (DF to FF or VF to FF) is always centripetal, whereas the feed-forward modulation (FF to DF or FF to VF) is always centrifugal. The centrifugal BD shifts shape the highly selective neural representation of a specific target-distance, whereas the centripetal BD shifts result in the expanded representation of the selected specific distance. Therefore, we hypothesize that the feed-forward modulations increase the contrast in neural representation and the feedback modulations play a role in focusing the neural processing on target information at a specific distance represented in the FF area.

396 The Cholinergic Basal Forebrain in the Ferret and Its Inputs to the Auditory Cortex

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Cholinergic inputs from the basal forebrain to the auditory cortex are thought to modulate auditory processing and regulate cortical plasticity. The aim of the present work was to describe in the ferret, an increasingly popular species for studies of cortical processing and plasticity, the distribution of cholinergic neurons in the basal forebrain and their inputs to the auditory cortex.

Standard histochemical techniques were used to examine myelin and acetylcholinesterase staining along with choline acetyltransferase (ChAT) and p75NRT immunocytochemistry on coronal brain sections. Morphometric analysis was performed using Neurolucida with StereoInvestigator software.

Cholinergic neurons in the ferret basal forebrain were found to extend from the septum rostrally, through the diagonal band of Broca, to the nucleus basalis magnocellularis (NB) caudally. These cells were strongly ChAT and p75NRT immunopositive. The p75NRT antibody specifically labelled cholinergic neurons in the basal forebrain, whereas ChAT immunopositive elements were found throughout the entire extension of the caudate/putamen complex. In the NB, ChAT and p75NRT immunopositive cells were limited medially by the internal capsule and laterally by the caudal corner of the putamen nucleus.

Tracer injections in the auditory cortex from previous studies revealed retrogradely-labelled cells in the NB that resembled in their location and morphology the neurons that showed ChAT and p75NRT immunoreactivity. No retrogradely-labelled cells were observed in the diagonal band of Broca or in the septum.

Density analysis of the cholinergic fibres showed a heavy innervation in the ectosylvian gyrus, where the auditory cortex is located. The innervation was largest in the posterior region of the gyrus, where the secondary cortical areas are located. The distribution of cholinergic fibres was homogenous in the infragranular layers, with no differences between layers V and VI, whereas a more heterogeneous pattern was found in the supragranular layers, with layers II-III being the most heavily targeted.

397 Behavioural and Neural Measurements of Timbre Perception in Ferrets

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Two ferrets were trained in a 2-alternative forced-choice task, which required them to discriminate the artificial vowels "/u/" (formant frequencies at 460, 1105, 2735 and 4052 Hz) and "/ae/" (formant frequencies at 730, 2058, 2979 and 4294 Hz). Stimuli were band-pass filtered click trains with the pitch being determined by the repetition rate of the click train, and timbre determined by the location of the band-pass filters. Upon initiating a trial, ferrets heard two repetitions of the vowel sound, each of duration 300 ms, with a 500 ms inter-stimulus-interval. The ferrets were trained to respond at one of two lateral response spouts contingent on which vowel they perceived. After ~30 days of behavioural training, both animals could reliably discriminate these sounds (performance exceeded 90% correct). Once animals had reached this criterion level of performance, the stimulus pitch was randomly varied between trials over a 150 – 500 Hz range of fundamental frequencies. Both animals could generalize timbre discrimination across this range of pitches and did so from the very first testing session. This suggests that the animals were recognizing differences in the spectral envelope and performing a genuine timbre task. By systematically altering the position of the first and second formant frequencies, we created a continuous series of 8 morphed vowels. The animals reliably classified vowels that were more similar to the trained stimuli, and performance fell to chance levels for the 50% morphs. Electrophysiogical recordings to the same stimuli were made in awake, passively listening ferrets. Changes in stimulus timbre or vowel identity at a constant pitch frequently altered both the firing rate and temporal characteristics of single neuron responses.

398 Asymmetric Crossed and Uncrossed Auditory Tectothalamic Projections

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How is information from the cat inferior colliculus (IC) represented and transformed in the medial geniculate body (MG)? Is the tectothalamic (TT) projection as strongly ipsilateral as early work suggests? We found a significant contralateral projection that suggests a revised TT arrangement. Moreover, the crossed TT projection does not always have the same targets as the ipsilateral TT projection. The IC subdivisions have been identified by cytoarchitectonic, connectional, and immunohistochemical methods. The central nucleus (CN), lateral cortex (LC), and dorsal cortex (DC) each project to the MG. We identified TT termination of pathways by depositing tracers, WGA-HRP (wheat germ agglutinin conjugated to horseradish peroxidase) and BDA (biotinylated dextran amines), in the IC that labeled the terminal fields in the MG. Projections were analyzed qualitatively. Deposits in CN, LC, and/or DC labeled terminal fields in multiple nuclei

of both the ipsi- and contralateral MG. Our major findings are: 1) some projections of CN, LC, and DC in the ipsi- and contralateral MG are asymmetric; 2) deposits in LC and/or DC consistently labeled the contralateral medial division (MGM) while CN deposits did not; and 3) the majority of labeling in the dorsal division (MGD) is from deposits in or invading the LC. These findings raise questions about the function of the TT system. First, do these ipsi- and contralateral TT asymmetries imply that acoustic or nonauditory inputs processed at the level of the thalamus are lateralized? Second, we find that the LC projects to ipsi- and contralateral MGM and MGD. Previous studies suggest that the LC and MGM receive somatosensory input; thus, the LC may be a source of IC multisensory integration. Finally, the contralateral pathways from an IC subdivision are not always a subset of the ipsilateral pathway. Is there a topography, tonotopy, or functional asymmetry unique to the contralateral thalamus that might support lateralization? Supported by NIH grant R01DC02319-29.

399 Amygdalar Axons Terminate Throughout the Inferior Colliculus in the Mustached Bat

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The amygdala is known to be a center of emotion, learning, and memory. Although it is not typically thought of as an auditory structure, the amygdala receives input from the medial geniculate body and auditory cortex, responds to auditory stimuli, and projects back to both the inferior colliculus and auditory cortex. To begin to identify how amygdala terminals influence auditory processing in the inferior colliculus, we placed an anterograde tracer in the amygdala and examined the location of amygdalo-collicular terminals in the inferior colliculus. Our results indicate that amygdalar axons have both en passant and terminal boutons that are widely distributed throughout the dorsal, central, and external nuclei of the inferior colliculus. Both the axons and boutons are very small (axons <0.2 µm and boutons < 0.5 µm in diameter). Termination patterns are evenly distributed, not clumped, throughout the nuclei. Similar termination patterns have been shown to have modulatory functions in other projections (e.g., cholinergic terminals).

We have previously shown that amygdalar stimulation alters the spike rate, latency, and background discharge of collicular neurons to acoustic stimulation. The effects seem to be differential based on the best frequency of the neuron and the acoustic stimulus presented. We hypothesize that the amygdala may alter the firing properties on neurons in the inferior colliculus, based on the significance of the auditory stimulus, to alter the attention of the animal to relevant stimuli.

Collicular neurons targeted by amygdalar projections have not been identified. It is possible that these projections influence neurons with ascending and/or descending projections. Future experiments need to examine the projections of the contacted neurons as well as the functional role of amygdalar projections on downstream auditory nuclei.

400 Effects of Salicylate on Central Auditory Temporal Processing

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High doses of sodium salicylate cause transient hearing loss and tinnitus; however, the effects of salicylate on the central auditory system are poorly understood. Our recent studies indicate that salicylate suppresses the neural output of the cochlea. In contrast, the neural output of the auditory cortex (AC) is enhanced suggesting that salicylate increases the gain of the central auditory pathway. Since the central auditory system plays an important role in temporal processing, which is critical for the perception of rapid time varying stimuli such as speech, we speculated that high doses of salicylate would impair temporal processing in the AC. To test this hypothesis, we implanted chronic electrodes in the AC and inferior colliculus (IC) of awake rats. Temporal processing was assessed by measuring the neural response to a gap (0.5 to 100 ms) embedded in an otherwise continuous noise. Gap duration was varied from 0.5 to 100 ms and the gap detection threshold (GDT), defined as the shortest gap that could induce a detectable response was measured before and after salicylate treatment. The average GDT in the AC increased from 1.4 ms before salicylate treatment to 2.1 ms 1 h post-treatment; this increase was statistically significant (t-test, $P = 0.01$). In the IC, the average GDT decreased from 0.55 ms ($n = 4$) before salicylate injection to 0.50 ms 1 h post-treatment; this difference was not significant (t-test, $P > 0.05$). These data suggest that high doses of salicylate impair the temporal response properties of AC, but not the IC. Supported in part by grants from the NIH (R03DC008685, R01DC00630 and R01DC009091)

401 Effect of Salicylate on Neural Response in Auditory Cortex

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High doses of sodium salicylate are often used to induce tinnitus in animal models. Our previous studies showed that 250 mg/kg of salicylate, a dose that reliably induces tinnitus in rats, decreased the neural output of the cochlea as reflected in the compound action potential. However, salicylate unexpectedly increased the amplitude of the sound-evoked field potential in auditory cortex (AC) of awake rats; this enhancement effect was abolished by isoflurane anesthesia. These results suggest that

salicylate increases the gain of the central auditory system in awake rats. Along with the increase in AC amplitude, we also found that salicylate increased the amplitude of acoustic startle reflex, behavior consistent with hyperacusis. To further explore these effects, we measured the effect of salicylate on the spontaneous and sound-driven activity in AC neurons. Single and multiunit responses were recorded from the AC of awake rats using chronically implanted high impedance electrodes. The mean spontaneous discharge rate measured 1-3 h after salicylate treatment (250 mg/kg) decreased by approximately 25% and then totally recovered 1 day post-treatment. A significant increase (~100%) in the onset response of the post-stimulus time histogram (PSTH) was also observed 1-3 h after salicylate treatment; the amplitude of the onset response recovered to normal 2-3 days post-treatment. On the other hand, the amplitude of offset response of the PSTH histograms showed a significant decrease 1-3 h after salicylate injection. Since the AC onset and offset response may arise from different neural inputs involving excitatory and inhibitory transmitter systems, it is possible that salicylate exerts different effects on the excitatory and inhibitory inputs to the AC. To investigate this possibility, salicylate was co-administered with vigabatrin (250 mg/kg, i.p.), an anti-seizure drug that increases GABA concentration in the brain. Vigabatrin blocked the salicylate-induced decrease in spontaneous activity and the decrease in the offset response; however, it did not alter the onset response. Collectively, these data suggest that high doses of salicylate may modulate GABA-mediated inhibition along the auditory pathway thereby altering the response of the AC. Supported by grants from NIH (R03DC008685, R01DC009219) and ATA.

402 GABA^B Receptors Presynaptically Inhibit Collicular and Cortical Inputs to the Medial Geniculate Body

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The medial geniculate body (MGB) receives both ascending inputs (colliculogeniculate or tectothalamic projections) from the inferior colliculus and descending inputs (corticogeniculate or corticothalamic projections) from the auditory cortex. In the present study, we wanted to determine whether presynaptic $GABA_B$ receptors modulate the colliculogeniculate and corticogeniculate neurotransmissions with whole-cell patch-clamp recordings from brain slice in rats. The ascending and descending afferent inputs to MGB neurons were electrically activated with two pairs of bipolar electrodes placed on the brachium of the inferior colliculus and on the superior thalamic radiations, respectively. Postsynaptic $GABA_B$ receptors were blocked with cesium (130 mM) and QX-314 (5 mM) added in the recording pipette solution. Picrotoxin (100 μM) was added in the bath solution to block $GABA_A$ receptors for recording evoked excitatory postsynaptic currents (eEPSCs) and kynurenic acid (4 mM) was added to block ionotropic glutamate receptors for recording evoked inhibitory postsynaptic currents (eIPSCs) from MGB neurons. We found that baclofen, a $GABA_B$ receptor agonist, reversibly suppressed both eEPSCs and eIPSCs in a concentration dependent manner. CGP 35348 (100 μM), a $GABA_B$ receptor antagonist, reversed the suppression produced by baclofen. The paired pulse ratio of eEPSCs and eIPSCs was significantly increased by baclofen (3 μM), confirming that presynaptic $GABA_B$ receptors inhibit eEPSCs and eIPSCs. There was no significant difference in the effect of baclofen on eEPSCs or eIPSCs between the ascending and descending pathways; however, baclofen had stronger suppression on eEPSCs than eIPSCs in either ascending or descending pathways, suggesting a possible difference in sensitivity or quantity between $GABA_B$ receptors on excitatory terminals and those on inhibitory terminals within the MGB. Our study demonstrates that the presynaptic activation of $GABA_B$ receptors can inhibit both ascending and descending neurotransmissions in the MGB and reveals a novel mechanism for auditory information processing in the MGB. This work was supported by the National Natural Science Foundation of China (Grants 30470560 and 30730041), the National Basic Research Program of China (Grant 2007CB512306) and the CAS Knowledge Innovation Project (Grant KSCX1-YW-R-36).

403 Investigating Cortical Descending Control of the Peripheral Auditory System

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Corticofugal projections may modulate auditory signal processing in many subcortical nuclei including the thalamus, inferior colliculus (IC) and superior olivary complex. Originating in the olivary complex the olivocochlear bundle, consisting of the lateral and medial (MOC) systems, extends descending control to the cochlea. Here we investigated the effect of reversible cortical inactivation on the guinea pig cochlea. A cooling technique was used to deactivate the cortex in anaesthetized guinea pigs, while a round window electrode monitored the cochlear microphonic (CM) and cochlear action potential (CAP).

Classically, activation of the MOC system leads to an increase in the CM and a decrease in the CAP amplitude, a result replicated in studies either directly stimulating the MOC or indirectly via IC stimulation. During cortical inactivation CM and CAP thresholds were elevated, most prominently in the 3-7 kHz range. The effects on the CM were generally greater than on the CAP. Reactivation of the cortex led to a recovery of both CM and CAP to control levels. A reduction and subsequent recovery in CM output at suprathreshold levels was also apparent, manifesting itself at various times and frequencies during the study.

These results indicate that even in the anaesthetized animal the cortex is exercising some control over the function of the cochlea.

404 Behavioural and Neural Pitch Discrimination Thresholds as a Function of Task Design

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Previous studies of pitch perception in animals have typically measured discrimination performance on "go/no-go" tasks in which the subject is required to respond to a change in tonal frequency. We have expanded on this work by demonstrating that ferrets can be trained to judge the direction (i.e. high/low) of periodicity changes in artificial vowel sounds on a two-alternative forced choice (2AFC) task. The discrimination thresholds of ferrets on this task were poorer than those of animals in the aforementioned studies. Two factors may explain this reduced performance: distinguishing the pitch of complex sounds may be perceptually more difficult than pure tone frequency discrimination, or classical "go/no-go" tasks in which the subject is required merely to detect a pitch change may be cognitively less demanding than our 2AFC task in which the ferrets were required in addition to judge the direction of a pitch change. To distinguish between these explanations, we trained ferrets on a "go/no-go" pitch task. This task was similar in design to that of previous authors, but it used artificial vowel stimuli like those in our 2AFC task. Pitch discrimination thresholds were substantially better on the change detection task than on the 2AFC task. We also designed "neurometric" algorithms to discriminate the pitch of artificial vowels based on the responses of auditory cortical neurons in anaesthetised and unanaesthetised, passively listening ferrets. We show that neural populations also support better pitch discrimination performance on change detection tasks than on analogous pitch direction judgment tasks. The results emphasize the importance of task design in auditory discrimination studies, in both psychophysical experiments and interpretations of neurophysiological responses.

405 Local Field Potentials Reveal Cortical Network Activity During Auditory Behavior

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The local field potential (LFP) measures large-scale activity of neural populations. Changes in spectral power of LFP can reveal behaviorally-driven changes in neural activity in different regions of the brain, and coherence of LFP between brain areas can indicate how information is transmitted between them.

We measured LFP in ferret primary auditory cortex and dorsal prefrontal cortex during two auditory behaviors: a conditioned avoidance task, in which animals responded to a target sound among distracters in order to avoid a small tail shock, and a positive reinforcement task, in which animals responded to the target sound in order to receive a liquid reward. We compared these data to baseline LFP data recorded during passive presentation of identical acoustic stimuli. In both cortical areas and during both behaviors we found the same basic changes from baseline: Power in the beta region of the LFP spectrum (10-30 Hz) was decreased while power in the gamma region (30-100 Hz) was increased. These changes are typical effects of increased arousal and selective attention. However, the magnitude of the shifts in power depended on the task. During conditioned avoidance, gamma increases were generally larger during the reference phase of each trial. During positive reinforcement, they were larger during the target phase. We also observed shifts in the coherence between auditory and prefrontal cortices during conditioned avoidance: Coherence in the beta band was decreased during behavior, suggesting a change in the information flow between the two areas. Taken together, the modulation of LFP power and coherence during auditory behavior suggests that large-scale activity in cortex is highly dependent on auditory attention and on the motor contingencies and/or reward values associated with different task stimuli.

406 Dual Pitch Processing Mechanisms in Primate Auditory Cortex

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Pitch is a crucial component of music and speech. Theoretically, pitch can be extracted using either spectral or temporal information within the acoustic signal. However, after more than a century of research, the exact neural mechanisms used by the auditory system for pitch extraction are still being debated. More recently, a neural representation of pitch has been identified in the auditory cortex of humans and monkeys. We have examined whether spectral and/or temporal information is used for pitch extraction by pitch-selective neurons in marmoset auditory cortex. We find evidence that temporal information is used for fundamental frequencies below 450 Hz, while spectral information is used to extract pitch for higher fundamental frequencies. Based on the spectral resolvability of neurons within auditory cortex, our data support the hybrid model of pitch processing proposed by psychophysicists, in which resolved harmonics are processed by a spectrally-based pitch mechanism whereas a temporally-based mechanism is used to extract the pitch from unresolved harmonics.

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407 Effects of Cortical Inactivation Upon the Responses of MGB Neurons to Broadband Noise as a Function of Interaural Level Difference (ILD)

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We have previously shown that for inferior colliculus neurons the ILD value that gives 50% of the maximum response (ILD50) can change during cortical inactivation. Here we present data from 135 MGB neurons showing that the sensitivity to ILD sensitivity at different average binaural levels changed during cortical inactivation. Broadband noise bursts of varying Average Binaural Level

(ABL) and Inter-aural Level Difference (ILD) were delivered to urethane anaesthetised guinea pigs and the responses of MGB neurons were recorded with multi-electrode arrays. Reversible cortical inactivation was achieved by cooling auditory cortex with a cryoloop. Inactivation and recovery of the cortex were monitored with a single electrode in the deeper layers. In the MGB shifts in ILD50 were associated with one of two patterns of changes in a neuron's receptive field. In the majority of neurons (128/135 or 94.8%) the receptive field changed in overall area, growing or shrinking in a manner consistent with facilitatory or supressive effects of cortical inactivation. In the minority of neurons (7/135 or 5.2%) the inactivation caused a translational shift of the receptive field as a whole that requires a more complex mechanism underlying the change induced by cortical inactivation than simple facilitation or suppression.

408 Behavioral Gating of Responses to Auditory Stimuli in Ferret Prefrontal Cortex

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One of the defining features of neurons in the prefrontal cortex (PFC) is selective encoding of task-relevant information. How is salient information identified and acquired by PFC and modified during changing behavioral task conditions? What are the responses of PFC neurons in non-task, quiescent states to previously relevant stimuli? Guided by a recent neuroanatomical study (Duque et al., 2009) that mapped out ferret PFC, we initiated neurophysiological studies in behaving and quiescent ferrets. They were trained on multiple auditory detection and discrimination tasks using conditioned avoidance techniques. We recorded from cells (n=760) in dorsal PFC of four head-fixed ferrets and observed responses that were highly adaptive, selective, and categorical and often gated by behavior. While some PFC neurons showed responses in the quiescent state, others showed no responses in non-task conditions, but displayed marked behavioral gating, particularly for sustained responses. We also found that responses to target stimuli initially persisted in post-behavioral testing in a quiescent "non-task" condition but then gradually extinguished with a variable half-life of ~5-100 minutes. Our results suggest that the PFC rapidly resets responses in a state-dependent manner, tracking attended task-relevant information in changing task conditions. However, in passive non-task conditions, responses to incoming information may be shaped by a fading memory of the behaviorally relevant target from the most recent task. Since the time course of acquisition and extinction of target representations in PFC paralleled the temporal trajectory for task-related receptive field plasticity in A1, primary auditory cortex (Fritz et al., 2003, 2007), we have also begun simultaneous recording in PFC and A1. Our observations suggest the presence of adaptive coding and behavioral gating of current task-relevant information in PFC that may contribute to top-down modulation of A1 responses.

409 Local and Large-Scale Organization of Auditory Cortex Probed with in Vivo Ca <Imaging

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Encoding of stimuli in the primary auditory cortex (A1) is thought to be sparse, with a multitude of complex overlaid maps of stimulus features. The dominant feature is a tonotopic map, which is best seen at near-threshold sound levels. One limiting factor in determining cortical maps is the challenge to record from large numbers of neurons simultaneously. In vivo 2-photon Ca2+ imaging allows for the functional probing of large numbers of neurons with single cell resolution.

Thus, to probe for the existence of multiple maps in mouse A1, we bulk loaded A1 neurons with the Ca2+ indicator OGB-1 and then imaged auditory evoked Ca2+ responses with in vivo 2-photon Ca2+ imaging. OGB-1 presumably reports sub-threshold as well as supra threshold voltage changes, thus widening the scope of probing organization, relative to extra-cellular recordings, which reflect only the suprathreshold responses. We find that in vivo 2-photon Ca2+ imaging reliably detects auditory responses. As expected, A1 neurons were found to be frequency selective. We find that locally, neurons were found to have similar stimulus selectivity and that the preferred frequency varies in a predictable manner. We are currently exploring the possible organization of feature selectivity with parameterized broadband stimuli.

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410 Functional Organization of Spectrotemporal Receptive Fields in Zebra Finch Auditory Forebrain

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Apart from tonotopy, the functional organization of auditory cortex remains poorly understood. Songbirds provide a useful model to study processing of complex sounds because they must learn and discriminate complex communication sounds. Probing with a computationally tractable stimulus that mimics the spectro-temporal correlations of zebra finch vocalizations, we have found a significant degree of spatial organization in the songbird equivalent of primary auditory cortex, field L.

Using a multi-electrode array, we systematically mapped adult zebra finch field L under urethane anesthesia. Multiunit activity at each recording site was used to compute spectro-temporal receptive fields (STRFs), revealing the neurons' preferred temporal and spectral modulations. We generated a 2D map of STRFs that spans much of mediolateral (5 birds) or anteroposterior (10 birds) cross-sections of field L. Tuning was quantified by measuring the spectral and temporal width of the positive peaks of the STRFs.

We found that neurons in the medial part of the primary thalamorecipient layer (L2a) were tuned to modulations

rapid both in frequency and time (~0.3 octaves; ~4 msec; width at 37% of the peak). Neurons in more lateral L2a exhibited broader tuning in frequency (> 1 octave) but maintained sensitivity to rapid temporal modulations (< 10 msec). In the secondary layers (L1/L3), which are reciprocally connected with L2a and project to higher areas, cells were sensitive to strikingly slow temporal modulations (80-100 msec), and their spectral tuning broadened laterally, as in L2a (~0.6 octave medial to > 1 oct lateral). These results demonstrate a clear layer-dependent clustering of differential temporal processing and a mediolateral gradient of spectral tuning in the auditory forebrain.
Our data provide a coherent picture of the first steps of forebrain representation of complex sounds and lay the groundwork for understanding how these STRFs arise along the auditory processing hierarchy.

411 Behavioral and Electrophysiological Measures of Pitch Learning

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Multiple-hour training on a fundamental frequency (F0) discrimination task dramatically decreases the threshold for detecting an F0 difference between two tones. In this study we investigated whether these performance improvements can be accounted for by neural plasticity at early pre-attentive stages of processing, using two electrophysiological measures of pitch processing, the frequency following response (FFR) (brainstem level), and the mismatch negativity (MMN) (auditory cortex level). 24 participants were trained for 12 hours on an F0 discrimination task using one of four different tones, differing in pitch and harmonic resolvability. F0 difference limens (F0DLs) along with FFR and MMN measures of pitch processing were assessed before and after training for all four tones. The change in performance was compared to that of two control groups, one trained on a level discrimination task, and one without any training. Training significantly decreased F0DLs but did not affect the FFR and MMN measures. This suggests that the behavioral performance improvements resulting from multiple-hour training may be mediated by changes at later processing stages.

412 Auditory Learning Using Tone, Speech- And Tone-In-Noise Training Stimuli in Children

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Previous research has shown that sentence-in-noise perception and phonological processing can be improved through auditory training. We examined whether these skills could be improved using auditory training in normally hearing and typically developing 8-10 year old children (n = 41). The children were all from one school, where testing and training occurred, and were divided into four groups. Groups 1 to 3 trained for twelve x 30-minute sessions over four weeks on different auditory tasks: 1) pure-tone frequency discrimination (FD), 2) FD in an amplitude-modulated (8 Hz), speech-shaped noise (FDN) or, 3) mono-syllabic words in the same noise (WN). Group 4 was an untrained control. Training tasks were delivered using a 3I-3AFC paradigm in which the tone frequency (Group 1) or tone / speech level (Groups 2 and 3 respectively) was varied adaptively. All children completed pre- and post-training tests of sentence perception in modulated (SMN) and unmodulated (SUN) speech-shaped noise, performance on each of the 3 training tasks, and phonological awareness. Both the noise-trained groups (FDN and WN) improved significantly more than the Control group on the SMN measure. However, although the WN and the Control group showed a significant improvement on the SUN measure, the WN did not improve significantly more than the Control group. Both the FD and FDN groups improved significantly more that the Control group on both FD and FDN. No group improved more than the Control group on the WN task. All groups showed improvements on phonological processing but, again, no group improved significantly more than the Control group. The results showed that, among the training stimuli, use of both a speech target and a noise masker produced the best transfer of learning. The improvement of the Control group on SUN was suggestive of a test-retest effect, but this effect was not present in the FD and FDN groups. Training on target tones in a noise masker transferred to improved sentence perception in noise, but only if the same noise was used in training and testing. To obtain improved speech-in-noise performance, training on a speech-in-noise task was thus shown to be the most effective. The improvement by the groups on phonological awareness was suggestive of test-retest effects, as no group improved more than the Control group.

413 Learning Frequency Discrimination While Practicing Temporal-Interval Discrimination with a Different Stimulus

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Can you learn one auditory skill while practicing another? We have been addressing this question by examining how frequency-discrimination performance is affected by training on temporal-interval discrimination. We previously established that interval training can contribute to learning on frequency discrimination in a 7-day training regimen, but only when the two tasks are trained closely in time in each session. In those experiments, the standard stimulus was the same for both tasks (two 1-kHz tone pips separated by a 100-ms onset-to-onset temporal interval). Here we asked whether interval training would still benefit frequency discrimination if the two tasks were trained with different standards. We trained two groups of listeners using the previously successful frequency/interval regimen, but used different standards for the two tasks. Frequency discrimination improved significantly when the standards differed between the two tasks only in the interval (350 vs. 100 ms, $p<0.01$), but not when they differed only in the frequency (4 vs. 1 kHz, $p=0.96$). Thus, frequency discrimination can be improved by the

subsequent practice of interval discrimination when the interval task employs stimuli with the same frequency as the initial frequency training, while the interval of those stimuli appears to be irrelevant. We speculate that, to learn on a task, practicing that task itself selects the neural processes to be modified and that within a certain time window around the selection, any stimulation that activates those processes helps bring about the modification. According to this view, frequency training selected a frequency-specific process and the following interval training activated that process only when the stimuli contained the frequency to which the selected process was tuned. These results open the possibility of learning multiple auditory skills simultaneously without incurring greater cost in time or effort.[Supported by NIH]

414 Distinct Phases of Auditory Learning Identified by Differences in Vulnerability to Intervening Events

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Learning has often been conceptualized as occurring over two phases, the actual period of training (acquisition) and the transfer of learning to long-term memory after training ends (consolidation). To explore the relationship between these two stages, we examined how auditory learning on a target condition over 6-7 daily training sessions was affected by training on a non-target condition in the same sessions. In four experiments, we trained the two conditions either consecutively, with the practice on the non-target condition presumably occurring during the consolidation phase of the target condition, or interleaved, with each condition practiced during the acquisition phase of the other. The target condition was a temporal-interval discrimination task with a 100-ms 1-kHz standard stimulus. The non-target condition differed from the target one either in the standard stimulus (350 ms, 1 kHz), but not in the task (temporal-interval discrimination), or in the task (frequency discrimination), but not the stimulus (100 ms, 1 kHz). In the consecutive regimen, listeners improved on the target condition only when the non-target condition employed a different stimulus, implying that practice on the different task disrupted the consolidation of learning on the target condition. In contrast, in the interleaved regimen, listeners learned on the target condition only when the non-target condition employed a different task, suggesting that practice with the different stimulus disrupted the acquisition of learning on the target condition. These data demonstrate that auditory learning is vulnerable to different intervening events during acquisition and consolidation. At least for temporal-interval discrimination, it appears that acquisition depends on resources that are shared by neural processes that encode different stimuli on the same task, while consolidation depends on resources shared by processes that encode different tasks with the same stimulus. [Supported by NIH]

415 Development of Learning Ability on Tone Detection in Backward Masking During Adolescence

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While adults can improve their performance on a wide range of perceptual tasks with training, little is known about when this learning ability emerges during development or how the development of learning ability relates to the development of naïve performance. We previously observed that learning ability on a temporal-interval discrimination task continues to develop well into adolescence, even after adult-like naïve performance has been reached (Huyck & Wright, 2008, Assoc. Res. Otolaryngol. Abs.: 158). Here we report a similar pattern of results for another basic auditory task: tone detection in backward masking. We trained listeners in three different age groups (11-year-olds, 14-year-olds, and adults) to detect a brief 1-kHz tone immediately preceding a broadband noise. The younger listeners were specifically selected because they began with adult-like naïve performance on this task (± 2 SD of adult mean). During training, all listeners completed ~30 detection-threshold estimates (900 trials) each day for 10 days. Nearly every adult (5 of 6) improved significantly across the training sessions, but fewer than half of the 11-year-olds (4 of 9) and 14-year-olds (2 of 7) did. Correspondingly, as a group, the adults improved significantly, but the 11- and 14-year-old groups did not. Thus, on average, for tone detection in backward masking, just as for temporal-interval discrimination, learning ability continues to develop beyond 14 years of age, even in listeners for whom the development of naïve performance is complete. These results demonstrate that the developmental course of learning ability can extend well into adolescence, and suggest that naïve performance and learning ability may be governed by different neural mechanisms. [Supported by NIH/NIDCD.]

416 Electrocorticographic and Pupillary Dilatation Response to Pure Tones in Auditory Cued Classical Conditioning in Human

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In classical conditioning paradigms, the response of auditory cortical neurons to identical sounds can show a systematic dependence upon the subject's state. Imaging studies in human subjects have also showed learning related, highly task-sensitive response changes. We investigated auditory cortical responses with an auditory cued classical conditioning paradigm in neurosurgical

subjects undergoing invasive electrocorticographic (ECoG) monitoring using direct intracranial recordings.
Pure tones (4sec. duration) of two different frequencies (800 and 2800Hz) were used as conditioned stimuli (CS+ and CS-) in a differential conditioning paradigm. The unconditioned stimulus (US) was an electric shock to the index finger. The experiment included adaptation, conditioning, and retention session. ECoG was recorded simultaneously from multiple electrode sites within Heschl's gyrus (HG) and superior temporal coertex.
Pupillary responses to CS tones showed an initial orienting dilation response followed by a late differential response. Initial onset latency was as short as 250ms, with the response peaking at 500-600ms after the onset of tones. The late sustained component of the pupillary response differentiated CS(+) and CS(-) most clearly within 1 sec. prior to the time of the US onset. Pupillary responses were also clearly seen in response to the US onset with a similar latency. The spectral content of onset responses to the CS tones was analyzed using a Thomson's multitaper spectral estimation method. Non-parametric local (third order) polynomial regression analysis of baseline-corrected theta, alpha and ripple frequency band power showed a differentiation between CS(+) and CS(-) sound during and after conditioning within HG and superior temporal gyrus. Results were compared with pure tone (250-8 kHz) responses to examine learning-related modulation of auditory cortical tonotopic representations.

417 Auditory Learning in Juvenile and Adult Animals

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The developing nervous system is thought to be particularly sensitive to environmental stimulation. Auditory deprivation can result in rapid degenerative changes, while supplemental acoustic stimulation can affect the maturation of auditory coding properties. However, the effect of auditory experience on behavioral performance has not been properly assessed in immature animals, particularly the influence of training. Therefore, we compared the ability of juvenile and adult gerbils to improve on an auditory perceptual task over the course of repeated testing. Animals were trained to detect sinusoidally amplitude modulated (sAM) noise as early juveniles (P25-37), late juveniles (P40-55) and as adults (P70-110). Detection thresholds were obtained initially with a broad range of sAM depths (0-100%) delivered in random sequence, followed by 4 additional days of testing with sAM depths that bracketed an animal's detection threshold from the previous day (i.e., method of descending limits). The learning displayed by adult gerbils was correlated with their initial thresholds: higher initial thresholds were associated with the greatest improvement over repeated testing. In contrast, approximately 50% of juvenile animals displayed poor learning that was independent of initial threshold. When re-tested as adults, animals displayed a positive effect of juvenile training, and this was independent of the learning that they displayed as juveniles. Both sAM detection thresholds and asymptotic performance were superior to those displayed by control animals which were trained and tested on two occasions as adults. sAM detection thresholds were 17±1% for adults with previous training as early or late *juveniles*; in comparison, they were 24±1% for adults with previous training as *adults*, and 24±2% for adult controls *without any* previous training). Thus, juveniles are uniquely sensitive to auditory training, ultimately displaying better performance in comparison with adults under the same regimen.

418 Stimulus Familiarity Affects Temporal Induction in the European Starling (Sturnus Vulgaris)

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In our everyday communication speech often is disrupted by masking noise. Nevertheless, we are able to understand the masked portion of speech and perceive the speech as continuing through the masker (e.g., Warren 1972). The brain appears to fill in a masked or even a missing part of the speech signal as long as the masker level is sufficient. This phenomenon is known as temporal induction (TI, see review by King 2007). TI in humans depends on the familiarity with the stimuli (e.g., Warren 1972). Here we present data from a songbird that learns its song in a way that parallels human speech learning and investigate whether similar effects of familiarity on TI exist in this animal model.
European starlings (Sturnus vulgaris) were trained in an operant Go/NoGo-procedure with food rewards to detect the deviator from a repeated background. To test whether TI takes place in the starling, three stimulus modifications of well-known-songs (WKS) were presented: C: complete song syllable; G: song syllable with silent gaps, 75 ms stimulus alternating with 50 ms gap; N: song syllable with gaps filled with band-pass noise (0.5 – 22.5 kHz). To test whether the familiarity with the stimuli does have an effect on the TI, we presented unknown song (UKS) syllables with the same modifications. Reaction times for detecting the deviator were analyzed using multidimensional scaling to evaluate the perception of the birds. For the WKS condition the multidimensional scaling grouped N significantly closer to C as G to C. In contrast to these findings no significant differences could be observed in the UKS condition. The results demonstrate that TI takes place in the starling and, similarly to results obtained from humans, familiarity with the stimuli has an effect in restoring destroyed auditory objects.
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419 Knowledge Transfer Depending on Task Difficulty

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The ability of NMRI mice to discriminate pairs of pure tones (PT) or amplitude modulated (AM) pure tones was tested in an aversive shuttle-box GO-NOGO paradigm. Whereas mice easily learned to discriminate PT of different frequencies within about two training sessions,

discrimination learning of AM tones took longer (5 to 10 sessions) to reach significant discrimination performance. When transfer of knowledge was tested in animals that were consecutively trained to both PT and AM tone discrimination (transfer between stimulus classes), we found that (1) beneficial transfer of knowledge was only seen when the demanding AM tone training was followed by the easy PT training but not when order of training was reversed, and (2) confusion of conditioned stimuli occurred when the AM tones used shared spectral components with the PT training. We conclude from these results that the type of knowledge transferred between training paradigms is likely to be procedural knowledge about shuttle-box training rather than possible stimulus generalizations. Obviously, as the benefit of knowledge transfer was small, it was only seen when an easy task followed a demanding one. It was not sufficient to over-compensate for the increased stimulus processing difficulty when a demanding task followed an easy one.

420 Efficient Encoding of Correlated Complex Acoustic Dimensions

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Early sensory processing is long thought to extract and exploit redundancy in the input, but perceptual evidence supporting this hypothesis has been limited. We report experiments that demonstrate highly efficient encoding of multiple acoustic attributes resulting in extreme reorganization of perceptual dimensionality. Stimuli varied across two complex, independent dimensions: attack/decay (AD) and spectral shape (SS). A subset of 24 stimuli captured a near-perfect correlation between these dimensions. Participants passively listened to 25 presentations of each of these stimuli over 7.5 minutes before discriminating sound pairs without feedback. Relative to control conditions without passive exposure, listeners retained their ability to discriminate trials consistent with the correlation. Impressively, listeners' discrimination of equal-magnitude stimulus pairs orthogonal to the experienced correlation dramatically improved, while becoming significantly worse on trials where only SS or AD varied. Passive listening to stimuli with correlated attributes resulted in reorganization of two physically and perceptually distinct stimulus dimensions as to efficiently encode exposure covariance, exploiting redundancy to optimize information transmission. Data are consistent with anti-Hebbian (decorrelation) models of cortical adaptation, optimizing sensitivity to information orthogonal to experienced covariation. Alternative Hebbian models that converge to the principal component (correlation) via dimensionality reduction poorly fit the data. [Supported by NIDCD]

421 Learning Sources from Mixtures

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Auditory scene analysis is ill-posed, and can only be solved via assumptions, or priors, about what sounds tend to be like. Some of these assumptions may be built in to the auditory system, but many of them are likely learned from experience. One challenge in acquiring this prior knowledge is that organisms often do not encounter sound sources in isolation. Rather, they are exposed to mixtures of sounds, and from these mixtures must learn the properties of individual sources. To study this process, we presented listeners with mixtures of novel sounds, and tested under what conditions they could segment sources from mixtures.

To generate novel sounds with some statistical similarity to real sounds, we modeled the log-energy time-frequency decomposition of a sound as a multivariate Gaussian, the correlation matrix of which was measured in a set of real sounds. The energy in cells that were nearby in the time-frequency decomposition was highly correlated; this correlation declined with separation in time or frequency. Time-frequency grids were drawn from this distribution and applied to samples of white noise to yield a set of novel sounds. To test whether listeners could segment these sounds from mixtures, we presented them with mixtures followed by a probe - either one of the sounds in the mixture, or a different sound that was also physically consistent with the mixture. We found that when presented with a single mixture, listeners were unable to identify the original sounds composing the mixture, indicating that their general, "bottom-up" priors were insufficient. However, when presented with multiple mixtures of one sound with various others, over time the repeating sound became apparent, indicating that in some cases, listeners can use regularities in sequences of sound mixtures to infer the properties of individual sources.

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422 Temporal Characteristics of Task-Dependent Contextual Shifts in Sound Localization

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A previous study of sound localization with a preceding distractor found that the responses were biased away from the distractor location even on the interleaved baseline trials on which the target was preceded by no distractor [Kopco et al., JASA, 121, 420-432, 2007].

The current study measured the temporal characteristics of this contextual plasticity. Subjects localized 2-ms frozen noise bursts presented either in the left (-11° to -79°) or the right (11° to 79°) hemifield of the frontal horizontal plane, preceded on some trials by an identical distractor coming from directly ahead of the listener (0°). Each 189-trial block used one randomly chosen combination of the target presentation hemifield (left or right), the percentage of non-distractor trials (50%, 25%, or 10%), and the distractor-to-target stimulus onset asynchrony (SOA of 25, 100, or 400 ms). Performance was compared to baseline blocks that only contained no-distractor trials.

Contextual shifts up to 4° away from the distractor location were observed in all conditions, with only small decreases at the longest SOA or when the percentage of distractor trials was the lowest. The contextual shifts were observed

at all target speaker locations and the build-up of the shifts was fast, reaching the maximum (or disappearing) within the first 40 trials after the onset (or the offset) of the distractor trials. The general character and the quick build-up of the effect suggest that the task-specific context is a top-down factor and that it can influence localization performance in a variety of experimental and everyday conditions.
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423 Room Adaptation Effects on Speech Intelligibility as a Function of Room Reverberation Time

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Results from recent studies suggest that prior listening exposure to the acoustics of a reverberant listening environment can facilitate speech intelligibility in the same environment. Currently unknown is the extent to which these adaptive speech perception abilities depend on specific aspects of a room's acoustics. Here speech intelligibility was tested in five room environments, with broadband reverberation times (T_{60}) ranging from < 0.01 s (anechoic) to 3 s. Virtual auditory space techniques were used to simulate all room environments and reverberation time was varied by changing the absorptive properties of the reflecting surfaces in the virtual room (dimensions: 5.7 m × 4.3 m × 2.6 m). Two listening conditions were tested in each room: one in which listeners were presented with only the color/number targets from the CRM speech corpus (C/N condition), and one in which listeners were presented with a two-sentence carrier phrase preceding the color/number targets (DC condition). The room adaptation effect was defined as the difference (improvement) in performance between these two conditions. In all conditions the speech signals were presented at a location directly in front of the listener (1.4 m source distance) and a competing masker (broadband noise) was presented opposite the listener's right ear. Signal-to-noise ratio (SNR) was varied over a range from -28 to +4 dB. In general, threshold SNR increased as T_{60} increased. The differences in thresholds between C/N and DC conditions (adaptation) were near zero for both the least and most reverberant rooms, but increased as a function of T_{60} for the moderately reverberant rooms, with a peak of greater than 3 dB (an improvement of greater than 20% correct) for most listeners in the room with T_{60} = 1 s. These results suggest that the magnitude of the room adaptation effect depends critically on room reverberation time, and that the effect may be most beneficial to speech intelligibility in rooms with moderate reverberation times ($0.3 \leq T_{60} \leq 1$ s). [Work supported by NIH DC008168]

424 Processing of Binaural Pitch Stimuli in Hearing-Impaired Listeners

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Binaural pitch is a tonal sensation produced by introducing a frequency-dependent interaural phase shift in binaurally-presented white noise. As no spectral cues are present in the physical stimulus, binaural pitch perception is assumed to rely on accurate temporal fine structure coding and intact binaural integration mechanisms. This study investigated to what extent basic auditory measures of binaural processing as well as cognitive abilities are correlated with the ability of hearing-impaired listeners to perceive binaural pitch. Subjects from three groups (1: normal-hearing; 2: cochlear hearing-loss; 3: retro-cochlear impairment) were asked to identify the pitch contour of series of five notes of equal duration, ranging from 523 to 784 Hz, played either with Huggins' binaural pitch stimuli (BP) or perceptually similar, but monaurally detectable, pitches (MP). All subjects from groups 1 and 2 and some from group 3 could hear both MP and BP in more than 80% of all presentations; these subjects obtained similar contour identification scores for MP and BP. Other subjects from group 3 could hear most MP but none of the BP pitches. These other subjects obtained significantly lower binaural masking level differences and binaural intelligibility level differences than subjects from group 1, but did not necessarily show reduced scores in a lexical decision task and a reading span test. Overall, these findings confirm that binaural pitch perception is either immediate or absent in hearing-impaired listeners, and that its absence is not due to a general difficulty extracting tonal objects from noise; they also suggest that the absence of binaural pitch percept might be an indicator of retro-cochlear impairment. A total absence of binaural pitch percept was found to coexist with impaired low-level binaural processing, but not to imply reduced cognitive function.

425 Binaural Pitch-Matching and Fusion Range in Patients with Asymmetrical Hearing Loss

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The purpose of this study is to examine the amount of frequency difference between ears that the human brain can adapt to and still perceive a fused image with a single pitch. In normal hearing listeners, two identical-frequency tones which are perceived as having different pitches between ears when listened to sequentially (diplacusis) can still be fused as a single perceived pitch when presented simultaneously (Thurlow and Berstein, 1957; Ward, 1970; Van den Brink, 1976). Listeners with normal hearing and those with asymmetrical hearing loss were recruited to measure the frequency difference that could be fused across ears into a single pitch percept for simultaneously presented tones (fusion band). The pitch mismatch between ears for sequentially-presented tones (diplacusis) was also measured. It was found that listeners with hearing loss have much greater amounts of diplacusis than do listeners with normal hearing. The pitch mismatch between ears generally falls within the fusion band for each subject. These results suggest that a large pitch discrepancy between ears in listeners with asymmetrical hearing loss can still be fused to have the same pitch percept. This implies that, following hearing

loss, the central auditory system can adapt to large discrepancies between ears in order to provide a single unitary perception of pitch.
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426 Effect of Reverberant Energy on Binaural Loudness

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The loudness of sound fields has typically been investigated in anechoic environments free from reflections, or in the presence of only moderate amounts of reverberation. Despite the sparsity of investigated acoustic environments, loudness models make no distinction in processing direct sound and reverberation: it is assumed that loudness is solely determined by the signals superimposed at the listener's ears. Several perceptual effects of reverberation in rooms, however, have been reported. These include e.g., increased spaciousness and more accurate distance localization, which may be attributed to decorrelation and varying direct-to-reverberant energy ratio of the signals at the ears, respectively. The extent to which reverberant energy affects binaural loudness has received relatively little attention, and it is unclear whether inputs other than at-the-ear spectra are needed in modeling the loudness of reverberant sound fields.
The aim of this study is to investigate the effect of reverberant energy on binaural loudness perception. A room model is utilized to generate binaural room impulse responses (BRIRs) with varying amounts of reverberation. Loudness matches between a reference sound and stimuli processed through the BRIRs are obtained from listeners. Results and implications to modeling the loudness of reverberant sound fields will be discussed. [Work supported by NIH DC008168, the Academy of Finland and Emil Aaltonen's Foundation]

427 Manipulations of Temporal Features of the Envelopes of High-Frequency Stimuli That Affect Sensitivity to Ongoing Interaural Temporal Disparities

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Listener's threshold ITDs were measured using an adaptive two-alternative paradigm employing "raised-sine" stimuli [John et al., Ear and Hearing **23**, 106-117 (2002)]. Such stimuli permit one to independently vary frequency of modulation, depth of modulation depth, and "peakedness". Threshold-ITDs were measured while varying the peakedness of the envelope for stimuli having modulation frequencies between 32 and 256 Hz. Graded *increases* in the peakedness led to graded *decreases* in envelope-based threshold ITDs. Threshold ITDs were also measured while parametrically varying peakedness and modulation depth. Overall, threshold ITDs decreased with increases in the modulation depth. Unexpectedly, increases in the peakedness of the raised sine led to especially large decreases in threshold ITD when the modulation depth was 25%. An interaural correlation-based model was generally able to capture changes in threshold ITD stemming from changes in the peakedness, depth of modulation, and frequency of modulation of the raised-sine stimuli. Several efforts to account, quantitatively, for the unexpected interaction between the peakedness and modulation depth were unsuccessful. [Work supported by NIH DC04147].

428 Frequency Invariant Combination of Across-Frequency and Across-Ear Cues

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Thresholds of a sinusoidal signal masked by a broadband masker can markedly be improved when the masker is comodulated (CM) compared to a masker where the intensity fluctuations are uncorrelated (UN) in different frequency regions. This release from masking is commonly referred to as comodulation masking release (CMR(UN-CM)). Another release from masking can be observed when a phase difference (IPD) is introduced in a signal masked by a diotic noise. This release from masking is commonly referred to as binaural masking level difference (BMLD). At a previous ARO we showed for a signal frequency of 700 Hz that the masking release for combined across-frequency and across-ear cues is equivalent to the sum of the monaural and binaural masking releases (Verhey & Epp, ARO 2007). The present study investigates if this superposition of masking releases is found for signal frequencies of 200 Hz and 3 kHz. A similar experimental paradigm was used with one masker band centred at the signal frequency and two additional masker bands above and below the signal frequency with a minimum distance of two critical bands. Additionally a transposed stimulus was used to increase the BMLD at 3 kHz. Thresholds were measured for various IPDs. While the magnitude of the single effects CMR(UN-CM) and BMLD varies with signal frequency, the total masking release can consistently be described as the sum of the two masking releases CMR(UN-CM) and the BMLD. This data discussed considering the bandwidth sensitivity of the binaural system supports the hypothesis that the masking release due to comodulation in dichotic listening conditions is frequency-invariant and can be described as the sum of CMR(UN-CM) and the BMLD.

429 Antagonists of Neural Nitric Oxide Synthase Affect Auditory Behaviors in Mice: A Study of the Acoustic Startle Reflex (ASR) and Its Inhibition by Gaps in Noise and a Change in Sound Source Location

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Rationale: The neuronal form of nitric oxide synthase (nNOS) is expressed at each stage of both monaural and binaural transmission of auditory information from the cochlea to neocortex, and expressed also in serotonergic

and cholinergic systems that regulate afferent activity. Recent evidence indicates that NMDAR-mediated NO-cGMP signaling affects brainstem auditory processing, suggesting that a specific inhibitor of nNOS (7-Nitroindazole, 7-NI) should then alter auditory-guided behaviors.

Design: Three experiments were conducted in which: (A) The ASR was elicited by WBN pips presented at 80 to 130 dB SPL, in a quiet background or in 70 dB WBN (0, 50, and 100 mg/kg 7-NI suspended in peanut oil, *ip*). (B) The ASR was elicited by 110 dB WBN pips in 70 dB WBN presented from a speaker 22.5° right of center, or 1 to 300 ms after WBN was shifted 45° to the left (0, 50, 100, and 160 mg/kg 7-NI); (C) The ASR to 110 dB WBN pips was elicited in 70 dB WBN, 60 ms after the offset of a quiet gap in the noise, 0 to 15 ms in duration (0 and 160 mg/kg 7-NI). We measured non-specific spontaneous activity prior to each startle trial.

Results: (A) Noise masked weak stimuli but potentiated the ASR to intense stimuli, and 7-NI did not affect these ASR results. (B) Changes in location and (C) gaps in noise inhibited the ASR: peak levels of inhibition increased with 7-NI dose but the timing of inhibition did not vary. Spontaneous motor activity was reduced with increasing 7-NI.

Conclusions: These data indicate that NO signaling affects some but not all aspects of brainstem auditory processing, but the unexpected finding that the test stimuli were more effective when NO levels were reduced by 7-NI requires explanation. 7-NI may directly affect intrinsic auditory processing or may influence this activity via non-specific systems (e.g., for attention, arousal, or motor control). *In vivo* and *in vitro* recording and focal application of 7-NI in specific auditory nuclei will resolve these issues.

430 Psychophysical Characteristics of the Franssen Illusion in Humans and Birds: Effects of Time and Intensity

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The auditory illusion known as the Franssen Effect (FE) is similar to the Precedence Effect but has received far less attention in the literature. This auditory illusion has so far been characterized in humans, cats, and more recently in non-mammalian birds. To elicit the FE, listeners are generally presented with a signal which has been split into a transient component with an abrupt onset and ramped offset which is separated in space from the second component which has a slowly rising onset and longer overall duration. When these two signals are played to human and cat listeners simultaneously, under certain conditions (where listening environment is difficult) the perception is that of a long-duration steady state tone being played at the location of the transient component even though the sound is no longer there. The current experiments aim to extend these general findings by manipulating aspects of time and intensity to more precisely determine the influence of these physical parameters on the incidence of the FE. We examined the FE in human listeners and two species of birds: budgerigars and zebra finches. Both humans and birds were tested on the exact stimuli and each participant served as their own control in each of the six experiments in the study. Both humans and birds exhibited the FE to varying degrees across conditions; some manipulations of time and intensity weakened the illusion, while others seemed to strengthen the illusion. The implications of these data and comparisons between birds and humans regarding the incidence of the FE will be discussed.

431 The Localization of Speech in "Precedence-Effect" Situations by Normal and Hearing-Impaired Adults in Quiet and in Babble

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The "precedence effect" is commonly regarded as useful when localizing sounds in rooms, as it enables a listener to make a decision on the direction of a source using only the first-arriving (direct) sound while ignoring subsequent echoes and reverberation. Any difficulties with the precedence effect will thus be of practical importance as they will interfere with sound localization: affected listeners might be expected to report the sound to be in a direction different to the actual direction of the source. The goal of this experiment was to determine if such mis-locations occur amongst elderly normal and hearing-impaired listeners and, if so, to estimate how severe they are. Listeners were required to report the direction that they thought a target sound came from. The target sound (a single word) was made-up of a "lead" signal quickly followed by a "lag" copy (4 ms later, and at the same level) that was 60 degrees apart. All the signals were presented using a horizontal ring of 24 loudspeakers, each separated by 15 degrees, and in which the participant sat in the center. A large combination of directions were randomly intermingled during the experiment. The stimuli were presented in either silence or in a diffuse babble, at a signal-to-nose ratio of 0, 6, or 12 dB. Currently 10 normal-hearing and 26 hearing-impaired listeners have completed the test (for these, mean age = 62; mean better-ear hearing-loss = 33 dB). The results show that most responses are not exactly on the lead sound but are biased towards the lag. In quiet, 20/35 listeners gave a mean bias of 0-10 degrees; 12/35 a bias of 10-20 degrees, and 3/35 a bias larger than 20 degrees. The biases were larger in babble: 1/35 listeners gave a mean bias of 0-10 degrees; 25/35 a bias of 10-20 degrees, and 8/35 a bias larger than 20 degrees. These results confirm that some listeners (in babble, at least 20%) would suffer noticeable mis-locations in precedence-effect situations.

432 Effects of Spectral Variability on Monaural Azimuthal Localization

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To localize a sound, monaural listeners must rely on the information that the head shadow introduces into the overall level and spectral shape. The overall level and

spectral shape at the ear, however, depend on both the source location and the overall level and spectral shape of the source. To investigate the effects of spectral variability on monaural localization, we measured the ability of normal-hearing individuals listening monaurally to discriminate between two virtual source locations (±30° from the midline). The stimulus had a random overall level and a random spectral shape (250 ms multi-tone stimulus with 3 components per octave between 500 and 8000 Hz). The covariance matrix of the level of each component can be written as $\sigma_E^2\mathbf{I} + \sigma_L^2\mathbf{U}$, where σ_E and σ_L are the standard deviations of the component and overall level perturbations, respectively, and **I** and **U** are the identity and unit matrices, respectively. The value of σ_L was fixed at 8 dB and σ_E was parametrically varied between 0 and 8 dB. The measured d′ systematically decreased as σ_E increased. Subjects were still able to reliably discriminate (values of d′ near unity) between the two source locations with σ_E equal to 4 dB (and in some cases 8 dB). Although there were only small individual differences in sensitivity, the subjects' relative weighting of each frequency component revealed differences in their decision strategies. The weighting patterns, however, were nearly independent of σ_E, indicating that each subject had a stable strategy. The overall efficiency, which accounts for both non-optimal weighting and internal noise, of the subjects was low suggesting that it is difficult to use the complicated changes in the spectral shape to determine the location of a sound source. In spite of the low efficiency, discrimination between two locations was still possible with spectrally variable stimuli. [Supported by NIH DC002012 and DC 009384]

433 Factors Affecting Distance Discrimination in a Territorial Songbird

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In acoustic communication it is advantageous for the receiver to assess the distance to the sender, e.g. a territorial bird benefits from assessing if another male sings inside or outside its territory. During transmission echoes are imposed on acoustical signals and distort its original amplitude and time pattern. Different distances lead to different echo patterns. The ability to distinguish different echo patterns is a prerequisite to use reverberation for distant assessment.

In the present study a territorial song bird, the great tit (Parus major), was used to test the influence of background noise on the ability to discriminate signals with different echo patterns. Reverberations were imposed on synthesized great tit song elements equivalent to sound transmission distances of between 5 and 160 m simulated in a "virtual forest". Great tits were trained in an operant Go/NoGo procedure with repeating reference to discriminate between song elements in which echo patterns simulated different distances. The response latencies of the great tits to all possible reference-test differences were recorded. Shorter response latencies indicate more salient differences for the subjects. Songs were composed of 2 or 3 elements. Signals were presented in silence or in a continuous background noise consisting of a natural dawn chorus.

A repeated measures ANOVA of data showed that response latencies were significantly dependent on the virtual distance and on the background noise. Response latency for the shortest virtual distance (5 m) were on average about 670 ms shorter than for the longest virtual distance (155 m) tested in this study (preliminary data from 4 individuals). In the continuous dawn chorus background response latencies were on average about 220 ms longer than in silence. The results suggest that perception of sound degradation may allow the assessing of the distance of another singer, but this ability is compromised by background noise.

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434 Environment-Optimized Noise Suppression for Cochlear Implants

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A previous study indicated that a noise-suppression strategy basedon Ideal Binary Mask (IdBM) has the potential of restoring speech intelligibility for cochlear implant users in noisy environments.

Compared to the commercially available ACE strategy that uses the maximum amplitude criterion to select the stimulation channels, IdBM applies a new selection criterion based on the signal-to-noise ratio (SNR) of individual channels. This new criterion picks target-dominant (SNR>=0 dB) channels and discards masker-dominated (SNR<0 dB) channels.

To apply this new noise-suppression strategy, a reliable SNR estimation algorithm needs to be developed. In the present study, we focus on a neural network (NN) based method that is trained to estimate the SNR values for a particular noisy environment. The NN uses speech features based on amplitude modulation spectrograms and is trained to learn the mapping of the extracted features to the target SNR values in each channel.

The performance of the proposed noise-suppression approach was evaluated in three different types of noisy environments: babble, train and exhibition hall. A different neural network was trained for each environment. Corrupted IEEE sentences at 5 and 10 dB SNR were input to the trained neural network to estimate the SNR in each channel. Channels with estimated SNR larger than a preset threshold were retained and channels with SNR smaller than the threshold were discarded. Sentences processed using the above SNR selection criterion was presented to seven cochlear implant users for identification. Results indicated significant improvement in performance with the proposed noise-suppression strategy compared to the implant users' daily strategy. The present study demonstrated the feasibility of noise suppression algorithms that are optimized to a specific noisy environment. This will allow cochlear implant users to switch to environment-specific noise-suppression programs for improved speech intelligibility.

435 Improvement of Speech Indelibility of the Hearing Impaired with Speech Enhancement Algorithm

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There is a persistent complaint among the hearing impaired (HI) of difficulties in understanding speech in background noise. Although most of the hearing assistive devices (hearing aids as well as cochlear implants) include some sort of advanced digital speech enhancement algorithm, the outcome so far is not satisfactory.

The purpose of the present study was to evaluate the effectiveness of a new speech enhancement algorithm which is based on a cochlear model including the role of the outer hair cells. This was evaluated in HI and normal hearing (NH)) listeners. Stimuli consisted of monosyllabic words (AB words) which were embedded in Gaussian noise at several signal to noise ratios. The results demonstrate that the use of the present algorithm significantly improved speech intelligibility in noise of the hearing impaired, in particular those who use cochlear implants, or those who use hearing-aids for a long time. No benefit, however, was demonstrated when using this algorithm in the NH. It is possible that hearing impaired listeners benefit from this algorithm because they developed speech processing strategies that are compatible with those used in the speech enhancement technique. This may suggest that speech enhancement algorithms need to be tailored to the HI individual.

436 Effects of Experience with Cochlear-Implant-Induced Spectral Shifts on Electric Pitch Perception

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Recent experiments indicate that pitch perceived through a Hybrid (short-electrode) cochlear implant can shift after 1-5 years of experience with the implant, by as much as 2 octaves (Reiss et al., 2007). One hypothesis is that the changes are driven by spectral discrepancies between ears introduced by the patient's speech processor frequency-to-electrode allocation, or MAP.

To test this hypothesis, we recruited patients who were previously provided with a narrow, high frequency MAP (e.g. 688-7938 Hz) to wear a new broad, low frequency MAP (188-7938 Hz) for at least 3 months. Electric pitch sensations were matched to acoustic pitch sensations in the non-implanted ear after experience with the new MAP. Preliminary data for one patient, after 1 month with the new MAP, shows that the pitch matches shifted to lower frequencies, as predicted.

We also present data obtained from another patient who had bilateral cochlear implants. This patient was initially given a Hybrid cochlear implant, but was subsequently given a long-electrode implant in the opposite ear after a bilateral loss of hearing from auto-immune causes. Both devices were programmed with a 188-7938 Hz MAP, despite the large differences in insertion depth and cochlear place frequency. After 1 year of experience with these MAPs, pitch matches were conducted by comparing the electrodes between ears. Pitch matches between the two devices were not aligned by cochlear position, but by the similarity of the MAP frequencies allocated to each electrode.

These findings suggest that over time, cochlear implant patients can adapt to the spectral mismatch introduced by MAPs by aligning pitch between the two ears according to input frequency, rather than cochlear place. Therefore, deep electrode insertion into the cochlea may not be necessary if there are sufficient neural elements in the cochlear base. Overall, experience-dependent changes may be more important in cochlear implant perception than previously thought.

437 Cochlear Implant-Mediated Perception of Polyphonic Pitch

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The ability of cochlear implant (CI) users to perceive music remains severely limited. While many studies have examined inaccuracies of pitch mapping in CI users, no studies have examined the perception of polyphony, which is fundamental to the perception of harmony. We hypothesized that CI subjects would show decreased ability to differentiate between single vs. multiple pitches in comparison to normal listeners. In this study, twelve post-lingually deafened CI subjects and twelve normal hearing (NH) subjects were recruited. Subjects were presented with a listening task in which one, two, or three pitches within a single octave(F0 range from 261-523 Hz) were presented simultaneously, using both pure tones and piano tones. Subjects were asked to identify the number of pitches heard. The NH group demonstrated statistically significant advantages in mean performance than the CI group (NH 66.9±9.4%; CI 43.1±12.3%; t test $p<0.001$). The CI group performed significantly poorer in perception of intervals and chords than NH listeners (CI 1-note 69.1±18.6%, 2-notes 29.1±14.1%, 3-notes 30.9±20.0%; NH 1-note 90.6±9.9%, 2-notes 60.4±14.8%, 3-notes 49.6±13.0%; t test: 1-note $p=0.0026$, 2-notes $p<0.001$, 3-notes $p=0.0136$). Both groups performed slightly better with piano tones than pure tones.

These results demonstrate that perception of polyphony is poor in CI users. Limitations in polyphonic pitch separation underlie poor perception of harmony in CI users and require further attention in order to improve implant-mediated perception of music.

438 Cochlear Implant-Mediated Perception of Musical Timbre Using Instrumental Chimeras

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The perception of musical timbre is a formidable challenge for cochlear implant (CI) users. Using the Hilbert transform, complex sounds of varying timbre can be

mathematically deconstructed into the two components of envelope and fine structure. In this study, we created "instrumental chimeras" that systematically combined variable amounts of envelope and fine structure in 25% increments from pairs of musical instruments. We hypothesized that poor perception of temporal fine structure was responsible for poor timbre perception in CI users. Four instruments (piano, flute, guitar, trumpet) playing an identical eight-note melody were used as the sources for all chimeras. Of these four instruments, two had percussive envelopes (piano and guitar) while two had sustained envelopes (flute and trumpet). Instrumental chimeras were presented to CI users (n=12) and normal hearing listeners (NH; n=14). During testing, subjects were given a choice of two instruments that represented the source pair from which the chimeras were created (eg. piano/flute). They were then randomly presented with all chimeras generated from that pair (6 pairs x 25 chimeras/pair = 150 total chimeras). After stimulus presentation, subjects were asked to determine which original instrument the chimera more closely resembled in a single-interval two alternative forced choice task. Our results show that NH controls and CI subjects vary significantly in their use of envelope and fine structure information for chimeras made from instrument pairs with similar envelopes vs. dissimilar envelopes. When chimeras were created from similar envelope instrument pairs, reducing the value of envelope as a cue, control subjects relied heavily on fine structure information to make timbre judgments (one-way ANOVA, $p=0.006$). CI subjects, by comparison, were unable to utilize fine structure or envelope in these cases and showed a random distribution to their timbre judgments (one-way ANOVA, $p=0.856$ envelope; $p=0.281$, fine structure), regardless of the ratio of fine structure information provided. When chimeras were created from dissimilar envelope instrument pairs, NH controls utilized a mix of envelope and fine structure information to make timbre judgments (one-way ANOVA, $p<0.001$ envelope; $p<0.001$ fine structure). In contrast, CI users utilized envelope information almost exclusively to make timbre judgments and ignored fine structure information (one-way ANOVA, $p<0.001$ envelope; $p=0.086$ fine structure). These findings demonstrate that reduced perception of fine structure underlies poor performance on timbre tasks in CI users.

439 Accurate Tuning of a Guitar by a Cochlear Implant Musician

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Modern multi-electrode cochlear implants (CI) can restore essentially normal speech recognition in quiet and yet still fail to provide adequate music perception, particularly in pitch related tasks. A high-performing CI user can converse on the phone but cannot recognize simple nursery melodies. Despite this, there are musicians that continue to perform even after implantation of a cochlear prosthesis. This study documents one such CI musician who is able to tune a guitar quickly and precisely without the aid of an electronic tuner, starting with a reference string and using harmonics to produce the same note across two strings. After plucking each string and allowing them to ring together, the subject was able to judge when the two notes were matched in frequency and if not, adjust one of them accordingly. When tested under controlled conditions with digitally generated pure tones (1s duration, comfortable loudness), tone frequencies could be matched to a randomized reference frequency (in the range of 100-1600 Hz) with less than 1 Hz error when the two tones were presented simultaneously. When the tones were presented sequentially, the error was much larger, indicating that the cues used in the previous task were beats that resulted from slightly mismatched tones. This was confirmed directly in the output of the subject's own speech processor. The strength and frequency of beating was reflected in the depth and frequency of amplitude modulation of the electrical stimulation pulses. In this case, tuning an instrument becomes a temporal discrimination task based on amplitude modulation detection rather than spectral. The observations in this report demonstrate that despite limited spectral resolution imposed by the physical properties of CI, users can take unexpected advantage of their processors to accomplish tasks that are normally considered difficult in the absence of fine pitch discrimination ability.

440 Vibrotactile Stimulation Enhances Cochlear-Implant Music Perception But Not Speech Perception

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While cochlear implants (CI) can restore speech recognition in most of their users, they produce poor music perception. Vibrotactile stimulation has been shown to facilitate lip-reading in hearing-impaired listeners, but its effect on music and speech perception remains unexplored in CI users. Here we tested a group of CI users in familiar melody recognition with or without the rhythm cue, in sentence and word recognition with the hearing in noise test (HINT), and in consonant and vowel perception under auditory-only, vibrotactile-only, and combined auditory-vibrotactile stimulation conditions. The original stimuli were presented to CI subjects via loudspeakers. The low-pass (< 500 Hz) filtered stimuli were used to drive a vibrotactile stimulator attached to the fingertip of the CI subject. The vibrotactile stimulation significantly enhanced CI melody recognition with or without the rhythm cue, but didn't enhance CI speech perception. The reason for the observed differential effects of vibrotactile stimulation on CI music and speech recognition is not clear. Together with our previous results showing enhancement of vibrotactile stimulation on Mandarin tone recognition in Chinese-speaking CI users, the present results suggest that vibrotactile stimulation can be added to a CI processor to improve CI music and pitch perception.

441 Perception of Spanish-American Accented English in Cochlear Implant Users

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Although cochlear implant technology has benefited from advances in device design and processing capabilities, assessment methods have changed relatively little since the introduction of multichannel implants. The speech-testing protocol typically utilized in the U.S. includes recorded tests of monosyllabic words and sentences spoken by male talkers of General American English (GAE). The limited scope of these test materials does not reflect the varied listening situations and talker types that implant users encounter on a daily basis, thus minimizing the ability of clinicians to appropriately program devices and counsel recipients regarding "real-world" benefit. The literature suggests that stimulus variability should be introduced into the test battery. One source of variability is the degree of accented speech. Given that the Spanish speaking population is the fastest growing language group in the U.S, implant users are increasingly exposed to Spanish-American accented English (SAE). The purpose of this study was to compare the performance of postlingually deafened adult implant users in tests of both SAE and GAE. We hypothesized that all implant subjects would perform significantly poorer when listening to Spanish-American accented language stimuli. We constructed new recordings of the HINT sentences spoken by three GAE and three SAE talkers (both male), and presented these tests to twenty adult implant recipients and twenty normal-hearing (NH) controls. Implant users demonstrated poorer speech perception for SAE in comparison to controls. Median GAE scores were -2.96 dB SNR for the NH group and 7.59 dB SNR for the implant group. Median SAE scores were -1.18 dB SNR for the normal hearing group and 11.35 dB SNR for the implant group. These results suggest that the presence of an accent degrades speech performance for implant users. Test material should be diversified to reflect real-world listening situations in a more ecologically valid manner.

442 Use of Fundamental Frequency and Duration Information in Lexical Tone Perception and Production by Cochlear Implant and Normal Hearing Children

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This study examined the utilization of fundamental frequency (F0) and duration patterns in the perception and production of lexical tones by prelingually deafened, pediatric cochlear implant (CI) recipients and their normal-hearing (NH) peers. All participants are native speakers of Mandarin Chinese. In the perception task, acoustic cues for lexical tones, including F0 and duration patterns were manipulated orthogonally between the high level tone (Tone 1) and the high-falling tone (Tone 4) of a disyllabic word, yan-jing. In a single-interval, two-alternative forced-choice task, each participant identified whether each stimulus sounded like yan3-jing1 ('eye') or yan3-jing4 ('eyeglasses'). In the production task, lexical tones were recorded from each participant. The preliminary results indicate that: (a) CI users' reliance on the F0 cue in lexical tone perception was much less pronounced than that of NH listeners; (b) CI users demonstrated systematic utilization of duration cues in lexical tone perception, whereas NH listeners did not; and (c) unlike NH listeners who primarily contrasted F0 patterns in lexical tone production, CI users contrasted both F0 and duration patterns. These results suggest a direct link between the perception and production of the acoustic information critical for lexical tones. [Supported by Chi-Mei Medical Center Research Fundation & NIDCD-R01DC04786]

443 F0-Based Speech Intonation Recognition by Hearing and Cochlear-Implanted Children

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Relatively little is known about the ability of young children to process the specific acoustic cues involved in speech intonation recognition, which plays an important role in speech perception. Further, to what extent early-implanted children can detect F0-based speech intonation patterns, is unknown. In this study, we investigated the ability of normally hearing (NH) adults, NH children, and cochlear-implanted (CI) children to identify different F0 contours (rising, falling or flat) in a single bisyllabic word ("popcorn") as question-like ("asking") or statement-like ("telling"). Results were obtained as "percent question" and plotted against the change in F0 from the beginning to the end of the contour. All children were six to eight years of age. The NH children demonstrated shallower psychometric functions than the adults. However, the children's asymptotic performance was similar to that of the adults. The CI children performed significantly more poorly than their NH peers in a sentence-recognition-in-noise task. Surprisingly, the results indicate no significant difference between the performances of the two groups of children in the speech intonation recognition task. Implications of the results for the processing of speech and prosody by NH and CI children will be discussed.

444 A Quantitative Estimate of the Contribution of Voice Fundamental Frequency Variations to Sentence Intelligibility in Tone and Non-Tone Languages

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Languages differ greatly in the acoustic and phonological features they exploit in conveying meaning. One interesting difference concerns the use of voice fundamental frequency variations, which underlie voice pitch or melody. In so-called 'tone languages', variations in voice pitch signal differences in meaning that are important in the same way as a change in a consonant or a vowel. Pitch variations convey important information in non-tonal languages too, but they do not serve this lexical function.

Voice pitch variations must, then, contribute more to sentence intelligibility in tonal languages than in non-tonal ones. However, this contribution is difficult to quantify because eliminating tone whilst preserving all other acoustic features leaves speech from even a tonal language highly intelligible, not least because of contextual cues. Here, we estimate the contribution tonal variation makes to sentence intelligibility in terms of a common metric - degree of spectral detail. Vocoding was used to manipulate the degree of spectral resolution (1, 2, 4, 8 and 16 channels) and the presence or absence of voice pitch variations in simple sentences (the natural contour vs. a neutral falling one). Listeners were asked to repeat back as much of the sentence as possible, with scoring based on the correct identification of key words. A logistic regression model of the proportion of key words correct was constructed with predictors of the $\log_2$ of the number of channels (a unit we refer to as a 'doubling') and the presence or absence of natural voice pitch variations. Comparing the coefficients in the model results in a measure of the 'worth' of tonal variations in terms of spectral detail. Using this measure for results obtained with 2 non-tonal languages (English and Lithuanian), we find that tone is 'worth' 0.1-0.4 doublings, depending upon the particular talker. In contrast, tone is 'worth' 0.6-1.0 doublings in the 3 tonal languages we studied (Mandarin, Cantonese and Yoruba). The fact that tone can improve intelligibility by the same degree as doubling the number of channels in tone languages strongly supports the notion that improvements in the transmission of tonal variations in cochlear implants would be a great boon for users, especially for speakers of tonal languages.

445 Relative Contributions of Pitch and Intelligibility to Improved Speech Perception in Noise Under Electro-Acoustic Stimulation

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While many cochlear implant (CI) users perform as well as normal hearing listeners at speech recognition in quiet, they have great difficulty understanding speech in noise. The addition of a hearing aid (HA) or even the introduction of fundamental frequency (F0) has been shown to improve CI speech recognition in noise for those with residual hearing. The benefit from this combined electric and acoustic stimulation is attributed to either intelligibility addition or pitch enhancement. These two mechanisms are fundamentally different and their relative contributions have yet to be fully explored. Here we tested 10 normal hearing subjects using a 4-channel CI simulation along with a HA simulation (<500 Hz low pass) or the F0 cue. The subjects were tested in vowel and consonant recognition tasks in both quiet and in noise (signal-to-noise ratio 0 dB) under five listening conditions: 1) CI-only, 2) HA-only, 3) F0-only, 4) CI and HA, and 5) CI and F0. The phoneme tokens and noise were presented simultaneously to the right ear via headphones at 70 dB SPL. Both the overall percent scores and the confusion matrices were obtained for each subject under each condition. The HA simulation significantly enhanced CI phoneme perception in quiet and in noise, while the addition of F0 significantly enhanced CI phoneme recognition in noise only. The Sequential Information Analysis (SINFA) of the confusion matrices showed that the HA increased the transfer of information related to both pitch and intelligibility, whereas the F0 mostly enhanced the transfer of pitch information.

446 Melodic Contour Identification Training by Mandarin-Speaking Pediatric Cochlear Implant Listeners

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Contemporary cochlear implant (CI) devices provide only limited pitch cues, which are important for music perception and tonal languages such as Mandarin Chinese. Previous studies have shown that a closed-set melodic contour identification (MCI) task could be used to quantify adult CI users' melodic pitch perception, and that MCI performance could be improved with moderate auditory training. In the present study, MCI performance was measured in 14 Mandarin-speaking pediatric CI users; MCI was also trained in 6 of these subjects. For the MCI task, test stimuli were melodic contours composed of 3 or 5 notes of equal duration whose frequencies corresponded to musical intervals. The musical "instruments" were either a 3-tone complex or a piano sample (MIDI synthesis). Similar to previous studies, results showed great inter-subject variability in MCI performance, with scores ranging from chance level to nearly perfect. On average, subjects

were able to identify 33.3% contours. There were no significant effects for contour length (3 or 5 notes) or for instrument type. Six subjects received MCI training using the 3-tone complex/5-note contours; subjects were trained using different pitch ranges than those used for testing. Subjects trained for half hours/day, 5 days/week for 10 weeks. After training, mean MCI performance improved from 24.7% to 76.1% correct. Results showed that the training improved not only the performance for the 3-tone complex/5-note contours, but also for the remaining untrained instruments and contour lengths. These preliminary data indicate that Mandarin-speaking pediatric CI patients exhibited a greater capacity to learn melodic pitch than reported in previous studies with English-speaking adult CI users, possibly because of the greater importance of pitch cues to tonal language. MCI training may be useful for improving Mandarin-speaking pediatric CI users' music perception and speech perception.

447 The Effect of Amplitude Envelope on Mandarin Tone and Sentence Recognition in Users of Cochlear Implants

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One major limitation in current cochlear implant devices is in providing voice fundamental frequency (f0). This causes special difficulty in users of tonal languages in which pitch variations are used to convey lexical meanings. Recent studies have reported that other acoustic cues such as amplitude envelope and duration can contribute to the recognition of tonal contrasts in Mandarin when f0 information was degraded or completely absent. The present study examined the effect of amplitude manipulation by either eliminating the natural variation of amplitude envelope or enhancing the amplitude envelope cue. The investigations were conducted in both implant users and normal-hearing listener using acoustic simulations of 4-channel vocoded speech. Experiment I examined the effects of flattening the speech amplitude envelope and of removing variations in syllable duration on tone recognition for isolated syllables. Results showed that tone recognition decreased considerably when the amplitude cue was neutralised. As it has been suggested that the contribution of amplitude envelope might arise from its similarity to the f0 contour, experiment II directly manipulated the amplitude envelope to closely resemble the f0 contour. Both isolated syllables and syllables in a sentence context were examined. The enhancement of the amplitude cue to follow f0 had only small effects on tone recognition, mainly in the rising tone. The effect of this modification of the amplitude envelope in sentence context was negligible. [Part of this work supported by NSC96-2413-H-133-011-MY2]

448 Pitch Matching Between Acoustic and Electric Stimulation by Cochlear Implant Patients with Normal Hearing in the Unimplanted Ear

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As part of a study investigating the effects of cochlear implants (CIs) on tinnitus in patients with unilateral hearing loss (Baguley *et al*, this meeting), we have performed pitch comparisons between electrical stimulation of a CI and acoustic sounds presented to the contralateral, normal-hearing ear. In most cases, these matches were obtained at switch-on, thereby avoiding effects arising from acclimatisation to the frequency-to-electrode allocation in a patient's everyday device. In addition to comparing pure tones to high-rate (1031-pps) pulse trains, similar to other studies, we asked patients to compare 25-pps electric pulse trains to 25-pps acoustic trains. This latter paradigm allowed us to vary place pitch by changing either the electrode or the acoustic bandpass filter frequency, while presenting temporal patterns of stimulation that were very similar in the two ears. Both pitch matching and the method of constant stimuli were used; in the latter case either one or two possible electrodes could be stimulated in each block of trials, each of which was compared to seven or eight acoustic stimuli. We showed that a) some, but not all, patients report strikingly similar percepts for 25-pps acoustic and pitch-matched electric pulse trains, b) no substantial differences between the values of matches obtained with 25-pps electric vs acoustic pulse trains compared to 1033-pps trains vs pure tones, c) contrary to some previous reports, pitch matches were roughly similar to the predictions of Greenwood's frequency-to-place function and of a model that takes into account cochlear current flow and neural anatomy. We also showed that the range of comparison stimuli used can substantially influence the matches obtained, and strongly recommend that matches should always be checked using different paradigms and/or stimulus sets.

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449 Detection of Tinnitus by MEG Uisng Coherence Imaging

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Tinnitus is ringing, buzzing or any sound perceived to be coming from the head or ears without an external sound source. Although no cure for tinnitus exists there are many treatments most of which provide only limited relief. The primary purpose of this study was to determine if MEG can

detect cortical activity correlated with severity or location of tinnitus. Coherence is a measure of synchronization between brain regions. Synchronized activity within a neuronal network is determined by the strength of network connections. How well two or more brain regions are connected can be determined by measuring the coherence between these regions. We collected spontaneous MEG from patients with tinnitus and control subjects. MEG data were collected at 508Hz from 0.1-100 Hz for 10 minutes then digitally filtered 1-50 Hz. All subjects wore ear plugs to eliminate outside sounds. Subjects keep their eyes open and fixated on point on the ceiling of the room. Tinnitus patients with unilateral tinnitus participated in this study. In these subjects MEG imaging showed highly coherent brain activity in the auditory cortex, contralateral to their perceived tinnitus. The control subjects had MEG coherence maps that displayed multiple brain areas active but no particular areas were found that were highly coherent during their 10 minute scan. We determined that specific cortical areas in the auditory cortex, which may be responsible for tinnitus, are detectable using coherence mapping of the MEG signals. MEG can provide a technique that enables us to detect cortical neuronal activity that will be useful in the diagnosis of tinnitus, the detection of improvements in the symptoms of tinnitus after different treatments, and possibly assist in the determination of site of perception of the tinnitus that could be targeted with electrical or chemical therapies to mitigate the tinnitus. The main outcome of this study will be to establish an effective clinically diagnostic tool for the detection and severity of tinnitus, additionally; it may assist us in the development of future interventional strategies to alleviate tinnitus. Research supported by NIH/NINDS Grant RO1-NS30914.

450 Treatment of Tinnitus by Lidocaine Using Inner Ear Drug Delivery System

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Tinnitus is a major and unpleasant symptom caused by inner ear disorders. The quality of life of the patient is greatly reduced due to its continuous and/or repetitive sound, which can never be recognized by others. At hospitals, there are some treatment options including medications and rehabilitations, however, each option has only limited effectiveness. Among these, a local anesthetic, lidocaine, is known to be effective by intravenous or intratympanic application. Although the intravenous lidocaine was shown to be effective by double blind tests, its effect lasts only for a few hours and has considerable risk of arrythmia and allergic reaction. The intratympanic lidocaine is also known to be effective, however, it causes severe vertigo. Lidocaine has not been a realistic option for a treatment of tinnitus for these reasons. To solve these problems and realize treatment of tinnitus by lidocaine, we developed a new drug delivery system to deliver lidocaine into inner ear for a longer duration in low concentrations. Poly lactic/glycolic acid (PLGA) polymer is a biocompatible material, which dissolves slowly in tissues and is utilized as a material of absorbable surgical threads. We prepared microparticles of PLGA encapsulating lidocaine. These particles released lidocaine for several weeks in vitro. When these particles were placed on the round window membrane (RWM) of guinea pigs, lidocaine was detectable in the perilymph of the cochlea at the highest concentration 3 days after the application, and was still detectable after 7 days. As for adverse events after the application of these particles, hearing levels by ABR thresholds did not change significantly, nystagmus was not detectable, and minor inflammation in the middle ear (otitis media) was observed in a limited area. These prompt us for a clinical trial against cochlear tinnitus using lidocaine drug delivery system, which is now in preparation.

451 The Bordeaux-Cambridge Programme for Unilateral Sudden Deafness with Disabling Tinnitus

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Background: Previous studies have demonstrated that tinnitus following sudden unilateral sensorineural hearing loss (SSNHL) can be severe and intractable. Clinical interventions for this symptom are limited in scope and efficacy. In this study we utilized cochlear implants in patients with unilateral SSNHL with the aim of amelioration of their severe tinnitus.

Purpose: This study aims (1) to determine whether cochlear implantation on the affected side is compatible with normal hearing in the other ear; (2) to establish which cochlear implant fitting strategy is most effective for tinnitus alleviation.

Study design: This prospective longitudinal study was based on measurements of (a) inventories including the *Tinnitus Handicap Inventory* and the *Speech Spatial Qualities of Hearing Scale*; (b) hyperacusis handicap; (c) tinnitus pitch and loudness matching. Four adults implanted with the Advanced Bionics HiRes, either in Bordeaux or Cambridge, were included. Patients were fitted alternatively with two strategies according to a cross-over randomized paradigm, (a) one strategy, based on fine pitch matching between acoustic and electric stimulation (*see poster* by Carlyon et al) intended to promote speech understanding in noise and restore some localization skills ("speech strategy"), (b) the other strategy attempted to stimulate the auditory nerve with customized sounds in order to specifically alleviate tinnitus suffering ("tinnitus strategy"). The study hypothesis is that speech strategy based on pitch matching shows greater efficiency in tinnitus relief and better acceptance in everyday life.

Results: Results will be reported concerning (1) the ability to use cochlear implant stimulation with contralateral normal hearing; (2) the relative efficacy of speech stimulation and tinnitus sound stimulation in tinnitus alleviation.

Study sponsor: Advanced Bionics

452 Improvement of Auditory Discrimination Learning by Ginkgo Biloba Extract EGb761®

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The effect of oral application of Ginkgo biloba extract EGb761® on auditory discrimination learning in young adult male Mongolian gerbils was investigated in an aversive shuttle-box GO-NOGO paradigm using discrimination tasks with three different degrees of difficulty (cf. Schulze and Scheich, 1999, Neurosci. Lett. 261: 13-16; Kaernbach and Schulze, 2002, Neurosci. Lett, 329: 37-40), and two protocols for drug administration starting 2 weeks prior to or at the beginning of training. In comparison to placebo-treated controls we observed statistically significant improvement of learning performance in treated animals both in easy as well as in more demanding discrimination tasks. EGb761® has been reported to increase the extracellular concentration of dopamine in the prefrontal cortex of rats (Kehr et al., 2006, Planta Medica 2006; 72: 1083 (P 347)) which plays a major role in the type of learning used in the present study. We, therefore, suppose that EGb761® improves discrimination learning through its effect on the dopaminergic system.

453 Effect of Prednisolone on Acoustic Trauma at Inactive Phase of HPA Axis in Mice

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Background and Objectives: Corticosterone concentrations of the BALB/c mice hypothalamic-pituitary-adrenal (HPA) axis exhibit circadian variation with lower levels at the onset of the light (inactive) phase and higher levels at the onset of the dark (active) phase. The present study aimed to evaluate the effect of the exogenous prednisolone supply at inactive phase of HPA axis activity with comparing the hearing changes after noise exposure following oral prednisolone intake or not at inactive phase of HPA axis activity in BALB/c mice. Materials and Methods: Each 3 mice for blood sampling to measure serum corticosterone level were killed at 8:00, at 8:30, at 9:00, at 11:00 after oral several prednisolone doses (1 mg/kg, 2 mg/kg, control) supply at 8:00. The hearing levels of sixteen mice were analyzed before noise exposure, and from 1 hour to 10 days after noise exposure (8:00-11:00) in prednisolone (2 mg/kg) supply group (n=8) and control (n=8) at 7:30. Results: The serum corticosterone value is higher than 300 ng/ml in 2 mg/kg prednisolone supply group from 30 to 180 minutes. The control group showed statistically elevated threshold shifts at after noise exposure, compared with prednisolone 2 mg/kg supply group ($p<0.05$). Conclusion: Exogenous prednisolone suppy before noise exposure at inactive phase of HPA axis activity may have protective effect on hearing against noise exposure to make temporary threshold shift.

Key words: BALB/c mouse, HPA axis, Corticosterone, Noise, Hearing

454 Round Window Application of the Floating Mass Transducer – Evaluation of Potential Candidates

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In patients with cronic ear disease which had undergone multiple ear procedures as canal wall down tympanoplasty for cholesteatoma, hearing loss is still a challenge to address. These patients often do not benefit from conventional hearing aids, as they cause moist cavities with inflammation or insufficient amplification. Bone anchored hearing aids (baha) are often limited in their maximum amplification. Since 2005 the round window placement of the floating mass transducer (Vibrant MedEl) has been reported in cases of limited ossicular movement.
To determine the number of potential candidates we scanned our audiological data base of our tertiary referral center. 43292 patients were searched for combined hearing loss which had to fall into the candidacy criteria of the manufacturer.
Of these, 362 patients were identified and contacted by phone interview. We could talk to 241 patients or their household members. Of these, 77 did show general interest in the application and requested further information as a brochure. With 35 patients, an appointment for further workup was made. We report on the outcome of this evaluation.

455 Wideband Middle-Ear Muscle Reflex Test in a Test Battery to Predict Transient Middle-Ear Dysfunction in Neonates

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A wideband (WB) test battery of middle-ear status, including acoustic reflex threshold (ART), acoustic transfer function (ATF) (i.e., reflectance), and 1-kHz tympanometry tests were assessed in relation to outcomes on a newborn hearing screening (NHS) test based on otoacoustic emissions. The ipsilateral reflex test stimulus was a sequence of four broadband-noise activator pulses alternating with five clicks presented before, between and after the pulses. Measurements were performed over 10 activator levels (4 dB steps). The reflex shift was defined as the difference between final and initial click responses. Reflex shifts were quantified by objective, maximum-likelihood estimates of ART, separately calculated for low frequencies (ART-L from 0.8-2.8 kHz) and high (ART-H from 2.8-8 kHz). Thresholds were present in 98% of newborns passing the NHS, and 87% at high frequencies up to 8 kHz. The mean ART-L was elevated by 14 dB in NHS refers compared to passes. An optimal combination of ATF and ART tests performed better than either test alone in predicting NHS outcomes, and either WB test performed better than 1-kHz tympanometry. These results suggest that adding an ART test to NHS protocols may

help improve identification of hearing loss. Applications to older children and adults will be described. (Research supported by NIDCD DC003784, DC006607, DC00013, DC004662)

456 Test Re-Test Reliability in Recording Toneburst-Evoked Cochlear Microphonics at the Ear Canal

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Acoustic noise often conceals low frequency otoacoustic emissions (OAEs) in the measurement. A faked "OAE" can be elicited from a dead cochlea by a high-intensity stimulus. Cochlear microphonic (CM) measurements do not have these two limitations. Therefore, measurements of toneburst-evoked CMs may become an alternative to OAE measurements in assessing the cochlear conditions. This became potentially feasible because recording place-specific derived CMs were reported by Ponton, Don, and Eggermont. To record CMs, the least invasive technique is to use an ear canal electrode. However, the signal to noise ratio in ear-canal recorded CMs is small. A small ratio may affect the test re-test reliability. The reliability is critical for any audiological test when the test is used to monitor changes in hearing caused by treatments or by disorders. We investigated the test re-test reliability in recording toneburst-evoked CMs using an ear canal electrode in normal hearing subjects. The supra-threshold recordings were performed at different times to evaluate the test re-test reliability. The intervals between two recordings ranged from a few seconds to ten days. All potential artifacts were carefully avoided by using techniques that were previously reported by Ferraro, et al. The Fourier transformation was used to extract the response amplitudes from the toneburst-evoked CMs. The frequency components could be clearly measured from the toneburst-evoked CMs recorded at the ear canal. The mean differences between the amplitudes of the CM frequency components recorded at different times were similar to those in the studies using a tympanic membrane electrode that were previously reported. Therefore, the results indicate that a reasonable reliability can be achieved using an ear canal electrode. This may lead to a possibility of using toneburst-evoked CMs as an alternative to the low frequency OAE measurements.

457 Tele-ASSR System

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Auditory Steady-State Response (ASSR) is one of the promising techniques in electrophysiology. It is known to have better frequency-selectivity than Auditory Brainstem Response (ABR). There are some commercial products on ASSR, and they have been widely used in clinic. Also, we have been developing a new ASSR system, which makes it possible to do an interactive remote ASSR test.

The tele-ASSR system is composed of two computer applications. One is a client program for the patient who is at remote site, and the other is a server program for the audiologist or medical doctor at the hospital. Surely, if there is a professional at remote site, it is possible to do a face-to-face ASSR test using only client program. In order to do an interactive tele-ASSR test, someone who helps the test for patient should attach several electrodes to the patients and connect to the server program. After connecting, the audiologist or medical doctor can do an ASSR test. It is real-time and interactive.

NI PCI-4461 (National Instruments) was used for generating 40-Hz amplitude modulated sound and for acquiring EEG data. ER-3A (Etymotic Research) insert earphone was used for stimulating sound to ears. Phase coherence was used as an automatic response detection algorithm.

We compared hearing thresholds in a face-to-face ASSR test to those in a tele-ASSR test. Four subjects are all twenties and they have normal hearing. They were required to be awake during experiment. The Audera® (VIASYS Healthcare) system is used for face-to-face test, and the test was done at Hearing Clinic in Seoul National University Hospital. After finishing the test, tele-ASSR test was done at Clinical Research Centre 1km away from Hearing Clinic. Four frequencies – 500, 1k, 2k and 4kHz – are used, and intensity has 5 dB steps.

According to the results of only four subjects, the mean differences on four frequencies are 3.75, 1.25, 1.25 and 0 dB. The hearing thresholds at 500Hz in tele-ASSR test are a little higher than conventional face-to-face test. We estimate that the reason is caused by the slight difference between two sound-proof booths in Hearing Clinic and Clinical Research Centre. The wall of sound-proof booth in Hearing Clinic is double-sided, whereas that in Clinical Research Centre is single-sided. However, it does not affect the result in high frequencies.

458 Behavioral Audiograms Measured in Humans Using a Multipurpose Instrument

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We are developing a system to administer a number of diagnostic measures of peripheral auditory function using one ear-canal probe assembly in humans. The current prototype is based on an Etymotic ER-10B+ otoacoustic emission probe with custom designed sound sources and is capable of delivering high quality stimuli of at least 80 dB SPL over the entire hearing range. We report here our initial success in measuring behavioral thresholds over the extended frequency range of 125 Hz to 20 kHz. We are on schedule to complete the first phase of our study of 400 subjects to guide our selection of a final set of tests that would have the highest diagnostic value in a typical clinical test session. We have employed seven relatively inexperienced testers, recruited mainly from Northwestern's AuD program. The audiograms are measured using a modified Bekesy tracking algorithm and use an insertion depth compensated calibration procedure in an IEC-711 ear simulator.

Behavioral audiograms grouped in five age categories appear quantitatively similar to the previous report of

Stelmachowicz, et al. (JASA **86**:1384-1391, 1989) that used a device specifically designed to measure audiometric thresholds at high frequencies. The consistency of the results in the youngest subjects is encouraging, given the number and relative inexperience of the testers. This is a good indication that our method will be reliable and practical in a typical clinical setting.
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459 Relative Effects of Spectral and Temporal Cues for Korean Phoneme Recognition

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Spectral and temporal cues are important features in recognizing the phoneme. In this study, the authors aimed to investigate the relative effects of spectral and temporal cues systematically varying the amount of spectral and temporal information through simulations of cochlear implant processors using a vocoder. Eleven normal-hearing native Korean-speaking listeners whose pure-tone averages of either ear were ≤20 dB HL participated in the study. Consonant stimulus set consisted of 18 syllables presented in a consonant-/a/ context and vowel stimulus set consisted of 17 vowels presented in an /h/-vowel-/d/ context. Spectral information was controlled by varying the number of channels between 1 and 16, and temporal information was controlled by varying the lowpass cutoff frequencies of the envelope extractors from 1 to 512 Hz. The speech signal was mixed with the noise at Signal to noise (S/N) levels of 24 dB, 18 dB, 12 dB, 6 dB, 0 dB, -3 dB, -6 dB, -9 dB, -12 dB, and -15 dB. Consonants and vowels processed were presented to eleven listeners for identification. The results were analyzed and compared with the documented results in English. Phoneme recognition improved as a function of the number of channels and low-pass cutoff frequencies for consonants. For vowels, phoneme recognition improved as a function of the number of channels, but the effects of low-pass cutoff frequencies was not significant. Reviewing the result for each individual phoneme, each consonant showed diverse features with respect to the channel number and the low-pass cutoff frequency. Listeners got higher phoneme recognition scores in vowel test than in consonant test. Phoneme recognition improved as a function of S/N ratio. Within the above-mentioned ranges of low-pass cutoff frequency and number of channels, both spectral and temporal cues were important for consonant and vowel recognition with the spectral cues having a greater effect than the temporal cues, which is similar in English. Listeners showed less degree of recognition in consonant compared to in vowel, which is not a documented feature in English.

460 Musicianship Enhances the Ability to Discriminate Speech and Music in Background Noise

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Approximately 17% of American adults report some of degree difficulty hearing in a noisy environment. It has been hypothesized that the olivocochlear (OC) efferent system, which provides efferent neural feedback from the midbrain to the inner ear, improves hearing in the presence of background noise. Recent findings suggest that the medial OC efferent system is enhanced in professional musicians compared to the general population, yet this study did not restrict enrollment to young adults (≤29 yrs). We hypothesized that if musicians have greater OC efferent feedback, and OC efferent feedback facilitates hearing in the presence of background noise, then musicians should have an enhanced ability to discriminate speech and music in the presence of background noises. To test this hypothesis, we recruited adults between 18-29 yrs. and tested: i) standard audiometric thresholds; ii) speech-in-noise intelligibility using the Hearing in Noise Test (HINT); iii) music perception-in-noise using the same protocol used for the HINT, but replacing the speech with questions from the Montreal Battery for Evaluation of Amusia (MBEA); iv) baseline transient (TrOAEs) and distortion-product otoacoustic emissions (DPOAEs); v) medial OC efferent strength by measuring the change in the amplitude of TrOAEs and DPOAEs when a binaural suppressor was added. All subjects completed a survey of their musical training history and were given the tonal imagery melody portion of the musical aptitude profile (MAP T1) to access their musical aptitudes. Our results confirm previous findings that higher musicianship correlates with stronger OC efferent feedback, and provide evidence that this more robust OC efferent feedback response results in a functional hearing advantage: enhanced ability to discriminate both speech and music in the presence of background noise.
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461 Auditory Health Beliefs of Young Adults in the Context of the Health Belief Model

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A Health Belief Model has been previously applied to preventative health. The model proposes that the likelihood of taking recommended preventative health action depends on three major elements: 1. Individual perceptions related to perceived susceptibility to and seriousness of disease. 2. Modifying factors such as perceived threat of disease. 3. Perceived benefits of preventative action minus perceived barriers to preventative action. A survey was designed to examine some elements of the Health Belief Model with reference to noise induced hearing loss. The survey was completed by 40 men and 198 women. With reference to perceived

invulnerability to hearing loss, majority (75.62%) of the young adults surveyed believed that they would not lose their hearing until they are older. With reference to perceived threat of noise induced hearing loss, only 10% of the participants agreed with the statement "I believe that hearing loss does not occur because of loud music". With reference to perceived seriousness of hearing loss, only 4% of the participants agreed with the statement "I don't mind having a hearing loss." However, with reference to perceived effectiveness of treatment, 44% of the participants believed that hearing loss could be "fixed" with hearing aids. With reference to potential internal triggers such as tinnitus serving as a motivator for preventative action, approximately 58% of the participants agreed with the statement "Although my ears ring after a social activity, the ringing goes away and I feel that I do not have to worry". With reference to perceived barriers to the use of hearing protection devices during exposure to loud music, approximately 46% students agreed with the statement "I don't wear hearing protection during loud activities such as a concert, because I feel the music is more difficult to hear with hearing protection". Overall, these results suggest a need for better education about noise induced hearing loss.

462 Sound Localization in Ferrets with Unilateral and Bilateral Cochlear Implants

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A behavioral model of bilateral cochlear implantation (CI) has been developed in the ferret to maximize potential benefits of bilateral CI in humans, whilst utilizing CI to study effects of unilateral and bilateral electrical stimulation on binaural development (Hartley et al. 2006). In chronic implantation studies, early (postnatal day (P) 30) and late onset (P>300) hearing loss was induced with daily subcutaneous neomycin injections (25-50 mg·Kg^{-1} for 10-21 days). Deafness was confirmed with no auditory brainstem response (ABR) to clicks (>95 dB SPL). Subsequently, custom-made intracochlear arrays (7 active electrodes) and an extracochlear ball electrode were implanted in one (n=2) or both ears (n=2). Daily electrode impedance measurements (mean 9.4 KΩ; range ≤1 KΩ) and electrically-evoked compound action potentials (ECAPs; mean threshold 253 μA; SD 23 μA) confirmed the integrity of all electrodes (duration of implantation 3-5 months, to date). ECAPs, electrically-evoked ABRs and behavioral assessments of comfort and threshold levels, were used to program ESPrit 3G speech processors (Cochlear Ltd) with a continuous interleaved sampling strategy. Ferrets received daily monopolar intracochlear stimulation (mean 8 hours/day) with biphasic current pulses (101 μs/phase, 500 pps/channel) by connecting their multichannel arrays, through percutaneous lead wires, to speech processors and modified stimulator-receivers carried in a custom-made jacket that preserved binaural cues. A free-field task based on a positive conditioning paradigm was used to assess sound localization to broadband noise bursts. Preliminary results indicate that bilateral CI enhances sound localization compared with unilateral CI.

Hartley DEH, Xu J, Shial A, Clarke M, Ahmed B, Schnupp JWH, Shepherd RK, King AJ (2006) Bilateral cochlear implantation in the ferret (*Mustela putorius*). *ARO Abstracts* 85.

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463 Effect of Simulated Spectral Holes on Speech Intelligibility and Spatial Release from Masking Under Binaural and Monaural Listening

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A recently growing clinical approach is to provide cochlear implant (CI) users with bilateral devices in order to improve their performance in adverse listening situations and their sound localization abilities. Although bilateral CI users perform more similarly to normal hearing listeners when using two CIs under some listening conditions, the gap remains large. One factor thought to be responsible for this difference is the presence of "dead regions", or "spectral holes" in information received by CI users. Using a CI vocoder, this study examined effect of spectral holes on speech reception thresholds (SRTs) and spatial release from masking (SRM) in adverse listening conditions under binaural and monaural listening. Prior to processing, stimuli were convolved through head-related transfer functions (HRTFs) to provide listeners with free-field directional cues. Target stimuli were recorded with a male voice and were presented from 0°; masker stimuli were recorded with two-female talkers and presented from either 0°, 90°, or -90°. Stimuli were presented over headphones under binaural or monaural (R ear) conditions. Spectral holes were created by dropping frequency regions with variable size (6 mm and 10 mm) and location (base, middle, and apex) along the simulated electrode array. Results suggest that spectral holes are detrimental to SRTs and SRM; however, the effect size depends on the location and size of the spectral holes. Spectral holes created in the middle of the simulated array were the most disruptive to subjects' performance. When target and masker stimuli were spatially separated, performance improved significantly in conditions with apical holes but not with middle or basal holes suggesting the importance of the dropped basal and middle information for benefits that depend on spatial cues. Listeners in the binaural conditions performed better than those in monaural conditions; however, the presence of spectral holes mitigated the binaural advantages.

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464 Relationship of Monaural and Binaural Channel Interaction Effects in Bilateral Cochlear Implant Users

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Improved localization and understanding of speech in noise are observed with two cochlear implants versus one, but performance remains poor relative to normal hearing listeners. In particular, sensitivity to interaural timing differences (ITDs) in the low tens of microseconds is observed in normal hearing listeners but not in bilateral cochlear implant (BICI) users listening through commercially available speech processors. With a bilaterally coordinated research processor, however, sensitivity to ITDs in the tens of microseconds has been achieved by several adult BICI users with postlingual onset of deafness when single electrode pairs are stimulated at low pulse rates or low modulation rates. An important next step is to test performance under more realistic listening conditions, in which there is stimulation by more than one electrode pair or where more than one ITD may be presented to the auditory system simultaneously.

In the unilateral cochlear implant literature changes in sensitivity due to stimulation of one or more additional electrodes on the same cochlear array are known as effects of "channel interactions." In our current work we are extending measures of channel interactions to binaural stimulation in order to: 1) examine possible effects on binaural sensitivity, and 2) determine whether such effects, if any, are likely due in whole or in part to channel interactions at the monaural level. Specifically, we are comparing performance in an ITD discrimination task when two left-right electrode pairs are activated to performance in a monaural probe detection task when two electrodes are activated in a single implant. Preliminary results indicate that 1) there are substantial effects of channel interactions on binaural sensitivity, and 2) these effects may have little dependence on monaural channel interactions except at quite small electrode separations.

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465 The Emergence of Spatial Hearing Skills in Toddlers Who Use Bilateral Cochlear Implants

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Previous work from our lab has shown that most children who receive sequential bilateral cochlear implants (BI-CIs) develop some spatial hearing abilities, with performance tending to improve with increased bilateral experience. However, many of these children perform significantly worse than their age-matched peers who have normal acoustic hearing (Grieco et al., 2007, ARO #464). Recent trends to provide BI-CIs by 2 years of age allow us to study the emergence of spatial hearing abilities in a younger population of children, for whom the duration of auditory deprivation prior to implantation is short, and the duration of unilateral exposure is either short or non-existent. This study tested the hypothesis that, unlike older children, the performance gap between children who receive BI-CIs by age 2 and their age-matched peers with normal hearing will be small. We used the observer-based psychophysical method (Olsho et al., 1987; Grieco-Calub et al., 2008) to assess spatial hearing skills on a 2-alternative-forced-choice, right-left discrimination task. Children were 2-3 years old and either used BI-CIs [N=25], unilateral CIs [N=10] or had normal hearing [N=8]. Results show that children who use BI-CIs perform significantly better when using both CIs versus their first device alone, with many reaching performance levels of their acoustic hearing peers. In addition, performance of the BI-CI group is strikingly better than that of the unilateral group, who were unable to perform the task. These results suggest that bilateral CI input at a young age promotes the development of spatial hearing. Although duration of bilateral experience appears to result in better spatial hearing skills, it does not appear to be the only determinant in this study. Other variables that contribute to performance on this spatial hearing task will be discussed.

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466 Lateralization of Inter-Implant Level and Timing Differences in Children with Sequential Bilateral Cochlear Implants

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Auditory development driven by one cochlear implant may compromise a child's ability to interpret inter-implant level and timing cues once a second implant is provided. We asked whether these children: 1) can detect changes in inter-implant level and timing and 2) respond differently to these cues than their normally hearing peers.

Auditory brainstem responses were evoked by electrical pulses in 15 children provided with a second cochlear implant 4.2±2.0 years after the first and by clicks in 9 children (age 10±2.9 years) with normal hearing. Regression analyses were used to obtain levels at which wave eV from both sides were most similar. At these levels, bilateral stimuli (500 ms trains) were presented with inter-implant timing differences (±400, ±1000, and ±2000 us) and children indicated whether the sound could be heard as coming from the left, right, middle or from both sides. The same task was completed in response to simultaneous presentations of bilateral stimuli which varied in level (0, ±10 and ±20 current units (CU) or dB). All stimuli, including control (unilateral) stimuli, were presented randomly.

In the normal hearing group, perceptual shifts from left to middle to right were observed as expected for both timing and level differences. In contrast, children using implants responded randomly to timing differences and few

indicated a perception of “middle”. Level differences were perceived with greater accuracy. The perceived shift from left to right occurred at various level differences between the two devices and was significantly correlated with levels evoking similar left and right evoked auditory brainstem response amplitudes.
Children receiving bilateral implants sequentially may not perceive a fused sound image from the two devices and often cannot interpret inter-implant timing differences. Level differences, on the other hand, provide some bilateral cues. Calibration of level between implants could be important to promote functional use of these cues and may be possible by using auditory brainstem responses.

467 Bilateral Cochlear Implants: Interaural Pitch and Binaural Abilities

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Clinical fitting of bilateral cochlear implants has not been designed to maximize binaural abilities, nor has research determined that fitting modifications would deliver significant binaural enhancements with today's processors. One measure that could be used to guide bilateral fitting is interaural pitch comparison. Studies have examined the relation of interaural pitch to binaural sensitivity in a small number of subjects (e.g., van Hoesel RJ and Clark GM. J Acoust Soc Am. 1997 Jul;102(1):495-507; Long CJ, Eddington DK, Colburn HS, Rabinowitz WM. J Acoust Soc Am. 2003 Sep;114(3):1565-74.). But, systematic measures of interaural pitch comparisons on an electrode-by-electrode basis with a large number of subjects with relation to other interaural abilities are lacking. In addition, the effect of exposure to a given frequency mapping on interaural comparisons remains unclear. This study aims to address the above issues.
Thus, the present study examines electrode-by-electrode interaural pitch-matching and pitch-ordering for users of Nucleus cochlear implants for every functioning electrode. It relates the pitch results to (1) the frequency allocation tables these recipients use in everyday life; (2) measures of interaural time difference sensitivity and binaural fusion; (3) and measures of monaural sensitivity.
At least some subjects in this study have shown dramatic differences between their pitch ordering and the frequency allocation tables they have used. The results will be discussed in terms of the relations between the different measures; the utility of pitch comparisons in probing the binaural system; and the effect of adjustments to frequency allocation tables on speech perception and subjective judgments.

468 ITD Coding with Bilateral Cochlear Implants: Effects of Congenital Deafness and Binaurally-Coherent Jitter

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Human bilateral cochlear implant users do poorly on tasks involving interaural time differences (ITD), a cue which provides important benefits in challenging acoustic environments in the normal hearing. Yet the precision of neural ITD coding in acutely deafened, bilaterally-implanted cats is essentially normal (Smith & Delgutte, J Neurosci 27:6740).

One explanation for this discrepancy is that extended periods of binaural deprivation degrade neural ITD sensitivity. We tested this hypothesis by recording from single units in inferior colliculus (IC) in two groups of bilaterally-implanted, anesthetized cats: acutely-deafened cats (ADC), which had normal binaural hearing until experimentation, and congenitally deaf white cats (DWC), which had no binaural experience. Stimuli were low-rate pulse trains (~40 pps) varied in ITD. In ADC, 80% of neurons had firing rates significantly modulated by ITD, compared to only 39% in DWC. For neurons that were ITD sensitive, distributions of best ITD and sharpness of tuning were similar in the two groups. Thus, ITD sensitivity is only half as prevalent in cats lacking binaural experience, but appears otherwise normal.

In both groups, sustained firing rates to periodic pulse trains drop steeply for pulse rates above ~300 pps. Inspired by recent behavioral findings that introducing binaurally-coherent jitter improves ITD sensitivity for high-rate pulse trains (Laback et al., PNAS 105:814), we measured responses of IC neurons as a function of pulse rate, pulse jitter, and ITD. Above ~300 pps, sustained firing rates were greater when the pulse intervals were randomly jittered by 90% of the mean, up to the highest pulse rate tested (1280 pps). Neurons stimulated with jittered high-rate pulse trains showed ITD sensitivity comparable to that produced by low-rate pulse trains. Thus, jitter appears to unmask neural ITD sensitivity by restoring sustained firing in IC neurons.
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469 Using Acoustic Models to Predict Sound Localization Performance of Bilateral Cochlear Implant Users

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Studies have shown that bilateral cochlear implants have the potential to improve performance in sound localization and hearing in noise tasks (van Hoesel and Tyler 2003; Litovsky et al. 2004). Further investigation is required to fully understand the perceptual effects of bilateral electrical stimulation and the ways in which sound processing strategies should be implemented and coordinated to improve performance. However, confounding factors and a

small test population make it difficult to draw conclusions from investigations with cochlear implant users. Researchers have shown that acoustic models may be used to predict trends in unilateral cochlear implants (e.g., Dorman and Loizou 1997) while avoiding confounding factors that are normally associated with electrical stimulation and small test populations. However, the utility of acoustic models for simulating bilateral cochlear implant conditions has not yet been thoroughly investigated. The goal of this study is to establish the validity of binaural acoustic models for predicting performance of bilateral cochlear implant users on a sound localization task. A bilateral cochlear implant sound localization experiment with adults (Litovsky et al. 2004) was replicated with normal hearing listeners. A virtual acoustic source was synthesized using digital filters constructed from head-related transfer functions (HRTFs) to model the free-field stimuli used by Litovsky et al. (2004). A secondary objective of this study was to explore how multi-rate algorithms (Nie et al. 2005; Throckmorton et al. 2006) might improve sound localization performance with bilateral cochlear implants. Results suggest that binaural acoustic simulations of bilateral cochlear implants may be useful in predicting trends in cochlear implant sound localization ability. Acoustic model data also suggest that when compared to single rate strategies, multi-rate strategies may not have a significant effect on binaural sound localization performance.

470 Novel Measures in Bilateral Cochlear Implant Users: Sound Localization and Speech Perception in Conditions with Unpredictable Background Interferers

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An increasing number of persons with severe to profound hearing loss are opting for bilateral cochlear implants (BiCI) in an attempt to access the benefits offered by listening through both ears. Numerous studies have demonstrated that bilateral implants provide benefits for sound localization in quiet acoustic environments. However, bilateral benefits have not been studied in complex listening environments, known to pose difficulties for BiCI listeners. We conducted a series of experiments on 9 BiCI users. First, sound localization and speech perception were studied in the presence of two-speech interferers at signal to noise ratios varying between +10 dB and -5 dB SNR. Second, sensitivity to interaural time difference (ITD) and interaural level difference (ILD) cues was measured with stimulation of electrically pulsed signals presented directly to pitch-matched electrodes (bypassing the processors). Third, results were compared with those obtained from 10 listeners with normal hearing (NH). In the BiCI group sound localization was significantly affected by the presence of interferers; performance degraded steeply as a function of decreasing SNR. Equivalent performance for listeners with NH and BiCI was seen only when BiCI listeners were tested at a SNR of 0 dB and NH listeners were evaluated at -20 dB SNR. On the speech perception tasks a similar relationship between the groups as a function of SNR was seen. Finally, in BiCI users localization ability correlated significantly with ITD sensitivity, but not with ILD sensitivity. The large gap in performance between NH and BiCI listeners will be discussed in relation to existing issues that include lack of coordinate input at the two ears with speech processors, lack of fine structure in the stimuli and the temporal smearing of signals that is caused by the clinical processors.

Work supported by NIH-NIDCD (R01 DC003083)

471 Processing of Interaural Time Differences in the Mongolian Gerbil (Meriones Unguiculatus) – a Comparison Between Acoustical and Electrical Stimulation

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The present study directly compares responses of the same neurons in the dorsal nucleus of the lateral lemniscus (DNLL) to both acoustic and electric interaural time differences (ITDs).

Normal hearing, adult gerbils were bilaterally implanted with round window electrodes, and an earphone was sealed to the ear canals for acoustic stimulation. To estimate acoustic ITD-sensitivity, pure-tone stimuli (duration 200 ms) were presented at five frequencies around the neuron's characteristic frequency with varying ITDs (step size 50-100 µs) at 20 dB above minimum threshold. To more closely mimic the broad excitation pattern evoked by pulsatile electrical stimulation of the cochlea, also bursts of Gaussian noise (duration 50 ms) were presented at different ITDs. For electric stimulation, biphasic constant current square-wave pulses (phase duration 80 µs) with varying ITDs were presented at 0-4 dB above the neuron's threshold. Responses were recorded extracellularly from single DNLL neurons.

Using acoustic tones, most neurons typically respond maximally to ITDs ('best ITDs') outside the physiological range for gerbils (+/-120 µs) and with the contralateral stimulus leading. There was a trend for noise stimulation to result in shorter ITDs than tonal stimulation. However, this difference was not significant. In contrast, using electrical pulses, the best ITDs were significantly closer to 0 µs than those in response to acoustic stimulation with both tones and noise. In some neurons, broadband stimulation with noise and, in particular, with electrical pulses resulted in a shift of best ITDs into the physiological range. However, the majority of best ITDs for all three kinds of stimulation (tone, noise, electrical pulses) remained outside the physiological range.

The lack of cochlear delays may have contributed to the shift of electrical best ITDs towards 0 µs. However, the findings that the electrical best ITDs were not closely centered around 0 µs but were mostly located outside the physiological range suggest that there are factors other than cochlear delays contributing to ITD processing and underscore the potential role of glycinergic inhibitory inputs to the medial superior olive.

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472 Animal Research and Proactive Education

Peter Santi[1]
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Biomedical scientists use animals for research, as an ethical and efficient way to generate new knowledge that has a high potential for improving the quality of life in humans and other animals. For most scientists, the ethical equation involving animal rights vs. human rights has been solved, and strongly favors human rights. The same equation has been solved for animal rights activists, but favors the rights of animals over humans. This dichotomy has resulted in controversy. Since the animal rights' position is in the minority, some activists have escalated their activities from peaceful protests and letter writing to illegal acts of violence. The scientific community has responded by increased regulation of the use of animals for research and the securing of its facilities. They have also encouraged new legislation to increase the punishment of illegal activities directed against animal research personnel and facilities. However, neither more animal welfare rules nor increased punishment and enforcement seems to have dissuaded animal rights activists from pursuing their agenda of the abolition of animal research. What can a scientist do besides closely adhering to IACUC protocols and performing high quality research? I think that we need to be more proactive in informing the public, who funds both our research and the treasury of animal rights organizations, about how biomedical research will benefit humans and animals. A frontal attack on animal extremism is less effective compared to describing the benefits of your research. In this talk I will suggest ways and nontraditional educational venues that biomedical researchers may use to get a positive message out about the need and benefits of animal research.

473 Use of Animals in Research: An Overview of the Ethical Issue

Taylor Bennett[1]
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There is no single ethical theory or procedure by which we can evaluate the use of animals in research. Several ethical theories have been proposed for that purpose, but in reality the current processes and procedures used by Institutional Animal Care and Use Committees (IACUC) to review and approve the use of animals in research, teaching and testing are a combination of those theories. This presentation will review those theories and discuss how they apply to the deliberations of an IACUC.

474 Public Education Strategy for Discussing Animal Research Utilizing K-12 Schools

William Cameron[1]
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It is difficult to find the time to engage the public in dialogue concerning the importance of animal research. To optimize our impact, the biomedical researcher might ask what the best target audiences are. Currently, the animal rights organizations are focusing much of their efforts on young adolescent hoping to recruit lifetime believers that all research on animals is immoral. To balance the dialogue around this emotionally charged topic, we designed a program for a middle school audience focusing on teacher professional development, creating the Teacher Institute for the Experience of Science (http://ties.ohsu.edu/). It is recognized that students learn more about science when it is placed in the current social context. The impact on student attitudes generated by such discussions is greatly enhanced by the teacher's skill in navigating controversial topics in the classroom. We received funding from the NIH's Science Education Partnership Award (SEPA) program to develop these skills in both science and non-science teachers through our institute. Over the past three summers of the program, we have refined our approach to talking about animal research with our teachers. The Oregon Health & Science University is a prime target for anti-animal research groups due in large part to our affiliation with the Oregon National Primate Research Center. For decades, the primate center has conducted tours for several thousand visitors annually consisting predominantly of secondary school students, their teachers and chaperones without measures of their impact. An integral part of the middle school program is an evaluation of the teachers along the students that they instruct. In this talk, I will discuss lessons learned from different approaches to the topic of animals in research for a middle school audience and present evidence for a positive impact in student attitudes as a result of these approaches.

475 Cortical Plasticity in Adult Cochlear Implant Users

Emily Tobey[1], Michael Devous[2], Peter Roland[2]
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Jack Cullen epitomized a translational scientist before the current notions of "Bench to Bedside" approaches. His science probed important questions in speech perception regarding how the central nervous system processes speech and non-speech sounds, how best to deliver a meaningful signal to a compromised auditory system, and how auditory processing disorders are reflected in sensory systems. This talk will review the cortical activations of adult cochlear implant users under a variety of listening conditions, including monaural vs. binaural presentations and spectrally degraded signals acquired using single photon emission computed tomography. Cortical plasticity will be evaluated in pre versus post-treatment data obtained for CI subjects undergoing an 8-week pharmacologically enhanced aural rehabilitation program. Data indicate aural rehabilitation alone produces a 12% increase in auditory-only speech tracking scores with increase activations of primary cortex. Pharmacologically enhanced aural rehabilitation resulted in nearly 40% increases in auditory-only speech tracking scores with substantial reorganization of primary and associative auditory cortices. Data suggest electrical signals from

cochlear implants, in conjunction, with intensive pharmacologically enhanced aural rehabilitation reflect cortical plasticity evident in behavioral measures of auditory-only speech tracking. Supported by NIH.

476 Defining the Cochlear Nucleus by Its Non-Cochlear Inputs – Signal Coding and Deafness-Induced Transformations

Withdrawn

477 What Is the Contribution of Noise to Hearing Loss Burden?

Robert Dobie[1]
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A model of hearing impairment in American adults was constructed using data from the Census Bureau, from international standard ISO-1999, from the AMA method of determining hearing impairment, and from estimates of the distributions of noise exposure in different age and sex groups. Occupational noise exposure probably accounts for 5 – 10% of the burden of adult hearing loss in the USA. Most of this is attributable to unprotected exposures above 95 dBA, and becomes apparent in middle age, when age-related threshold shifts are added to prior noise-induced shifts, resulting in clinically significant impairment.

478 Research Labs and Cocktail Parties - What Do They Have in Common?

Joan Besing[1]
[1]*Montclair*

Jack Cullen's approach of bringing knowledge gained in the lab to solve real-world problems serves as an important model for applied research. As we know, a problem for listeners with and without hearing loss is to understand one or more conversation partners in the face of other competing talkers and background noise in environments where echoes and noise sources abound. Not only are these challenges that need to be solved for our patients, they are an important concern for individuals using cell phones and those working in distributed environments.

The nexus of these different fields of inquiry provides a unique opportunity to exploit ideas from non-audiology fields to facilitate listening in difficult environments. The goal of this talk is to consider ways in which information from research efforts outside of audiology may provide potential solutions to real-world problems faced by listeners with hearing loss.

479 Understanding Speech Perception by Cochlear Implant Users

Mario Svirsky[1]
[1]*NYU School of Medicine*

Although better known for his work in auditory physiology and psychophysics, Jack Cullen was also a pioneer of the scientific study of cochlear implants (CIs) and he stressed some basic principles that are still valid today. For example, Jack stressed the importance of ensuring that speech sounds remain discriminable after being processed by the CI, as discrimination of speech sounds is a prerequisite for their identification. Jack also recognized the importance of the study of acoustic phonetics, as it can inform us about which dimensions are important for speech perception. Finally, Jack recognized the plasticity of the human auditory system, and that perceptual learning in adults is possible. One consequence of these principles is that postlingually deaf listeners with CIs may be able to adjust their expectations about how different phonemes should sound, as long as those phonemes are discriminable from each other.

This presentation will describe a mathematical model of phoneme identification by CI users. This model is inspired on these basic principles put forth by Jack, and provides a quantitative account of the influence of auditory discrimination and perceptual learning on speech perception by CI users. Finally, the model provides a parsimonious explanation for several different results from the literature.

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480 The Story of Ultra-Audiometric Hearing in 1975 and the Construction of the World's First Upward Shifting Translator Ultimately Used for More Than 300 Such Patients

Charles Berlin[1]
[1]*University of South Florida*

In 1975, using a system designed to study hearing in mice, I found a patient who had been (mis-)diagnosed as having an hysterical loss because she detected speech at 30 dB SPL but had a 3 frequency pure tone average of 80 dB HL. We ultimately found a sharply rising pure tone audiogram that peaked to 20 dB SPL at 12 kHz, questioning von Bekesy's traveling wave theory and presaging OHC tuning. Jack, Mead Killion, Henry Halpern, then a medical student at LSU and I designed and built an upward shifting translator, described in Time Magazine and distributed to 300 patients.

481 Dichotic Listening and Auditory Processing Disorders

Deborah Moncrieff[1]
[1]*University of Pittsburgh*

For several decades, dichotic listening tests have been used to probe hemispheric lateralization for language in normal and disordered populations. Performance on dichotic listening tests depends upon a listener's ability to successfully engage stimulus-driven activations throughout the ascending auditory system together with instruction-driven activations that tap into attention and working memory. Dynamic interactions between bottom-up and top-down processes have been shown to produce variable results within one test across different listeners and across different test stimuli or instructions within the same listener. Normal performance is characterized by similar scores in both ears with a slightly superior score in one ear that is defined as a right- or left-ear advantage. Poor

performance in both ears during a dichotic listening test can be attributed to disorders of language, attention, or verbal working memory, but it is difficult to attribute a deficit in only one ear to those global, non-auditory factors. A unilateral deficit may be more likely to occur from deficits in binaural integration, a stimulus-driven process that engages multiple structures of the ascending auditory nervous system from the periphery to the cortex. Children with learning and reading disabilities have demonstrated significantly poorer than normal performance in one ear during dichotic listening tests, leading to speculation that weaknesses in binaural integration may interfere with learning. Emerging evidence of more normally symmetrical dichotic listening performance following intensive training suggests that clinical diagnosis and remediation of this unilateral deficit may be especially important in school-age children. Relevant evidence will be reviewed as support for new standards of clinical practice.

482 Development of a Diagnostic Test Battery for APD Based on a Population Study of Hearing, Listening and Cognition in Children

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Definitions of auditory processing disorder (APD) point to a primary auditory sensory deficit. The IHR multi-centre study of auditory processing (IMAP) aimed to separate sensory and cognitive contributions to some 'typical' presenting symptoms of APD by testing, in schools, 1638 children (6-11 y.o.) from the general population. The one-hour test battery included spectral and temporal auditory processing (AP), speech intelligibility (VCV in speech-shaped noise) and cognition (nonverbal IQ (NVIQ)), memory, reading and language). Parental report on communication and listening abilities was assessed by the Children's Communication Checklist (CCC-2) and Children's Auditory Performance Scale (CHAPS). Results of 1471 (89.8%) children who had normal hearing (≤25 dBHL 1 and 4kHz bilaterally) are reported here.

Poorer AP was defined as task threshold > z = 1.5 (age adjusted). For each individual AP task (backward masking with a tone-masker gap of 0 or 50ms; simultaneous masking with or without a spectral notch; frequency discrimination (FD)), poorer AP performers showed significantly poorer cognitive abilities ($p<0.05$) across the board (NVIQ: $p<0.001$). Correlations between individual AP tasks and speech intelligibility, communication, and listening, the 'typical' presenting symptoms of APD, were highly significant but of mostly modest explanatory value ($r=0.1–0.3$, $p<0.001$). However, NVIQ explained 9% of the variance for the correlation between FD and a composite measure of individual AP tasks. By contrast, for derived AP tasks (spectral and temporal resolution) that serve to cancel out procedural and nonsensory factors, correlations with the presenting symptoms had little or no explanatory value (r= 0-0.1). Poor auditory sensory processing, as evidenced by the derived measures of temporal and frequency resolution, thus failed to account for the symptoms of APD. We are turning our attention to poor auditory cognition as the primary identifiable contributor to APD.

483 APD and Language Disorders: Essentially Linked or Independent?

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Much current interest in APD is centred on the impact an APD may have on the development of language and literacy. Strong claims have been made that two of the most important language disorders, dyslexia and SLI (specific language impairment) arise as a direct consequence of an APD. I will review the nearly 4 decades of work in this area, with a particular focus on the nature of the language disorders, their linguistic and auditory components, and the results of a number of auditory intervention schemes for rehabilitation. It appears that APD is more common in people with dyslexia and SLI, but appears to play little or no causative role in their language disorders. Therefore, rehabilitation schemes aimed at rectifying the APD through low-level auditory interventions have no impact on the language disorder, whatever other effects they may have.

484 Brain Correlates of APD and Their Plasticity

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Speech and music sounds consist of *pitch* (F_0), *timbre* (harmonics/formants) and *timing* components. It is possible to access the subcortical neural transcription of these fundamental elements objectively, non-invasively and with great fidelity with scalp electrodes in humans. Brainstem responses to speech provide a biological marker for a subset of auditory processing disorders. [a]

Reading and listening (speech-in-noise) are associated with neural representation of *timing* and *timbre* – important for phonological processing – but not with *pitch*. This subcortical transcription can be considered part of the language processing network and is likely shaped by our extensive corticofugal circuitry during development. [b]

Lifelong language and music experience and short-term auditory training can shape the neural encoding of *pitch, timing* and *timbre* early in the sensory processing stream. [c] Such malleability – stemming from sensory-cognitive linkages – is likely what contributes to the success of auditory training in some individuals.

(a) Banai et al. J Neursosci 2005;25:9850-7.
(b) Suga & Ma Nature Rev Neurosci 2003;4:783-94; Johnson et al. J Neurosci 2008;28:4000-7.
(c) Krishnan et al. Cogn Brain Res 2005;25:161-8; Musacchia et al. Proc Nat Acad Sci 2007;104:15894-8; Wong et al. Nature Neurosci 2007;10:420-2; Song et al. J Cogn Neurosci 2008;20:1892-1902; Russo et al. Behav Brain Res 2005;156:95-103.

http://www.brainvolts.northwestern.edu

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485 A Population-Based Analysis of Familiality in Auditory Processing Disorder

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Auditory Processing Disorder (APD) is a diagnosis with an unknown genetic predisposition. Combining a computerized Utah genealogy with diagnosis data from a hospital serving 20% of the state we examined familial clustering of APD and studied genetic contribution to predisposition. We used a set of ICD-9 diagnostic codes (suggested by audiologists and speech pathologists, the ASHA, and Aetna) to identify 397 APD cases from 1994-2006 who also are included in the Utah genealogy. We identified all genetic relationships between cases to assess relatedness of cases and to estimate relative risks (RR) for APD among the relatives of cases. We tested the hypothesis of no excess relatedness by comparing the average relatedness of all pairs of APD cases (via the coefficient of kinship) to the expected relatedness of 1000 sets of sex- and age-matched controls selected randomly from all hospital patients with genealogy data. We estimated RR in relatives as the case/control odds ratio. We selected 5 sets of controls for each case, and compared the observed number of APD cases among the relatives of the cases to the observed number of APD cases among the relatives of the 5 sets of controls. The relatedness of APD cases was reliably higher than that of 1000 sets of controls (p=0.008). When 1st and 2nd degree relationships were ignored, there was no excess relatedness (p=0.792). Relative risks in 1st and 2nd degree relatives were significantly elevated; 1st-degree RR = 15.65, 95% CI 3.16,77.58; 2nd-degree RR=4.12, 95% CI 1.11, 15.35. The RR for 3rd-degree relatives was not significantly elevated (RR=0.65, 95% CI 0.15, 2.83). The small sample size (n=387, including only cases from 1994 to 2006 in one Utah hospital) limited our power and resulted in RR estimates with very large confidence limits. The significant excess relatedness observed and the elevated risks in 1st and 2nd degree relatives, however, strongly support some genetic contribution to predisposition to APD.

486 Acoustic Modifications for Supporting Learning and Attention

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An evaluation of the installation and use of sound amplification systems in mainstream and special schools in England has been carried out to investigate the impact of classroom modifications on teaching and learning in elementary school classrooms. The evaluation included noise surveys of classrooms, a questionnaire survey of pupils and teachers and experimental testing of children in with and without the use of sound amplification. Teachers' and pupils' perceptions are compared with objective data evaluating change in performance when amplification is used for cognitive tasks. The results are discussed in terms of effective learning and listening environments in elementary schools.

487 Auditory Training as Management for APD

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Auditory processing disorder (APD) is a complex problem that may be due more to inattention than to impaired sensory processing (Moore et al. Hear. Res. 2008; Ferguson et al. ARO abs. 2008). The two approaches to APD management most often cited are improved acoustic environment (including FM/amplification devices) and auditory training. However, neither of these approaches has been subject to rigorous testing, due in part to poor specification of APD and consequent measurement difficulties. A requirement for appropriate outcome measures is being addressed by our IMAP study (see Ferguson et al.). Parallel research in auditory learning also informs our selection of training methods. To promote long periods of training, adaptive, child-friendly training programmes used in the lab are made available for home-based training. To facilitate compliance, research examines the time requirements for training, and participants are rewarded for completing an entire course. Test-retest reliability and consistency of outcome measures are major difficulties (McArthur, Dyslexia 2007). Auditory learning research relevant to these goals and requirements includes the following results: (1) Significant auditory perceptual learning can occur rapidly, within minutes of starting training (Hawkey et al. Nat. Neurosci. 2004); (2) Children generally do not benefit from more than 10-20 mins training in one session (Halliday et al. JASA 2008); (3) Training in the company of peers promotes better learning (Halliday et al. unpublished); (4) Many studies have shown improved language and literacy in standard tests following retesting without training; (5) Retest effects can be reduced by choosing tests with different items for each use; (6) Single session auditory training is more influenced by top-down than by bottom-up processing (Amitay et al. Nat. Neurosci. 2006), suggesting that deficits in sensory attention may be appropriately managed using auditory training.

488 Mouse Foxi3 is Necessary for the Induction of the Otic Placode

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The cranial sensory organs including the inner ear develop from a border region between the neural plate and surface ectoderm. A variety of experiments show that much of this region is competent to give rise to different cranial placodes if grafted to the appropriate location (Jacobson, J. Exp. Zool. 1963; Jacobson, Science 1966; Baker et al., Development 1999; Groves and Bronner-Fraser, Development 2000). A number of genes have been shown to mark the border between the neural plate and epidermis (Yang et al., J. Neurosci. 1998; Quint et al., J. Exp. Zool. 2000; Streit, Dev. Biol. 2002). We have identified and analyzed the expression pattern of mouse Foxi3 (Ohyama and Groves, Dev. Dyn. 2004). Foxi3 is broadly expressed in the pre-placodal domain at the pre-somite stage and

later the expression becomes restricted to the branchial pouches. This observation suggests that Foxi3 is a novel pre-placodal marker gene. To understand the function of Foxi3, we disrupted the Foxi3 gene by using gene-targeting method and the resulting mutant mice did not form the otic placode. Howerver, other cranial sensory placodes such as the lens, olfactory, trigeminal and the epibranchial placodes are induced although some of them are reduced in size. These results strongly suggest that mouse Foxi3 has crucial roles in cell fate determination of the otic placode within the pre-placodal domain.

489 Dorsal-Ventral Patterning of the Developing Inner Ear

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The inner ear develops from a simple ectodermal thickening known as the otic placode. We recently demonstrated an embryological manipulation in the frog Xenopus, the physical removal or ablation of either the anterior (A) or posterior (P) half of the placode, which results in a high percentage of mirror image ears. Removal of the posterior half results in mirror anterior duplications while removal of the anterior half results in mirror posterior duplications. In contrast, dorsal (D) or ventral (V) half ablations never result in mirror duplications. These ablations result in the loss of corresponding structures and suggest that there is less regenerative potential along the D-V axis. In terms of the signaling molecules potentially involved in axial patterning, studies in zebrafish have demonstrated a role for hedgehog (Hh) signaling in A-P patterning of the developing ear. In mouse and chick, Hh signaling is required for ventral pattering while Wnt signaling is important for dorsal patterning. We have found that blocking Hh signaling results in mirror duplications of anterior structures, suggesting that Hh signaling is necessary for posterior half identity and making Xenopus more like zebrafish. Here we explore the role of canonical Wnt signaling on axial patterning in Xenopus with gain and loss of function experiments. Conditional blocking of canonical Wnt signaling results in severe reductions in the number of sensory organs and semicircular canals within the inner ear. Of the two most common phenotypes, one resembles ears with mirror posterior duplications, suggesting that Wnt signaling may play a role in anterior half identity. By placing beads containing Wnt3a protein on inner ears in which the anterior, posterior, dorsal or ventral half was ablated, we are testing to see if Wnt signaling is sufficient to rescue the loss of any of these tissues.

490 Retinoid Signaling in the Inner Ear: A "Goldilocks" Phenomenon

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Retinoic acid (RA) is a metabolite of vitamin A that is crucial for inner ear development. The normal function of RA is achieved only at optimal homeostatic concentrations, with RA excess or deficiency leading to otic dysmorphogenesis. We previously showed that in utero exposure to excess RA disrupts expression of two members of the Fibroblast growth factor family, (*Fgf3* and *Fgf10*). We now show that *Fgf3* and *Fgf10* are modified under conditions of retinoid deficiency. Expression of RA synthetic enzymes and retinoid receptors within the otic epithelium suggests that RA may act directly on this tissue. However, absence of RA in loss-of-function mouse models affects rhombomere development, raising the possibility that perturbation in hindbrain patterning, and thus an indirect effect on the otocyst, may come into play. We have begun to test for possible mode(s) of RA action to distinguish between direct and indirect influences on the inner ear. We developed a model in which pharmacological inhibitors of retinoid signaling are administered subsequent to the timepoint when hindbrain patterning is already fixed. Our findings show that treatment with these reagents leads to the formation of small hypoplastic otocysts which recapitulate otocyst phenotype in vitamin A deficient mouse embryos. Furthermore, we show that expression of *Fgf3* and *Fgf10* is modified when RA signaling from the otic epithelium is blocked with pharmacological reagents. Retinoid receptors are also expressed in periotic mesenchyme, indicating that RA synthesized in the otic epithelium may act indirectly on periotic mesenchyme. We tested in vitro interactions between otic epithelium and periotic mesenchyme and showed that RA synthesized in the otic epithelium affects interactions with periotic mesenchyme. Taken together, our findings support a direct influence of RA on *Fgf* signaling in the inner ear.

491 The Role of Sox2 in Neuron Formation in the Developing Mammalian Cochlea

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In the cochlea, spiral ganglion neurons have a critical function in hearing as they are responsible for transmitting sound representations from mechanosensory hair cells to the cochlear nucleus in the brainstem. The proneural basic helix-loop-helix transcription factors Neurogenin1 (Neurog1) and NeuroD1 have been shown to be essential for the development of otocyst-derived inner ear sensory neurons. Here we demonstrate neural competence of non-sensory epithelial cells in the cochlea as ectopic expression of either Neurog1 or NeuroD1 results in the formation of neuronal cells. We show that Sox2, an HMG transcription factor implicated in neurogenesis, is expressed in otocyst-derived neural precursor cells and later in the spiral ganglion neurons along with Neurog1 and NeuroD1; therefore, we utilized both loss- and gain-of-function experiments to examine the potential role of Sox2 in cochlear neuron formation. Spiral ganglion neurons were absent in cochleae from Sox2Lcc/Lcc mutant mice, signifying that Sox2 is required for neuronal formation in the cochlea. Furthermore, ectopic expression of Sox2 resulted in the production of neurons, suggesting that Sox2 is sufficient for the induction of neuronal fate in non-sensory epithelial cells. These ectopic neurons became positive for Tuj1 and morphological analysis revealed the presence of neurites and growth cones. In addition, these

neurons were able to survive for at least 15 days after transfection. While follow-up studies are required to determine long term survival and whether these neurons become physiologically functional, our study provides evidence for the first time that non-sensory epithelial cells in the embryonic cochlea are competent to develop into neurons.

492 The Transcription Factor *six1a* Acts as an Activator in the Sensory Lineage and as a Repressor in the Neuronal Lineage of the Developing Zebrafish (*Danio Rerio*) Inner Ear

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In the developing inner ear, the mechano-sensory hair cells and the neurons of the stato-acoustic ganglion that innervate them are all derived from the otic epithelium. However, the genetic programs underlying their formation and relative numbers remain unclear. We recently demonstrated that *six1a*, a member of the *sine oculis* transcription factor family, could play opposing roles in the sensory and neuronal lineages by promoting hair cell fate and, conversely, inhibiting neuronal fate by differentially affecting cell proliferation and cell death in these lineages. The question that remains is how the same gene can have opposite effects. Members of the SINE OCULIS family have been shown to act either as transcriptional activators or transcriptional repressors, depending on the partners with which they interact. In order to assay whether Six1a could act as a repressor in one lineage and as an activator in the other, we assayed the effects of mutating known protein-protein interaction or DNA binding sites in the Six1a sequence on its function both in vitro as well as in the developing zebrafish inner ear. We show that Six1a can act as part of a transcriptional activator complex in the sensory lineage and transcriptional inhibitor complex in the neuronal lineage.

493 The Cytokine Macrophage Migration Inhibitory Factor (MIF) Is the Major Bioactive Component of the Embryonic Otocyst-Derived Factor (ODF) and Acts as a Neurotrophin in Early Inner Ear Development

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Sensory hair cell (HC) and/or spiral ganglion/vestibular ganglion (SGN/VG) loss in the inner ear are major causes of human deafness, tinnitus and balance disorders, especially in the ageing population. The molecular bases for neuronal development, nerve cell loss and possible retention, re-growth or replacement after injury are not understood. We are testing the hypothesis that mammalian spiral ganglion neurons (SGN) can be maintained, repaired or induced to re-grow in the adult inner ear if they can respond to the embryonic otocyst derived factor(s) (ODF) that promote(s) the development and survival of early inner ear statoacoustic ganglion (SAG) neurons. Therapeutically applied ODF or its component factors could therefore aid SGN retention or regrowth as well as potentiate the function of a cochlear implant. ODF contains a bioactive mixture of cytokines/chemokines, glycoproteins already known to be critical for neurogenesis, neuronal regeneration and immune system function. They include: macrophage chemoattractant protein 1 (MCP1), made by hair cells (HC) (Bianchi et al., 2005),) and macrophage migration inhibitory factor (MIF), made by supporting cells (SC). We are investigating the instructional role for these cytokines in embryonic inner ear development, in SGN maintenance and in regeneration after noise or ototoxic drug-induced injury. Mice null for MIF, MCP1, CCR2 (the MCP1 receptor exhibit selective and severe hearing loss at 48KHz (by ABR measurements) as early as 4 weeks of age and have significant loss of both SGN and HC in the basal (high frequency) cochlea. MIF alone at picogram levels can support the directional outgrowth of mouse and chick SAG neurites, supports the survival of dissociated SAG neurons and evokes a neuronal phenotype from mouse embryonic stem cells. Such ES-derived neurons could be used to replace damaged or missing neurons of the SG. The receptors for both MIF (CD74) and MCP1 (CCR2) are retained on adult mouse SGN.

494 Notch Signaling Specifies Prosensory Regions in the Inner Ear

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Notch signaling plays a role in several aspects of inner ear development. However, an early role for Notch as an inducer of prosensory domains has remained unclear in part because of discrepancies between the phenotypes of mice with deletions of single Notch related genes. One explanation for these discrepancies is functional compensation by other Notch receptors or ligands. Therefore, to determine the effects of complete elimination of Notch signaling, we generated an inner ear specific knockout of the *Rbpsuh* gene. RBP-J protein, which is encoded by *Rbpsuh* gene, is a transcriptional co-activator for all Notch molecules and thus deletion of this protein inhibits all Notch signaling.

Foxg1^{Cre} mice were crossed with *Rbpsuh*^{flox} mice to delete the *Rbpsuh* gene in the otocyst. To examine the effects of differences in deletion efficiency, both *Rbpsuh*$^{-/flox}$ and *Rbpsuh*$^{flox/flox}$ mice were used. The inner ear phenotypes were determined at various developmental stages.

When deletion efficiency was low hair cells were present in the cochlea, however the number of inner hair cells increased while that of outer hair cells was decreased. This phenotype is similar to the phenotype reported previously for the *Foxg1*^{Cre} line mediated knock-out of the notch ligand *Jag1*. In contrast, when deletion efficiency

was high, the cochlea contained a small patch of hair cells located at the extreme apex. To determine the signaling pathways regulated by Notch signaling, expression of prosensory and patterning molecules were examined. The expression of *BMP4, Fgf10, Lfng* and *Atoh1* were comparable between control and *Rbpsuh* mutants at E13 but by E15 most were significantly down-regulated in the mutants.
These results indicate that Notch signaling mediates several aspects of inner ear development. Early in inner ear formation, Notch signaling specifies the future sensory epithelia by regulating the formation of prosensory patches while at later time points, Notch signaling determines the number of cells that will develop as hair or supporting cells within the prosensory domains.

495 Prosensory Domain Formation in the Absence of Notch Signaling

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In the mammalian cochlea, the mechanosensory hair cells responsible for hearing are arranged in a highly organized pattern consisting of one row of inner hair cells and three rows of outer hair cells. Each hair cell is surrounded by supporting cells and these two cell types form the sensory epithelium known as the organ of Corti, extending along the basal to apical axis of the cochlea. Hair cells and supporting cells originate from a population of common cochlear progenitor cells in the prosensory region of the cochlear epithelium during embryonic development. These progenitors differentiate into either hair cells or supporting cells shortly after they exit the cell cycle. There is strong evidence that Notch mediated lateral inhibition is involved in the postmitotic fate decision to differentiate as a hair cell or a supporting cell. However, it is unclear whether the Notch signaling pathway is also involved prior to this event, in the patterning of the prosensory domain. To address this question, we have analyzed inner ear specific conditional knock outs of the Notch pathway and our preliminary results suggest that Notch signaling is not required for the prosensory domain formation. We are currently performing gain of function experiments, where the active intracellular domain of Notch is overexpressed in “naïve” portions of the cochlea, or in the whole cochlea. We hope to determine whether overactivation of the Notch pathway is sufficient to elicit hair cell or supporting cell fates in the non sensory epithelium, without previously inducing a progenitor fate.

496 Dlx3b-Dlx4b Specify Otic Sensory Epithelia

Withdrawn

497 The Cytokine Macrophage Migratory Inhibitory Factor (Mif) Plays Critical Roles in the Development of Zebrafish Inner Ear Sensory and Nerve Cells

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Macrophage migratory inhibitory factor (MIF) was first identified as inhibiting the migration of guinea pig peritoneal exudate cells, mainly macrophages. The roles of MIF in the immune system, tumorigenesis, and the neuroendocrine axis have been extensively studied; it is also one of the most prominent â€œinflammatoryâ€ cytokines. MIF is expressed in the developing CNS and PNS neurons, astrocytes, Schwann cells, the ear and the eye and in human, mouse, chick, zebrafish and Xenopus embryos. In Xenopus, the knockdown of Mif expression using antisense morpholino oligonucleotides (MO) results in a complete lack of neural tissues, including brain, spinal cord, eye, and otic vesicle (Suzuki et al., 2004). This dramatic effect of Mif MOs was the first indication of the importance of Mif in Xenopus nervous system development. We have cloned and examined the expression patterns of 2 mif genes in zebrafish and investigated the effects of MOs against mif, its related protein mif-like, and the orthologues of the mammalian Mif receptor (invariant chain like proteins, iclp-1, -2) on developing zebrafish embryos. Knockdown of mif & mif-like caused loss of hair cells (HC) in the saccular macula, failure of semicircular canals (SCC) to form completely and increased cell death in the brain, resulting in smaller anterior brains and eyes. The loss of HC and SCC abnormalities could be partially rescued by injection of capped RNA. Studies with an antibody to zebrafish neurons (Zn5) demonstrated that a knock-down of mif resulted in fewer neurons in statoacoustic ganglia (SAG) and other head ganglia. Similar to the effect of mif MOs, the application of iclp-1 MO resulted in a smaller brain and eye. Although the mif pathway does not control neural axis formation or the initial morphogenesis of the brain and the sensory organs as it does in Xenopus, it is critical for the normal development of these systems and particularly of HC, SCC and neurons in the zebrafish inner ear.

498 Requirement for Lmo4 in Mouse Vestibular Morphogenesis

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In vertebrate inner ear, auditory and vestibular components are derived from defined compartments within the otocyst. The vestibular apparatus, including three semicircular canals, saccule, utricle, and their sensory organs, are responsible to detect angular and linear acceleration of the head and relay the information along vestibular neurons to vestibular nuclei in the brainstem. How the early development events manifest vestibular structures at the molecular level is largely unknown. Here, we show that LMO4, a LIM-domain-only transcriptional regulator, is required for the formation of semicircular canals and their associated sensory cristae. Targeted

disruption of Lmo4 results in the semicircular system dysmorphogenesis including an absence of three semicircular canals, anterior and posterior cristae. In the Lmo4-null otocyst, the formation of canal outpouches failed to form and the cell proliferation is largely reduced in the dorsolateral region. We also found that Lmo4 is essential for the normal expression of several genes in the dorsolateral domain of the otocyst, whereas the initial compartmentalization of the otocyst remains largely unchanged. Our results demonstrate that Lmo4 facilitates semicircular cristae and their canals formation by two mechanisms: regulating the expression of otic sepecific genes and stimulating proliferation of the dorsolateral part of the otocyst, in which later will develop to be semicircular system.

499 Chromodomain Protein 7 (CHD7) Regulates Multiple Developmental Signaling Pathways During Inner Ear Morphogenesis

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Morphogenesis of the developing inner ear is highly coordinated and requires precise temporal and spatial control of cellular proliferation, differentiation, survival, and gene expression. Several signaling pathways control inner ear morphogenesis in the mouse and chick, including Shh, Fgf, Bmp, Wnt, and retinoic acid; however, the genetic mechanisms regulating these signals are not well understood. Heterozygous chromodomain protein 7 (*Chd7*) deficient ($Chd7^{Gt/+}$) mice exhibit highly penetrant but variable defects in lateral and posterior semicircular canals, and in posterior cristae innervation, despite the presence of intact hair cells. Based on its proposed role in chromatin remodeling and gene transcription, we hypothesized that *Chd7* regulates expression of genes that are critical for semicircular canal formation and development of neuronal projections to the posterior cristae. In this study, we analyzed Eya1, Fgf, Bmp, Netrin, and Neurotrophin signaling in the developing $Chd7^{Gt/+}$ mouse ear. *Bmp2* expression was absent, and *Netrin1* and its receptors, *Unc5b* and *Unc5c,* were reduced in the developing $Chd7^{Gt/+}$ lateral canal region. *Bmp4* and *Fgf10* expression were reduced in developing $Chd7^{Gt/+}$ cristae, yet there were no defects in $Chd7^{Gt/+}$ cell proliferation or survival in the e10.5-e12.5 canal epithelium, mesenchyme or cochleovestibular ganglion, suggesting that Chd7 may primarily activate genes involved in one or more aspects of cellular differentiation. Using the neuroanatomical tracer DiI, we found abnormalities in innervation to the posterior cristae in postnatal $Chd7^{Gt/+}$ ears. *Fgf10*, *Eya1,* and *TrkB* were also reduced in $Chd7^{Gt/+}$ vestibular ganglia. These studies indicate that Chd7 regulated Eya1, Fgf, Bmp, Netrin, and Neurotrophin signaling are critical for proper semicircular canal and vestibular ganglion morphogenesis, and suggest a general role for regulated chromatin remodeling in the developing inner ear. Supported by NOHR, the A. Alfred Taubman Medical Research Institute, the Berte and Alan Hirschfield Foundation and NIH/NIDCD grant P30 DC05188.

500 Regulation of Otoconia Formation by Otopetrin 1

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Otoconia are complex calcium carbonate (CaCO3) biominerals that are required for the sensation of gravity. Degeneration of otoconia is thought to contribute significantly to balance disorders and to the displacement or ectopic formation of otoconia that occur in patients suffering from benign positional vertigo (BPV). In addition, commonly used aminoglycoside antibiotics can lead to disruption of otoconial structure and function. Despite the prevalence of balance disorders, little is known about the mechanisms regulating the development and pathology of the vestibular mechanosensory apparatus.

Tlt and Mlh mice have a severe balance disorder due to the congenital absence of otoconia. By positional cloning we identified mutations in Otopetrin 1 (Otop1) as the genetic etiology of the Tlt and Mlh mouse phenotype. Knockout of the Otop1 gene, by insertion of beta-galactosidase, also resulted in otoconia agenesis and provided a tool to track expression of Otop1. This analysis revealed Otop1 expression in the supporting (precursor) cells of the macula as early as embryonic day 13.5 and in adult mice, suggesting a role for Otop1 in both the formation and maintenance of Otoconia. Otop1 is a multi-transmembrane domain protein containing three evolutionarily conserved domains of unknown function. Examination of the phenotypes of animals with mutations or deficiencies in Otop1 suggests a direct role for Otop1 in the initiation of extracellular biomineralization, possibly through the regulation of intracellular Ca2+. We demonstrate localization of Otop1 to the apical surface of macular supporting cells and both, in vitro and, in vivo regulation of cellular Ca2+ in response to purinergic stimuli.

501 Dissecting the Role of the Small GTPase Rac1 in Cochlear Development

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Highly organized remodeling of the actin cytoskeleton is critical for morphogenesis of the mammalian inner ear, particularly for the formation and patterning of the hair cell stereociliary bundle. Despite recent advances, the factors that coordinate cytoskeletal remodeling within the organ of Corti are poorly understood. As molecular switches, Rho GTPases (Rho, Rac, Cdc42) are widely known for their role in regulating the actin cytoskeleton using cell-based models. Of the three Rac family members in mammals, Rac1 is the most highly studied and widely expressed. We therefore investigated the role of Rac1 in auditory hair cell development and bundle formation during embryogenesis in the mouse. Using a Rac1 conditional allele (Glogauer et al., 2003) and a Cre-loxP conditional gene targeting strategy, we generated mutants where Rac1 is deleted

within the early developing inner ear. Immunohistochemical and ultrastructural analyses of Rac1 mutant cochleae revealed severe, highly penetrant defects in cochlear morphogenesis, hair bundle morphology, and organ of Corti cellular organization. We noted dramatic reduction of cochlear length and hair cell number in the Rac1 mutant organ of Corti. Cellular organization and spacing was also disrupted. We also observed a wide range of structural and polarity defects in the mutant hair bundles. A high percentage of bundles appeared shorter and lacked a discernable or crescent-shaped organization. Furthermore, the kinocilia of malformed bundles were frequently absent or mispositioned within the bundle. Some bundles were also misoriented, reminiscent of the reported PCP (Planar Cell Polarity) mutant phenotype. These data reveal a critical function for Rac1 during inner ear development. To identify downstream effectors of Rac1 during hair bundle formation, we are currently examining the role of Rac1 in regulating known hair bundle resident/regulatory proteins, many of which are implicated in deafness disorders such as Usher syndrome.

502 Developmental Refinement of Inhibitory Connections in the Auditory Brainstem Depends on Cholinergic Transmission in the Cochlea

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During development, the inhibitory pathway from the medial nucleus of the trapezoid body (MNTB) to the lateral superior olive (LSO) is refined, becoming more tonotopically precise. *Before* hearing onset, most MNTB-LSO connections in rats are silenced, such that a given LSO neuron receives input from fewer MNTB neurons confined to a smaller frequency region (Kim and Kandler, 2003). *After* hearing onset, MNTB axon branches in gerbils are pruned, a process that requires auditory experience (Sanes and Takacs, 1993). We hypothesized that the functional map created well before hearing acts as a template that instructs the pattern of axonal pruning that occurs during auditory experience.

To test this hypothesis we characterized the morphology of MNTB axon terminals in the LSO in mutant mice (α9 KOs) that lack the AChR α9 subunit necessary for cholinergic transmission in the cochlea (Vetter at al., 1999). These mice show impaired functional refinement before hearing onset, but have normal hearing (May et al., 2002).

At hearing onset (P12-14), we observed no difference in the number of MNTB boutons in the LSO or spread of boutons along the frequency axis, indicating that at P12-14 structural MNTB-LSO connectivity in α9 KOs does not differ from wildtype mice (WT) (WT, n = 6, KO n = 4). Therefore, despite the fact that *functional* MNTB-LSO maps are twice as large in α9 KOs than in WTs, the size of *structural* maps is the same in α9 KOs and WTs. During the first week of hearing, MNTB axon branches in the LSO were significantly pruned in WT mice but no pruning was observed in α9 KOs (P19-21, WT n = 5, KO n = 4).

We conclude that early cholinergic innervation of cochlear hair cells is important for the specific silencing of inhibitory MNTB-LSO connections before hearing and that the resulting functional map acts as a powerful template for the subsequent axonal pruning that takes place in the presence of auditory experience.

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503 Identifying Components of the Hair-Cell Interactome Involved in Cochlear Amplification

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Although outer hair cells (OHCs) play a key role in cochlear amplification, it is not fully understood how they amplify sound signals. In fact, two competing or possibly complementary mechanisms, stereocilia-based and somatic electromotility-based amplification, have been considered. Unfortunately, lacking knowledge about the exceptionally rich protein networks in the OHC plasma membrane, as well as related protein-protein interactions, limits our understanding of cochlear function. Therefore, we focused on finding protein partners for two important membrane proteins: Cadherin 23 (CDH23) and prestin. CDH23 is one of the tip-link proteins involved in mechano-electric transduction (MET) and stereocilia-based amplification, while prestin is the basolateral membrane protein responsible for OHC somatic electromotility. Using a membrane based yeast two-hybrid system to screen a newly built cDNA library, we identified two completely different groups of potential protein partners using prestin and CDH23 as bait. These include both membrane bound and cytoplasmic proteins with 12 being de novo gene products with unknown function. Some of these genes are closely associated with deafness loci, implying their potentially important function in hearing. The most abundant potential partners for prestin (38% of all identified prestin prey) compose a group of proteins involved in electron transport and possibly playing a role in OHC survival. The most abundant group of CDH23 prey (55%) contained calcium-binding domains. Since calcium plays a crucial role in hair cell mechano-electric transduction, understanding the interactions between CDH23 and calcium-binding proteins will likely increase our knowledge of hair cell function at the molecular level. These two discoveries, using this new methodology, open potentially fruitful lines of investigation into MET function and OHC death (Work supported by NIH Grants DC00089, DC006412 and The Hugh Knowles Center Leadership Fund).

504 Quantitation of the Hair-Bundle Protein Constellation

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To estimate the concentrations and bundle enrichment of several hundred proteins present in our preparation of purified chicken vestibular hair bundles, we used liquid chromatography-tandem mass spectrometry (LC-MS/MS)

and label-free quantitation methods. One dataset was generated from eight bundle preparations, each from 100 chicken ears, and sixteen vestibular epithelium preparations of comparable protein amounts; tissues were directly digested by trypsin prior to LC-MS/MS. More recently, we have achieved greater sensitivity by initially separating bundle or epithelium proteins by SDS-PAGE, followed by tryptic digestion of gel slices and LC-MS/MS. In both cases, we quantified proteins in each run by calculating their intensity factors; the intensity factor is derived from the ion-current intensity for the summed peptide MS/MS fragments assigned to a given protein, weighted by molecular mass. The intensity factor represents the apparent molar concentration of each protein present in the preparation and has been validated by comparison to other label-free quantitation methods, such as spectral counting and extracted-ion chromatogram integration. Moreover, in hair bundles, not only did individual proteins in complexes of known stoichiometry (e.g., alpha and beta tubulin, heterotrimeric G-protein subunits alpha and Gbeta, and F-acting capping protein alpha and beta subunits) had very similar intensity factors, but intensity factors gave abundances similar to those obtained by protein immunoblotting. Comparison of intensity factors of each bundle protein in the hair bundle preparation (~95% pure) and in the epithelium preparation (~0.4% bundles) allowed us to determine which proteins were enriched in bundles. Abundant proteins that were more than 50-fold enriched in bundles included (among others) espin, CLIC5, radixin, NHERF2, fimbrin, PTPRQ, and PMCA2. Other proteins were present at comparable concentrations in bundles and whole epithelium; these too are authentic bundle proteins. Finally, many proteins were present in the epithelium preparation at much higher concentrations than in bundles; these are contaminating proteins, not bundle proteins. Measurement of bundle enrichment thus allows us to sift through the list of proteins present in the bundle preparation and focus on those proteins actually located in hair bundles. Moreover, the accuracy of intensity factor quantitation allows us to suggest possible protein complexes based strictly on their similar stoichiometries in hair bundles.

505 Cadherin Dynamics and Molecular Mechanisms of Hereditary Deafness

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The hair-cell tip link, an essential component of the mechanotransduction apparatus in the inner ear, has been proposed to be formed by cadherin-23 (CDH23) and protocadherin-15 (PCDH15). Both molecules belong to the cadherin superfamily of adhesion proteins and are mutated in hereditary deafness. Uncertainties remain about their function, however: 1. Calcium is required for the formation of cadherin-cadherin interactions and also for maintaining tip link integrity, but the molecular mechanisms underlying the influence of calcium on tip-link function are not well understood. 2. Both theory and micromechanical measurements of hair cell transduction indicated the presence of an elastic gating spring in series with the channel, but it is unclear whether tip links are responsible for this elasticity. 3. Certain mutations in CDH23 and PCDH15 target calcium-binding sites and cause deafness, but it is not known how.
Here we report molecular dynamics simulations of classical cadherins type I (C-cadherin) and type II (cadherin-8 & cadherin-11) as possible models for the tip-link cadherins CDH23 and PCDH15, whose structures are unknown. These simulations show how calcium ions control the structural integrity of cadherin's linker regions, thereby allosterically regulating cadherin's equilibrium dynamics and the availability of key residues involved in cell-cell adhesion. By application of force in molecular dynamics simulations, protein elasticity and breaking strength can be inferred. Under the simulated conditions, cadherin is much stiffer than expected for the gating spring. The simulations also revealed that deafness-producing mutations at linker regions can modulate the strength of cadherin proteins. The results illustrate the general principles of linker-mediated elasticity of modular proteins relevant for cell-cell adhesion and sound transduction.

506 Adapting the Tip Link: A Detailed Map of Cadherin 23-Harmonin Interactions

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We report novel findings on the binding interaction between two proteins of the Usher “interactome”: cadherin 23 (Cdh23; USH1D), which contributes to stereociliary tip links and transient lateral links, and harmonin (USH1C), its three-PDZ-domain cytoplasmic adaptor protein. Our results were obtained using in vivo and in vitro binding assays. The in vivo assay involved co-transfections in LLC-PK1-CL4 epithelial cells, in which GFP-harmonin constructs efficiently co-localize with FLAG-tagged Cdh23 constructs in the apical (microvillar) plasma membrane. These results were confirmed in direct biochemical binding assays examining purified 6xHis-tagged harmonin constructs and GST-Cdh23 tail constructs immobilized on glutathione-agarose beads. Our results indicate that the binding interaction between Cdh23 and harmonin differs in two major ways from that proposed by earlier investigators and featured in models for the Usher interactome. First, we found no roles for the -ITEL Class-I PDZ-binding motif at the C-terminal end of the Cdh23 cytoplasmic tail or the PDZ2 domain of harmonin. This was the case with or without inclusion of the exon-68 peptide of Cdh23. For example, elimination of segments of the Cdh23 cytoplasmic tail up to and including the exon-68 peptide did not decrease harmonin binding. Using this mutagenesis approach, we mapped the single harmonin binding site to a short internal peptide between the membrane insertion site and the exon 68 peptide of Cdh23. Curiously, mutation of potential PDZ-binding “pseudopeptides” present in this internal peptide failed to reduce harmonin binding. Second, the harmonin PDZ1 domain proved to be necessary and sufficient to bind the Cdh23 tail, but quite unexpectedly we discovered that mutation or elimination of amino acids that form the PDZ1

domain's predicted binding pocket had no effect. This raises the surprising possibility that harmonin binds Cdh23 via a mechanism other than a conventional PDZ interaction. (DC004314, JRB)

507 The Roles of Espins in Regulating Stereocilium Width and Length: New Insights from Stereociliary Defects in Extrastriolar Vestibular Hair Cells of Espin-Deficient Jerker Mice

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The espin actin-bundling proteins, which have been implicated as regulators of stereocilium length and width, also provide calcium ion-insensitive cross-links that likely stabilize the stereocilium's parallel-actin-bundle scaffold during signal transduction. To further elucidate the roles of espins, we are using scanning electron microscopy to examine the stereocilia of homozygous jerker mice, which we have shown to lack espin proteins owing to a point mutation in the espin gene. Here, we report on an informative group of region-specific stereociliary defects in the vestibular hair cells of jerker mice in the CBA/CaJ genetic background. The stereocilia of hair cells in the striola of the utricular macula and the central zone of the cristae were found to be dramatically shorter and thinner than controls. These defects were evident early in postnatal life, and by P40 few stereocilia remained. This pattern of stereocilia degeneration and loss is reminiscent of that observed for cochlear hair cells in these mice at P10 and beyond. In contrast, the stereocilia of hair cells in the extrastriolar region of the utricular macula and peripheral zone of the cristae showed milder, albeit significant, defects in length and width and degenerated much more slowly. These extrastriolar stereocilia, which grew to become about one-half as long as wild-type counterparts, were retained beyond P40. Although organized staircases did not form, a slight length gradient was evident. Remarkably, we detected a pronounced decrease in stereocilium diameter proceeding tipward from just above the ankle region. We propose that stereociliary degeneration in the striolar/central regions of the vestibular system (and the cochlea) is accelerated by an activity-dependent process. Importantly, the defects observed for stereocilia in the extrastriolar/peripheral regions of the vestibular system may allow a more accurate assessment of the roles of espins in stereocilium morphogenesis. (DC004314, JB)

508 Candidate Protein for the Tectorial Membrane Attachment Crown

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The tectorial membrane (TM) attachment "crown," first visualized by electron microscropy (Goodyear et al., 2005), was suggested to be the link that connects the tip of the tallest outer hair cell (OHC) stereocilia to the TM. In mice, the TM attachment crown first becomes visible two days after birth (P2) and is observed at the top of all three rows of stereocilia. Gradually, the crown disappears from the two shortest rows, and by P19 remains only at the tops of the tallest stereocilia. Although the TM attachment crown can be removed by the protease subtilisin, the molecular basis of the "crown" remains unknown.

We have identified a *de novo* protein exclusively expressed in OHCs (Miller et al., 2008). It belongs to the CEACAM (Carcinoembryonic antigen-related cell-cell adhesion molecule) family, a group of glycoproteins with diverse functions. The location of this *de novo* protein during different developmental stages (P0, P4 and adult) was investigated using immunofluorescence. Our preliminary data show that the expression pattern of ceacam protein appears to fit the timescale of "crown" appearance as described above. In addition, when cochleae were treated with subtilisin, ceacam protein staining was significantly decreased. Together, these data indicate that ceacam is likely a component of the "crown". The specific location of this protein indicates its potentially important role in hearing, especially since connections between the tallest row of OHC stereocilia and the TM are essential for cochlear amplification (Legan et al. 2000). (Work supported by NIH Grants DC00089 to PD, and DC006412 and the American Hearing Research Foundation to JZ.)

509 Calcium Transients in Single Stereocilia of Mammalian Cochlear Hair Bundles

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Transduction of auditory stimuli in the mammalian cochlea occurs by deflection of hair cell stereociliary (hair) bundles which activates mechanotransducer (MT) channels sited in the hair bundle. Although much data exists to suggest the MT channels are near the tops of the stereocilia, the exact location of the channels remains to be determined. MT channels are highly permeable to calcium, so monitoring calcium entry can be used to identify channel location. Perfusion of the fluo-4 family of calcium indicator dyes into neonatal rat inner and outer hair cells via patch pipettes while deflecting hair bundles with a fluid jet allowed the tracking of calcium through open MT channels. To follow fast changes in calcium in each row of stereocilia, a swept field confocal microscope (Prairie Technologies) attached to a Redshirt camera that could acquire at speeds of at least 500 frames per second was used. With a 100X objective (pixel size 0.1 microns), it was possible to see 'spots' corresponding to calcium signals in single 0.5 micron diameter stereocilia of inner hair cells. Stereociliary fluorescence changes were abolished by hair cell depolarization and with MT channel blockers streptomycin or curare indicating the fluorescent signal was directly linked to calcium influx through the channels. The numbers of 'spots' visible was proportional to MT current amplitude which ranged up to 1 nA. Measurements at three focal planes in the bundle showed changes in calcium concentration were up to ten times larger and substantially

faster in the second or third stereociliary rows than in the first row. Calcium signals were also measured in individual rows of smaller diameter stereocilia from outer hair cells with similar results. There was a smaller slower calcium elevation in the first row as compared to the other two rows. These findings suggest there may be no MT channels in the first row stereocilia of inner or outer hair cells. Supported by NIDCD 03896 (AR) and 01362 (RF).

510 Locating MET Channels in Outer Hair Cells Near the Lower End of the Tip Link

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The goal of the present study was to localize mechanoelectrical transduction (MET) channels in hair-cell stereocilia (SC). Since MET channels are permeant to Ca^{2+}, their location was investigated using fluorescent calcium imaging. Outer hair cells isolated from the adult guinea-pig cochlea were used. Ca^{2+} transients in the SC were evoked by fluid-jet stimuli with [Ca^{2+}] of 4 mM in the ejection micropipette and 100 µM in the bathing medium. The time constant of the fluid jet near the SC was 14 ms. Intracellular [Ca^{2+}] changes were monitored using the fluo-3 dye.

The time course of the Ca^{2+} onset transient was described by a single exponential function. The time constant (τ) was 29.7 ± 2.4 ms in the top region of the middle SC. In contrast, in the longest SC, in the region above the shorter SC, τ was an order of magnitude longer and increased with distance from the cuticular plate (CP). Application of channel blockers (100-µM dihydrostreptomycin; 10-µM Gd^{3+}) had no observable effect on the signals in the longest SC, but in the shorter SC τ increased to the value observed in controls for the longest SC. Depolarizing the cell from -70 mV to 0 mV reduced the Ca^{2+} signal in the shorter SC, but had no detectable effect for the longest SC.

To reveal underlying processes, the passive diffusion model of Lumpkin and Hudspeth (1998) for a single SC was adapted to a long and a short SC connected by passive diffusion through the CP. The simulation was carried out assuming MET channels at one or both ends of the tip link (TL). Only placement of the channel at the lower end of the TL yielded agreement with the spatiotemporal properties of the experimentally observed Ca^{2+} signals.

Both the experimental data and the simulation suggest that Ca^{2+} enters the shorter SC through the MET channels and that the signal recorded in the longest SC derives from passive diffusion through the CP. Therefore, we conclude that the MET channel is located at the lower end of the TL.

511 Potential Roles of Pkd1 in Mechanoelectrotransduction of Mouse Hair Cells

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The components of the mechanoelectrotransduction (MET) channel in hair cells are unknown. We hypothesize that as a member of the TRP family, polycystic kidney disease (Pkd1), is part of the MET channel in hair cells because of its role in fluid-flow sensation and Ca^{2+} uptake in the cilia of the kidney. Pkd1 is also known to form cation channels with other TRP members with a conductance similar to that of MET channels. We have created and analyzed two mutant mouse models with independent alleles of *Pkd1*: a knockin model (*Pkd1T3041V*) that disrupts the normal cleavage of the protein abolishing its function and a hair cell specific conditional knockout model (*Pkd1cko*) using an Atoh1-CreER.

In wildtype (WT) cochleae, we have localized the mRNA of Pkd1 to both inner and outer hair cells through RT-PCR from laser-captured individual hair cells. Also qRT-PCR indicates Pkd1 mRNA in the postnatal cochlea. In both *Pkd1T3041V* and *Pkd1cko* models, auditory brainstem response tests revealed a 25-30 dB hearing loss compared to WT littermates. In addition, SEM imaging displayed similar abnormal stereocilia morphology in both models; however, there is no significant difference in the number of stereocilia in the two models as compared to WT. FM1-43 dye uptake experiments in our PKD1 mutants show similar results to WT. Preliminary electrophysiology results of the *Pkd1T3041V* model indicate normal amplitude, operating range and adaptation time constants of the MET current. Analysis of vestibular hair cells, including gross morphology, rotarod test and electrophysiology, indicates no significant differences from WT.

Analysis of two PKD1 mouse models corroboratively demonstrates that the hearing loss is specifically due to a phenotype of the hair cells of the inner ear. These results do not exclude Pkd1 from having a role in the MET channel; however, it is more plausible that Pkd1 is part of a heteromeric MET channel and/or plays a role in maintaining stereocilia structure.

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512 Coupled Hair Cells in the Bullfrog Sacculus

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Auditory and vestibular systems have remarkable sensitivities that cannot be explained by the response of a single cell. We use a high-speed (10,000 fps) CMOS camera to record multiple hair cells in the bullfrog sacculus. This system allows us to record stereocilary motion in an *in vitro* preparation with the otolithic membrane left intact and to track many hair bundles simultaneously. We applied mechanical stimuli to the otolithic membrane and measured the entrained responses of the cells. We have measured the decay of amplitude, phase locking, and correlations between hair cells over an approximately 300 x 45 μm^2 area of the sacculus. The space constant for the amplitude decay has been found to lie in the range of a few hundred microns; resulting in approximately 20 cells coupled by moderate stimuli. Our technique can be applied to other systems.

513 Tuning of the Control Parameter in Hair Cells of the Bullfrog Sacculus

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Free-standing hair bundles of *in vitro* preparations of the bullfrog sacculus have been shown to exhibit limit cycle oscillations. A number of theoretical studies have modeled this as indicative of a nonlinear system whose internal feedback mechanism has driven it across the Hopf bifurcation and into an unstable regime. In this talk, we will present results from several studies that examine potential biological control parameters and their effects on hair cell oscillations. First, we applied strong mechanical signals to the hair bundles, mimicking the effects of loud sound, and measured the time of recovery to original oscillation frequency. Secondly, we varied calcium concentration over a significant range, sufficient to drive the system outside of the oscillatory regime. And finally, we applied sustained voltage signals, and found them to shift frequencies of spontaneous oscillation, frequently in irreversible fashion.

514 Developmental Mechanisms Underlying Placode Formation

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The peripheral nervous system is derived from two embryonic sources: neural crest and cranial ectodermal placodes. Placodes give rise to portions of the sensory organs--eyes, nose and ears--and components of the Vth, VIIth, IXth, and Xth cranial nerves. Unlike neural crest, little is known about formation of cranial ectodermal placodes. Here we examine the role of growth factors in induction, specification, and neurogenesis of the largest cranial ganglion, the trigeminal. The trigeminal ganglion is composed of cells from the neural crest and trigeminal placode. The trigeminal placode arises in the ectoderm adjacent to the midbrain via an interaction mediated by secreted factor(s) from the dorsal neural tube. To identify candidate inducers, we performed an RT-PCR screen of growth factor receptors present in the presumptive trigeminal placode. One potential ligand, Platelet Derived Growth Factor (PDGF) was found to be required for trigeminal placode induction both in vitro and in vivo. However, PDGF alone was not sufficient to induce the trigeminal placode, suggesting that this is a multifactorial process. Next, we determined genes upregulated in response to trigeminal placode induction, thus providing a catalog of putative molecular players involved in its specification. Experiments are in progress to study the role of several of these genes, including CD151 and Frzb in development of the trigeminal placode into the ganglion.

515 Molecular Determinants of Atoh1 (Math1) Specified Lineages

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Atoh1 (Math1) is a basic helix-loop-helix (bHLH) transcription factor that specifies proprioceptive pathway neurons in the central nervous system (CNS) as well as sensory hair cells in the inner ear, Merkel cells in the skin, and secretory cells in the intestine. Genetic evidence (knockout mice and overexpression systems) demonstrates the essential function of Atoh1 in the formation of these distinct cell types. An enhancer that directs expression of reporter genes such as GFP and Cre to all these Atoh1-specific lineages has been localized to a 1.4 kb non-coding sequence 3' of the Atoh1 gene. The fidelity of this enhancer is likely due to the presence of at least one Atoh1 autoregulatory binding site, a sequence 100% conserved from mouse to fish. The specificity of this site is striking in that transgenic reporter genes containing the 26-bp sequence are restricted to an Atoh1 expression pattern. However, this sequence is not obviously found in other putative Atoh1 targets, and thus, the understanding of Atoh1 function at a molecular level is lacking. We are addressing this issue using microarray analysis to identify and compare genes expressed in the different Atoh1 populations isolated from Atoh1-nGFP transgenic mice. This analysis has revealed known genes in Atoh1 lineages such as Lhx2, Lhx9, Barhl1, and Barhl2 as well as genes not known to be distinct in these lineages such as TrkC, a neurotrophin receptor. Regulatory regions of genes identified in the Atoh1 lineages, that are also dependent on Atoh1 for expression, are being analyzed to reveal a cis-regulatory code for Atoh1 targets to provide mechanistic insight into the function of this essential regulatory factor.

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516 Role of Sox2 in Retinogenesis

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Our long-term goal is to dissect the transcriptional regulatory circuitry underlying neural progenitor competence. SOX2 is an HMG box transcription factor

that acts as a master regulator of stem cell identity. In the vertebrate central nervous system SOX2 is expressed in all neural progenitor cells and is downregulated coincident with cell fate restriction and terminal differentiation of progenitor cells. We focus on the retina as a model system to genetically dissect SOX2 biological functions and mechanisms of action. As an isolated and well-defined structure of the central nervous system, the retina has become a well-characterized model for studies of molecular mechanisms of neurogenesis. Only six major types of neurons develop within the retina, along with a single type of glial cells. These cells are readily distinguished from one another by morphology, laminar position and defined cell-type specific molecular markers. Moreover, the retina is one area of the developing nervous system where SOX2 is expressed in the absence of other SOXB1 sub-family members. Finally, SOX2 mutations have been implicated in several hereditary eye conditions in humans.¬ During the course of retinal development, progenitor cells transition through stages of competence – the process leading to generation of diverse retinal cell types. Most, if not all neural retinal progenitors maintain expression of SOX2 from the onset until the latest stages of retinal differentiation. However the levels of SOX2 vary between subsets of neural retinal progenitor cells. Through the generation of an allelic series of Sox2 mutations in the mouse we demonstrated the molecular and cell biological basis for these hereditary conditions (Taranova et al., 2006). SOX2 is required for proliferation and differentiation of early retinal neural progenitors and lowering expression levels of SOX2 (below 40%) results in aberrant neuronal differentiation leading to anophthalmia and microaphthalmia. We will discuss the use of these genetic tools coupled with a protocol that we have developed of culturing and electroporating embryonic and postnatal mouse retinas in order to: (i) to conditionally inactivate and (ii) reduce the levels of expression of Sox2 in subsets of retinal progenitors at distinct developmental stages.

517 4 Vertebrate Notch Receptors: 4 Qualitative, or 1 Quantitative Signal?

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Notch2 signaling is essential for formation of mature proximal tubules (PT) and podocytes during nephrogenesis. Reduction in the canonical Notch signaling after nephron segmentation disrupts PT epithelial monolayer in a dose-dependent manner, with multiple PT cysts forming following conditional inactivation of Notch1, Notch2, both or RBP-J in murine renal epithelial progenitors. Some Notch-signaling-deficient PT epithelia retained apical-basal polarity, but delayed exit from the cell cycle, and stratified into focal hyperproliferative polyps within cysts. Focal hyperplasia progresses to papillary microadenomas resembling a precursor state for papillary renal cell carcinoma (PRCC) in one third of the Notch-signaling-deficient kidneys. These findings provide a mechanistic hypothesis for PRCC pathogenesis and points out that therapies aimed at inhibiting Notch signaling in patients suffering from diabetic nephropathy carries a potential risk of renal cyst formation, PT dysfunction and PRCC.

518 Principles of Otic Induction and Development – Applied to Guiding Embryonic and Somatic Stem Cells Toward Otic Fate and Hair Cell Differentiation

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Research done by many laboratories has revealed quite a number of basic principles of otic induction, as well as the development of the inner ear's sensory organs and accessory structures. My laboratory utilizes these principles and applies them toward the stepwise in vitro conversion of embryonic stem cells and induced pluripotent stem cells into early and mature inner ear cell types. At the core of this guidance procedure is the concept of mimicking environmental cues, which have to be applied with the proper timing and correct order to achieve a directed development of pluripotent cells toward the otic lineage. The first step along this path is the generation of an early ectoderm that is responsive to otic induction, assayed by occurrence of early otic markers. We subsequently expose the otic marker-expressing cell populations to cues that further specify the otic and sensory lineage to guide development into sensory epithelial-like cell populations. Here, I will attempt to place in vitro guidance results into the context of what is known about otic development, an endeavor that reveals the strengths as well as the limitations of this in vitro guidance approach.

In a parallel study, we are interested in propagation and guidance of progenitor/stem cells isolated from neonatal vestibular and cochlear sensory epithelia. Although these isolated sphere-forming progenitors have no direct developmental equivalent, they appear to respond to developmental cues in a similar fashion as developing otic sensory epithelia.

519 Notch Signaling Involvement in Prosensory Specification and Maintenance in the Mammalian Inner Ear

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The mammalian inner ear consists of six separate sensory regions—five for balance and one for audition. Each sensory area is composed of three basic cell types: the sensory hair cells, supporting cells and innervating neurons, all arising from the otocyst. Developmental studies have demonstrated that hair cells and supporting cells arise from a common progenitor and that otic neurons can also share in this lineage. Previously we demonstrated that the transcription factor SOX2 is required for the development of the sensory lineage. Two different *Sox2* mouse alleles showed little or no sensory development in the ear and markers for both hair cells and supporting cells were absent in the more severe allele, suggesting SOX2 is important for the specification and/or maintenance of the sensory progenitors. More recently, we identified another factor, the Notch ligand Jagged1 (JAG1) that is also important for the development of the sensory lineage. Conditional inactivation of JAG1 in the developing otocyst

leads to severely compromised sensory development. To gain a more mechanistic understanding of the role of JAG1-mediated Notch signaling, we are investigating whether JAG1 is required for the specification or maintenance of the sensory progenitors. Our results show that the SOX2 domain is smaller in the JAG1 mutants. In addition, proliferation is reduced in the JAG1 mutant inner ears. We have also undertaken a gain-of-function approach using an activated form of the Notch receptor (NICD) to determine whether expression of activated Notch can initiate sensory development in non-sensory regions of the mammalian ear. Our results indicate that ectopic sensory regions can be induced in the non-sensory regions of the cochlea, and involves early upregulation of SOX2. Our results support a role for JAG1-mediated Notch signaling in the specification of the sensory progenitors, although Notch may also be important for maintaining proliferation in the sensory regions.

520 Sox2 Signaling in the Specification of Prosensory and Hair Cell Fate in the Developing Mouse Cochlea

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A key step in the development of inner ear sensory epithelia is the commitment of multipotent progenitor cells located in the ventromedial domain of the otocyst to a prosensory fate, defined as the ability to give rise to either hair cells or support cells. However, the molecular factors that confer prosensory fate to otic progenitor cells are only beginning to be identified. Previous studies by Kiernan et al (2005, 2006) have demonstrated that deletion of either the Notch ligand, Jagged1, or the HMG transcription factor Sox2 results in a significant (Jagged1) or complete (Sox2) loss of prosensory cells. To determine whether either of these factors is sufficient to induce prosensory specification, gene transfer was used to activate both pathways in non-sensory inner ear cells. Forced expression of the intracellular domain of Notch (Notch-ICD) was sufficient to induce ectopic expression of Sox2 implying that the Notch signaling pathway acts upstream and that expression of Jagged1 is the first indication of prosensory identity. Forced expression of Notch-ICD, Sox2, or Prox1 failed to induce hair cell formation. However, forced expression of Sox2 was sufficient to induce Prox1 expression. In addition, our results demonstrated that Sox2 and Prox1 actually act to inhibit hair cell formation through antagonism of Atoh1. Moreover, our results suggest that in the outer hair cell domain, Prox1 is the key regulator of Atoh1 antagonism, but since Prox1 is not expressed in the inner hair cell domain, Sox2 inhibits Atoh1 directly. These results are consistent with the hypothesis that prosensory domain is specified by Jagged1-Notch interactions which act to induce Sox2 expression. However, subsequent hair cell formation is dependent on down-regulation of Sox2 suggesting a complex role for Sox2 in inner ear development.

521 Sprouty Gene Function in Otic Placode Induction

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Sprouty (Spry) genes encode antagonists of receptor tyrosine kinase signaling, including Fibroblast Growth Factor (FGF) signaling. We have previously shown that Sprouty2 (Spry2) is a mouse deafness gene that antagonizes FGF8 signaling to establish both proper cellular patterning of the auditory sensory epithelium and normal hearing (Shim et al. Dev. Cell 2005). We have found that embryos missing both the Spry1 and Spry2 genes (Spry1-/-; Spry2-/- double mutants) have severe malformations of the inner ear epithelium. Here we demonstrate that defects in these mutants can be found as early as otic placode stages, and that the Dlx5 expression domain, which marks the otic placode, is expanded in Spry1-/-; Spry2-/- double mutants. Consistent with a putative role in otic placode induction, we find that both Spry1 and Spry2 are co-expressed in the pre-otic ectoderm and underlying mesenchyme. FGFs have been shown to induce otic placode formation in multiple species including mouse, chick, and zebrafish. In the mouse, double mutant combinations of Fgf3 and Fgf10 or Fgf8 and Fgf3 result in the absence or dramatic reduction of the otic placode (Alvarez et al., Dev. 2003, Ladher et al., Genes and Dev. 2005, Wright and Mansour, Dev. 2003, Zelarayan et al., Dev. Bio. 2007). Our observation that the Dlx5+ otic domain is expanded in Spry1-/-; Spry2-/- double mutants is consistent with the possibility that Spry1 and Spry2 negatively regulate FGF-mediated induction of the otic placode. To test the possibility that Spry1 and Spry2 antagonize FGF signaling during otic placode induction, we have begun genetic interaction experiments between the Spry1, Spry2, and Fgf10 genes. Preliminary results from these genetic interaction experiments will also be presented.

522 Differential Requirements of the Hindbrain and Mesenchyme on Inner Ear Patterning

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The ability of the vertebrate inner ear to functionally detect and process sensory information related to balance and hearing is dependent on the proper patterning of its three dimensional structure. Signals from surrounding tissues, such as the hindbrain, notochord, and mesenchyme, are thought to play critical roles in this developmental process. To elucidate the specific requirements of these tissues on inner ear patterning, we performed microsurgical manipulations in ovo of the chick hindbrain and mesenchyme adjacent to the developing ear. Previous reports suggested the necessity of Sonic Hedgehog (Shh) signaling from the floorplate of the hindbrain and/or notochord in cochlear formation. Interestingly, when a segment of hindbrain and notochord directly adjacent to the otocyst was rotated along its anteroposterior (AP) axis, which presumably should not affect the level of Shh that is being provided to the inner ear, the

cochlea was stunted or malformed. This result suggests that other signals from the hindbrain and notochord in addition to Shh are required for cochlear outgrowth. Or, alternatively, the level of Shh is differentially distributed along the AP axis of the hindbrain and such a gradient is important for proper cochlear development. In contrast to the hindbrain manipulations that mainly affected the cochlea, semicircular canal development was affected when the mesenchyme was disturbed. Replacement of mesenchymal tissue posterior to the otocyst with an equivalent size of tissue anterior to the otocyst resulted in the posterior canal to adapt some anterior canal characteristics. Taken together, our results suggest that the correct AP orientation of the hindbrain and the underlying notochord is important for proper patterning of the cochlear duct, while the mesenchymal tissue adjacent to the anterior or posterior side of the otocyst appears to be important for providing AP axial identities to the semicircular canals.

523 Maintaining Borders Between Sensory, Nonsensory, and Neuronal Tissues: A Role for Lmx1a in the Mouse Inner Ear

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The inner ear develops from a seemingly homogeneous pocket of ectoderm into an intricate patchwork of sensory, non-sensory, and neuronal tissues. Delineating and maintaining the borders among these tissues requires several levels of interactions between and within cells. Transcription factors are excellent candidates for acting as the master switch for these developmental processes. LIM homeodomain (LIM-hd) proteins form a subfamily of transcription factors that contain two amino-terminal LIM motifs and a downstream homeodomain. LIM-hd proteins participate in heteromeric transcriptional complexes in a tissue-specific manner. These transcriptional complexes are critical to many cell fate decisions and patterning of organs (Trends Genet; 16:75-83, 2000).

In this study, we show that the Lim-hd gene *Lmx1a* is a key molecule in maintaining proper borders among tissues within the inner ear. In an *Lmx1a* functional null mutant mouse, *dreher* (Nature; 403:764-9, 2000), the inner ears lack an endolymphatic duct and the membranous labyrinth is poorly developed, consistent with its role as a selector gene in other systems. More importantly, while all three primary fates of the inner ear – neural, sensory and non-sensory are induced in the *dr/dr* inner ears, normal boundaries among these tissues are often violated. The boundary between vestibular and auditory neurogenic domains is disorganized, marked by ectopic *Fgf3* expression and an enlarged vestibular ganglion. Moreover, the size and shape of various sensory organs are aberrant. Most notably, vestibular-like hair cells are found in the basal region of the cochlear duct.

524 Over-Expression of Hoxb3 Affects Sensory Neuron Differentiation and Inner Ear Morphogenesis

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Mutations of several *Hox* genes, including *Hoxa1, Hoxb1* and *Hoxa2*, have been shown to cause sensorineural deafness and abnormal inner ear development in human and mice. However, the pathogenesis mechanisms and the functions of *Hox* genes during inner ear morphogenesis remain unclear. Using a *Hoxb2* enhancer element to over-express *Hoxb3* in the otic vesicle, we generated a transgenic mouse mutant $Hoxb3^{Tg}$. The $Hoxb3^{Tg}$ transgenic mice were deaf and had abnormal circling behavior. In wildtype embryos, *Hoxb3* is expressed in the otic epithelium at E9.5, by E11.5 the expression is restricted to the superior vestibular ganglion. In $Hoxb3^{Tg}$ embryos, we found that as a result of over-expression of *Hoxb3*, the expression of *Hoxb1* was down-regulated. The expression of *Ngn1, Six1* and *NeuroD* which are required for neuroblast specification, delamination and differentiation were reduced in $Hoxb3^{Tg/+}$ mutants, indicating that *Hox* genes are involved in sensory neuron specification.

The $Hoxb3^{Tg/Tg}$ homozygotes developed dysmorphic inner ears with poor differentiation of sensory epithelium and had almost no sensory neurons, except for a diminished projection to the posterior cristae. The $Hoxb3^{Tg/+}$ heterozygotes lacked or had a reduced utricle whereas the horizontal and anterior crista and saccule were less affected. These defects correlate with a severe reduction of sensory neurons in the superior vestibular ganglion, specifically the innervation to the utricle. $Hoxb3^{Tg/+}$ mutants had a shortened cochlea with multiple rows of hair cells and supporting cells and fibers overshooting the organ of Corti. The innervation defects were similar to those of *Foxg1, Fgf10, Lmx1A, Ngn1, Gata3* and *Eya1/Six1* mutants. The expression of these genes in cochlea was altered and the expression of *Sox2* in prosensory domain was reduced in $Hoxb3^{Tg/+}$ mutants. We show that over-expression of *Hoxb3* affects prosensory, sensory neurons, and inner ear development in a dosage dependent fashion.

525 Forced Expression of Tlx3 Results in Altered Gene Expression in an Otic Neuroblast Cell Line

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Our previous studies have demonstrated that canonical Wnt signaling and a combination of Sonic hedgehog (Shh) and retinoic acid (RA) regulate expression of distinct sets of sensory neuron markers in somatic pluripotent stem

cells after neural differentiation. To elucidate the effects of these signaling molecules on otic progenitor cell differentiation, we used the ventral otocyst-neuroblast cell line clone 33 (VOT-N33) that was established from the ventral otocyst of E10.5 immortomice. Our initial molecular characterization revealed that members of Shh, RA and Wnt receptors as well as their downstream effectors are constitutively expressed in the VOT-N33 cell line. After 2-3 days of expansion under proliferative conditions (33 °C, γ-interferon), VOT-N33 cells were grown at 39 °C in a neural induction medium supplemented either with Shh/RA or Wnt1, a canonical Wnt ligand, for up to 14 days. Shh/RA had no effect on the expression level of GATA3, which was up-regulated 300-fold by Shh/RA in somatic stem cells. In contrast, Wnt1 induced a significant up-regulation of Tlx3 and Ngn1 in VOT-N33 cells, a consistent trend observed in somatic stem cells. Since Tlx3 is known as a glutamatergic selector gene, we wished to test the effect of Tlx3 expression on VOT-N33 cell differentiation. Undifferentiated VOT-N33 cells were transfected with a Tlx3 expression or control vector using the lipofectamine 2000 kit. High Tlx3 expression was detected in VOT-N33 cells expressing the expression vector, but not in those expressing the control construct. VOT-N33 cells stably expressing Tlx3 were selected, expanded and subject to neural induction. Gene expression profiling in VOT-N33 cells in the presence or absence of Tlx3 and before or after neural induction is currently underway and the results will be discussed.

526 Wnts and Axon Guidance in the Chick Inner Ear

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Mechanosensory hair cells in the chicken inner ear are innervated by the peripheral processes of statoacoustic ganglion (SAG) neurons. Understanding how SAG projections are guided to their sensory targets could improve treatments of sensorineural hearing loss. Current studies are aimed at investigating the Wnt family of signaling molecules as possible axon guidance cues in the chicken inner ear. Wnt ligands are known to attract or repel axons in a variety of systems. However, the role of Wnt signaling in axon guidance in the ear has not been studied. Previously, our lab reported the expression of several Fz transcripts in chick SAG neurons, suggesting that the axons may be Wnt responsive during outgrowth. The expression of transcripts for various Wnt ligands in non-innervated areas of the chicken inner ear, and their absence in the innervated prosensory domains, suggests that Wnts may serve as repulsive axon guidance cues in the otic ectoderm. To test this hypothesis, three-dimensional collagen cultures are used to grow E3-E5 chick SAG neurons in the presence of cells transfected with genes for Wnt ligands. Cell aggregates should serve as point sources to create a Wnt gradient within the collagen gel. SAG explants are cultured in serum-free conditions for 24 hours at a fixed distance from Wnt-secreting cells, fixed, immunostained, and imaged with the confocal microscope. We then quantify length and density of SAG axons to determine whether axons are shorter on the side of the explant facing Wnt-expressing cells. Three-dimensional collagen gel cultures serve as a convenient method for screening candidate Wnts before performing *in vivo* studies of SAG axon outgrowth in conditions that alter Wnt signaling.

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527 Disruption of EphB Signaling Alters Normal Spiral Ganglion Cell Projections and Innervation Patterns in the Cochlea

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The mammalian cochlea is finely structured with precise tonotopic organization to facilitate the intricate audition of complex sounds. The molecular cues that guide axons of spiral ganglion neurons to appropriate hair cells to establish this tonotopic organization that effectively translates the mechanical stimuli of sound to the auditory nervous system are poorly understood. Previous studies in our laboratory with ephrin and Eph knock out mice have demonstrated deficits in the auditory and vestibular systems. Specifically, evaluation of distortion product otoacoustic emissions (DPOAE) revealed diminished levels in EphB1 and EphB3 receptor knockout mice relative to wild-type littermates. We have used EphB Lac Z mutant mice and ß-gal staining to determine expression patterns of EphB receptors in the cochlea. In addition, we have used Neurovue lipophilic dyes placed in the internal auditory canal to fluorescently label the spiral ganglion nerve fibers as they track thru the modiolus and innervate the organ of Corti in early post natal (P8) and adult (>P30) quadruple EphB1, EphB2, EphB3, and EphB6 knockout mice. From these experiments, we have characterized cochlear innervation patterns in EphB receptor knockouts compared to age matched wild-type control CD1 mice. The Neurovue labeled nerve fibers were analyzed by confocal microscopy and demonstrate that deletions of EphB1, B2, B3, and B6 result in irregular innervation of the organ of Corti in early post natal mice and substantially more nerve fibers innervating the cochlea in adult mice. These results suggest a role for EphB receptors in establishing and fine tuning cochlear innervation patterns during development.

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528 The Role of NeuroD in the Development of Auditory and Vestibular Neurons in Chick Inner Ear

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NeuroD is a basic helix-loop-helix (bHLH) transcription factor that is expressed in developing neurons of the inner ear. In mice, deletion of NeuroD results in deafness and head tilting/circling behavior due to the loss of cochlear and vestibular neurons, respectively (Liu et al., 2000; Kim et. al., 2001). The aim of this study was to examine the

role of NeuroD in the development of the afferent neurons of the avian cochlea and vestibular organs. Previous results from studies in Xenopus indicate that NeuroD is capable of converting epidermal cells into neurons, but the ability of this gene to specify cochlear and/or vestibular neurons during inner ear development is unknown (Lee et. al., 1995). In order to address this issue, we are currently over-expressing NeuroD in the developing chick ear in ovo. Initial observations show that cells transfected with NeuroD develop into neurons within the auditory and vestibular ganglia. In addition, transfected cells in the surrounding mesenchyme are positive for neuronal labels and have projections that extend toward the inner ear sensory epithelia. Those observations suggest that NeuroD is sufficient for cochlear and vestibular neuron development. Additional studies have focused on whether the peripheral projections of NeuroD-transfected cells innervate appropriate targets. Preliminary data show that peripheral projections of NeuroD-transfected cells within the vestibular ganglion terminate in the vestibular sensory organs. Similarly, NeuroD-transfected cells within the cochlear ganglion send peripheral projections toward the basilar papilla. These data suggest that ectopic expression of NeuroD does not disrupt peripheral innervation patterns. Further studies will investigate the central projections of NeuroD-transfected cells. Those data will allow us to determine the role of NeuroD in neuronal development and/or neuronal pathfinding.

529 Expression of the IGF2 Regulator Imp2 in the Developing Mouse Inner Ear

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IGF signalling has been shown to play important roles in growth and differentiation in the developing embryo. In the developing inner ear, early neurogenesis and the development of otic neuroblasts involves IGF1. Addition of either IGF1 or IGF2 to cultures of otic epithelium stimulates proliferation in vitro. In man, sensorineural deafness has been associated with an IGF1 mutation, and Igf1 mutant mice exhibit aberrant cochlear innervation.
The control of IGF signalling is likely to be complex, and involve multiple levels of regulation. For example, Igf2 is controlled at a transcriptional level through *cis*-acting enhancers. IGF signalling is also regulated at a post-translational level by the IGFBP (insulin-like growth factor binding protein) proteins, that bind both IGF1 and IGF2 proteins with high affinity. However, an additional layer of IGF regulation is available to the developing embryo through the IMP (IGF2-mRNA binding protein) family of RNA binding proteins, that are able to bind to untranslated regions of the mRNA for Igf2. It is thought that IMP binding could regulate the IGF pathway by either directly controlling translation and/or localisation of Igf2 mRNA.
In a previous study, we isolated Imp2 from the developing mouse otic vesicle. In order to begin to understand how and when Imp2 may control IGF signalling in the developing inner ear, and what affects this has on the developing inner ear, it is important to understand the precise temporal and cellular expression of Imp2 during inner ear development. We will present expression data during ear development for the mouse Imp2 gene.
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530 Expression of PSA NCAM, Class III Beta-Tubulin, Calbindin, and S100 in the Developing Spiral Ganglion in Mice

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The spiral ganglion develops in the mouse from the otocyst through the cochlear-vestibular ganglion (CVG), reaching its almost-final form at P10. We were interested in the roles of four selected markers during spiral ganglion development: polysialic acid attached to neural cell adhesion molecule (PSA NCAM), class III beta-tubulin (TB), calbindin (CB) and S100. Therefore, we investigated their expression patterns from embryonic day 11 (E11) to postnatal day 10 (P10) in C3H mice using immunohistochemistry. Double immunofluorescent staining and grey level analysis were used in the study. PSA NCAM and CB were expressed in the otocyst and CVG from E11. Both markers were also detected in the cochlear ganglion. TB expression appeared in the neurons of the cochlear ganglion and their fibers invading the epithelial cells of the cochlear duct from E13. Spiral ganglion neurons (SGNs) and their dendrites were distinctly TB immunoreactive from E17. PSA NCAM and CB immunolabeling collocalised in some SGNs during late embryogenesis. In addition, S100 immunostaining started to be apparent in SGNs at E17. Interestingly, the S100-positive population of SGNs differed from those immunolabelled for PSA NCAM and CB. Early postnatally, SGNs and their afferent nerve fibres still expressed PSA NCAM and CB; at the same time, TB immunostaining of SGNs was attenuated. S100 immunostaining was still present in a few SGNs at P4. However, S100 immunostaining exclusively marked developed Schwann cells later in postnatal development. The temporal and spatial patterns of PSA NCAM and CB expression correlated with the growth of dendrites and the establishment of the synaptic contacts of hair cells. TB positivity marked differentiated neurons, while S100 labelling was later restricted to Schwann cells only.
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531 The Robo Genes Are Expressed in Distinct Patterns During Inner Ear Development

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The Slit/Robo signaling pathway plays a role in axon guidance, axonal and dendritic branching and neuronal migration in the developing central and peripheral nervous system. However, little is known regarding Robo distribution and function in the developing mammalian inner ear. Using a combination of in situ hybridization and immunohistochemistry, we describe the temporo-spatial

expression of Robos 1-3 in the mouse embryonic, early postnatal and adult inner ear.
The results demonstrate that Robo1, Robo2 and Robo3 are differentially expressed in the embryonic cochlea, particularly at E16 when Robo1 is present in the spiral ganglion neurons whereas Robo2 and Robo3 are restricted to the apical aspect of the cochlear epithelium. By the early post-natal period, Robo1 and Robo2 are expressed in overlapping regions including the spiral ganglion neurons and the organ of Corti. Interestingly, this expression persists into adulthood. The embryonic expression of the Robo genes and proteins suggest a potential role for this signaling pathway in mediating auditory connectivity. Continued expression of the Robo genes in adulthood suggests that this signaling pathway has the potential to be modulated to enhance neuronal connectivity as new therapies for sensorineural hearing loss are developed.

532 Deafness in Mice Lacking the T-Box Transcription Factor Tbx18 in Otic Fibrocytes

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In the cochlea, fibrocytes play important physiological roles, including the maintenance of the ionic composition of the endolymph. Human deafness upon fibrocyte alterations witnesses their crucial role for hearing. We demonstrate that differentiation of otic fibrocytes requires the T-box transcription factor gene *Tbx18*. *Tbx18* expression during inner ear development is restricted to the sub-region of otic mesenchyme that is fated to differentiate into fibrocytes. We rescued the somitic defect that underlies the perinatal lethality of *Tbx18*-mutant mice by a transgenic approach, and measured auditory brainstem responses. Adult *Tbx18*-deficient mice showed profound deafness and a complete disruption of the endocochlear potential that is essential for the transduction of sound by sensory hair cells. The differentiation of otic fibrocytes of the spiral ligament was severely compromised. Tissue architecture of the stria vascularis of the lateral wall was disrupted, exhibiting an almost complete absence of the basal cell layer, and a reduction and changes of intermediate and marginal cells, respectively. Stria vascularis defects resulted from the failure of *Tbx18*-mutant otic fibrocytes to generate the basal cell layer by a mesenchymal-epithelial transition. Defects in otic fibrocyte differentiation may be subordinate to a primary role of *Tbx18* in early compartmentalization of the otic mesenchyme, as lineage restriction and boundary formation between otic fibrocytes and the surrounding otic capsule were severely affected in the mutant. Our study sheds light on the genetic control of patterning and differentiation of the otic mesenchyme, uncovers distinct steps of stria vascularis formation and illuminates the importance of non-epithelially-derived otic cell types for normal hearing and the etiology of deafness.

533 Involvement of PDGFR-β in Maintenance of Mesenchyme of the Neonatal Mouse Inner Ear

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Platelet-derived growth factor receptor (PDGFR) signaling has been demonstrated to play a pivotal role in early embryonic development. Although the expression of PDGF in the inner ear has been studied by RT-PCR, how PDGFR is involved there remains largely unclear. In the current study, we used the antagonistic anti-PDGFR-β antibody, APB5, to investigate the role of PDGFR-β in the neonatal mouse inner ear. PDGFR-β was detected immunohistochemically in the mesenchymal tissue adjacent to the sensory epithelium of the inner ear, but partially in the sensory epithelium. To determine whether this expression plays a functional role, we injected APB5 into neonates to block the function of PDGFR-β from the postnatal day 0 (P0) to P8. Mesenchymal tissue defects and abnormal capillaries with irregular shapes, especially in the cochlear lateral wall, were detected in APB5-treated mice. In the case of mice euthanized at P9 after the administration of APB5, the incidence of abnormalities in the cochlea and saccule of the group injected APB5 from P0 to P4 was significantly higher than that of the control. Mice injected with APB5 for the last 5 days showed no significant differences in mesenchymal defects of the inner ear compared with the control group. We speculate that, within several days after birth, the cochlea and saccule are more sensitive to APB5 and it takes a few days to develop mesenchymal defects after the first administration of APB5. To study further the effect of APB5 administration on the sensory epithelium of the inner ear, we conducted an *in situ* TUNEL assay. Not only the adjacent mesenchymal cells but also the sensory epithelial cells underwent cell death. These results indicate that PDGFR-β signals are required for the survival of the capillary and mesenchymal cells in the neonatal mouse inner ear and also indirectly implicate these signals in the survival of the sensory epithelium.

534 The Otic Capsule as a System of Chondro-Osseous Channels Replenished from the Fissula Antefenestram. a Developmental Study

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We studied the normal development of the otic capsule to clarify our recent observation of changes in its vestibular "arch" region in Ménière's disease (Michaels et al. Acta Otolaryngol in press). We examined stained step sections of 49 temporal bones from 27 fetuses between 8 and 40w and 24 temporal bones from 20 post-partum children and adults between 1 and 52 years.
Cartilage canals (CCs) are channels in the developing cartilage model of bones. They convey stem cells and

blood vessels derived from the perichondrium to the avascular cartilage interior to initiate ossification there. At 11w large CCs are seen in the vestibular capsule and a single CC, the fissula antefenestram, in the cochlear capsule. Both sites start to ossify at 16w but the fissula persists throughout life and produces, at all ages, new skeletal tissue around it. Stages of ossification are similar to those in other endochondral bones except that mineralized channels of cartilage do not disappear, but persist as cochlear capsule network, containing mesenchymal, cartilage and ossified cells ("globuli ossei") throughout life.

At about 20w a thin wall of compressed mesenchymal cells covers the interface with the perichondrium of the membranous labyrinth and, at the external surface of the spiral ligament, and especially at the perichondrium of the vestibular arch, forms new mineralized cartilage lacunae and mesenchymal cells.

From 34w there appear basophilic modified Haversian-like channels. These form bone on their exterior and their lumina link up with those of the chondro-osseous channels.

Otic capsule is thus a system of skeletal channels containing loose globuli ossei, with a background of bone. New channels formed from the fissula seem to replenish the system throughout life. This arrangement may be a means by which important soluble substances can form as a result of apoptosis of cells in the channels and diffuse through to the nearby endolymph.

535 Genomic DNA of Pou3f3/Brn-1 Is Highly Methylated in the CpG Island at the 3'-Flanking Region in the Developing Auditory Epithelium

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One of the gene regulatory mechanisms is enforced by methylation of cytosines on the GC-rich expression control regions (CpG islands, CGIs), which results in inhibition of transcription factors to associate their binding regions. In the previous meeting, by using PCR-based screening method called amplification of inter-methylated sites (AIMS), we demonstrated that multiple genomic regions were differentially methylated in the rat auditory epithelium between postnatal day 1(P1) and P14, before and after the onset of hearing. We identified one of the P1-specific DNA fragments as the 3'-flanking region of transcription factor Pou3f3/Brn-1, and demonstrated that Pou3f3 was expressed in the supporting cells, mesenchymal cells including tympanic covering layer, but not in the hair cells, ganglion cells, or stria vascularis in the cochlea. In this meeting, we further analyzed the changes in methylation frequency in the 3 CGIs in the genomic DNA of Pou3f3 during postnatal development. Methylation-specific PCR proved that the CGI located at the 3'-flanking region was highly methylated at P14 compared with P1. The other two CGIs, one at the 5'-flanking region and the other at the 3'-untranslated region of Pou3f3, did not show significant increase in the methylation status. During development, Pou3f3 expression was measured to be significantly decreased by using TaqMan-quantitative RT-PCR. DNA methyltransferases Dnmt3a and 3b, responsible for de novo methylation of genomic DNA, were found to be expressed in the developing auditory epithelium. Association of genomic methylation and repression of Pou3f3 will be discussed.

536 Differential Effects of Efferent Stimulation by Contralateral Bandpass Noise on the Two Major Components of Distortion Product Otoacoustic Emissions

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Contralateral acoustic stimulation (CAS) modification of otoacoustic emissions (OAEs) are thought to be mediated by the medial olivocochlear (MOC) fibers, which synapse directly on the outer hairs cells. Distortion product otoacoustic emissions (DPOAE) are the sum of at least two different components coming from different cochlear regions and generated by different mechanisms. The generator component stems from the nonlinear properties at the region of maximal primary overlap, and the reflection component is a linear reflection of energy from the DPOAE characteristic frequency. Consequently it is expected that the two components will be differentially sensitive to CAS stimulation. We recorded DPOAEs using the scissors paradigm (Kummer et. al., 1998) by varying L2 (35-60 dB SPL) and the bandpass noise CAS (50-70 dB) in 5 dB steps. In addition to extracting the total DPOAE (generator and reflection components together), which gives rise to DPOAE fine structure, we also extracted the generator and reflection components using a modified Least Squares Fit procedure that adapts to changes in latency with frequency. Consistent reduction of the total DPOAE level was only seen at high CAS levels (60-70 dB SPL). The extraction of the two major components revealed that the reflection component was more susceptible to CAS stimulation than the generator component. While the amplitude of the generator component was reduced only at high levels (60-70 dB SPL) in a manner similar to changes in the total DPOAE, the reflection component was already reduced at moderate CAS levels (as little as 50 dB SPL). Finding the optimal primary and CAS levels to measure the suppression effects of the two major DPOAE components has the potential to provide additional information to enhance the clinical and research evaluation of MOC function (This work was partially supported by funded by the National Organization for Hearing Research, and Grant No. H133E03006 from the National Institute on Disability and Rehabilitation Research, U.S. Department of Education).

537 Measuring the MOC Reflex at DPOAE Fine Structure Maxima in Human Adults

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In humans, when the medial olivocochlear (MOC) pathway is activated by broadband noise in the opposite ear, changes in distortion product otoacoustic emission (DPOAE) level can be recorded in the test ear. Recent developments in our understanding of DPOAE generation, along with some emerging literature, suggest that the choice of DPOAE frequency and where it falls on the alternating pattern of level maxima and minima, i.e. fine structure, affects direction (suppression vs enhancement) of these level changes. In this study, DPOAEs were recorded at fine frequency intervals from 500-2500 Hz, with and without 60 dB SPL of contralateral noise in a group of 15 normal hearing adults. To calculate the MOC reflex, noise-induced changes in DPOAE level were recorded at frequencies corresponding to peaks in the fine structure only. Additionally, Inverse FFT was conducted to evaluate the impact of contralateral noise on individual DP components (overlap and CF). Results show: 1) the MOC reflex was stable and reliable across 2 trials in the same individual, 2) the MOC reflex ranged from 1.9 to 2.5 dB and 97% of data points reflect suppression (vs enhancement), 3) contralateral noise reduced the CF component level more than the overlap component level and 4) a peak frequency shift of between 5-10 Hz (upward in frequency) was observed with the presentation of contralateral noise. Results indicate that recording the MOC reflex at DPOAE frequencies corresponding to peaks in fine structure, when dual components of the response are in-phase, results in a stable, robust measure of the MOC reflex and eliminates observations of level enhancement. We argue that DPOAE level enhancement evoked by contralateral noise is not a true MOC effect, but primarily a result of component mixing in the ear canal. When this factor is controlled by recording at maxima only, a more accurate and stable measure of the MOC reflex can be recorded.

538 The Effects of Contralateral Stimulation on Distortion Product Otoacoustic Emission (DPOAE) Fine Structure

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The amplitude of the $2f_1$-f_2 DPOAE in humans is typically altered by a contralateral acoustic stimulation (CAS) most probably mediated by the medial olivocochlear reflex. The DPOAE amplitude changes due to CAS show large interindividual variability [1], different characteristics in DP-Gram maxima and minima [2] and large changes from suppression to enhancement for only small changes of the primary levels [3,4]. The underlying mechanisms of these effects are still not fully understood. We hypothesize that the two interacting DPOAE sources might be differently affected by the CAS. If our hypothesis is true, CAS will cause specific changes in the DPOAE fine structure. Therefore, DPOAE fine structures were measured in seven subjects using frequency-modulated primaries (log frequency sweeps, f2: 1500- 3000 Hz) which maintain a constant frequency ratio f_2/f_1 of 1.2 [5]. Data was obtained for primary levels L2 of 60dB SPL and three different L1 (58, 63, 68dB SPL) without and during broadband CAS (50dB SPL). The DPOAE were computed at 1840 frequency steps, permitting a detailed analysis of changes and shifts in DPOAE fine structure according to CAS. Furthermore the DPOAE data were analyzed using a latency windowing technique to separate the contributions from the two interacting DPOAE sources investigating the influence of CAS on the two DPOAE sources separately.
Almost all of the effects of CAS on DPOAE described in the literature mentioned above can be explained based on the findings in the current study. In addition, the data indicate, e.g., that there is most probably no “true” enhancement in terms of enhanced cochlear activity during broadband CAS. Rather there are slight frequency shifts in DPOAE fine structure due to changes in the relative contribution of the two DPOAE sources - both sources being slightly suppressed. This shift can lead to a “pseudo” enhancement of up to 20dB for isolated frequencies.

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539 Objective Hearing Threshold Estimation in Humans Using the DPOAEs Primary-Source Component

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Objective estimation of hearing threshold in humans using distortion product otoacoustic emissions (DPOAE) can be performed by extrapolating the input-output (I/O) function of their cubic component. The method is nevertheless strongly influenced by the DPOAE generation mechanism, namely by its secondary source. This source appears to be partially responsible for inaccuracies in the estimation of hearing threshold.
The goal of our study was to remove the contribution of the secondary source and to establish the accuracy of the threshold estimation method using only the primary-source component of the DPOAE. For this purpose, DPOAE I/O functions were measured using a pulsed f2 primary paradigm in normal hearing subjects. The stimulus parameters were: f2/f1 = 1.2, f2 = 1.8–2.5 kHz swept in 20 Hz steps, L1 = 0.4L2 + 39 dB and L2 = 25-65 dB SPL. Investigation of the DPOAE time-signal after the f2 primary onset showed that the primary source is near its steady-state within 8-9 ms after the f2 onset, while the secondary source is just starting to build up. Consequently, by taking the time-signal of the DPOAE 8 ms after the f2 onset, the primary-source component can be obtained and the secondary-source component eliminated.

Hearing threshold was subsequently estimated by extrapolating the DPOAE I/O functions constructed solely from the primary-source component. For comparison, hearing thresholds were also estimated using the conventional steady-state DPOAE. We show that the threshold estimated using the pulsed f2 paradigm mirrors the audiometrical threshold and that the number of I/O functions included in the analysis improves from 77% for the conventional steady-state DPOAE to 95% for the pulsed paradigm. Moreover, the standard deviation of the threshold estimated using the pulsed f2 paradigm (6 dB) was smaller than that obtained with the conventional steady-state DPOAE (14 dB).
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540 Speech-Evoked Otoacoustic Emissions Elicited by Speech in Quiet and in Noise

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Speech-evoked otoacoustic emission (SPOAE) responses were measured in human ear canals with recorded speech as the stimulus using a nonlinear mutual suppression procedure. SPOAEs provide a non-invasive measure of cochlear nonlinearity in response to coarticulated speech that has significance for theories of speech perception. Stimuli were presented at a moderate level with an interstimulus interval of 1.4 s and were comprised of voiced and unvoiced stop consonants and vowel /a/ in a vowel-consonant-vowel context. SPOAEs for speech-in-quiet showed adaptation in some ears, with an increase in SPL approximately 100-150 ms after stimulus onset. SPOAEs were also recorded for speech in the presence of either ipsilateral or contralateral continuous, broadband noise at 10 dB SNR, gated on 200 ms before speech onset. SPOAEs for speech in noise were larger than for speech in quiet, consistent with noise-induced adaptation. The temporal progression of adaptation of the in-quiet and in-noise SPOAE responses is consistent with the action of medial olivocochlear efferents on outer-hair-cell functioning. SPOAE level adaptation is ~10 dB, much larger than adaptation measured using other OAE types. Results using other speech and non-speech stimuli to elicit OAEs and further study their adaptation properties will be described. (Research supported by NIDCD DC003784, DC00013, DC004662)

541 Stimulus Compensation Method for Improved Transient-Evoked Otoacoustic Emissions Recording

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The meatus (auditory canal) plays a role in altering the timbre and waveform of incident sound, acting as a Helmholtz resonator that peaks around 3 kHz. The stimulus incurs both amplitude and phase distortions. Often in transient-evoked otoacoustic emission (TEOAE) recording protocols, a 100 µs click is utilized to elicit a click-evoked response. TEOAEs are recorded by a probe microphone placed in the meatus, and last for about 20 ms. Time-domain ringing in the meatal response (MR) creates a ringing stimulus artifact that lasts up to 5 ms, obscuring early latency TEOAEs. In an ideal recording of TEOAEs, it is desirable to transmit a broadband transient of extremely short duration.
This research is motivated by the need for a real-time, ear and probe placement dependent method for minimizing the distortions of the meatus. The MR is first obtained, from which an inverse compensation filter is created. The MR can be obtained by a direct impulse response method, or via a swept-sine stimulus method. The inverse filter uses an inverted MR magnitude in tandem with a phase equalizer. The meatal compensation filter is subject and session dependent, and needs to be recomputed before any recording session. All stimuli should be run through the filter prior to presentation to the subject in order to minimize the distortions of the meatus, and provide a more consistent input to the ear.
Results from normal adult subjects show an improvement to the flatness of the magnitude response and linearization of the phase response. Furthermore, a reduction in effective duration of the MR is found, reducing the meatal artifact by about half for click stimuli. The high frequency TEOAE content found in the early latencies of the response that is typically obscured by the MR artifact are revealed with the use of the compensation filter.

542 The Effect of Changes in Ear Canal Pressure on the Transient and Distortion Product Otoacoustic Emissions in Normal Hearing Adults

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Existing literature describes a simple effect of external ear canal pressure on otoacoustic emissions (OAEs) where pressure has a systematic high pass effect (Osterhammel, Nielsen & Rasmussen, 1993; Plinkert, Bootz & Vobiek, 1994 and Naeve et al, 1992), however, these studies pool data across individuals and only investigate large changes in pressures (100 daPa steps). Here we investigated the effect of small changes (25 daPa steps) in ear canal pressure in normal-hearing individuals. Six normal hearing subjects (seven ears) with an age range of 18-38 years were tested with a special integrated probe that was devised to allow for simultaneous alteration of middle ear pressure while measuring the OAEs. Six trial sets of recording of both Transient and Distortion-Product OAEs with randomly presented small changes in middle ear pressure in 25daPa steps within a range of ±100daPa;totaling 126 recordings/subject were made. Results showed that even small changes in pressure can have a significant effect on transient and especially on distortion-product OAEs magnitudes (reducing them by up to 7-10dB). OAE magnitudes were generally largest at atmospheric pressure (0daPa) rather than tympanometric peak pressure and decreased with increasing or decreasing pressures particularly for OAEs at frequencies below 2 kHz. However, not all subjects displayed this trend and surprisingly some displayed the opposite trend, namely a reduction of OAE magnitude near 0, at 3 and

6kHz. In all, we have found an underappreciated complex interaction of ear canal pressure and OAE magnitude exists. This unexpected nature suggests that ear canal pressure could be altering the reflection coefficient at the oval window thus effecting OAE generation within the cochlea. A goal for many researchers had been to use the relative magnitudes of OAEs to say something about hearing health, but the complicated natures of OAEs in how they are generated and now how they interact with small changes in ear canal pressure need to be understood before those metrics can be used.

543 3D Reconstruction of the Inner Ear in Three Strains of the Zebrafish

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The zebrafish offers many experimental advantages over mammals for investigating developmental and genetic studies of auditory and vestibular disorders. It is relatively easy to maintain, breed, manipulate its gene expression, and it has large inner ears that are accessible in externally-developing embryos and in the newly-developed transparent adult fish (Casper mutant). Due to its lack of pigmentation (except for the eyes) the Casper mutant has a high potential for future research projects, and it would be useful to investigate the morphology of its inner ear. Inner ear morphology was investigated in three strains of zebrafish (wild-type, Roy Orbison, Casper). Fish were fixed by immersion in paraformaldehyde and prepared for Thin-Sheet Laser Imaging Microscopy (TSLIM). Whole heads were bleached with hydrogen peroxide, decalcified, dehydrated, cleared with methyl salicylate/benzol benzoate, and stained with rhodamine isothiocyanate. Serial, transverse, optical sections of the head of each fish were obtained at 20 micrometer intervals. Inner ear structures were segmented and 3D renderings prepared using the Amira program. The following inner ear structures were resolved and segmented: semicircular canals with ampullae, utricle, saccule, lagena, otoliths, nerves, transverse canal, and the sinus impar. The Roy and Casper mutant fish displayed several inner ear morphological variations, including lack of the transverse canal and thinning of the common crus. Morphometric measurements of the segmented structures are being performed and will be reported. TSLIM is an ideal tool that can be used to obtain a z-stack of well-aligned, high resolution optical sections of the inner ear and brain of the zebrafish. Virtual resectioning and 3D reconstruction of inner ear structures and their connections to the brain can be reliably obtained by TSLIM, without having the artifacts produced by mechanical tissue sectioning. Further investigation of these and other mutant zebrafish inner ears will provide a better understanding of auditory and vestibular deficits in humans.

544 A Novel Method to Image Cochlear Soft Tissue at the Cellular Level Without Opening the Cochlea

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The cochlea is a difficult organ system to study, given its convoluted soft tissue and bony structure. Techniques including conventional X-rays, CT, and MRI lack the spatial resolution to visualize microstructures within the closed cochlea. Microscopic techniques require opening the cochlea, causing damage and shrinkage of delicate structures and leading to artifacts of micromechanical measurements. To non-invasively study the cochlea, we have been developing a technique using inline phase contrast X-ray imaging.

At the Advanced Photon Source (APS) of Argonne National Laboratory, images of cochleae from mice and gerbils were taken with hard X-rays at full field. X-ray phase and absorption radiographs were taken at photon energies of 15-31.5 keV, using synchrotron radiation. The images were compared with corresponding histologic sections, absorption contrast x-ray images, and lower energy phase contrast x-ray images from previous experiments. At higher energies, different structures in a decalcified closed cochlea can be distinguished, including outer pillar cells, inner pillar cells, the inner spiral sulcus, the tectorial membrane, the basilar membrane, and Reissner's membrane. The images show superior quality over those taken at lower energy.

The results demonstrate the ability of hard X-rays to noninvasively image cochlear structures at an unprecedented cellular level through the decalcified bony capsule.

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545 Estimation of Total Number of Spiral Ganglion Neurons Using the Optical Fractionator

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Quantitative estimates like number, length and volume of cells and structures are of great value in inner ear research. Not only in characterization of knock-out models but also in evaluation of different experimental manipulations expected to affect cell number or volume. Design-based stereological methods do not assume

anything about the size, shape or distribution of the particles of interest and includes systematic sampling at several levels, which ensures an un-biased estimate.
The present study implements the optical fractionator on plastic-embedded cochleas from normal guinea pig in order to estimate total number of spiral ganglion neurons. The cochleas were embedded in Technovit 7100, a clear plastic that assures transparency in thick sections. The cochleas were sectioned in 24 µm thick sections and every 10th section was collected resulting in a section sampling fraction (SSF) of 10%. When counting spiral ganglion neurons, a counting frame of 1600 µm2 was moved over the section in steps 126.5 µm in both x and y direction resulting in an area sampling fraction (ASF) of 10%. At each sampling position cells were counted in a 12 µm disector and the total thickness of the section was measured. The height sampling fraction (HSF) varied between 57 – 66% depending on the actual height of the section. The z-axis distribution of cells was determined to ascertain that the cell density was relatively constant in the disector height. According to the fractionator principle the total number of spiral ganglion neurons can be obtained by multiplying the inverse sampling fraction at each level with the number of SGN counted. This approach resulted in the total number of spiral ganglion neurons in the guinea pig cochlea being 30 000 (±3089) with the variation of the method lying at 7%. This optical fractionator method proved to be reliable and efficient for estimating total numbers of spiral ganglion neurons in plastic embedded cochleas.

546 3D-Computer Model of Endolymphatic and Perilymphatic Spaces

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Objective: 3D-model of inner ear of hydrops will be helpful to understand the underlying pathology of Meniere fs diasese and distribution pattern of locally applied drugs such as gentamicin and steroid. We attempted to make 3D-model of inner ear with or without hydrops using temporal bone sections from autopsy.
Methods: We used two ears with endolymphatic hydrops and four ears without hydrops from temporal bone collections at Minnesota University. Every tenth from 20µm-thick temporal bone sections was collected. Using ZedView, an analytical software, 3D-model of inner ear was obtained by reconstruction of these sections. Endolymphatic volume (EV) and perilymphatic volume (PV) were calculated in each part of the cochlea and vestibular apparatus including semicircular canals.
Results: In one hydropic ear, cochlear EV was 17.5µl and cochlear PV was 30.7µl. Vestibular EV was 42.5µl and vestibular PV was 33.4µl. In the other hydropic ear, cochlear EV was 31.2µl and cochlear PV was 30.1µl. Vestibular EV was 25.6µl and vestibular PV was 71.8µl. On the other hand, in the control ears, averages of cochlear EV and cochlear PV were 4.8µl and 39.3µl, respectively. Averages of vestibular EV and vestibular PV were 22.9µl and 69.7µl, respectively.
Conclusions: 3D-model of endolymphatic and perilymphatic spaces enables us to get volumes of each space and gives us useful information such as composition of hydrops and distribution of locally applied drugs.

547 The Utility of CT Scanning Prior to Processing Human Temporal Bones in Celloidin

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Standard light microscopy for human temporal bones involves fixation, decalcification, embedment, serial sectioning and staining. We have added an additional step recently: CT scan after fixation and before decalcification. CT images are acquired using a standard clinical protocol for temporal bone imaging in live patients. The images are obtained in the axial plane with 0.5 or 0.6 mm collimation and a 3D volume data set is generated which can then be reformatted in multiple planes.
The CT information is valuable in ascertaining anatomy and pathology within a given specimen, thereby allowing one to optimize its subsequent processing. Examples include: 1) One can ascertain the adequacy of removal (is the specimen worth processing?) 2) It permits detection of pathologies such as superior canal dehiscence or tegmen defects which are best studied by sectioning in the vertical plane rather than the standard axial plane. 3) In cases with a cochlear implant, the CT shows its depth of insertion, thus determining whether to process the specimen in araldite (for full insertion) or in celloidin (for partial or no insertion). 4) Extent of tumor and similar pathology can be evaluated by CT scan. Tumors are difficult to infiltrate with celloidin and knowing the size enables one to optimize the time for decalcification and infiltrate in various concentrations of celloidin for longer times, thus decreasing the need for re-embedding. 5) Knowing that ossicles are missing due to artifact or disease alerts one to the need to use other landmarks for obtaining a proper cutting angle.
Another benefit of CT is the ability to perform radiologic and pathologic correlations on one and the same specimen, thereby deepening our understanding of how to interpret radiologic images in live patients. Examples include radiologic diagnosis of congenital abnormalities and anatomical variants. Such correlations also afford better understanding of pathology such as otosclerosis.
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548 Visualization of Cochlear Pericytes in the Capillaries of Spiral Ligament in Vivo

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Pericytes, mural cells located on microvessels, are considered to play an important role in the formation of the vasculature and the regulation of local blood flow in some

organs. Little is known about the physiological cochlear pericytes. In order to investigate the function of cochlear pericytes, we developed a method to visualize cochlear pericytes *in vivo* using diaminofluorescein-2 diacetate (DAF-2DA) and intravital fluorescence microscopy. This method can permit the study of the effect of vasoactive agents on pericytes under *in vivo* and normal physiological condition. The specificity of the labeling method was verified by the immunofluorescent labeling of desmin, one of the pericyte marker proteins. Superfused K^+ and Ca^{2+} to cochlear lateral wall resulted in localized constriction of capillaries by pericytes, while there was no obvious change in cochlear capillary diameters when administrated with adrenergic neurotransmitters such as noradrenaline. The results show our method could be a routine way to visualize cochlear pericytes and microvessels *in vivo*. Moreover, we demonstrate for the first time that cochlear pericytes have contractibility, which may be important for cochlear blood flow regulation *in vivo*.

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549 Characterisation of Cochlear Fibrocyte Cultures from Rats and Mice

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Five main fibrocyte types are distinguishable in the spiral ligament by their morphology, location and relative expression of specific proteins: types III and IV express caldesmon, type I also S-100, whilst types II and V both plus Na^+/K^+ATPase and the glutamate transporter, GLAST. Fibrocyte cultures could allow development of cellular transplantation therapy to ameliorate strial presbyacusis and have previously been grown from gerbils and mice. The aim was to characterise fibrocyte cultures from adult Sprague-Dawley rats, CD-1 and CBA/CaJ mice. Cochleae were excised in MEM-α with antibiotic-antimycotic. Lateral wall segments were placed in a drop of medium containing 10% foetal calf serum and 1% Insulin-Transferrin-G in collagen I coated wells, coverslipped and left overnight, after which the coverslip was removed. Adherent segments were incubated for 2-3 weeks, forming primary cultures, trypsinized and reseeded for secondary culturing. Secondary cultures grown for 2-3 weeks on coverslips were fixed in 4% formaldehyde and labelled for caldesmon, S-100, Na^+/K^+ATPase and GLAST for confocal microscopy or prepared for scanning electron microscopy. Primary cultures grown on Aclar plastic sheets were fixed in 2.5% glutaraldehyde and 1% osmium tetroxide, dehydrated and embedded in Spurr resin for transmission electron microscopy. Fibrocyte cultures grew best from rats and CD-1 mice. The majority of cells were broad and flat, but small cells with relatively extensive processes were also observed. All cells expressed all the proteins but smaller cells expressed Na^+/K^+ATPase more than broader flatter cells, and less caldesmon. S-100 was strongly present, and GLAST only weakly. The cells had normal organelles, including mitochondria that in adult CD-1 mice have been found to be damaged. We conclude that culture cells express proteins characteristic of fibrocytes in situ but they do not exhibit clear characteristics of a particular fibrocyte type.

550 Development and Application of New Sample Preparation Methods for Quantitative Proteomic Analyses of Human Temporal Bones

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Objectives: 1) develop and optimize sample preparation workflow from formalin-fixed mouse liver to extract, measure and identify peptide sequences and complementary proteins for quantitative proteomics applications, and 2) apply these methods to extract and analyze proteins from archival human temporal bones.

Study Design: Two variables and their effects on yield and identity of proteins isolated from formalin-fixed samples were determined; 1) extraction buffers – QProteome (QP – Qiagen, Inc) and citraconic anhydride (CA – Pierce Inc.), and 2) heat. Mouse liver samples were fixed in 10% buffered formalin. GFP was added to the samples and served as an exogenous standard. Proteins were identified and semi-quantitated using Mass Spectrometry and liquid chromatography.

Methods: Samples were homogenized in either QP or CA buffer and GFP added. Total protein was extracted and quantitated. Each sample was divided in half and subjected to either heat or placed on ice. Extracted proteins were subjected to an overnight trypsin-digestion at 37ºC. Analyses were performed using a Q-Trap 4000 (ABI) coupled to a 2D nanoLC (Eksigent, Inc.). The resulting data were analyzed using the Analyst software and bioinformatics performed using MASCOT database.

Results: Fixed samples treated with CA/heat resulted in more identifiable peptide sequences (97) and probable protein matches (345) compared to fixed samples treated with QP/heat (32 peptide sequences and 185 probable protein matches). Fixed samples treated with CA/ no heat produced 68 peptide sequences with 295 protein matches compared to fixed samples treated with QP/ no heat (32 peptide sequences and 198 probable protein matches).

Conclusions: Samples treated with CA and heat result in more peptide (97) and potential protein matches (345) than samples treated with QP and heat.

Key Words: proteomics, LC-MS/MS, proteins, celloidin-embedded human temporal bones.

551 Mitochondrial Variants/haplogroups Modulate the Phenotypic Manifestation of Deafness-Associated 12S RRNA A1555G Mutation

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The mitochondrial 12S rRNA A1555G mutation has been found to be responsible for both aminoglycoside-induced and nonsyndromic hearing loss in many families worldwide. Here, we reported the clinical, genetic and molecular characterization of 69 Chinese families carrying the A1555G mutation. These Chinese families exhibit a wide range of penetrance and expressivities of hearing.

The average penetrances of hearing loss in 69 Chinese pedigrees were 29.4% and 17.6%, respectively, when aminoglycoside-induced deafness was included or exclude. Furthermore, the average age-of-onset for hearing loss in the absence of aminoglycoside exposure ranged from 5 and 30 years old, with the average of 14.5 years old in these 69 Chinese families. The sequence analysis of the complete mitochondrial genomes in these families identified the identical homoplasmic A1555G mutation and distinct sets of mtDNA polymorphisms belonging to eight different haplogroups. The frequencies of mtDNA haplogroups B, D, F, G, H, M, N and Y in hearing-impaired families were 15.9%, 40.6%, 18.8%, 1.4%, 7.2%, 5.8%, 8.7% and 1.4%, respectively. In particular, the frequency of haplogroup D in these hearing-impaired families was much higher than in Chinese controls, while the haplogroup B modulates the phenotypic manifestation of the A1555G mutation. In addition, we showed that secondary mtDNA mutations: tRNACys T5802C and G5821A , ND5 T12338C, tRNAGlu A14693G, CO1/tRNASer(UCN) G7444A, tRNAThr G15927A and tRNASer(AGY) C12224C variants may increase the penetrance of hearing loss in these Chinese families.

552 A Histological and Radiological Study of Scala Communis

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Scala communis (interscalar septum defect, under-segmentation defect of cochlea) is a developmental abnormality of the cochlea characterized by a dehiscence in the bony partition separating the turns of the cochlea. Scala communis is the commonest cochlear abnormality observed in radiologic and histopathologic studies of the temporal bone. The goal of the present study is to review this anomaly and describe its characteristics using the collection of temporal bones at the Massachusetts Eye and Ear Infirmary (MEEI) and using CT scans done on live patients in the Department of Radiology, also at MEEI.

Preliminary histopathological and radiological analyses indicate that scala communis can occur in cochleae of normal length or in shortened cochleae (in which case it is part of a broader inner ear anomaly such as Mondini dysplasia). Scala communis occurs most often between the middle and apical turns of the cochlea. It is often accompanied by hypoplasia of the modiolus, a flattening of the interscalar ridge and an increase in the area of some scalae.

This study will explore radiologic and histologic correlations in detail with respect to the occurrence, frequency and diagnosis of scala communis using qualitative and quantitative data. Methods to improve the accuracy of radiologic diagnoses in live patients will be investigated.

We will also examine the embryological basis for the scala communis anomaly. Preliminary studies indicate that the anomaly cannot be explained solely on the basis of an epithelial arrest during embryogenesis. It appears to result from an abnormality of mesodermal tissues such as excessive resorption in the formation of the scalae.

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553 Jagged1 May Regulate the Boundary of Mammalian Prosensory Patch Through Notch 3

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Approximately 250 million people worldwide suffer from hearing impairment, and about half of these cases are presumed to have a genetic cause. Nowadays the effect of treatment depending on prosthetic devices such as hearing aid or cochlear implants is still limited. The sensory epithelia of balance and auditory organs contain mechanosensory hair cells(HCs). The common precursor is called „prosensory patches“, which can be initially identified in the otocyst in the mouse at E12.5.

Cells destined to become HCs express Notch ligands like Delta1 (Dl1) and Jagged2(Jag2).

Jag1flox/flox mice, gift from Thomas Gridley, were kept on the C57BL/6J genetic background. They were crossbred with Pax8cre+/- mice, genotyping was performed. ABR measurements were done and after sacrificing the mice, histology with H&E staining was performed.

The wildtype mice were larger than the conditional ko (cko) siblings. The cko mice were overactive and cycling around their axis. The hearing threshold of the cko mice was 10-20 dB higher than that of controls. In H&E stained slides, sacular and utricular macula and ampullae were present in control. In the slides of Jag1-cko, sacular macula can be found and there was no obvious malformation. However, utricular macula and ampullae were absent. This coincides with the previous studies.

554 Postnatal Development of the Organ of Corti in a Dominant-Negative Gjb2 Transgenic Mice

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Hereditary hearing loss is one of the most prevalent inherited human birth defects, affecting 1 in 2,000. A strikingly high proportion (50%) of congenital bilateral nonsyndromic sensorineural deafness cases have been linked to mutations in the *GJB2* coding for the connexin26. It has been hypothesized that gap junctions in the cochlea, especially connexin26, provide an intercellular passage by which K+ are transported to maintain high levels of the endocochlear potential essential for sensory hair cell excitation. We previously reported the generation of a mouse model carrying human connexin26 with R75W mutation (R75W+ mice). The present study attempted to evaluate postnatal development of the organ of Corti in the R75W+ mice. R75W+ mice have never shown ABR

waveforms throughout postnatal development, indicating the disturbance of auditory organ development. Histological observations at P5-14 were characterized by i) absence of tunnel of Corti, Nuel's space, or spaces surrounding the outer hair cells, ii) significantly small numbers of microtubules in inner pillar cells, iii) shortening of height of the organ of Corti, and vi) increase of the cross-sectional area of the cells of the organ of Corti. Thus, morphological observations confirmed that a dominant-negative *Gjb2* mutation showed incomplete development of the cochlear supporting cells. On the other hand, the development of the sensory hair cells, at least from P5 to P12, was not affected. The present study suggests that *Gjb2* is indispensable in the postnatal development of the organ of Corti and normal hearing.

555 Role of Connexin 32, 36, and 43 in Inner Ear Development and Function

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Mutations in genes encoding gap junctional proteins in the inner ear account for a great majority of genetic hearing loss. Gap junctions, comprised of connexin (Cx) proteins, are sites of intercellular coupling, involved in tissue growth and differentiation, cell signaling, and ion recycling. At least 20 Cx isotypes have been identified in mammals. Cxs 26, 30, 31, and 43 have been identified in the cochlea, and Cxs 26, 30, and 43 in the vestibular organ. The importance of Cxs in auditory function is well established by the implications of Cx 26 mutation as the leading cause of non-syndromic hereditary sensorineural hearing loss, although the precise pathophysiology remains unclear. However, the role of other potentially important Cxs in inner ear development and function remains largely unknown. The present study aims to gain further understanding into the role of Cxs 32, 36, and 43 in inner ear development and function by using respective Cx knockout and heterozygote mice. Wild type males of the C57 and CD1 strain ranging in age from 5 to 15 months served as controls for this study. Male C57 Cx 32-/- homozygote knockouts, CD1 Cx 36 -/+ heterozygotes, and CD1 Cx 43 -/+ heterozygotes served as the study populations. Cxs 32, 36, and 43 distribution pattern and expression level differ compared to Cx26 and 30. Normal hair cell development was observed in Cx32KO, Cx36 HT, and Cx43 HT mice by 5 months. From 11-15 months, greater hair cell loss was observed in comparison to age matched WT mice. Cx43 HT showed the greatest degree of hair cell loss with absent ABR by 15 months, followed by Cx32KO, then Cx36HT. Our study suggests that although Cxs 32, 36, and 43 does not effect early inner ear development, they are important for maintaining hair cell survival and inner ear function.

556 Connexin26 and Connexin30 Null Mice Display Dramatically Different Pattern and Time Course of Cell Death in the Cochlea

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A significant portion (20-50%) of inherited prelingual non-syndromic deafness in humans is caused by genetic mutations in connexin26 (Cx26) and Cx30. To investigate underlying molecular mechanisms, we compared the time course and pattern of cellular degeneration in the cochlea of Cx30 null and conditional Cx26 (cCx26) null mice.

To facilitate long-term observation of the cochlear morphology, we back crossed the Gjb6-/- mutation into the CBA/CaJ genetic background by >8 generations of back cross breeding. Two types of cCx26 null mice were obtained by cross breed two genetically-engineered mice in which Cre recombinase expression is directed by either foxg1 or pax2 respectively, with another genetically-modified mice in which the Gjb2 gene are flanked by the loxp sequence. These mouse models were validated by their non-syndromic deafness phenotype, and further confirmed by immunolabeling, Western blot and elelctrophysiological methods.

In the Cx30 null mice, the pattern of hair cell loss showed a base to apex, outer hair cell to inner hair cell gradient. In the cCx26 null mice, cellular degeneration started in the middle turn and gradually spread to the basal turn. Cell death in the cochlea of cCx26 null mice is more rapid and widespread. By P30, all types of cells in the organ of Corti of the middle turn of cCx26 null mice are degenerated. Cell death in the Cx30 null mice generally is restricted to the hair cells and takes a much longer time course. Correspondingly, 51% of spiral ganglion neurons (SGNs) in Cx30 null mice survived after 18 month of deafness. This is in sharp contrast to a >90% loss of SGNs at P30 in the middle cochlear turn of the cCx26 null mice. By P60, most SGNs in the middle and basal cochlear turns of the cCx26 null mice were lost.

Results obtained from Cx30 and cCx26 null mice demonstrated a dramatically different pattern and time course of cell degeneration in the cochlea, suggesting fundamentally different underlying mechanisms.

557 Endocochlear Potential Depends on Chloride Channels: Deafness in Bartter Syndrome IV

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Human Bartter syndrome IV is an autosomal recessive disorder characterized by congenital deafness and severe renal salt and fluid loss. It is caused by mutations in BSND, which encodes barttin, a β-subunit of ClC-K1 and ClCK2 chloride channels, corresponding to ClC-Ka and ClC-Kb in humans.

A conditional Bsnd knockout mouse ($Bsnd^{lox/lox}$) was generated by flanking exon 2 with loxP sites and the Sox10-Cre line (Matsuoka et al, 2005) was identified as deleting in the inner ear without causing a renal phenotype. $Bsnd^{lox/lox}$ Sox10::Cre mice had a normal lifespan, showed no collapse of Reissner's membrane and while hearing in Sox10::Cre controls was not affected. Vestibular effects were minor and the renal expression appeared to be normal.

Barttin and ClC-K were expressed in the basolateral membranes of strial marginal cells in the WT, while the staining for barttin was completely abolished in marginal cells of $Bsnd^{lox/lox}$ Sox10::Cre mice. Although retaining all cell types and intact tight junctions, the thickness of the stria was reduced early on and outer hair cells degenerated over a span of several months.

ABR hearing assessment showed a hearing loss of ~60dB that remained stable over time.

The normal endolymph volume in $Bsnd^{lox/lox}$ Sox10::Cre mice allowed the measurement of the potential and [K+]. While no difference in [K+] was found, the EP was drastically reduced (from 104.1±2.1mV (n=10) WT to 16.7±2.1mV (n=15), 20 - 30 days of age) in the conditional KO. Consequently DPOAEs at all frequencies were abolished in 3-4 week old KO mice when OHCs were still present and could be stained for prestin.

These results strongly suggest that the drop in EP, by reducing the transduction current, severely impairs the ability of OHCs to respond electromechanically to sound entailing a profound congenital hearing loss. $BsndBsnd^{-/-}$ mice thus demonstrate the function of Cl^- channels in generating the EP and reveal the mechanism leading to deafness in human Bartter syndrome IV.

558 Inner Ear Protein Networks and Biomarkers in a Mouse Model for Deafness in Usher Syndrome 1F and Nonsyndromic Deafness DFNB23

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Mouse mutants have served as excellent models to study the genetic basis of deafness in humans. This report identifies candidate biomarkers and protein networks associated with cochlear pathogenesis in the Ames waltzer (av) mouse, a model for deafness in Usher syndrome 1F (USH1F) and DFNB23. Cochlear proteins from normal and av mice at postnatal day 30, a time point were cochlear pathology is well established in this model, were compared. The soluble proteins extracted from the cochlea were analyzed by 2D-DIGE followed by mass spectrometry. The analytic gel resolved ~ 2300 protein spots and over 20 protein spots showed significant changes in expression levels. In all, 43 proteins were found to be up-regulated and 26 down-regulated in the cochlea of av mice compared to the control. Cochlin protein was identified in 20 peptide spots, some of which were up-regulated while others were down-regulated. Significant differential expression of cochlin in the av model makes it an interesting candidate biomarker for USH1F and DFNB23. Network analysis performed with Metacore software yielded 7 relevant and statistically significant candidate protein networks predicted to be active in the affected cochlea. Embedded in these networks are factors known to be associated with apoptosis and degeneration of hair cells and spiral ganglion cells, including c-JUN, p53, and caspase-3. Focused analysis confirmed increased caspase-3 enzyme activity and upregulation of p53 mRNA in av cochlea compared to controls. Identification of protein markers in the apoptosis pathway shows proof of principle that this approach could be used to identify protein networks associated with ear pathology in the av model.

559 The Expression of SLC4A11 in the Murine Inner Ear as the Gene Responsible for Harboyan Syndrome

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The solute carrier4 (SLC4) family is known to encode bicarbonate transporters, which play important roles in the absorption or secretion of H^+ or HCO_3^- by several epithelia as well as the regulation of cell volume and intracellular pH. In the inner ear, active ion transportation and acid-base homeostasis are essential for cochlear and vestibular functions. Recently, the mutations of SLC4A11 gene have been identified in patients with Harboyan syndrome, which consists of congenital corneal endothelial dystrophy and progressive perceptive deafness. Likewise, a mutant SLC4A7 gene also causes visual and auditory impairments and is considered as one of the potential candidate genes for Usher syndrome.

Here we describe the expression of SLC4A11 in the murine inner ear and the comparative quantification in SLC4A7 and SLC4A11 between the inner ear and the other organs including the eyes and the kidney. We detected that SLC4A11 is expressed in the inner ear at the transcription and protein level. Its expression was widely distributed in various cells in the cochlea, including the stria vascularis, the organ of Corti, the spiral ligament and spiral ganglion cells, as well as sensory hair cell in the vestibule. At the transcription level, the expression of SLC4A11 in the inner ear was higher than other organs, whereas that of SLC4A7 was quantitatively similar to the other organs. These results suggest that the presence of SLC4A11is qualitatively and quantitatively indispensable for the inner ear.

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560 Merlin Knockdown in Human Schwann Cells as an in Vitro Model for Vestibular Schwannoma Tumorigenesis

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NF2 and sporadic VS are associated with loss of functional merlin (schwannomin) in the Schwann cell. Following loss of merlin expression, the initial steps toward VS tumorigenesis are unknown. Furthermore, the predilection for Schwann cell tumorigenesis along the vestibular nerve is not understood. Additionally, there is no ideal animal model for vestibular schwannoma tumorigenesis, since the conditional NF2-knockout mouse does not develop vestibular schwannomas.

Merlin, a putative tumor suppressor protein, interacts with many cellular proteins, regulating their function. Among these are receptor tyrosine kinases, including the ErbB family receptors, EGFR and ErbB2, possibly down-regulating their proliferation and survival signaling through the ERK1/2 and AKT pathways. Elucidation of the role of merlin and ErbB receptor tyrosine kinases in the pathogenesis of VS will be a critical step in developing pharmacologic agents to eradicate VS.

In order to elucidate the molecular progression towards VS tumorigenesis, we investigated an *in vitro* model of vestibular schwannoma tumorigenesis by siRNA knockdown of merlin. Confirmation of merlin expression knockdown was performed by real-time quantitative PCR and Western blotting for merlin. Microarray analysis was performed to compare expression profiles between normal human Schwann cells and those deficient in merlin expression, in order to identify the early effects of merlin deficiency on gene expression profiles. We report those findings.

Additionally, we, as well as others, have previously identified a potential role for certain receptor tyrosine kinases as potential therapeutic targets in VS, such as EGFR, ErbB2, and ErbB3. Therefore, we assessed changes in expression of the ErbB receptor family members, epidermal growth factor receptor (EGFR), ErbB2, and ErbB3 using real-time quantitative polymerase chain reaction (qPCR), confocal microscopy, and Western blotting techniques in our merlin-deficient VS tumorigenesis model. We found a robust time-dependent upregulation of EGFR, as well as a modest upregulation of ErbB2, as merlin protein became depleted in human Schwann cells after siRNA transfection. We propose that merlin deregulation of ErbB receptor signaling is a critical step towards vestibular schwannoma tumorigenesis.

561 Ultrastructural Changes That Precede Hearing Loss in Osteoprotegerin-Deficient Mouse - An Animal Model of Otosclerosis

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Osteoprotegrin (OPG), a soluble decoy receptor for receptor activator of nuclear kappa ligand, plays a critical role in preventing bone remodeling. We have previously reported that OPG is highly expressed in multiple cochlear cell types, and in perilymph. We have also shown that OPK knockout (ko) mice exhibit pathologic bone remodeling reminiscent of otosclerosis – one of the most common causes of acquired hearing loss in humans. Most recently, we have shown that hearing loss in OPG ko mice has a substantial sensorineural component, in addition to a mild, mid-frequency conductive component. The hearing loss is apparent at the earliest tested age (8 weeks) and progresses thereafter. To better understand cellular origins of sensorineural hearing loss in OPG ko mice, we studied ultrastructural alterations in OPG ko mice prior to the onset of hearing loss. Two μm sections of araldite-embedded cochleas of OPG ko mice and wild type (wt) controls at 1, 3, 5 and 8 weeks of age were analyzed. The earliest ultrastructural abnormalities were evident at 1 week of age, and included degeneration of interdental cells, root cells and fibrocytes of the spiral limbus. By 3 weeks of age, degenerative edema of strial interdental cells was noted. By 8 weeks of age, type I fibrocytes were decimated. The degenerating cell types in the OPG ko mouse include the same cell types that express OPG in the wt mouse, thus demonstrating that OPG is required for function and survival of these cells. Ultrastructural changes in cells of the cochlear membranous labyrinth preceded ultrastructural changes in the otic capsule. In summary, we have identified cochlear ultrastructural changes that precede gross histological changes and hearing loss in OPG ko mouse. Future therapies aimed at preventing such degenerative changes have a potential to treat presently untreatable sensorineural hearing loss that affects 20-30% of patients with otosclerosis.

562 Structural Differences in Mitochondria Among Hair Cells in the Belgian Waterslager and Non-Belgian Waterslager Canary Basilar Papillae

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The Belgian Waterslager (BWS) canary has a genetic basis for its high frequency hearing loss. At both surface and subsurface levels, examination of BWS canary basilar papillae (BP) demonstrates hair cell damage and loss as well as ganglion cell loss.

These observations raise the question as to how genetic mutations can cause changes among hair cells, leading to elevated auditory thresholds. In an attempt to resolve this question, we performed transmission electron microscope examinations of hair cells of both the BWS and non-BWS BP at 20%, 50%, and 80% distances from the basal tip of the BP.

Two sets of data on mitochondria among hair cells were obtained. One, from non-BWS BP, showed that most mitochondria had a clear matrix and well-defined cristae that ran parallel to the long axis of the mitochondria. These cristae exhibited typical variability in their number and packing. In contrast, in all the mitochondria of the BWS hair cells the matrix was much denser than that of the hair cells in non-BWS canaries, even when the hair cells showed little, if any, structural evidence of apoptosis. The mitochondrial matrix was so dense as to almost demolish its cristae, but the cristae themselves looked very similar to non-BWS ones. There were also mitochondria with obliterated and/or disorganized cristae (several of which contained swollen inner membranes), and pronounced intracristal inclusions that ran almost the entire length of one or two cristae in the mid-region of the affected mitochondria.

The altered mitochondrial structures among the hair cells of the BWS BP suggest a possible mitochondrial role in BWS hearing loss. It remains to be seen as to whether these changes are a result of mitochondrial DNA mutations or a secondary effect of events that cause structural damage along the sensory epithelium of the BWS canary.

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563 Characterization of the Brainbow Transgenic Labeling System in the Mouse Cochlea

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The development of new technology which is capable of specifically labeling cells. The Brainbow mouse system has shown that labeling individual neurons can be performed on a large scale in vivo using Cre/LoxP recombination (Livet et al., Nature (2007) 450: 56-62). The Brainbow transgenic mouse system utilizes a CMV/thy1 promoter scheme. The Thy1 promoter is known for its activity primarily in neurons; however, it is unknown whether this Brainbow transgene cassette is expressed in the cochlea. Here, we characterized the three available Brainbow transgenic mouse lines (BB1.0H, BB1.0L, and BB1.1) for their specific expression in the cochlea. In the postnatal cochlea, Atoh1 expression is restricted to hair cells and Prox1 expression is only localized to supporting cells; thus, we crossed the Brainbow mice with both Atoh1-CreER and Prox1-CreER lines. We found that the Brainbow gene is detected in a small number of spiral ganglion cells, but not in supporting cells or hair cells. In the future, it will be beneficial to develop a ubiquitous Brainbow transgenic system or a cell-type specific Brainbow transgenic system for gene manipulation in mouse cochleae.

Atoh1-CreER and Prox1-CreER mice were gifts from Drs. S. Baker and G. Oliver, respectively. This work is supported by the ALSAC and NIH grants DC006471, DC008800, and CA21765.

564 Novel POU3F4 Mutation Patients with Cochlear Implant: Molecular Analysis and Clinical Features

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DFN3, an X-linked deafness type 3, is the most common type of X-linked hearing loss in humans and result from mutations in the POU3F4 gene. In the present study, molecular analysis was executed in two X-linked SNHL families including 3 affected males and their intraoperative findings, surgical complications, and postoperative speech performances after cochlear implantation were noted. And medical, surgical, and genetic considerations for patients with POU3F4 mutation will also be discussed. Two different novel POU3F4 mutations, 383delG and L208X, were identified in these families, respectively. Three children with an X-linked anomaly in temporal bone CT had severe hearing loss about 90 dB of average of PTA and over 90 dB in ABR and underwent CI. The speech performance after CI showed good results in all patients. The clinicians should consider the uneventful surgical problems in deaf patients with POU3F4 mutations during the cochlear implantation and also provide the genetic counseling for the patients with X-linked deafness about the cause of hearing loss, medical consideration, and chance of recurrence, rehabilitation, and follow-up with assistance and recognition of the emotional aspect of the associated family.

565 SLC26A4 Mutations Are Common Causes of Non-Syndromic Hearing Loss with Enlargement of the Vestibular Aqueduct (EVA) in Chinese Population

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The enlarged vestibular aqueduct (EVA) is the most common type of inner ear malformation. Although many diseases have been reported to be associated with EVA, non-syndromic hereditary deafness DFNB4 and Pendred syndrome (PS), a syndromic form of sensorineural hearing loss (SNHL), account for the majority of the cases. PS is differentiated from non-syndromic HL with EVA by the presence of goiter, which is usually not evident until the second decade of life. Recessive mutations of *SLC26A4*,

which encodes the anion transporter pendrin, are the cause of both classical PS and deafness associated with EVA. We performed *SLC26A4* sequence analysis of 32 Chinese patients (3 from multiplex and 29 from simplex families) with bilateral moderate-severe to profound SNHL and EVA as well as 100 race matched normal controls. None of the patients presented with goiter. Overall, 100% of subjects were found having at least one possible pathogenic variant in *SLC26A4*. Thirteen different mutations distributed along the *SLC26A4* gene were identified, five of which are novel. A total of 87.5 % (28/32) of the patients harbored biallelic mutations; eleven of them were homozygotes, seventeen were compound heterozygotes. One case had complex allele with three variants. Four patients were found to carry a single *SLC26A4* mutation. The IVS7-2A>G and p.H723R were the two most frequent mutations, accounting for 59.0% and 14.8 % of the mutant alleles, respectively. Our results further confirm that mutations in the *SLC26A4* gene are common causes of deafness associated with EVA in Chinese population**.**

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566 Clinical and Molecular Genetic Analysis of a Family with Macrothrombocytopenia (MTCP) and Early Onset Sensorineural Hearing Loss (SNHL)

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A kindred with inherited macrothrombocytopenia (MTCP) and sensorineural hearing loss (SNHL) from Ghent, Belgium was identified. Currently, mutations within MYH9 alone have been linked to joint expression of MTCP and hearing loss. Thus, we tested the hypothesis that a mutation in MYH9 is responsible for the autosomal dominant inheritance of MTCP and hearing loss in the Ghent family. A mutation screen of MYH9 coding region including its intron-exon junctions, as well as common hearing loss genes GJB2, GJB3, and GJB6, was performed. However, no pathogenic sequence alteration was identified. Patients' neutrophils were free of Döhle-like inclusions that have been linked with MYH9-related disease, further corroborating the absence of a subtle but pathogenic MYH9 mutation. In addition, western blot analysis with anti-NMHC-IIA antibody identified a single immunoreactive band, expected mw of 220 kDa, and demonstrated a normal expression level of NMHC-IIA within the platelets and leukocytes of the affected family members. The immunoblot analysis eliminates the possibility of a large deletion within MYH9 that can escape detection by direct sequencing. Collectively, these results suggest that molecular-genetic etiology of the Ghent family disorder may be due to as yet unidentified gene whose mutation(s) yields a phenocopy of the MYH9-related disease.

567 Digenic Inheritance of Non-Syndromic Deafness Caused by Mutations at the Gap Junction Proteins Cx26 and Cx31

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Mutations in the genes coding for connexin 26 (Cx26) and connexin 31 (Cx31) cause non-syndromic deafness. Here, we provide evidence that mutations at these two connexin genes can interact to cause hearing loss in digenic heterozygotes in humans. We have screened 108 *GJB2* heterozygous Chinese patients for mutations in *GJB3* by sequencing. We have excluded the possibility that mutations in exon 1 of *GJB2* and the deletion of *GJB6* are the second mutant allele in these Chinese heterozygous probands. Two different *GJB3* mutations (N166S and A194T) occurring in compound heterozygosity with the 235delC and 299delAT of *GJB2* were identified in three unrelated families (235delC/N166S, 235delC/A194T and 299delAT/A194T). Neither of these mutations in *Cx31* was detected in DNA from 200 unrelated Chinese controls. Direct physical interaction of Cx26 with Cx31 is supported by data showing that Cx26 and Cx31 have overlapping expression patterns in the cochlea. In addition, by coimmunoprecipitation of mouse cochlear membrane proteins, we identified the presence of heteromeric Cx26/Cx31 connexons. Furthermore, by cotransfection of mCherry-tagged Cx26 and GFP-tagged Cx31 in human embryonic kidney-293 cells, we demonstrated that the two connexins were able to co-assemble *in vitro* in the same junction plaque. Together, our data indicated that a genetic interaction between these two connexin genes can lead to hearing loss.

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568 Pathogenesis in the Cochlea of Three Types of Conditional Connexin26 Null Mice Suggests That Connexin26 Plays Essential Roles in the Postnatal Development of the Organ of Corti

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Gap junctions (GJs) are intercellular channels extensively expressed in the cochlea. Mutations in the gene (Gjb2) coding for connexin26 (Cx26), which is a subunit constituting in cochlear GJs, cause a substantial portion of human non-syndromic hereditary deafness cases. To investigate molecular mechanisms of deafness caused by Cx26 mutations, we generated three types of conditional Cx26 (cCx26) null mice to circumvent the embryonic

lethality in Gjb2-/- mice. Targeted elimination of Gjb2 in either a spatially-specific or time-specific manner was achieved by foxg1- or pax2-directed, or by a tamoxifen-induced system for the expression of the Cre recombinase. These cCx26 null mouse models were validated by a multidisciplinary approach and confirmed by animals' profound non-syndromic deafness.
The three types of cCx26 null mice all showed normal gross histological features of the cochlea. Cell differentiation in the cochlea appeared to be completed at birth. However, the postnatal development of the organ of Corti was stalled as the tunnel of Corti and the Nuel space were never opened in cCx26 null mice. Starting at around P14, epithelial cells in the organ of Corti started to degenerate and all types of cells in the middle turn were completely lost by P30. The cell death gradually spread to the basal turn, while cells in the apical turn were largely spared. Correspondingly, spiral ganglion neurons in the middle and basal turns were substantially degenerated. Using an inducible Cx26 null mouse model, we observed that the deafness phenotype was readily induced if the Cx26 expression in the organ of Corti was substantially reduced before P4. Reducing Cx26 expression after P4, however, generated a phenotype more consistent with symptoms of early-onset age-dependent hearing loss. These results indicated that Cx26 plays essential roles in the postnatal maturation of the organ of Corti and is required for the survival of all types of cells resided on the basilar membrane.

569 Mutation of Matn1, But Not of Matn3, Causes a Mild Hearing Loss in Mice

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Matn1 and Matn3 belong to a gene family encoding matrilin proteins, a four-member family of oligomeric extracellular matrix proteins are thought to connect matrix components in cartilage to form macromolecular networks. Matrilin-1 and matrilin-3 are abundant in cartilage, whereas matrilin-2 and matrilin-4 show a wider tissue distribution. Matrilin-1 interacts with type-II collagen and aggrecan. Matrillin-1,3,4 associate with type-VI collagen and connect these networks to type-II collagen and aggrecan. Collagens are known to be important in inner ear function (eg Suzuki et al 2005; Meyer zum Gottesberge et al, 2008), and both aggrecan and type-II collagen have been found in the tectorial membrane (eg Thalmann et al 1993, Goodyear & Richardson 2002). We asked if matrilin-1 and -3 are required for normal cochlear function by analysing mice with each gene inactivated.
Wildtype and mutant Matn1 and Matn3 mice at 13 weeks old were anaesthetised using Ketamine and Xylazine and placed in front of a loudspeaker at a distance of 20cm. Subcutaneous needle electrodes were inserted to allow recording of the auditory brainstem response (ABR) to presentation of clicks and 6, 12, 18, 24 and 30kHz tone pips at levels from 10-85dB SPL. ABR thresholds recorded in homozygous Matn3 mutants were not significantly different from wildtype controls (ANOVA; $p>0.05$). However, homozygous Matn1 mutants showed ABR thresholds that were on average 5-9dB higher than wildtype controls. These elevations represent a mild but significant deterioration in hearing sensitivity (ANOVA; $p<0.05$). These data suggest that Matrilin-1 may play a role in hearing. Deficits in Matn1 expression may impair cochlear function and further investigations are underway.

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570 Explore Channel Properties of Deafness Gene THMS

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The gene of TMHS, also known as LHFPL5, has recently been identified to be involved in deafness as a missense mutation in hurry-scurry mice (Longo-Guess et al., 2005, PNAS, 102:7894-7899). Mutations of TMHS have also been found to cause autosomal recessive nonsyndromic hearing loss, DNFB67, in human (Kalay et al., 2006, Hum Mutat, 27:633-639; Shabbir et al., 2006, J Med Genet., 43:634-640). However, the TMHS function and pathological mechanisms underlying its mutations induced hearing loss remain unclear. The TMHS gene encodes a tetraspan transmembrane protein that is expressed at hair cell stereocilia. Like most channel proteins, the TMHS protein locates at the plasma membrane and possesses four transmembrane domains, two extracellular loops, one intracellular loop, and intracellular N- and C-termini. In this study, we investigated whether TMHS can form channels to function by patch clamp recording. The TMHS and four deafness mutants were trasnfected into HEK 293 cells and recorded under whole-cell configuration. We found that there was no significant evoked current as membrane voltage was stepped from -140 mV to 40 mV in the TMHS transfected cells. Application of ATP, a known cochlear function mediator existed in the endolymph and perilymph, could not evoke significant current. Alternation of membrane tension also could not evoke apparent current. There was no significant difference in input resistance between WT and the deafness mutants in patch clamp recording. Thus, TMHS may not form voltage-dependent channels at the plasma membrane to function. The data further support the conception that the TMHS may interact with other proteins at the stereocilia to form networks underlying the cohesion of the hair bundles.
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571 Characterization of *Tmie*, the Gene Affected in the Mouse Deafness Mutant Spinner and in Human DFNB6

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Mutations in the mouse and human *Tmie* ("transmembrane inner ear") genes result in congenital hearing loss, with mouse mutants also exhibiting associated vestibular dysfunction (Mitchem et al., Hum. Mol. Genet. 11:1887-1898, 2002; Naz et al., Amer. J. Hum. Genet. 71:632-636, 2002; Cho et al., Comp. Med. 56:476-81, 2006). Knockdown of zebrafish *Tmie* similarly results in inner ear and lateral line defects (Shen et al., Develop. Dynamics 237:941-52, 2008). *Tmie* genes encode a small, novel protein with a predicted signal peptide and at least one transmembrane domain. Localization using an anti-TMIE peptide antibody indicates immunoreactivity in stereocilia of sensory hair cells in the mouse cochlea, with an apparent base-to-apex gradient in relative expression levels. Together with the stereocilia pathology in affected spinner mice carrying *Tmie* null mutations, this localization is consistent with a local effect of TMIE protein on the normal maturation of stereocilia. Expression studies in cultured cells indicate that TMIE is associated with the plasma membrane and the cortical actin filament network, including actin filament-rich projections on the apical/dorsal surface. The C-terminus of TMIE is rich in charged amino acids and includes two clusters of lysine residues, similar to other transmembrane proteins implicated in anchoring the actin cytoskeleton to the membrane. We have engineered missense substitutions associated with nonsyndromic hearing loss in humans (*DFNB6*) into mouse *Tmie* constructs and evaluated localization of the encoded proteins after transfection into cultured cells. Each of the missense mutants appears to localize like wild type TMIE, suggesting that the mutants are defective in some other aspect of protein function.

572 Background Strain Influences the Hearing Loss Associated with Caveolin-1 Knockout Mouse

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Caveolin is an integral membrane protein involved in the formation of lipid microdomains. Functional roles for caveolins are still being elucidated, but they have been implicated in aberrant cell signaling, altered lipid metabolism, and changes in ion channel/synaptic function. Previously, we reported the expression of all three members of the caveolin gene family in chick and mouse cochlea (Chen et al., ARO, 2007; Duncan et al., ARO, 2008). We also showed that a genetic knockout for caveolin-1 (*Cav1* KO) exhibited higher ABR thresholds compared with age-matched control mice as early as 3 weeks of age. Shifts in DPOAE sensitivity seemed to account for the hearing loss, suggesting a deficit in OHCs and the cochlear amplifier. Genetic backgrounds of knockout and control lines available from Jackson Laboratories differ slightly, however. Both lines includ contributions from several inbred lines including C57BL/6, which exhibits age-related hearing loss (AHL) by 10 months of age. Control and *Cav1* KO mice were genotyped for SNP variants of *Cdh23* that are associated with susceptibility to hearing loss at the *Ahl* locus on chromosome 10. All *Cav1* KO mice were homozygous for the AHL-sensitive *Cdh23* allele, while the majority of control mice carried both sensitive and resistant alleles. To determine whether hearing loss could be definitively associated with loss of caveolin-1, we crossed *Cav1* KO mice with CBA/CaJ, a strain carrying AHL-resistant alleles of Cdh23. F2 mice carrying the AHL-resistant allele exhibited normal hearing comparable to wild-type CBA/CaJ, regardless of *Cav1* genotype. These data suggest that the hearing loss in *Cav1* KO mice is sensitive to genetic background. Further experiments are required to determine the nature of these background effects on loss of caveolin-1. (Supported by P30 DC0578188).

573 CD44 Expression in the Mouse Inner Ear

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The cluster of differentiation proteins (CD proteins) is a large and diverse group of cell surface proteins originally characterized in human leukocytes. We have previously used RNA extracted from early postnatal cochlear and vestibular sensory epithelia to compare the transcriptomes of these two closely related systems using Affymetrix microarrays. Our analysis focused on the expression of CD proteins due to their possible role in autoimmune inner ear disease, as well as their potential use as endogenous markers for cell sorting of the mouse inner ear sensory epithelia. While 106 CD proteins were detected as expressed in the mouse inner ear, only three were detected as preferentially expressed in the mouse cochlea (defined as a two fold or greater ratio). These three genes included *CD333* (*Fgfr3*) and *CD339* (*Jag1*), both previously characterized in the mouse inner ear, as well as *CD44*.

CD44 is an integral cell membrane glycoprotein with a diverse range of suggested functions. The principal ligand of CD44 is hyaluronic acid, an extracellular matrix protein. Other identified ligands include laminin, fibronectin, collagens, serglycin and osteoponin. *CD44* KO mice are viable and do not suffer from obvious developmental

defects but do exhibit specific alterations in their lymphocyte-dependent immune responses. Interestingly, *CD44* is localized to the linkage interval of autosomal recessive deafness locus DFNB51. We present a characterization of the spatiotemportal expression pattern of CD44 in the mouse inner ear, auditory characterization of the CD44 knockout mice and a mutation analysis of *CD44* in the DFNB51 families.

574 Laser Microdissection of Cytochrome C Oxidase Immunostained Archival Human Temporal Bone Tissue: A Technique for Studying the Distribution of Mitochondrial DNA Deletions in the Cochlea

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Somatic mitochondrial DNA (mtDNA) deletions have been associated with the process of aging in many post-mitotic tissues. While the available evidence suggests that mtDNA deletions are associated with presbycusis, their role in the development of this disease process remains unknown. In our laboratory, we have developed techniques to perform laser microdissection (LMD) on unstained archival human temporal bone tissue. Using this approach, structures within the cochlea have been isolated for analysis with real time polymerase chain reaction (RT-PCR) assays for mtDNA deletions. In this presentation we describe the use of a cytochrome c oxidase immunostaining technique to identify areas within the cochlea with mitochondrial enzyme deficiency for LMD and subsequent RT-PCR analysis. This approach has been designed to characterize and quantify the distribution of mtDNA deletions in the cochlea of individuals with presbycusis and correlate these findings with the observed pattern of hearing loss.

575 Application of Stepwise SNaPshot Multiplex Assays for Simultaneous Multi-Gene Mutation Screening in Patients with Idiopathic Sensorineural Hearing Impairment

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Genetic heterogeneity of idiopathic non-syndromic sensorineural hearing impairment precludes an efficient molecular diagnosis when patients are being approached. Several high through-put strategies, including the microarray technology and the Invader assay, have been developed to tackle the clinical difficulty by screening multiple genetic mutations simultaneously. Since different populations revealed different mutation spectrums in common deafness genes, each population might have its most suitable strategy for rapid genetic examination. Herein, we reported our initial experience in applying the SNaPshot multiplex assay to screen deafness mutations in a prospective cohort composed of 106 unrelated Taiwanese patients with idiopathic sensorineural hearing impairment. Based on our previous epidemiologic study, 20 most prevalent mutations in GJB2, SLC26A4 and the mitochondrial 12S rRNA gene were selected, and examined in two runs of SNaPshot multiplex assays with each run screening 10 common mutations. Theoretically, the design could detect > 98% mutated alleles of the known deafness mutations in Taiwanese. The results of the SNaPshot multiplex assays were then validated using direct sequencing of the corresponding exons by an independent investigator. Mutations in GJB2, SLC26A4 and the mitochondrial 12S rRNA gene were identified in 31 (29%), 12 (11%) and 9 (8%) patients, respectively. In total, the SNaPshot multiplex assays yielded an accuracy of > 99%. In addition to high efficacy and accuracy, the strengths of the SNaPshot multiplex assay include an extremely low cost (<10 US dollars for 2 runs of assays), flexibility (the examination panel can be easily expanded for additional mutations), and easy applicability for any institute with a DNA sequencer. Although only 20-30 mutations can be examined in 2-3 runs of the SNaPshot assays, the technology might still be suitable for screening deafness mutation in populations with a relatively homogeneous ethnic background.

576 Genetic Association Analysis of Familial Sensorineural Hearing Loss Resembling a DFNA2 Phenotype

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An extended family with inherited non-syndromic sensorineural hearing loss (NSHL) identified in our clinic underwent phenotypic and genotypic analyses. The pedigree of 118 family members consists of 35 nuclear families, with 16 living affected family members. NSHL is present in every generation and is transmitted by both genders, consistent with autosomal dominant inheritance. Hearing losses for all affected persons are similar in severity and configuration, with bilateral moderate-to-severe hearing loss and a severe-to-profound dip at 1000 Hz. In general, the hearing loss configurations of affected family members resemble those from one Dutch family with a known mutation in the KCNQ4 gene (locus DFNA2, chromosome 1p34), although the progression and severity of the hearing losses differ. DNA samples were obtained for 43 family members, and preliminary screening of GJB2 and GJB6 mutations were negative. Genomic DNA samples from 9 affected and 15 unaffected family members underwent genome-wide single nucleotide polymorphism (SNP) analysis using the Affymetrix

Genome-wide 6.0 array. Initial genetic association analysis of 23 SNPs across the KCNQ4 gene locus revealed no significant association with any of the variants, suggesting that another locus is responsible for NSHL in this family. Genome-wide high-density mapping and linkage analysis will be used to identify loci and genes responsible for this unique phenotype.
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577 Glutathione S-Transferase (GSTM1 and GSTT1), N-Acetyltransferase 2 (NAT2) Genes Polymorphisms and Presbycusis

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Hearing loss (HL) due to the aging process, referred to as presbycusis or age-related hearing loss (ARHL) is the most common sensory impairment in the elderly, affecting 50% of 80 year old people. ARHL is a complex disorder, highly heterogeneous and is a result of both genetic and environmental influences. We aimed to investigate whether genetic variation in the human protective antioxidant system is associated with susceptibility to ARHL. We analyzed the deletion polymorphisms in the GSTT1 and GSTM1 genes and polymorphisms at *NAT2**5A, *NAT2**6A/B, *NAT2**7A/B, and *NAT2**14A/B in 55 Caucasian adults with presbycusis (age 40 to 75 years) and 79 race-age-matched controls. Genotypes were determined using polymerase chain reaction (PCR)-based methods and direct sequencing analysis. We detected a significant difference in frequencies of the *GSTT1* deletion between subjects with ARHL (60%, 33/55) and the controls (27.8%, 22/79) (P=0.0003). No significant difference for *GSTM1* deletion, polymorphisms at *NAT2**5A, *NAT2**6A/B, *NAT2**7A /B, and *NAT2**14A/B could be found between the ARHL and control groups (P>0.05). Our results indicate that an increased risk to develop ARHI existed for subjects homozygous for *GSTT1* deletion. However, the sample size was relatively small, and further studies on a larger number of individuals and in other ethnic populations are required to determine whether susceptibility to ARHL is associated with polymorphisms in genes that are involved in the oxidative stress response.
The work is supported by NIH DC 05575

578 The Role of L-VDCC for Activity-Dependent BDNF Transcription: A Cell Culture Model

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Brain-derived neurotrophic factor (BDNF) plays a crucial role for activity-dependent plasticity, alteration of synaptic efficacy and balance of inhibition and excitation (Korte et al., 1995). A role of Calcium in BDNF mediated LTP and LTD responses are discussed in various studies (Tippens et al., 2008, Arundine et al., 2003, Tao et al., 2002, West et al., 2001; Ghosh et al., 1994). Recent studies point to a crucial role of BDNF/L-type calcium channels involvement for neuronal injury, as phantom pain (Shulga et al., 2008). Altered BDNF levels in the periphery of the cochlea post trauma (Rüttiger et al., 2007, Panford-Walsh et al, 2008), may be the trigger for pathological imbalances of neuronal activity in the central auditory system. Interested in the role of L-type calcium channels for BDNF-mediated changes in the auditory system post trauma, we generated a cell culture system in which we transfected YFP- and CFP-tagged BDNF transcripts (exon IV and exon VI), including their appropriate promoter regions. BDNF expression can be induced upon Pilocarpine and Kainate, indicating an endogenous appropriate signalling cascade that may mimic glutamatergic and cholinergic usage of BDNF promoter. Costransfection studies with Cav1.2/Cav1.3 plasmids, Ca2+ dependent trans-acting elements and siRNA studies will elucidate the usage of this in vitro cell system for analyse Ca2+ dependent signalling cascade upstream of the BDNF promoter.
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579 Interaction Between Sod1 and Cdh23ahl Genes in Age-Related Hair Cell Loss

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Cu/Zn Sod1, an antioxidant enzyme that eliminates the highly toxic superoxide anion, is abundantly expressed in the inner ear. Previously, we reported that deletion of the Sod1 gene accelerated age-related hair cell loss and hearing loss in Sod1-/- mice that were developed on a 129/CD-1 background. The effects of the Sod1 gene deletion in these mice, however, were confounded by the presence of an age-related hearing loss variant (ahl) that is thought to be a mutation in the cadherin 23 gene (Cdh23). To evaluate the effects of Sod1-/- and Cdh23ahl/ahl alone or in combination, we evaluated cochlear pathologies in 4 new mouse strains with the following genotypes: Sod1+/+ Cdh23+/+, Sod1+/+ Cdh23ahl/ahl, Sod1-/- Cdh23+/+ and Sod1-/- Cdh23ahl/ahl. As expected, OHC and IHC lesions were most severe in Sod1-/- Cdh23ahl/ahl mice followed closely by Sod1+/+ Cdh23ahl/ahl mice. The OHC and IHC lesions in these 2 strains were prominent at 6 months of age and spread from base to apex with advancing age. Despite the massive loss of cochlear hair cells, the spiral ganglion neurons and nerve fibers in the habenula perforata appeared reasonably normal at 15 months of age. In contrast, OHC lesions were much smaller in Sod1-/- Cdh23+/+ and Sod1+/+ Cdh23+/+ mice, developed later in life and tended to be larger in the apex than the base. IHC loss was negligible in Sod1-/- Cdh23+/+ mice; however, Sod1+/+ Cdh23+/+ mice unexpectedly developed moderate IHC lesions in the middle of the cochlea at 12-15 months of age. Despite extensive damage to cochlear hair cells, vestibular hair cells appeared remarkably normal in all strains, even those with Sod1 and Cdh23 deficiencies.

These results suggest that the ahl mutation in the Cdh23 gene affects the hair cells in the cochlea, but not the vestibular system. Supported in part by NIH grant R01 DC005827-05 to KJ.

580 Effect of Chronic Salicylate Treatment on Age-Related Cochlear Degeneration

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Salicylate (aspirin) is a widely used drug in clinics. Acute application of salicylate may cause reversible hearing loss, reduction of distortion product otoacoustic emission (DPOAE), and loss of outer hair cell (OHC) electromotility. A long-term application may cause tinnitus. Interestingly, it has been reported that a long-term salicylate application up-regulated expression of prestin, the OHC motor protein, consequently leading to an increase of OHC electromotility and DPOAE. Salicylate has also been shown to have protective effect on ototoxicity induced by noise, cisplatin and gentamicin. In the current study, aging Fischer 344 rats (18 months old) were treated with sodium salicylate at a dose of 100 mg/kg for 2 times per day for 5 days per week for 3 weeks. DPOAE and auditory brainstem response (ABR) were recorded and compared before and after the treatment. The OHC-related cochlear functions including cochlear microphonics (CM) and cochear amplification were also determined. Finally, prestin levels in OHCs were examined immunohistochemically. It appeared that the treatment delayed some aging processes.
This study was supported by NIOSH grant 1R01OH008113-01A1

581 Glycine Receptor Subunit Changes in DCN of Rats with Behavioral Evidence of Presbycusis

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Presbycusis, age-related hearing loss, can be considered a consequence of progressive peripheral auditory deafferentation. Previous studies suggest that temporal processing deficits observed in presbycusis may partially result from functional loss of the inhibitory glycinergic neurotransmission in dorsal cochlear nucleus (DCN). The present study assessed age-related behavioral gap detection and neurochemistry changes of postsynaptic glycine receptor (GlyR) subunits and their anchoring protein gephyrin over fusiform cells of young (7-8 month) and aged (28-29 month) Fischer Brown Norway (FBN) rats. Aged rats showed significantly (20-30dB) higher ABR thresholds across all tested frequencies and longer gap detection thresholds compared to young FBN rats. *In situ* hybridization and quantitative immunocytochemistry were used to measure GlyR subunit message and protein levels. There was a significant age-related increase in $GlyR\alpha_1$ subunit message but significant decreases in protein levels. $GlyR\alpha_2$ showed significant age-related decreases in both message and protein levels. Gephyrin message and protein were significantly increased in aged DCN fusiform cells. The pharmacologic consequences of these age-related subunit changes were assessed using [^{3}H] strychnine binding. In support of the age-related decrease of α_1 subunit protein, there was a significant age-related decrease in the number of GlyR binding sites with no significant change in affinity.
Previous studies suggest that gephyrin may act as a retrograde GlyR intracellular transporter contributing to an age-related decrease in $GlyR\alpha_1$ protein and binding in DCN. These changes may reflect an effort to re-establish a homeostatic balance between excitation and inhibition impacting on fusiform cells in aged animals by down-regulating glycinergic inhibition. This age-related down-regulation comes at the cost of accurate temporal acuity observed at the single cell and behavioral level.
Supported by NIH DC00151 and ATA.

582 Behavioral Effects of Unilateral Noise Exposure on Young and Aged Mice

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Young (n=12) and aged (n=13) mice were anesthetized with isoflurane and given unilateral noise exposure for one hour using the same acoustic parameters used in previous studies with rats (116 dB SPL, 16 kHz octave band signal). Age-matched control mice were sham exposed for one hour (n=5 young, n=7 aged). ABR thresholds were collected immediately before and after noise exposure for each ear. Mice were behaviorally tested before noise exposure and at 1, 3, 7 and 14 days after noise exposure. Behavioral measures included prepulse inhibition and gap-induced inhibition of the startle reflex. Previous studies have used such tests to assess tinnitus and hyperacusis-like behaviors in rats. Frequency bands tested included 1 kHz bandpass signals centered at 4, 8, 10, 12, 16, 20, 24, and 32 kHz, as well as broadband noise, each presented at 60 dB SPL. Preliminary analyses suggest young and aged mice responded differently to the noise exposure, but that in both cases the results were consistent with noise inducing a hyperactive auditory system. At two weeks post noise exposure, aged noise-exposed mice exhibited significant gap detection deficits at 8 kHz (as well as a trend at 10 kHz). However, young mice exhibited significant gap detection improvements at 20 kHz (as well as trends for better responses throughout the entire 12-24 kHz range). These age-related responses, and their temporal development following noise exposure, are discussed in the context of the possible relationship between tinnitus and hyperacusis.

583 *Cdh23*^{753A} Does Not Impact Functional Aging of Gravity Receptors in C57BL/6J Mice

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Cochlear changes with age have been well described and predisposing factors such as gender, environment, and genetics are widely studied. To date, nine loci and one genetic mutation (*Cdh23*^{753A}) contributing to age related hearing loss (Ahl) have been identified and the impact on cochlear aging is characterized. The purpose of this study was to characterize age related change in inner ear gravity receptor function for three strains harboring one or more *Ahl* mutations (C57BL/6J, CE/J, and NOD NON-H2^{nb1}/LtJ) and one control strain (CBA/CaJ). Vestibular evoked potentials (VsEPs) were used to assess macular function from 2 to 24 months of age. Auditory function was assessed with ABR and DPOAEs. No significant gender differences were found for VsEP response parameters. In line with published literature, hearing loss was mild for 24-month-old CBA, profound for C57 and CE/J by 14 months and NOD.NON by 3 months. At 24 months, VsEP thresholds were significantly elevated for CBA and CE/J strains. Linear regression revealed a 10 to 15 dB re:1.0g/ms decline representing a 50 to 60% loss of macular dynamic range. In contrast, C57 retained macular sensitivity with VsEP thresholds declining by only 3 dB over 24 months. Preliminary data for NOD.NON suggest that macular function remains normal at least to 12 months of age. These results suggest that *Ahl* mutations (particularly *Cdh23*^{753A}) do not affect the inner ear vestibular organs in predictable ways despite the fact that cadherin23 is expressed in both cochlear and vestibular hair cells and other *Cdh23* mutations have been shown to affect both sensory modalities. Indeed, vestibular aging appears to be minimal for C57 relative to CBA. The lack of gravity receptor functional aging in C57 is consistent with studies showing minimal loss of hair cells or ganglion in old C57 mice; however, mechanisms that minimize gravity receptor aging remain to be determined. Supported by Amer. Acad. Audiol. Found., NIH F31 DC008012 and RO1 DC006443.

584 Homeostasis Defects, Mitochondrial Dysfunction, Sign Age Related Hearing Impairment

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Presbycusis, or age-related hearing loss (ARHL) is the major form of deafness, and the predominant neurodegenerative disease of aging. Since the cellular and the molecular mechanisms of presbycusis are unknown, no preventative or therapeutic interventions have been developed. To investigate to impact of age on hearing, we compare ARHL in fast aging SAMP8 and in C57BL/6 mice which present normal aging process but premature auditory dysfunction. Combination of morphological and molecular approaches with compound action potential of the auditory nerve and distortion product otoacoustic emissions recordings shows that, in addition of sensory hair cell loss, the fast aging SAMP8 mouse additionally displayed metabolic defects and drastic death of auditory neurons that shares pathological features with tauopathies (i.e., mitochondrial dysfunction, hyperphosphorylated microtubule-associated protein tau). Macrophages invading the cochlear structures were observed in aged cochleae, SAMP8 cochleae been more affected than C57BL/6J cochleae from animals of the same age. Because hearing loss may have an impact on the cognitive and behavioral symptoms in Alzheimer patients, careful evaluation and management of hearing should be applied to all Alzheimer patients.

585 The Level of Neuregulin-1 Modulates Age-Related Hearing Loss

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Age-related synaptic loss is associated with functional decline of the nervous system. Whether this synaptic loss is the cause or the consequence of neural loss is still unknown. We directly address this question by establishing a conditional tissue-specific transgenic mouse model expressing neuregulin-1 (*NRG1*), a modulator of synaptic transmission, in spiral ganglion neurons (SGNs). Transgenic mice with *NRG1* over-expression in SGNs show improvements in hearing thresholds, while *NRG1* -/+ mice show a complementary worsening of hearing. We find, however, no dramatic changes of age-related SGN or hair cell loss in these mice compared to controls. Furthermore, the *NRG1* induced improvement of hearing thresholds, which we find to be the result of enhanced synaptic transmission between SGNs and hair cells, can be reversed by "turning-off" *NRG1* over-expression. These data therefore provide the first evidence that the enhancement of synaptic transmissions during aging cannot prevent age-related neural loss in the cochlea.

586 Prevention of Presbycusis by Anticonvulsant Medications

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One major cause of age-related hearing loss (presbycusis) is the loss of hair cells and spiral ganglion neurons (SGNs). Currently, there is no effective way to prevent presbycusis. Here we show that the anticonvulsants, trimethadione and ethosuximide, significantly delay presbycusis. Both drugs are blockers of a family of T-type calcium channels comprised of three main pore-forming α1 subunits (α1G, α1H, and αI). At the molecular level, trimethadione prevents presbycusis via the α1H subunit because it fails to attenuate presbycusis in the α1H null or heterozygous mice. At the cellular level, the same drug fails to prevent SGN death *in vitro*. At the system level, trimethadione delays presbycusis by lowering levels of corticosterone which has been shown to be strongly associated with the extent of presbycusis. Because elevated glucocorticoid levels contribute to neurodegenerative disorders, our study suggests potential therapeutic roles of T-type calcium channel blockers in treating these disorders.

587 Preliminary Study of Relationship Between Hearing Loss and Synapses Changes in C57BL/6 Mouse Hippocampus

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Objectives To explore the relationship between hearing loss and cognition changes by measuring ABR (auditory brainstem response), Morris water maze and synaptophysin. Methods C57BL/6 mouse are divided into three groups according to age: 2-4 months old group, 6-8 months old group and 10-12 months old group. CBA mouse serves as control. Hearing threshold was measured by auditory brainstem response. Cognitive behavior was evaluated by Morris water maze. The quantity and distribution of hippocampus synapses were represented by synaptophysin in immunohistochemistry. Results The hearing threshold elevated at 6 months old significantly, then developed to total deafness at 10-12months old. To our interesting, the performance of Morris water maze and the change of synaptophysin developed as same pattern as the hearing threshold. The performance of Morris water maze and the change of synaptophysin had a little change at 6-8months old, but obvious chang at 10-12months old. Conclusions: The hearing level of C57BL/6 decreased with age. At the same time, Cognitive behavior and change of synaptophysin in hippocampus also decreased, especially when the hearing level close to total deafness.

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588 PIP3-Related Pathways in Age-Related Hearing Loss

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Phosphatidylinositol 4,5-bisphosphate (PIP_2) and phosphatidylinositol 3,4,5-trisphosphate (PIP_3) are major signaling molecules, interconvertible by phosphorylation (by PI3-kinase) and dephosphorylation (by PTEN phosphatase). PIP_3 signaling, in particular, is associated with the control of cell survival and cell death. We have previously reported (ARO 2008) that PIP_3 was decreased in the aging cochlea of CBA/J mice due to an increase in PTEN. Consequently, the phosphorylation of Akt and the activity of associated cell survival pathways were compromised. Here, we expanded these observations to elucidate further details of downstream targets of phosphoinositide signaling.

Correlating with lower levels of PIP_3 there was a reduction in the phosphorylation of the protein kinase Akt in the cochlea at an age of 18 months. NF-κB levels were also attenuated, a fact consistent with reduced phospho-Akt and an upregulation of IκB-beta, an inhibitor of NF-κB activity. These data indicates that reduced phosphoinositide signaling via Akt, IKK and NF-kB in the aging inner ear may contribute to a lessened survival capacity of aging outer hair cells. In addition to the changes in PIP_3 signaling, nuclear PIP_2 in outer hair cells was increased at 18 months of age. While the role of nuclear PIP_2 is still speculative it may be linked to gene regulation via histone modifications and can therefore contribute to the overall effect of a disturbed PIP_2/PIP_3 balance.

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589 Antioxidants May Influence Age-Related Hearing Loss

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Oxidant stress has been linked to noise- and drug-induced hearing loss, primary evidence being that antioxidant supplementation can prevent the deterioration of auditory function and hair cell structure. Suggestive evidence that manipulation of antioxidant status can also affect the aging auditory system has been presented (e.g., Seidman and colleagues), but acetyl L-carnitine which should improve mitochondrial efficiency and reduce oxidative stress failed to influence age-related hearing loss in rats (Bielefeld et al., 2008).

In a currently ongoing study female CBA/J mice received dietary supplementation of vitamins A, C and E plus L-carnitine and lipoic acid to their regular chow. The feeding regimens began at an age of 9 months and the two groups (n = 50 each) had well matched ABR thresholds. Group 1: 54 $\pm$ 11 dB at 4 kHz, 15 $\pm$ 7 dB at 12 kHz, 14 $\pm$ 7 dB at 24 kHz, and 12 $\pm$ 6 dB at 48 kHz. Group 2: 53 $\pm$ 13 dB at 4 kHz, 12 $\pm$ 7 dB at 12 kHz, 12 $\pm$ 9 dB at 24 kHz, and 14 $\pm$ 13 dB at 48 kHz. By 18 months, thresholds had increased somewhat at all frequencies but no differences were apparent between the two groups. However, preliminary evaluation of a subset of the animals (n = 17 in each group) at 24 months of age showed that significant threshold shifts had developed and that differences between the two groups were significant at the highest frequencies: Group 1 had thresholds of 39 $\pm$ 21 dB at 24 kHz, and 41 $\pm$ 27 dB at 48 kHz while group 2 showed 24 $\pm$ 19 dB at 24 kHz and 25 $\pm$ 19 dB at 48 kHz. We will discuss the possible influence of antioxidant supplementation on age-related hearing loss.

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590 Decline of Heat Shock Response in Age Related Hearing Loss Model

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It is known that heat shock response(HSR) is necessary for cochlea function and hair cell survival when the mammalia is exposed to intense noise and during aging.

We previously reported that pharmacological upregulation of heat shock proteins (Hsps) attenuate these damage in cochlea. However the mechanism of protection has not been clarified. Here, we assessed whether HSR against noise injury altered in aged cochlea using age related hearing loss (ARHL) model. We used male C57BL/6 mice as ARHL model and control CBA mice. 24 hrs after sound exposure for three hours (130 dB octave band noise with a center frequency of 4 kHz), whole cochleae were removed. For western blotting assay, we used the primary antibody against Hsf1, Hsp 110, Hsp 70(inducible Hsp70), Hsp 27 and ß- actin. For functional assessment, we measured thresholds of the auditory brain stem response (ABR). ABR test showed that permanent threshold shift up 14 days after sound exposure in all animals. Western blot assay revealed that induction of Hsps both in CBA mice and C57BL/6 at 8 weeks of age when intense sound was exposed. But C57BL/6 at 40 weeks of age did not show upregulation of Hsps whereas CBA mice at 40 weeks of age did. These results indicate that the HSR in the cochlea of ARHL decline during aging and pharmacological enhancement of HSR is reasonable intervention to ARHL.

591 Maintenance of Hearing Requires Heat Shock Transcription Factor 1 in Mice

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Heat shock protein (Hsp) works as a chaperonin that protects cells against some kinds of stress, and heat shock transcription factor (Hsf) control this response at the transcription level. In otology area, it was reported that Hsf1 was indispensable to the ear protection against acoustic trauma by using Hsf1 knockout mouse (Sugahara, Hear Res. 2003). Moreover Hsp inducer, Geranylgeranylacetone (GGA), was able to protect inner ear against noise trauma (Mikuriya, Brain. Res.2005). Also, GGA can protect inner ear against age-related hearing loss in DBA/2J mouse known as one of the elderly hard hearing model animals (Mikuriya, Brain. Res.2008). However, there was no report related to age-related hearing loss and Hsf1. In the present study, we researched whether Hsf1 was necessary for maintaining hearing by using the Hsf1 deficient mouse.

Hsf1 deficient mouse backgrounds of the CBA mouse were used in this study. Hetero mouse and wild type mice were used as a control group. We assessed auditory brainstem response (ABR) threshold at 4, 20, and 36 weeks. ABR threshold were no difference between the Hsf1 knockout, and the hetero, and the wild type mouse group at 4 weeks. However, the threshold was significantly larger in the Hsf1 knockout group than in control group at 36 weeks.

592 The Protective Heat Shock Response in Age-Related Hearing Loss

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Hearing loss is a major health problem for both the young and old. Approaches for protecting the cochlea from noise damage throughout life would be highly desirable. Our research examines the role of a major protective mechanism controlled by the heat shock transcription factor 1 (HSF1). Many stressors can activate HSF1, leading to transcriptional activation, DNA binding, and induction of genes for heat shock proteins (Hsps). We are developing *Hsf1*$^{-/-}$ knockout (KO) and constitutively active HSF1 mouse models suitable for aging studies. We replaced the sensitive allele of the *Ahl* locus on Chr 10 with the resistant *Ahl* allele from CBA/CaJ and assessed ABR thresholds with age in *Hsf1*$^{+/-}$ heterozygotes and *Hsf1*$^{-/-}$ KO mice. An ANOVA-type analysis identified a statistically significant age x genotype interaction term at 48 kHz, suggesting that *Hsf1*$^{-/-}$ KO mice are more susceptible to age-related hearing loss at high frequencies. Attempts to develop a CBA/CaJ congenic strain were unsuccessful, because *Hsf1* KO mice have reduced viability on this genetic background. We also plan to develop a transgenic mouse model expressing a constitutively active form of HSF1 in the cochlea to assess directly the protective role of HSF1 with age. We are comparing the transcriptional activities of epitope-tagged alpha and beta isoforms of mouse HSF1 in NIH 3T3 cultured cells. Preliminary data suggest that the alpha isoform can activate HSP genes.

593 The Heat Shock Response in Young and Old CBA/J Mice

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Hearing loss is a major health problem for both the young and old. Excessive noise can cause either transient or permanent hearing loss. Approaches for protecting the cochlea from noise damage throughout life would be highly desirable. Our research has focused on the classic stress-inducible protective pathway regulated by heat shock transcription factor 1 (HSF1). The Hsf1 gene is constitutively expressed, but HSF1 monomers do not bind DNA. Many different stresses can activate the HSF1 protein via trimerization and hyperphosphorylation, leading to DNA binding and induction of genes for heat shock proteins (HSPs), the downstream targets of HSF1. To assess the role of HSF1 in the normal and aged cochlea, we examined the stress response in young (3 month) and old (18-20 month) CBA/J mice from the NIA Aged Rodent Colony. We used two different stressors – heat and noise – and assessed the activation state of HSF1 by measuring induction of several HSP genes. Each sample consisted of RNA isolated from the cochleae of a single mouse. Gene expression was measured by quantititative RT-PCR (qRT-

PCR) with gene-specific TaqMan probes. Surprisingly, we did not observe a significant decrease in activation of HSF1 by heat stress in the cochlea of wild-type CBA/J mice with age. We also examined the induction of HSPs following exposure to broadband noise (BBN) (2-20 kHz) presented for 2 hr at increasing intensities (98 dB, 106 dB, and 120 dB). Using noise as a stressor, we did observe a slight but significant decrease in induction of HSP genes in old vs. young CBA/J mice. We attempted to determine the sensitivity to noise in old vs young CBA/J mice by measuring ABR thresholds before and 2 weeks after noise exposure and calculating threshold shifts. These experiments were confounded by different ABR thresholds and different responses to noise observed in two batches of old CBA/J mice from NIA. Supported by NIH grants P01 AG025164 and P30 DC005880

594 Evidence for Structural Compromise of the Tympanic Membrane in the Aged Brown Norway Rat

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We are investigating the contribution of the outer and middle ear to presbycusis. Analysis of the tympanic membrane (TM) transfer function has shown an 8-10 dB loss of sound conduction in the mid to high frequencies of the aged Brown Norway rat (Gratton et al, 2008). Decreases in TM thickness associated with aging were suspected to result in the compromised TM transfer function (Gratton, Davis and Saunders, 2008).

In this study, we extended our findings to the histologic level. Matrix molecule expression patterns for elastin, collagens (type I, II and III) and proteoglycans (chondroitin sulfate (CSPG) and heparin sulfate (HSPG)) were examined in TMs and the annular ligament isolated from 6 young adult and 4 aged Brown Norway rats. Each TM was examined at an equivalent position in pars tensa just inferior to the contact point of the manubrium of the malleus with the medial side of the TM.

Each matrix molecules displayed a unique distribution of expression within the TM and annular ligament. Collagens type II and III were found in the mucosal and epidermal layers of the TM as well as in the annular ligament, but were absent from lamina propria. Collagen type I, CSPG and HSPG were present in at least one TM layer as well as the peripheral circumferential fibers of lamina propria.

In the aged TM, changes were found in all of the matrix molecules. Specifically noted was dissolution of layer-specific staining and dimunition-to-absence of circumferential fiber staining. Accompanying the altered expression patterns was an apparent compromise in the tension of the TM evidenced by “wrinkling”. The loss of structural rigidity or of elasticity contributes to the compromised TM structural integrity and the reduced responses to acoustic stimuli in the aged rats.

595 Auditory Function in a Nox3 Mutant Mouse Strain

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The purpose of the current study was to evaluate auditory function for the *Nox3* mutant mouse strain. *Nox3* is a gene that plays a role in the formation of a NADPH oxidase complex known to be critical in otoconia development and vestibular function. *Nox3* expression has been demonstrated in the cochlea; specifically the sensory epithelia and the spiral ganglia. However the role of *Nox3* in auditory function has not been studied extensively. To examine auditory function we measured auditory brainstem response (ABRs) and distortion product otoacoustic emissions (DPOAEs) in homozygous mice (n = 56) from 2 to 12 months of age. Vestibular evoked potentials (VsEPs) were used to confirm absent otolithic function. Data available from age-matched C57BL/6J mice served as background controls. Results from *Nox3* mutants revealed that DPOAE amplitudes were up to 15 dB smaller than C57 controls, particularly for low to mid-frequencies. As expected, DPOAEs diminished with advancing age; however age related declines occurred sooner for *Nox3* mice. Consistent with DPOAEs, ABR thresholds were significantly elevated for *Nox3* mutants. At 2 to 3 months of age, ABR thresholds for 8 kHz averaged 53 dB peSPL for *Nox3* compared to 36 dB peSPL for C57. Indeed, the number of animals with no responses was also greater for *Nox3* mutants when compared to controls at the frequencies of 16, 32, and 41 kHz across all ages tested. The present results contrast with previous studies and suggest that mutations in *Nox3* can affect cochlear function and hence, hearing. Alternatively, *Nox3* and *Cdh23*^{ahl} mutations (both present in the *Nox3* mutants) may interact to produce a more severe phenotype in the *Nox3* homozygotes. Research supported by the American Academy of Audiology Foundation, NIH R01 DC006443, NIH R01 DC00713.

596 Early Detection and Clinical Evaluation of Streptomycin Ototoxicity

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Despite its strong anti-tuberculous effect, streptomycin(SM) has been used restrictively because of its risk of ototoxicity. The authors evaluated 418 patients who had used SM for treatment of tuberculosis and tried to assess the rates and clinical pictures of its ototoxicity and how early-detection work for preservation of cochlear and vestibular function of the patients.

All patients got electronystagmography, bithermal caloric test, slow harmonic acceleration test, pure tone audiometry including 8kHz and 12kHz and oto-acoustic emission, as a baseline study before the injection. The patients were informed about possible symptoms of ototoxicity, and checked every time before injection. If the symptoms arose, further injection was stopped, Frenzel

goggle tests were performed and above lab tests were reevaluated.
30 patients (7.1%) complained vestibular symptoms. Among them, only 17 patients (4.1%) showed objective laboratory findings of vestibulopathy. No patients dropped into total bilateral vestibulopathy in follow-up caloric test. No patients could be restored caloric response, but symptom was improved after cessation of injection. 12 patients (2.8%) suffered from hearing symptoms such as tinnitus and subjective hearing impairment. However, no patient had decrease in threshold in pure tone audiogram even at high frequency. 6 of these patients (1.4%) suffered from cochlear symptoms along with vestibular dysfunction. Vestibular symptoms were occurred earlier (within 38 days) than cochlear symptoms (137 days) ($p<0.05$). Nobody suffer from cochlear symptoms after completion of injection.
In summary, our 10 year experience of SM injection for tuberculosis show 4.1% incidence of vestibular toxicity with 38days, and no cochlear toxicity. Early detection of ototoxicity could minimize the risk, preserve the function, and use enough therapeutic doses in 95.9% of patient.

597 T-817MA Attenuates Inner Ear Barotrauma in the Guinea Pig

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Objective: Inner ear barotrauma (IEB) that is caused by acute pressure changes can often lead to permanent severe sensorineural hearing loss (SNHL). However, the mechanism that causes IEB is still unknown and its treatment is not established. 1-{3-[2-(1-benzothiophen-5-yl)ethoxy]propyl}-azetidin-3-ol maleate (T-817MA) is a newly synthesized agent developed for treatment of neurodegenerative disease such as Alzheimer's disease. T-817MA not only has neurotrophic effects but also reduce oxidative stress. In the present study, we assessed the possibility of new drug treatment using T-817MA for IEB.
Methods: Healthy female guinea pigs with a normal Preyer's reflex were used in this study. Animals were randomly assigned to one of three groups: (1) group 1 received T-817MA-enhanced water with 0.7mg/ml (2) group 2 received T-817MA-enhanced water with 0.2mg/ml (3) group 3 received normal water (control group). Treatment was begun10 days prior to the intense pressure loading, which induced acute SNHL, and continued until day 21. To assess the efficacy of T-817MA, auditory brainstem responses (ABR) were measured on day 1 (10days pre-exposure), just after the pressure loading, and then at 3 day, 1, 2, and 5 weeks after the pressure loading. ABR threshold shifts were compared between the three groups at each time point.
Results: T-817MA significantly attenuated hearing loss at 1 and 2 weeks after the intense pressure loading ($p < 0.05$, Mann-Whitney's U test).
Conclusions: Treatment for IEB using T-817MA was proved to be effective. These findings indicate that T-817MA might be a promising therapeutic drug in IEB induced SNHL.

598 JNK Activation and Up-Regulation of Thioredoxin Reductase (TrxR) in the Rat Cochlea Following Styrene Exposure

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Styrene, a widely used industrial chemical, is ototoxic. It causes death of auditory hair cells after about 7 days of exposure (800 mg/kg/day) through apoptosis (Chen et al., 2007). This study was designed to determine styrene-induced alterations in the cochlea underlying the apoptotic cell death. Thioredoxin(Trx)/thioredoxin reductase(TrxR) system plays an important role in maintaining cellular redox balance and inhibiting JNK pathway, which leads to apoptotic cell death. Long Evans rats were exposed to styrene by gavage at a dose of 800 mg/kg in oil for 4 days or as controls by oil gavage alone. Cochlear tissues were sampled 1 hour after the last exposure. TrxR and p-JNK were determined by Western blot analysis. The results showed a remarkable increase of p-JNK in the styrene exposed group compared to the oil control group. However, TrxR level in the styrene exposed level was also up-regulated. The data indicated that styrene did not cause damage to the Trx/TrxR redox system. We speculate that styrene exposure results in over-production of reactive oxygen species (ROS), which activates stress signaling pathways leading to apoptotic cell death. The over-produced ROS can also oxidize Trx and the overloaded oxidized Trx may stimulate synthesis of TrxR.
This study was supported by NIOSH grant 1R01OH008113-01A1

599 Changes in the Structure and Function of the Inner Ear Caused by Burow's Solution

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We examined the changes in the hair cells of the inner ear following intratympanic injection of Burow's solution. Under general anesthesia, physiological saline was placed with a dropper upon the left round window membrane (RWM) of albino guinea pigs through a small hole made in the tympanic bulla. For experimental animals, Burow's solution (pH 3.7) was placed on the RWM for 30 min (30-min group), 1 h (1-h group) or 2 hs (2-h group). Five days later, animals were anesthetized and sacrificed by decapitation. ABRs at 4, 8 and 20 kHz were recorded immediately before surgery and decapitation. The left bony labyrinth was removed and then fixed with 4% paraformaldehyde at 4ºC. The cochlea and utricle were dissected were stained with rhodamine-phalloidin, and examined under a fluorescence microscope. The left RWM of additional animals in the 2-h group were embedded in Epoxy resin. On the operation day, all animals temporarily had a head tilt to the side of the surgery. In 30-min groups, no significant difference was observed between the postoperative ABRs and base-line thresholds. The post operative ABR thresholds at 20 kHz in the 1-h group and at 8 and 20 kHz in the 2-h group were significantly increased compared to base-line thresholds. Surface preparation of the organ of Corti showed no hair cell loss

in the 30-min group, outer hair cells loss in the lower half of the basal turn in 1/4 animals in the 1-h group, and outer hair cells loss of the base turn in almost all animals in the 2-h group. Micro-thin sections of RWM in the 2-h group showed the degeneration of the outer epithelium and many erythrocytes infiltration in the scala tympani adjacent to the RWM. Surface preparation of the utricular macula showed no hair cells loss in any groups. These findings suggest that the application of Burow's solution put on the RWM for 1 h or longer induces damage to the outer hair cells in the cochlea, but not the utricular hair cell, through the damaged RWM. Physicians should pay attention to this adverse effect of Burow's solution when applied into the middle ear cavity.

600 Proteomic Analysis of Mefloquine Ototoxicity

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In previous studies, we have shown that the anti-malarial drug mefloquine damages rat cochlear hair cells and auditory nerve fibers by apoptosis. Using RT-PCR apoptosis-focused gene arrays, we have reported changes in a wide range of apoptotic and anti-apoptotic signals in the basilar membrane (hair cells-supporting cells) and spiral ganglion regions of rat cochlear organotypic cultures treated with 100 μM of mefloquine for 3 h. Comparison of sensory and neuronal mRNA responses suggested both differences and tissue specific mechanisms induced during the early states of ototoxicity. In the present study, we assayed changes in this model (same dose and time point) in 200 cell signaling proteins using an antibody microarray. Proteomic analysis also revealed tissue specific regulation of gene expression. Changes in expression of signaling proteins were much less than changes in levels of mRNA indicating the possibility of important translational control of the balance between cell survival and cell death responses. Supported in part by NIH grant R01 DC06630.

601 Amelioration of Noise-Induced and Age-Related Hearing Loss in the Augmented Acoustic Environment (AAE)

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Augmented acoustic environment (AAE) is a paradigm in which animals are exposed to a non-traumatic noise and was first introduced by Turner and Willott (1998) to ameliorate progressive genetic hearing loss (HL) in mice by initiating the treatment around the time of the manifestation of HL. AAE is also known as "acoustic enrichment" which has been shown to ameliorate noise-induced hearing loss (NIHL, Norena and Eggermont, 2005; Niu et al., 2004). We have used the AAE paradigm to examine the protective effects against NIHL and age-related HL (ARHL) and the influence on outer hair cell (OHC) death.

To examine the AAE effects on NIHL and OHC death, chinchillas (n=5) were exposed to a traumatic noise (one octave-band with the center frequency at 4 kHz at 107 dB SPL) for 1 hour and then immediately exposed to a non-traumatic continuous noise (4 – 20 kHz at 80 dB SPL) for 3 days. An acoustically-deprived group (n=5) wore earplugs for 3 days starting immediately after the same noise exposure. The results showed that the AAE/acoustic enrichment group showed significantly smaller ABR threshold shifts at 4 and 8 kHz and fewer numbers of deteriorated OHC (apoptotic, necrotic, and missing OHCs) compared to the deprived animals.

In order to investigate whether the AAE/acoustic enrichment ameliorates ARHL when it was initiated after the manifestation of HL, 16-month-old Fischer 344/NHsd rats (n=5) were exposed to the non-traumatic noise (4-20 kHz and 80 dB SPL), for 12 hours/day for 3 months. Six unexposed rats were used as controls. ABR thresholds were obtained before the treatment, and re-tested at 2, 6, 9, and 13 weeks after the initiation of the treatment. The results showed that the "vector" for ARHL was essentially stopped by the introduction of AAE and by 13 weeks of the treatment, the control group had 10-20 dB larger ABR threshold shifts at 20-40 kHz compared to the AAE group. Additionally, fewer numbers of deteriorated OHCs were observed in the AAE group compared to the control group. In conclusion, our results from both studies demonstrated that non-traumatic noise exposure can prevent deterioration of OHCs caused by a traumatic noise and aging.

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602 Effect of Modulating Vitamin C Levels on Age-Related Hearing Loss

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The free radical theory of aging asserts that buildup of macromolecular damage from oxygen free radicals leads to the functional decline associated with aging in multicellular organisms. Age-related hearing loss (ARHL) has been considered to be related to excess generation of free radicals. For example, McFadden et al. (Neurobiol Aging 1999) have shown that copper/zinc superoxide dismutase deficiencies increase the vulnerability of the cochlea to damage associated with normal aging through metabolic pathways involving the superoxide radical. Vitamin C (VC) or L-ascorbate is an essential nutrient for a large number of higher primate. It is made internally by almost all organisms including mice, but not humans. It is known that VC deficiency causes scurvy in humans. The pharmacophore of VC is the ascorbate ion and in living organisms, ascorbate acts as an antioxidant since it protects the body against oxidative stress. We have established senescence marker protein 30 (SMP30)/

gluconolactonase (GNL) knockout (KO) mice, which are incapable of synthesizing VC in vivo, because SMP30/GNL is involved in the VC biosynthetic pathway (Kondo Y, et al. PNAS 2006). Amounts of SMP30 significantly decrease with aging in an androgen-independent manner, and the amino acid sequences of this 34-kDa protein are highly conserved among vertebrates.

Using these KO mice, we examined whether modulating VC affects ARHL. SMP30/GNL KO and WT (C57BL/6) mice were weaned at 30 days, at which time they were divided into the following four groups: WT and SMP30/GNL KO mice given sufficient (VCs) or insufficient VC (VCi). The VCs group had free access to water containing VC (1.5 g/L), whereas the VCi group had free access to water containing VC (3.75 mg/L). At 10 months of age, we measured auditory brainstem response (ABR) thresholds at 4, 8, 16, and 32 kHz. Then, animals were euthanized for evaluation of cochlear pathology in the left ear and measurement of VC levels in the right inner ear, liver, and plasma using a high-performance liquid chromatography-electrochemical detection method. At 10 months. these four groups looked similarly except that the VCi KO mice were alopecic around the mouth and eyes. The total VC levels in the inner ear, liver, and plasma were similar among VCs KO mice and VCs and VCi WT mice but significantly reduced in VCi KO mice (< 20% compared to other groups). ABR measurements, however, revealed no significant differences in ABR thresholds at any frequencies among groups. No significant differences were observed in cochlear pathology among groups. These findings suggest that significant reduction of VC level in the inner ear may not accelerate ARHL and that supplementing VC may not increase VC level in the inner ear or slow ARHL in C67BL/6 mice.

603 Effects of Repeated "Benign" Noise Overexposures in Young CBA Mice

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A surprising recent finding in mice demonstrates a sequence of events that ultimately lead to “primary” spiral ganglion degeneration without the mechanosensory hair cell loss after “benign” noise exposure, i.e., the exposure that does not cause permanent hearing threshold change. In animals exposed with noise that produces only temporally threshold shift (TTS), the following are seen: 1) full recovery of hearing thresholds measured with ABR after 2 weeks, 2) normal distortion product otoacoutic emission and total preservation of mechnosensory hair cells (both inner and outer), 3) acute IHC afferent terminal retraction and degeneration that persist over weeks and months, and 4) acute ganglion cell demyelination and eventual ganglion cell loss. With incomplete recovery from acute injury, cumulative effects from repeated TTS exposures may be inevitable, and their central consequences should not be underestimated. We, therefore, decided to test if repeated TTS exposures will eventually culminate in permanent threshold change and how that relates to inner ear histopathology. Two doses of sequential maximal TTS exposures do not result in permanent threshold changes. However, no benefits of prior exposure were seen because the acute TTS from the second dose is similar to that after the first dose. Even though full recovery of ABR thresholds is seen, the growth function of ABR wave1 amplitude (synchronized spiral ganglion cell activity) is greatly diminished indicating diffuse ganglion cell dysfunction consistent with observed ganglion cell pathology.

604 Pathological Sequelae of Ear-Kissing

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A 49 year old mom received a surprise, but vigorous kiss on the left ear by her four year old daughter. Unfortunately, the kiss landed directly on the aperture of the EAM causing considerable negative pressure to be applied to the EAM and tympanic membrane (TM). The suction resulted in an immediate and complete loss of the sense of hearing in the left ear. Fortunately, this profound effect was short lived, and was followed several hours later by: a more moderate, purely sensorineural loss of hearing, dysacusis, the perception of a screeching tinnitus, a hypersensitivity to all external auditory stimuli, and continuous unprovoked facial muscle spasms about the ear. A series of diagnostic procedures was performed including: audiometry, immittance, ipsilateral and contralateral acoustic reflex battery, ABLB, DP-OAEs, ABR, facial nerve testing, high resolution CT scan and MRI. The results revealed a mild, 35 dB HL, unilateral sensorineural hearing loss in the “kissed” ear; a paralyzed acoustic reflex in the presence of an intact ossicular chain with good facial nerve function, and documented hyperacusis. The potential contribution of a severed stapedial tendon in the paralysis of the acoustic reflex, hyperacusis and disacusis was explored. The relationship of ear suction and sensorineural hearing loss was examined, and the dangers of applying sudden negative pressure to the TM of adults and especially infants via “ear-kissing” were discussed.

605 Cellular Changes in the Spiral Ganglion *in Vivo* After Deafening

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After loss of hair cells, spiral ganglion neurons (SGNs) gradually degenerate. This involves several cellular changes including degeneration of the peripheral processes, reduced CREB phosphorylation and increased Jun phosphorylation. There are concomitant cellular changes in the spiral ganglion Schwann cells (SGSCs). Hurley et al. (Eur J Neurosci 26:1813, 2007) have shown loss of myelination markers in rats deafened by kanamycin/furosemide injection. In rats deafened by daily kanamycin injections postnatal day 8 (P8) - P16, we have shown expression of the neurotrophin receptor $p75^{NTR}$ in SGSCs and reentry of $p75^{NTR}$-expressing SGSCs into the cell cycle. Immunohistochemical studies of cochlea from

rats euthanized on P23, P32, P46, and P60, show $p75^{NTR}$ expression first appearing in the osseous spiral lamina (OSL) by P32. Subsequently, the number of $p75^{NTR}$-expressing SGSCs increases and expression extends into Rosenthal's canal. We quantified neural immunofluorescence (NF200/NF150/β3-tubulin) as a function of distance from the organ of Corti to the SGN somata. We find that degeneration of neurites in the OSL coincides with appearance of glial $p75^{NTR}$. This pattern suggests that neurite degeneration is a stimulus to upregulation of glial $p75^{NTR}$. We also asked whether there is loss of SGSCs in the OSL. We counted Schwann cell nuclei in the OSL and estimated cell density. We find no significant change at P60 (≈7 weeks post-deafening). We suggest that, although $p75^{NTR}$ signaling is often associated with apoptosis in glia, there is no net loss of glia at a time when $p75^{NTR}$ is upregulated. Possibly, this is due to cell division in $p75^{NTR}$-expressing SGSCs. Also, because by P60 about half of the SGNs have died while SGSC density is unchanged, loss of SGSCs is unlikely to be causal to SGN death. Finally, the presence of SGSCs long after deafening suggests that should means be found to induce neurite regeneration from surviving SGNs, glia will be available for their support.

606 Neurotrophin-3 (NT-3) Expression in Rat Organ of Corti (OC) After Deafening

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Spiral ganglion neurons (SGNs) die gradually after the loss of hair cells. It is not yet known what provides trophic support for the SGNs during this period and why it is ultimately insufficient to prevent SGN death. NT-3 is expressed in inner hair cells and in the immediately adjacent organ of Corti supporting cells (OCSCs): phalangeal and inner pillar cells. NT-3 is a trophic factor for SGNs and NT-3 knockout results in death of SGNs during prenatal development. It is not known whether NT-3 is necessary for SGN survival in the mature cochlea. Here, we test a hypothesis that OCSCs provide neurotrophic support to the SGNs. Specifically, we ask whether NT-3 expression is maintained in the OC after hair cell loss and, if so, whether it declines in parallel with SGN death. To answer this question, rats were deafened by kanamycin injection on postnatal day 8 (P8)-P16 and euthanized on P23, P60, and P90. These time points were chosen based on the time course of SGN death in rats deafened by these means: P23 is at the onset of SGN death, P60 is midway through the period of SGN death, and at P90 few SGNs remain. We isolated RNA from the OCs, and used quantitative PCR to determine NT-3 transcript levels. NT-3 has multiple alternatively-spliced transcripts (Sekimoto,1998) and we identify E1A, E1Aα1, and E1B as the major transcripts in the rat OC. BDNF transcripts are detectable but at such a low level (Ct>30) that BDNF may have at most only a modest role in SGN survival. By P23, the earliest time point post-deafening, NT-3 transcript levels have fallen to <10% of the age-matched control. NT-3 remains at this low level with no further decline throughout the period of SGN death. Given the low NT-3 expression after hair cell loss and the lack of correlation between NT-3 levels and SGN number, we conclude that OCSCs do not provide significant neurotrophic support to SGNs post-deafening and SGN death is not due to loss of OCSC-derived support.

607 Combinations of Metal Ions and Their Effect on Fibroblasts and Neuronal Cells

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Degeneration of spiral ganglion cells (SGC) after deafness and fibrous tissue growth around the electrode carrier after cochlear implantation are two of the major challenges in current cochlear implant research. As shown earlier, Silver ions in very low concentrations can have a neuroprotective effect on cultured spiral ganglion cells whereas on cultured fibroblasts and PC-12 cells there is first an anti-proliferating and at higher concentrations a toxic effect. Furthermore, when repeating experiments with Zink ions, the threshold concentration for anti-proliferative and toxic effects was higher for PC-12 cells than fibroblasts (Paasche et al, IEB 2008). Therefore, we now investigate whether the combination of both ions leads to any synergistic effects.

For the experiments five different mixing ratios (100:1; 10:1; 1:1; 1:10; 1:100) of Ag and Zn ions were used. Total metal ion concentrations between 0.03 and 1000 µmol/l were added to cultures of standard cell lines (NIH 3T3 and PC-12; seeded at 10000 cells/ well) to investigate toxicity and antiproliferative effects. After a cultivation time of 48 hours, cells were stained using a Neutral red assay and absorption was measured at 570 nm. Spiral ganglion cells were freshly isolated from newborn rats (p3-5). Cells were cultivated for 48 hours using medium supplemented with BDNF, stained with anti-neurofilament antibody and counted. In all experiments 5 wells were treated in the same manner and each experiment was repeated at least three times.

Results showed that with a higher number of silver ions in the solution, results were similar to the treatment with silver alone. Only at a ratio of 1:100 (Ag:Zn), the higher thresholds for anti-proliferative effects on PC-12 cells became detectable even though this effect was less pronounced than without Ag ions in the Zn solution.

With this combination of metal ions, the effect in cultured cells was dominated by silver ions, the ion with lower toxicity thresholds compared to Zink, but no increase in anti-proliferative effects could be found.

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608 ER Stress Signaling Is Involved in the Glutamate-Mediated Ototoxicity of VOT 33 Spiral Ganglion Cells

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Glutamate plays as an excitatory neurotransmitter in the cochlear, but also it shows toxic effects on organ of Corti in various pathological conditions. However, the cytotoxic effects of glutamate on auditory cells have not been fully understood. Therefore, this study was designed to elucidate the signaling mechanisms of glutamate toxicity in VOT33 spiral ganglion cells. Treatment with glutamate significantly decreased the viability of VOT33, which was accompanied with apparent apoptotic features and increased fraction of subGo/G1 phase. Treatment of VOT33 cells with glutamate resulted in a decrease in intracellular protein levels of reduced glutathione (GSH), GSH2, and superoxide dismutase. However, it increased the protein expression levels of cysteine transporter (xCT), catalase, and iNOS. These results eventually lead increased generation of NO and H2O2, which further causes the oxidative stress in VOT33 cells. Furthermore, accumulation of intracellular calcium resulted in endoplasmic reticulum (ER) stress, accompanying with increases in expression of Grp78/Bip and C/EBP homologues protein (CHOP) and PERK phosphorylation. Moreover, ectopic expression with HO-1 siRNA reduced the expression levels of CHOP and phosphorylated PERK proteins in glutamate-treated VOT33 cells. Taken together, these data suggested that the cytotoxicity of glutamate was ascribed to ROS generation, which would result in the modulation of the intracellular redox cycle and ER stress through calcium accumulation.

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609 Inner Hair Cell (IHC)-Spiral Ganglion Neuron (SGN) Synapse Regeneration Following Excitotoxic Trauma Is Deficient in Mice Lacking $P75^{NTR}$

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The pan-neurotrophin receptor $p75^{NTR}$ is not essential for the development of IHC-SGN synapses, nor is it expressed at significant levels in the mature cochlea. However, $p75^{NTR}$ is upregulated in the organ of Corti following excitotoxic trauma, suggesting a possible role in recovery. We have previously shown that brief exposure to kainic acid (KA) in vitro causes disruption of IHC-SGN synapses, followed by a reinnervation that only partially restores the initial innervation. To test the role of $p75^{NTR}$ in reinnervation, we exposed organotypic cochlear explant cultures from postnatal day 4-6 mice to KA and compared reinnervation between $p75^{-/-}$ mice and wild-type ($p75^{+/+}$) or heterozygous ($p75^{+/-}$) littermates. Contacts between SGN peripheral axons and IHCs were quantified and individual synapses (labeled with anti-PSD95 antibody) were counted. We found that regeneration after 3 days in vitro was $p75^{+/+} > p75^{+/-} > p75^{-/-}$ and very poor in $p75^{-/-}$ explants. These data indicate a requirement, at least in part, for $p75^{NTR}$ during synaptic regeneration following KA. To test whether $p75^{NTR}$ promotes regeneration, we applied a low concentration (<1 nM) of proNGF to selectively activate $p75^{NTR}$ but not the TrkB or TrkC neurotrophin receptors expressed on SGNs. proNGF significantly increased the number of peripheral axons regenerating and contacting IHCs and significantly increased the number of newly formed synapses in mouse explants. proNGF had no effects on explants from $p75^{-/-}$ mice. Furthermore, conditioned medium from proNGF-treated organs of Corti increased neurite growth in cultured dissociated SGNs, an effect partially inhibited by TrkC-IgG, a NT-3 antagonist. Interestingly, regeneration (with or without proNGF) is better in apical cultures, in which there is a higher level of endogenous NT-3. These data indicate that $p75^{NTR}$ signaling promotes reinnervation of IHCs by SGNs after excitotoxic trauma, in a manner dependent, at least in part, on NT-3.

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610 The Effect of in Vivo Intracochlear Electrical Stimulation on Apoptosis of Spiral Ganglion Neurons in the Rat: The JNK-Jun Signaling Pathway

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The c-Jun-N-Terminal Kinase (JNK)-Jun signaling pathway has been implicated in neuronal death as a consequence of neurotrophic factor removal, neurotoxic insults, or neurodegenerative disease. We have investigated JNK-Jun signaling in spiral ganglion neurons (SGN) death due to deafferentation. SGNs die as a result of loss of hair cells, their sole afferent input. We have previously shown that in rats in which hair cells were killed by aminoglycoside injection, JNK and Jun are phosphorylated in apoptotic SGNs (Alam et al, 2007). This implies that SGN membrane depolarization by afferent input should suppress activation of JNK. This has been confirmed in vitro by previous work in our lab examining the upstream effects of electrical stimulation on SGNs in culture. Here, we exploited the relative accessibility of the cochlea to test whether depolarization suppresses Jun phosphorylation in vivo. We implanted an electrode in the cochleae of kanamycin-deafened rats and showed that electrical stimulation (monopolar, biphasic pulses, 125 pulses/sec, amplitude twice eABR threshold) for four hours suppressed Jun phosphorylation in SGNs normally seen following deafferentation. Moreover, this suppression was

blocked by intracochlear perfusion of a calcium/calmodulin-dependent protein kinase II (CaMKII) inhibitor, KN93, implying that the same intracellular pathway described in vitro functions in vivo. Further work to distinguish between the effects of high pulse rate (5000 pulses/sec) and low pulse rate (125pulses/sec) stimulation is ongoing. Our technique provides evidence that the rat is a useful model to study the intracellular effects of electrical stimulation after deafness.

611 Understanding the Role of C-Jun-N-Terminal (JNK) Isoforms in Apoptosis and Neurite Growth of Spiral Ganglion Neurons (SGNs) *in Vitro*

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JNK is a member of the mitogen activated protein kinase (MAPK) family implicated in responses to cellular stress and in apoptosis. Activation of JNK requires JNK interacting protein (JIP-1), a scaffold protein necessary for formation of a protein complex containing JNK and its upstream activators. Indeed, we have previously reported that JNK is activated *in vivo* and *in vitro* in apoptotic SGNs and that JNK inhibition reduces apoptosis in SGNs. We inhibited JNK by using either the kinase inhibitor SP600125 or a cell membrane permeable peptide, I-JIP, that mimics the JIP-1 JNK binding domain and competitively inhibits JNK binding to JIP-1, preventing the activation of JNK. Bodmer et al. (2002) showed that the inhibition of mixed lineage kinase, an upstream activator of JNK, inhibits SGN neurite growth *in vitro*. We further show here that SP600125 strongly inhibits initiation of neurite growth from cultured SGNs and inhibits growth of neurites already established. Consistent with this, we detect JNK activation in SGN growth cones by immunofluorescent labeling with anti-phosphoJNK antibodies. In contrast, addition of the I-JIP peptide has only a small and insignificant effect of SGN neurite growth, One possibility is that a scaffold protein other than JIP-1, plays a role in JNK activation at the growth cone. The result implies caution when interpreting results obtained with pharmacological inhibitors. In oder to further investigate the mechanism by which SP600125 inhibits neurite growth, we assessed the effect of SP600125 on cell motility. SP600125 was added to motile A431 cells (a human carcinoma cell line) and cell motility quantified from time-lapse images. SP600125 caused a rapid and sustained reduction in cell movement along the laminin substrate although cell membrane ruffling and lamellopodial extension persisted. This suggests an effect on cell-substrate adhesion, a possibility we are testing with cell lines and SGN growth cones.

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612 Intracochlear Infusion of Brain-Derived Neurotrophic Factor (BDNF) Promotes Survival of Spiral Ganglion Neurons During Development in Cats Deafened as Neonates

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Postnatal development and survival of spiral ganglion (SG) neurons depend upon both neural activity and neurotrophic (NT) factors. Prior work in neonatally deafened cats has shown that intracochlear electrical stimulation only partially prevents SG degeneration after early deafness. Thus, NT agents that may be combined with stimulation to improve SG survival are of interest. Recent studies reporting that direct cochlear infusion of BDNF promotes SG survival after deafness and is additive to stimulation effects in reducing SG degeneration have been conducted in rodents and limited to fairly short durations. Our study evaluated BDNF infusion over a longer duration and in the developing auditory system of cats that may better model the slow progression of SG degeneration in the human cochlea. Kittens were deafened as neonates (neomycin, 60 mg/kg SID) and implanted at 4-5 wks. of age with a scala tympani electrode with a drug-delivery cannula connected to a mini-osmotic pump. Pumps were changed to allow continuous infusion of BDNF (94µg/ml; 0.25µl/hr) or artificial perilymph for 10 weeks.

Previously, SG survival was evaluated using volume density, an unbiased stereological method reflecting both cell size and number. In this study, SG cell somata in BDNF-treated cochleae had a mean cross-sectional area of 253µm^2, which was 29% larger than cells on the deafened control side (196µm^2). Thus, to accurately assess the density of surviving SG neurons, we used a physical disector stereological method to count SG nucleoli in serial 5µm plastic sections. Mean SG density in the BDNF-infused cochleae (77.8 cells/[100µm] ^{3}) was 24% higher than on the contralateral side (62.9 cells/[100µm] ^{3}), indicating a significant effect of BDNF (P=0.001) in promoting survival of SG neurons in these developing animals. Other interesting findings after BDNF infusion included a higher density of myelinated radial nerve fibers in the osseous spiral lamina and sprouting of fibers into the scala tympani.

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613 Intracochlear Infusion of Brain-Derived Neurotrophic Factor (BDNF) Results in Decreased EABR Thresholds and Increased Density of Radial Nerve Fibers in Cats Deafened as Neonates

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After the loss of cochlear hair cells in sensorineural hearing loss, the spiral ganglion (SG) neurons degenerate progressively, often over a prolonged period. The integrity and survival of SG neurons is assumed to be important to cochlear implant function, and many studies suggest that SG maintenance depends on both neural activity and neurotrophic molecular support. In this study, electrophysiological and histological data were evaluated to assess the effects of a neurotrophic agent (BDNF) in the developing auditory system of cats. Kittens were deafened as neonates and implanted unilaterally at 4-5 weeks of age with scala tympani electrodes containing 4-6 wires for electrical stimulation and an integrated drug-delivery system for infusion of neurotrophic agents. Electrically-evoked auditory brainstem responses (EABR) were recorded longitudinally while animals received 10 or 18 weeks of either BDNF or artificial perilymph (AP).

Comparison of initial and final EABR thresholds in animals that received BDNF (n = 6) demonstrated a significant decrease in thresholds (mean difference = - 5 dB; -143 µA, P = 0.001; paired t-test). Animals that received AP (n = 4) showed no significant shift in threshold (mean difference = +1 dB; +9 µA, P = 0.728; paired t-test). Further, neural thresholds recorded in the inferior colliculus of the same subjects were generally lower than and co-varied with final EABR thresholds.

Histological studies of BDNF-treated cochleae showed improved SG cell survival (reported elsewhere) and also revealed extensive populations of myelinated radial nerve fibers in the osseous spiral lamina and sprouting of fibers into the scala tympani. Fiber density was assessed using a pixel intensity scan of optical images. Measurements showed a significantly higher density in BDNF-treated cochleae as compared to the contralateral deafened cochleae (P = 0.001; unpaired t-test), suggesting an increase in fiber density and/or fiber size. The proliferation of radial nerve fibers may contribute to the improved EABR thresholds in these animals after BDNF infusion.

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614 Effects of Brain Derived Neurotrophic Factor (BDNF) and Electrical Stimulation on the Survival of Spiral Ganglion Cells in Cats

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Survival of spiral ganglion (SG) neurons is dependent upon both neural activity and neurotrophic (NT) factors expressed by SG neurons and supporting cells. In earlier studies we have shown that electrical stimulation promotes SG survival in vivo in cats deafened early in life, but SG density was still significantly below normal. In this study we explored the effect of prolonged intracochlear infusion of BDNF on SG cell survival. Kittens were deafened at 30 days of age by systemic administration of neomycin (60 mg/kg SID). After profound deafness was confirmed, animals were implanted (at ~8 wks of age) with scala tympani electrodes containing 4 wires for electrical stimulation and a drug-delivery cannula attached to an osmotic pump. The pump was changed 2-3 times to allow BDNF infusion for 12-14 weeks, either alone or combined with electrical stimulation.

BDNF promoted a marked increase in SG cell size, in contrast to the reduction in cell size caused by deafness in the non-implanted ears. The mean cross-sectional area of cells in the BDNF-treated cochleae was significantly (32%) larger than cells in contralateral non-treated cochleae (t-test, $p<0.01$). A similar 27% difference in SG cell size was observed in cochleae after combined electrical stimulation and intracochlear BDNF infusion. Due to these differences in cell size a physical disector method was used to estimate the number of surviving SG cells. The major effect of BDNF treatment on cell number was observed in the basal part of the cochlea, where the mean numerical density was 88 cells/[100µm]³. This is similar to the SG density in control subjects studied at the time of implantation (about 90 cells/[100µm]³), suggesting that most of the neurons in this region were maintained both by BDNF treatment alone and BDNF treatment combined with electrical stimulation. SG density differences in more apical regions were more variable and may be related to different electrode designs and site of BDNF delivery.

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615 Artemin Improves Survival of Spiral Ganglion Neurons in Vitro and in Vivo

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Introduction: The glial cell line-derived neurotrophic factor (GDNF)-family consists of four growth factors. In particular GDNF and artemin (ARTN) and their receptors GFRa1, GFRa2 and GFRa3 are up-regulated in the auditory nerve of deafened rats. This possibly represents an intrinsic protective mechanism against ototoxicity-related apoptosis. Hitherto, a neurotrophic effect of ARTN on

spiral ganglion neurons (SGN), however, has not been demonstrated. Therefore, aim of the study was to investigate the biological effects of ARTN on SGN in vitro and in vivo.
Methods: Spiral ganglion neurons were isolated from neonatal Sprague-Dawley rats (P 3-5) and cultivated in a serum-free medium for 48 hours. Culture media were supplemented with ARTN and/or BDNF. Survival rates and neurite lengths were determined after fixation of the cultures. Furthermore, using a miniosmotic pump ARTN was delivered to the inner ear of systemically deafened guinea pigs. The in vivo effects of ARTN on SGN density were compared with the effects of BDNF and artificial perilymph.
Results: Compared to the negative control survival rates were significantly ($p<0.01$) improved after cultivation of SGN in the presence of BDNF (50 ng/ml) or ARTN (75 ng/ml and 100 ng/ml). Combined treatment of SGN with BDNF and ARTN did not further increase the survival rate when compared to treatment with either factor alone. However, a combined treatment with both growth factors led to significant neurite outgrowth when compared to the negative control and to treatment with BDNF alone ($p < 0.01$). The biological effects of ARTN were also demonstrated in vivo.
Conclusion: As a conclusion, ARTN improves survival of isolated rat SGN and enhances neurite outgrowth when combined with BDNF. Also, ARTN acts in vivo on survival of SGN after aminoglycoside-induced degeneration.

616 Power-Law Adaptation in the IHC-AN Synapse Model Can Explain Neural Adaptation to Sound-Level Statistics

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Although rate-level functions of auditory neurons show a restricted dynamic range, the auditory system encodes sound levels with remarkable accuracy over a wide range of levels. Recently, Dean et. al. [Nature Neuroscience, 8(12), 1684-1689, 2005] showed that the responses in the auditory midbrain shift as a function of the distribution of stimulus sound levels. The shifts serve to improve the accuracy of the neural population code near the sound levels that were most probable in the distribution. This adaptation is characterized by a shift in the rate-level function with threshold approaching the most commonly occurring sound levels, and by a change in the slope of the rate-level function. However, the mechanism and origin of this adaptation along the auditory pathway remain unclear. We hypothesized that an auditory-nerve (AN) model with appropriate long-term dynamics in the inner-hair-cell (IHC)-AN synapse could account for this adaptation.
To better describe rate adaptation in the stimulus offset and several long-term response properties of AN fibers, we developed an AN model that includes both exponential and power-law adaptation rather than only exponential adaptation in the IHC-AN synapse complex. The much longer "tail" on the power-law function produces a longer memory for past responses than does exponential adaptation. We tested this model with stimuli similar to those used by Dean et. al., and found that the AN rate-level functions shifted toward the most commonly occurring sound levels with a decrease in slope and maximum rate. The time course of this adaptation also matched the data [Dean et. al., J. Neurosci., 28(25), 6430-6438, 2008].
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617 Threshold Tuning Curves of Chinchilla Auditory-Nerve Fibers Predict Cochlear Phase-Frequency Curves and Impulse-Response Frequency Glides

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Six properties of the responses of auditory-nerve fibers (ANFs) undergo transitions near the same characteristic frequency (CF), 1 kHz. The transition in one of these properties, (1) the asymmetrical shape of frequency-threshold tuning curves, FTCs (A. N. Temchin et al., *J. Neurophysiol.* 100, in press, 2008), accounts qualitatively for the transitions at the same CF of (2) the SPL dependence of rate-frequency curves (e.g., J. E. Rose et al., *J. Neurophys.* 34: 684-699, 1971) and, in combination with labile cochlear non-linearity, of (3) alterations of best frequency induced by systemic injection of furosemide (W. F. Sewell, *Hear. Res.* 14: 305-314, 1984). We now show that the transition in the shapes of FTCs probably also accounts for transitions in (4) the shapes of phase-vs.-frequency curves (A. N. Temchin and M. A. Ruggero, *A.R.O. M.W.M. Abst.* 24: 156, 2001) and in (5) the direction of the frequency glides (i.e., from low to high or vice versa) of impulse responses of ANFs at the same, 1-kHz, CF region. Furthermore, impulse responses derived from FTCs (Temchin et al., *op. cit.*) predict the main features of the impulse responses and the phase-vs.-frequency curves of ANFs, and probably also basilar-membrane vibrations, throughout the cochlea. A sixth property of ANF responses, the frequency dependence of compression (N. P. Cooper and G. K. Yates, *Hear. Res*. 78: 221-234, 1994), also undergoes a transition at a CF of 1 kHz. It is not clear whether that property is also related to the asymmetry of FTCs. *We were supported by NIH Grants DC-000110 and DC-000419.*

618 Phase-Frequency Functions for Chinchilla Auditory-Nerve Fibers with Characteristic Frequencies Beyond the Phase Locking Range

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M. van der Heijden and P. Joris (*J. Neurosci.* 23: 9194-9198, 2003) devised a method, based on stimulation with tone complexes (“zwuis stimuli”), that allows specifying the response phases of auditory-nerve fibers (ANFs) with characteristic frequencies (CFs) in the non-phase-locking frequency range (≥ 3-4 kHz). However, zwuis stimuli only yield *relative* phases, even when concatenating the responses of each ANF to multiple zwuis stimuli with partly

overlapping frequency ranges. We are conducting experiments in chinchilla in which recordings of ANF responses to zwuis stimuli are complemented by recordings of responses to low-frequency tones in the same ANFs. By combining responses to the two types of stimuli the otherwise relative phases of responses to zwuis stimuli are anchored by the phases of responses to low-frequency tones. After compensation for synaptic and neural delays, the resulting phase-frequency curves cluster around a constant lag of 2 periods relative to rarefaction at the eardrum and are very similar to phase-frequency curves for basilar-membrane (BM) responses at sites of the chinchilla cochlea with comparable CFs (e.g., 6-14 kHz: M. A. Ruggero et al., *J.A.S.A.* 101: 2151-2163, 1997; W. S. Rhode and A. Recio, *J.A.S.A.* 107: 3317-3332, 2000; W. S. Rhode*, J.A.S.A.* 121: 2792-2804, 2007). In conjunction with findings for chinchilla ANFs with CFs ≤ 3-4 kHz (A. N. Temchin and M. A. Ruggero, *A.R.O. M.W.M. Abst.* 24: 156, 2001), the present results suggest that BM response phases at CF are similar, about -2 periods re rarefaction, throughout most of the extent of the chinchilla cochlea, excepting the extreme apex. *We were supported by NIH Grant DC-000419.*

619 Rate Threshold of Auditory Nerve Fibers, Shape of Period Histogram and Spontaneous Activity Are Interrelated

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The relation between instantaneous sound pressure p(t) of pure-tone stimuli and instantaneous spike rate I(t) in period histograms can be described by the exponential relation $I(t)=I_0\exp(\alpha p(t))$ (Horst et al., *44th IEB-Workshop*, p83, 2007) in the region between spontaneous activity I_0 and rate threshold. This relation holds for low, medium and high-spontaneous rate fibers. The factor α describes the sensitivity of the nerve fiber: the higher the threshold, the smaller α. We investigated the relation between effective sound pressure p_{eff} and average spike rate I by means of simulation.

The spike rate was determined as the average of I(t) over one period of the stimulus $p(t)=p_0\sin(2\pi t/T)$. Using α as a parameter we calculated I as a function of p_0.

This average rate I increased monotonically with $p_{eff}=p_0/\sqrt{2}$. The relation was not linear, neither on linear scales nor on logarithmic scales. Variation of α produced a horizontal shift of the curves on a $\log(p_{eff})$-scale, i.e. a horizontal shift on a sound level scale: the smaller α the further the curve shifted to higher sound levels, the higher the rate threshold. Variation of I_0 produced a multiplication of the function on a linear I-scale. At threshold α and p_{eff} were inversely proportional, so on logarithmic scales the relation followed a line with slope -1. The smaller the instantaneous rate I_0 , the larger increase of p_{eff} was needed to reach threshold. So for a given value of α a higher threshold was found for smaller instantaneous rate. This is one possible explanation for the higher thresholds that are usually found for fibers with low spontaneous rates.

Using a fixed criterion we found that rate threshold depends on α in way that is nicely in agreement with our single-fiber data. The parsimonious description $I(t)=I_0\exp(\alpha p(t))$ provides simple relations between α, threshold and spontaneous activity, on a dB-scale:
Thr = 104.3 dB – $20.^{10}\log(\alpha)$ dB, for I_0 =1 spike/s
And Thr = 96 dB – $20.^{10}\log(\alpha)$ dB, for I_0 =20 spks/s.

620 Towards a Unifying Basis of Auditory Thresholds: The Shapes of Rate-Level Functions of Auditory-Nerve Fibers Revisited

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The seminal study of Sachs and Abbas (1974) modelled the discharge rate vs. sound level functions of auditory-nerve fibers (ANFs) as the result of the interaction of a 'mechanical stage', describing basilar membrane displacement as a function of sound amplitude, with a 'transducer stage', converting displacement into ANF discharge rate. The latter stage was modelled as a saturating power law with a power of 1.77. Spontaneous rate (SR) was assumed to simply add to the sound-driven rate. Later investigators proposed an integer power of 2 or powers varying between about 1 and 3, depending on SR.

Apart from the added complexity required to explain different powers, the suggested values are difficult to reconcile with a related phenomenon, the dependence of detection thresholds in quiet on sound duration. We showed that such thresholds can be understood as resulting from probability summation of detection events whose rate is proportional to the 3rd (but no lesser) power of sound amplitude (Heil & Neubauer 2003). Support for a power of 3 was also derived from the dependence of ANF first-spike timing on sound amplitude and rise time (Neubauer & Heil 2008; Heil et al. 2008).

Here, we present a physiologically plausible modification of the 'transducer stage' which assumes a saturating power law with a power of 3, independent of SR or characteristic frequency (CF). We show that it accounts well for cat ANF rate-level functions measured at frequencies well below CF, where the mechanics are linear (Robles & Ruggero 2001). Further, SR and its correlation with ANF sensitivity are emergent properties of the model. The model also captures phase-locking at low sound frequencies.

Our analysis suggests that the 'transducer stage' operates with power 3 in all inner hair cells, while its sensitivity differs among synapses or ANFs. Our model unites, for the first time, absolute thresholds at the perceptual level with the shapes of ANF rate-level functions.

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621 Rapid Dynamic Range Adaptation in the Auditory Nerve

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The auditory system operates with fine behavioral acuity over a vast range of sound pressure levels well exceeding the dynamic range of most auditory neurons. Dean et al. (Nat. Neurosci. 8:1684) have reported that the dynamic range of midbrain auditory neurons adapts to the distribution of sound levels in a dynamic stimulus by shifting towards the most probable level. Here we show that dynamic range adaptation also occurs in primary auditory neurons for tone and noise stimuli.

We recorded from auditory-nerve (AN) fibers in anesthetized cats in response to continuous dynamic stimuli in which the level was randomly drawn every 50 ms from a broad distribution containing a 12-dB wide high-probability region (HPR) in which levels occurred with 80% probability. We found that the steep parts of rate-level functions shift systematically towards the HPR mean level. This dynamic range adaptation differs from classic short-term adaptation, which would produce a constant rate decrement with no change in sensitivity. The rate of dynamic range shift with HPR mean level ranged from 0.1 to 0.5 dB/dB, and was not correlated with characteristic frequency or spontaneous rate. Using stimuli in which the HPR mean level switches between two values every 5 s, we further found that dynamic range adaptation occurs over just a few hundreds of milliseconds.

Dynamic range adaptation across the AN fiber population improved the precision of level coding within the HPR. However, this benefit was partially offset by increasing compression of the range of firing rates with increases in HPR mean level. Dynamic range adaptation in the AN was weaker than in the midbrain, and not sufficient to prevent a pronounced degradation in the precision of level coding above 60 dB SPL. These findings suggest that rapid adaptive processing to the sound level distribution first occurs in the auditory periphery and is enhanced along the auditory pathway.

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622 Effect of Reverberation on the Directional Sensitivity of Auditory Neurons: Peripheral Factors

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Reverberation poses a challenge to sound localization because the superposition of acoustic reflections on the direct wavefront degrades directional information by causing temporal fluctuations in the interaural time difference (ITD) and decorrelation of the ear-input signals. We previously showed that ITD-sensitive neurons in the auditory midbrain show more robust directional responses in the presence of reverberation than predicted by a binaural model based on the long-term cross-correlation of the two ear-input signals (Devore & Delgutte, Abs. Comp. Sys. Neurosci. 2006).

To determine the extent to which this robustness is of central or peripheral origin, we recorded responses of auditory-nerve (AN) fibers in anesthetized cats to broadband noise in simulated reverberant and anechoic environments. The spike trains were analyzed using the shuffled correlation technique (Louage & Joris, J. Neurophysiol. 91: 2051-2065) in order to simulate processing by a bank of ideal coincidence detectors receiving inputs from left and right AN fibers. Consistent with the midbrain results, we found that the directional information (as measured by the peak shuffled correlation) was degraded in reverberation, and that the degree of degradation increased with characteristic frequency.

We also analyzed how the shuffled correlation changes dynamically over the time course of the stimulus. For anechoic stimuli, the peak correlation remained relatively constant throughout the stimulus, while for reverberant stimuli the correlation was higher in the early portion of the stimulus, when the reverberant energy is still building up. Ongoing analyses are aimed at testing the hypothesis that short-term adaptation in the AN helps robust coding of directional information in reverberation by enhancing responses near the onset of the stimulus where the degree of coincidence is high.

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623 High Frequency Plateau in Gerbil Auditory Nerve Tuning Curves

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Ruggero *et al*. (PNAS 97(22) p.11744, 2000) compared threshold tuning curves of chinchilla basilar membrane (BM) vibrations and auditory nerve (AN) fibers. They noted that the AN tuning curves lacked the supra-CF frequency plateaus that are present in BM responses and suggested that BM vibrations do not translate into AN responses in the supra-CF frequency region. This observation is relevant to the relationship between BM motion (macromechanics) and hair cell stimulation (micromechanics). To further investigate the discrepancy, we recorded AN responses from the gerbil, concentrating on the supra-CF region of single AN fibers. We observed a supra-CF frequency plateau in AN responses at very high sound pressure levels (> 100dB SPL). This AN plateau was at least 10 to 15dB higher than what is predicted from BM plateaus.

However, at high sound pressure levels, we also recorded subharmonics in the acoustic signal in the ear canal. The subharmonics were not produced by the speaker and appeared to be produced in the auditory mechanics. Dallos and Linnell (JASA 40(3) p.561, 1966) studied similar subharmonics, and concluded that they were produced in the ear drum and in the cochlea. We still

need to determine whether the subharmonics are responsible for the supra-CF frequency plateaus in the AN responses or if the AN responses are due to the motion of the BM at the fundamental frequency.
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624 Predicting Auditory Nerve Status Using the Compound Action Potential

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The long-term goal of this research is to develop more precise techniques to characterize inner ear transduction and auditory nerve function. Future progress in hair cell regeneration and genetic therapy will require diagnostic tools that can identify the appropriate target within the cochlea and auditory nerve for delivery of therapeutic agents. The compound action potential (CAP) may be a useful tool for specifying the underlying pathophysiology in sensorineural hearing loss. Our approach has been to use Goldstein & Kiang's (1958) convolution model that includes a parameter representing the number of nerve fibers contributing to the CAP. The purpose of this study was to determine if this parameter (N) was correlated with the actual number of fibers in an animal model with auditory nerve lesions.

Mongolian gerbils with surgically-induced auditory nerve lesions (n=10) were compared to a control group of gerbils with normal ears (n=20). CAPs were recorded from an electrode placed on the round window with tone-burst stimuli ranging from 1000-16000 Hz at levels ranging from 10-100 dBSPL. Cubic distortion products were measured in all animals to ensure normal cochlear function. Animals were euthanized and histologic sections of the auditory nerve were prepared for stereologic examination.

For normal ears, the average fiber count was 16,880 (± 3,002 SD). For lesioned ears, our sampling technique to estimate the number of fibers was confounded by the lack of uniformity of damage throughout the nerve sections. Thus, we calculated the ratio between total damaged area and total nerve area and used percentage of nerve damage as our dependent variable. The percentage of nerve damage was inversely related to the N parameter at high signal levels across all stimulus frequencies. This relation suggests that the N parameter derived from the CAP may be able to predict the status of the auditory nerve.

625 Histochemical Analysis of Mitochondrial Enzyme Activity in Deafferented Chick Auditory Neurons

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Chick auditory neurons in nucleus magnocellularis (NM) receive excitatory input solely from eighth nerve afferents. Permanent cessation of these afferents via cochlea removal triggers a cascade of events within NM, including the death of 20-40% of neurons. Previous studies by our lab and others showed activation of apoptotic caspases throughout deafferented NM, in both dying and surviving neurons. TUNEL labeling of degraded DNA, a late event in the apoptotic pathway, was identified in the subpopulation of deafferented neurons undergoing death. Interestingly, TUNEL labeling accumulated in cellular cytoplasm, suggesting a role for mitochondrial DNA in cell death. Here, we evaluate activities of three mitochondrial enzymes within deafferented NM. P10-P14 hatchlings underwent unilateral cochlea removal. Contralateral NM neurons remained unperturbed and served as within-animal controls. Chicks were sacrificed 12, 24, 48, and 168 hours later. Brains were harvested, frozen in heptane cooled by dry ice, and sectioned on a cryostat. Serial sections through NM were mounted onto three series of slides and stained with cytochrome oxidase (CO), succinate dehydrogenase (SDH), and ATPase, respectively. A fourth series of adjacent NM sections was mounted and stained with thionin to assess ribosomal Nissl integrity, a known correlate of cell fate. Optical densities were measured in individual neurons and compared across time points. Twelve hours after cochlea removal, the CO histogram of deafferented NM shows a broader spectrum of optical densities than for contralateral neurons. By 48 hours, a small population of neurons with low CO activity persists, perhaps representing the thionin-negative, dying neurons. Examination of the SDH and ATPase histograms shows the emergence of two distinct subpopulations within deafferented NM by 12 hours. Mitochondrial enzyme activity varies across deafferented NM, implying that mitochondrial health may affect deafferentation-induced cell death.

626 Manipulating Neural Circuits of the Auditory Hindbrain with Light

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The lower auditory system is characterized by complex parallel processing performed by a large number of nuclei. To investigate the underlying neural circuits we are developing a method to manipulate and control single projections with a temporal resolution similar to electric stimulation, but with higher specificity and spatial resolution. Such a tool would allow to precisely control single components of the neural circuits and facilitate their investigation.

A promising approach to this aim is the use of light-sensitive ion channels such as Channelrhodopsin 2 (ChR2) from the green alga Chlamydomonas reinhardtii, or light-sensitive chloride pumps such as Halorhodopsin from the archaeon Natronomonas pharaonis. We obtained stable in vivo expression of those proteins in Mongolian gerbils (Meriones unguiculatus) by stereotactic injections of various viral constructs containing DNA for those proteins. Additionally, recordings in brain slices obtained from transfeced animals confirmed the physiological activity of these constructs in auditory neurons.

In one series of experiments, the light sensitive channels were introduced into auditory neurons via lentiviruses. These viruses are well suited for chronic expression of the desired channels, as well as cell type specific expression.

Therefore, lentiviruses are ideal tools for any type of experimental approach where manipulations of longer time scales are required. Alternatively, the same constructs were introduced into auditory brainstem neurons via Semiliki-Forest viruses, whose hallmark is extremely fast and non cell-specific expression of the target protein at very high levels. These constructs are ideal tools for experiments where long incubation periods are not desired. The availability of constructs based on various virus types with the same target construct establishes a set of tools for the manipulation of neural circuits in very specific and new ways.

627 Histological and Physiological Properties of Channelrhodopsin-2 in the Rat Dorsal Cochlear Nucleus

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Recently, channelopsin-2 (Chop2) has been cloned from Chlamydomonas reinhardtii and forms membrane cation channels directly gated by light. Application of channelrhodopsin-2 (ChR2, Chop2 with an attached chromophore) into the cell membrane and activation by light (450 nm + 25) can cause depolarization of neurons.

We injected an adeno-associated virus 2 construct containing the ChR2 and green fluourescent protein transcript into the fusiform layer of dorsal cochlear nucleus (DCN) to determine the efficacy of transfection in specific CN cell types. We used the stereotaxic coordinates consistent with Paxinos and Watson (AP 1.16 relative to bregma, L 0.32 and DV 0.49 from pia). Five weeks later we observed ChR2 labeled fusiform, giant, stellate and elongated cells.

Auditory brain responses (ABRs) were used to determine whether activation of ChR2 in DCN neurons was capable of transmitting neuronal signals throughout the auditory pathways. Intact animals produced ABR waveforms I - V. Preliminary data indicated that the surgical method for DCN exposure caused a 5 dB hearing loss in both non- and ChR2 injected animals. Exposing the DCN of animals with no ChR2 produced normal ABR waveforms. Exposing the DCN to light (Royal Blue LED compatible with ChR2 stimulation) 5 cm above the DCN for up to 110 seconds resulted in no ABR waveforms. Intact animals injected with ChR2 produced ABR waveforms that were similar to the non-ChR2 group. However, light stimulation of the DCN produced only one ABR waveform.

These results indicate that adeno-associated virus 2 is capable of infecting several cell types in the DCN with ChR2 and stimulation of DCN results in synchronous neuronal activity detectable by ABR. Further studies are needed to determine the concentration of adeno-associated virus 2 containing the ChR2 construct necessary for maximal transfection of DCN neurons, the time and intensity of light necessary for robust activation of DCN neurons.

628 Localization and Gene Expression Levels of Two Pore Domain Potassium Channels in Sound Activated Cochlear Nucleus Neurons

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Two pore domain potassium channels (K2PDs) allow background leakage of K+ thereby regulating intrinsic neuronal excitability. Previous studies identified K2PD leak currents, K2PD expression and subunit production in the CN (Holt et al., 2006; Kim et al., 1994; Pal et al., 2005 Rothman and Manis, 2003). These results implicate alteration of K2PD levels as a possible mechanism for the modulation of neuronal excitability in the CN.

In the current study, K2PD gene expression was examined in sound exposed (10 kHz 118 dB 4hrs) adult male rats 24 hours following exposure and compared with the normal hearing group. Subdivisions (DCN and VCN) and cell types of the CN were assessed in separate experiments. In addition two of the K2PD subunits were localized to specific cell types identified by back-filling CN neurons with injections of fluorogold into either the inferior colliculus, medial nucleus of the trapezoid body or the lateral superior olive.

In the DCN, TASK1 was expressed in fusiform cells and small somata within the deep core layer while in the AVCN stellate cells were immunoreactive for this isoform.

To determine the relationship between neuronal activity caused by sound and changes in K2PD gene expression, first we have compared the levels of gene expression in DCN and VCN following sound exposure. These experiments demonstrate increased levels of TASK1 and TASK3 in the DCN while TASK5 was decreased. Within the VCN, TASK1 and TASK5 showed decreases while TASK3 levels were increased. Second, we immunolabeled back-filled CN neurons with cFos (marker of neuronal activation) and compared expression levels of TASK1, TASK3 and TASK5 in activated versus non-activated neurons. Stellate cells within the AVCN appeared to be activated by sound as determined by colocalization with cFos and TASK3 was significantly decreased in these cells after sound exposure.

629 Hyperpolarization-Activated Conductances Are Reduced in Neurons of the Ventral Cochlear Nuclei of Mice That Lack the HCN1 α Subunit

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Hyperpolarization-activated conductances (gh) are prominent features of neurons in the ventral cochlear nucleus. They are exceptionally large in octopus cells and are also present in bushy and stellate cells. The ion channels that underly these conductances are tetrameric combinations of hyperpolarization and cyclic nucleotide gated (HCN) α subunits 1-4. HCN1, the subunit with the fastest kinetics and lowest cyclic nucleotide sensitivity, is

strongly expressed in the ventral cochlear nucleus including in the octopus cell area (Koch et al., Eur J Neurosci 20:79, 2004). To understand the role of gh in determining the biophysical properties of neurons in the ventral cochlear nucleus, whole-cell patch-clamp recordings were made from neurons in mice that lack the HCN1 α subunit. HCN-/- mice are healthy and their robust Preyer reflex indicates that they hear. In HCN1-/- mice, rates of activation and deactivation and the maximal amplitude of gh were reduced. The amplitude was reduced most, by about 60%, in octopus cells. The reduction was smaller in T stellate cells, about 30%. In bushy cells the reduction was difficult to measure because even in the wild type there is great variability. The half-activation voltages were more hyperpolarized in octopus and bushy cells, but not in T stellate cells, in HCN1 -/- mice than in wild type controls. The reduction in gh was accompanied by increases in the input resistance but not by changes in the resting potential. This work was supported by a grant from NIH DC00176.

630 Postsynaptic P2Y Receptors Mediate Ca^{2+} Signals at Developing Endbulb of Held Synapse

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Recent studies suggested a prominent role of purinergic signaling in the peripheral auditory system i.e. in the cochlea, which prompted us to investigate whether the signaling mediated by P2 receptors might also play a role at the large calyceal synapse (endbulb of Held) in the cochlear nucleus (CN) of Mongolian gerbil. To address this issue, we performed a Ca^{2+}-imaging study (partially in combination with whole cell recordings) on acute brainstem slices from gerbils P9-14 and recorded responses in the large spherical bushy cells (SBC). First, large Fura-2 positive cells from bulk labeled slices were electrophysiologically characterized as SBC by means of current clamp recordings. Further characterization, assessed *post hoc* by immunocytochemical labeling of the biocytin-filled neurons, confirmed morphological characteristics of large SBC. Next, the P2 receptor mediated Ca^{2+} responses were delineated pharmacologically. Extracellular ATP and ADP, applied per bath perfusion, increased $[Ca^{2+}]_i$ in a dose dependent manner. These Ca^{2+} transients were effectively blocked by PPADS and to a lesser extent by suramin. The responses to ADP showed no exclusive dependence on extracellular Ca^{2+}. They were inhibited by a PLC blocker U73122, and by a calcium ATPase blocker cyclopiazonic acid, suggesting the involvement of metabotropic P2Y receptors. Ca^{2+} signals were reliably evoked by more specific $P2Y_1$-receptor agonists ADPβS and 2-methyl-thio-ADP and the amplitudes of the responses were similar to those of ADP. Furthermore, the specific $P2Y_1$ receptor-antagonist MRS2179 inhibited ADP-induced and – to a lesser extent – ATP-induced Ca^{2+} signals in a dose-dependent manner. Immunohistochemical staining of $P2Y_1$ receptors confirmed the Ca^{2+}-imaging data. Thus, it is concluded that large SBC express P2 receptors of the $P2Y_1$ subtype which could be involved in the SBC signal transduction mechanisms during the initial phase of auditory signal processing.

631 Presynaptic Nicotinic Modulation of Endocannbinoid Signaling Controls Synaptic Strength and Plasticity

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Nicotinic AChRs mediate modulatory effects on synaptic transmission and plasticity. Here, we reveal a novel mechanism via which a nAChRs modulate endocannabinoid (EC) signaling in the dorsal cochlear nucleus. Our findings indicate that the effect of nAChRs on EC signaling determines synaptic strength, short-term plasticity, as well as the size and sign of long-term synaptic plasticity in an auditory brainstem nucleus. Moreover, the interaction between nAChRs and EC signaling shapes the timing window over which coincident pre- and postsynaptic activity is capable of inducing synaptic plasticity. Our findings link nicotinic and EC signaling and may uncover a general gating mechanism of EC signaling by nAChRs that regulates synaptic transmission and synaptic plasticity throughout the brain.

632 Cell-Specific Synaptic Plasticity in Feedforward Inhibitory Circuits Leads to Adaptive Temporal Integration of Synaptic Inputs

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Spike timing precision and the temporal resolution of neuronal integration depend on the period or window within which excitatory inputs summate to reach the threshold for spike generation (integration window, IW). Our recent work in the dorsal cochlear nucleus (DCN) has demonstrated unique, opposing forms of spike timing-dependent synaptic plasticity (STDP) at excitatory synapses onto principal (fusiform) and feedforward inhibitory (cartwheel) interneurons. In fusiform cells, spikes evoked 5 ms after parallel fiber EPSPs led to Hebbian LTP, while the same EPSP-spike protocol led to “anti-Hebbian” endocannabinoid-mediated, LTD in cartwheel cells. These findings are unique and suggest that cell-specific STDP, by shifting the balance of excitation and inhibition towards excitation, may provide a mechanism allowing modulation of IW and spike timing precision. To test the effect of cell-specific STDP on the IW, we performed two-pathway experiments in fusiform cells. First we determined the IW of fusiform cells under control conditions. Two pathways were adjusted to evoke a spike in 50% of trials when delivered synchronously, but subthreshold when delivered alone. Our experiments showed that the duration of the IW dropped significantly at 2-3 ms thus tracking the time course of the previous studies in other feedforward inhibitory loops. Then, we

examined the effect of cell-specific synaptic plasticity in the DCN. We induced LTP in path 1 by pairing an EPSP with action potentials. To induce LTD in cartwheel cells, which has been shown to be mediated by endocannabinoid signaling, we took advantage of the finding that 50 nM WIN-55,212-2 (cannabinoid receptor, CB1R agonist) depresses parallel fiber inputs to cartwheel cells, but does not affect parallel fiber inputs to fusiform cells. The combination of electrically-induced LTP in fusiform cells and application of 50 nM WIN led to increased EPSP and decreased disynaptic IPSP. After induction of LTP in the fusiform cell and after waiting for WIN application to reach a plateau, we measured the IW as previously described. The probability of spiking was shallower after the induction of LTP in fusiform cells and LTD-like depression in cartwheel cells. Thus, when cell-specific synaptic plasticity occurs, it is accompanied by a widening of the time window for action potential generation. Such modulation would have important implications for spike timing and temporal precision of sensory representation

633 Dynamics of Synaptic Inputs to Bushy and Stellate Cells of the Mouse Anterior Ventral Cochlear Nucleus

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Bushy and stellate cells of the anterior ventral cochlear nucleus (AVCN) are innervated not only by the excitatory synaptic inputs from the auditory nerve, but also inhibitory inputs from non-primary sources including the dorsal cochlear nucleus (DCN). To compare the dynamics of these different synaptic inputs, we recorded synaptic currents from bushy and stellate cells in parasagittal brain slices of CBA mouse cochlear nucleus while stimulating either the auditory nerve root, or the deep layer of the DCN. Short stimulus trains at 50, 100, 200 and 400 Hz were delivered, and post-tetanic responses examined from 5 to 2000 ms. EPSCs in both bushy and stellate cells depress during the stimulating trains at all frequencies, with stellate cells showing less depression at a given stimulus frequency. However, IPSCs are quite different between bushy and stellate cells. The IPSCs in bushy cell facilitate early in the train, and show less depression late in the train, in comparison to EPSCs. IPSCs in stellate cells, surprisingly, showed strong facilitation throughout the train, with depression only at higher frequencies. The recovery of EPSCs in both bushy and stellate cells is approximately exponential, reaching ~80% of the control amplitudes within 2 seconds. However, the recovery of IPSCs in bushy cells is much faster than that of EPSCs returning to control amplitudes in less than a second. IPSCs in stellate cells show significant variation in amplitude between trials and recovered from tetanic stimulation with either a slow decay following facilitation at lower frequencies, or with a slow recovery from depression after the stimulation at higher frequencies. Our results suggest that the synaptic dynamics of both excitatory and inhibitory synapses onto bushy and stellate cells are different, but complementary. These differences in synaptic release and recovery kinetics are likely to play important roles in the processing of dynamic acoustic stimuli.

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634 The Innervation of T-Stellate Multipolar Neurons in Ventral Cochlear Nucleus

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Morphological differences suggest the existence of two distinct populations of multipolar neurons in the ventral cochlear nucleus (VCN). T-stellate (planar) multipolar neurons have dendrites that are oriented to isofrequency laminae of the VCN, spanning a narrow frequency range. Relative to auditory nerve fibers (ANFs), spectral representations coded by T-stellate multipolar neurons extend across a wider range of sound pressure levels and have greater spectral contrast in the presence of background noise. According to the “selective listening” model, extended dynamic range is achieved in the T-stellate multipolar neurons by selectively integrating inputs from low-threshold ANFs at low sound levels and from high-threshold ANFs at high sound levels. Selective integration is enabled by inhibitory inputs that shunt excitatory input from low-threshold ANFs at high sound levels. Glycinergic D-stellate multipolar neurons, having dendritic fields that span a wide range of isofrequency laminae, are the supposed source of this inhibition. As a test of the hypothetical circuit, this study describes the distribution of inhibitory D-stellate multipolar synapses on the somata and dendrites of T-stellate multipolar neurons.

As a first step in describing this synaptic distribution we labeled T- and D-stellate multipolar neurons and used pathway tracing techniques to determine synaptic locations in VCN. A tracer was used to label T-stellate multipolar neuron somata and dendrites while a second tracer was used to label D-stellate multipolar neuron axons and axonal swellings. Different fluorophores were bound to each tracer so components of T-stellate multipolar neurons could be distinguished from those of D-stellate multipolar neurons. Axonal swellings in close apposition to somata and dendrites were considered to be putative presynaptic terminals. The data indicate that D-stellate multipolar neurons innervate T-stellate multipolar neuron somata and dendrites, and that dendritic synapses can be located far (tens of microns) from the soma.

These preliminary results support the selective listening hypothesis and other neural circuit models based on such innervation. In future studies we aim to further quantify the innervation distribution, extending the pathway tracing across multiple tissue sections and comparing the D-stellate multipolar neuron synaptic distribution with the distribution of glycinergic synapses. Discrepancies in these distributions will indicate the uniqueness of the hypothesized role played by D-stellate multipolar neuron inhibition of T-stellate multipolar neurons.

635 Bushy Cell Network in the Ventral Cochlear Nucleus of the Rhesus Monkey

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Bushy cells (BCs) of the ventral cochlear nucleus (VCN) encode temporal features of the acoustic waveform and convey this information to upper auditory structures. The VCN output signals exhibit enhanced synchronization compared to those of the auditory nerve fibers, thus BCs might serve as coincidence detector. The morphological structures responsible for this mechanism have not yet been established. However, recent rodent data from our laboratory showed the existence of a BC network that might represent the neuroanatomical basis for such a paradigm. This network consists of specialized plasma membrane junctions between BC dendrites and somata, and their synaptic connections. To determine whether this network also exists in higher non-human mammals, we investigated the rhesus monkey VCN. By using a combined histological, immunocytochemical and ultrastructural approaches, we determined that BCs and their neural-synaptic network are indeed present in the primate. These cells were found to be organized in clusters, with BC dendrites oriented towards neighboring BC soma. Additionally, BC soma and dendrites were fully decorated with endbulb-like VGLUT1 immunopositive endings. Ultrastructurally, BCs were shown to be connected by dendro-somatic and dendro-dendritic specialized membrane junctions. We also found that the auditory nerve made multiple synaptic contacts between different BCs. All of these observations are consistent with what has been described in the rat, indicating that the cytoarchitectural organization of the VCN is well conserved across mammalian species. We propose that the BC network constitutes the neuroanatomical basis for the processing of acoustical information by individual BCs and for the synchronization of the final enhanced VCN output signal.

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636 Primary-Like, Non-Prepotential Cells in the Low-Frequency, Anteroventral Cochlear Nucleus Are Likely to Project to the Inferior Colliculus

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Anatomically and physiologically the anteroventral cochlear nucleus (aVCN) has been described to contain a rather homogeneous cell population, dominated by spherical bushy cells (SBCs). However, fluorogold (FG) injections into the inferior colliculus (IC) retrogradely labeled a moderate number of large, round cell bodies in both aVCNs. Even though by shape and location these neurons resemble SBCs, there are several lines of evidence that they are in fact not. (a) Double staining of the retrograde labeled neurons with calretinin, revealed large endbulb synapses at SBCs, but not at FG labeled cells. (b) Extracellular single unit recordings from SBCs show the typical complex waveform containing one presynaptic (PP) and two postsynaptic components. In the present sample of units from the rostral aVCN 48/75 did indeed reveal complex waveforms identifying them as SBCs, but there is a population of cells (27/75) intermingled with the SBCs that do not show complex waveforms. (c) Analyzing the postsynaptic waveform showed significantly longer spikes in SBCs than in non-PP-cells ($p=0.003$; SBC: 0.30 ±0.05 ms; non-PP-cells: 0.26 ±0.07 ms). Only 14% of the non-PP-cells showed chopper responses and therefore qualify to be multipolar (stellate) cells, while the remaining 86% do not. Our recordings yielded a significant number of non-PP-cells in the rostralmost, low-frequency part of the aVCN suggesting that the cell population in the rostral aVCN of gerbils is not as homogeneous as we had assumed so far. Therefore, it will be necessary in future studies of SBCs to confirm the targeted cells are contacted by endbulbs of Held, either by documenting the complex waveforms of the recorded signals or by staining the endbulbs. These non-PP-cells are either SBCs which do not receive large endbulb endings or they are stellate cells which do not have a chopper response pattern. In either case these cells, whose function will have to be revealed, establish projections to the IC.

637 Vesicular Glutamate Transporter 2 Is Associated with the Cuneo-Cochlear Nucleus Pathway in Guinea Pigs

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The cochlear nucleus (CN) receives primary input from the auditory nerve as well as numerous non-auditory inputs, some of which originate in somatosensory brainstem nuclei. We have previously demonstrated that projections from the spinal trigeminal nucleus(SP5) in guinea pig terminate primarily in the CN granule cell domain (GCD) and are associated with vesicular glutamate transporter 2 (VGLUT2); whereas auditory nerve fibers are associated with VGLUT1 (Zhou and Shore, J. Neurosci. Res. 78: 901-7; Zhou et al., J. Comp. Neurol. 500:777-87). The cuneate nucleus, which is a first-order somatosensory nucleus, has been shown to send projections to the CN in the rat (Wright and Ryugo, J.Comp. Neurol., 365:159-72). Here, we confirm the cuneo-cochlear nucleus pathway in guinea pigs using anterograde and retrograde track tracing methods combined with immunocytochemical analysis of vesicular glutamate transporters.

Following injections of biotinylated dextran amine(BDA) into cuneate nucleus, anterograde labeling was found primarily in the ipsilateral CN GCD, and was co-localized with VGLUT2. No projections were co-localized with VGLUT1. A retrograde tracer, either FluoroGold or BDA, was injected into the cochlear nucleus (DCN) to elucidate the source of this pathway in the guinea pig. Labeled cells were found primarily in the dorsolateral edge of the ipsilateral cuneate nucleus.

These findings indicate that in addition to trigeminal inputs, inputs arising in dorsal column nuclei also project to the

guinea pig CN, and like Sp5 projections, are associated with VGLUT2. Trigeminal and dorsal column inputs to the CN may play an important role in the modulation and generation of somatosensory-based tinnitus (Pinchoff et. al., Am. J. Otol., 19: 785-9; Biesinger et al., HNO 56:673-7).
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638 The Boundary Cap Cell Survival and Differentiation in Monoculture and Co-Culture with Auditory Brain Stem Slice Culture

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The regeneration of the auditory neural system remains a challenge in hearing restoration. One strategy would be the replacement of degenerated spiral ganglion neurons and cochlear nucleus neurons using cell replacement therapy. Stem cells are interesting candidates for cell replacement therapy. The boundary cap (BC) cells are the transient neural crest-derived cells located in the dorsal root entry zone that have been shown to differentiate into sensory neurons and glia in vivo. These cells comprise a source of multipotent sensory specified stem cells. Here we cultured the BC cells in the conditioned medium, the medium collected from the brain slices. The results demonstrated the auditory brain stem slices released some factors that essential for BC cell survival and differentiation. In order to visualize and study the biological properties of neurite outgrowth, the BC cells were implanted into the brain stem slices by using Stoppini method. This in vitro method allows to manipulate the survival and differentiated factors and auditory neural connections. This study showed the survival of the slices co-cultured with BC cells for a week. The differentiated sensory neurons showed the neurite outgrowth toward the cochlear nucleus. Our data suggests that the BC cells would be the alternative source for the degenerative auditory neurons to enhance the functionality after injury.

639 Distribution of Perineuronal Nets in the Human Superior Olivary Complex

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Perineuronal nets are specialized constructs of the extracellular matrix within the central nervous that are associated with limited neuronal populations within the human central nervous system. Perineuronal nets typically cover the soma, primary dendrites and the initial axon segment and have been specifically associated with fast-spiking neurons but may also function in plasticity and maintaining the local ionic environment. We have used histochemical and Nissl staining techniques to investigate the localization of perineuronal nets within the human superior olivary complex. Our preliminary results indicate that, within the human superior olivary complex, perineuronal nets are largely restricted to the medially situated periolivary cell groups, namely the superior paraolivary nucleus, the medial and ventral nuclei of the trapezoid body and the posterior periolivary nucleus. In these nuclei, perineuronal nets surround a fairly high percentage of neurons. Perineuronal nets are also found occasionally within the contours of the lateral nucleus of the trapezoid body, but almost never within the medial or lateral superior olives. These preliminary data provide evidence of a limited, but highly specific distribution of perineuronal nets within the human superior olive.

640 Synaptic Terminals on Middle Ear Muscle Motoneurons: Vesicle Morphometry

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The stapedius and tensor tympani (TT) are the two middle ear muscles; in rats, both contract in response to sound. The central inputs to the motoneurons controlling these muscles are responsible for regulating their contraction, but the morphology of their synaptic inputs is barely known. We used electron microscopy to quantify features of synaptic vesicles in terminals on retrogradely labeled rat stapedius motoneurons (SMNs) and tensor tympani motoneurons (TTMs). A total of 29 synaptic terminals were studied on two SMNs and 58 terminals on three TTMs. Terminals were previously categorized using qualitative methods into three major types using synaptic vesicle size and shape: 1) “large round”, 2) “small round”, or 3) “pleomorphic” terminals. There were roughly equal numbers of these types. Morphometric measurements of synaptic vesicles indicated a distribution of circularities ($4\pi(\text{area}/\text{perimeter}^2)$) and areas for the vesicles in each terminal, from which mean vesicle circularity and mean vesicle area were calculated. Pleomorphic terminals were separated from other types because they had lower mean vesicle circularities (< 0.85). Round vesicle terminals were subdivided by vesicle area: small round terminals had mean vesicle areas < 1500 nm2 whereas large round terminals had larger mean vesicle areas. In SMNs, vesicle area also separated terminals that contained dense core vesicles (small round terminals contained them, but large round terminals did not). In TTMs, though, examples of dense core vesicles were found in all three types of terminals. The data support our previous subjective classification of terminal types, and indicate that TTMs and SMNs have similar types of inputs. The presence of more TTM terminals containing dense core vesicles (that are associated with very active synapses), might predict differences in physiological response compared with SMNs. Supported by: NIDCD RO1 DC01089 and KO8 DC06285.

641 Cholinergic Varicose Fibers and Muscarinic Receptor Subtypes: Regional Distribution in the Mouse Brainstem

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Vesicular acetylcholine transporter (VAChT) is involved in acetylcholine transport into synaptic vesicles and is

regarded as a reliable marker for cholinergic terminals and preterminal axons. In the mouse brain, VAChT immunoreactivity was seen in almost all major cholinergic cell groups including brainstem motoneurons. We have investigated the distribution of cholinergic terminal-like structures in the mouse precerebellar nuclei by VAChT immunohistochemistry. We found that the immunoreactive varicose fibers were especially dense in the pontine nuclei. By retrograde neuronal labeling combined with VAChT immunohistochemistry, we demonstrated that VAChT-immunoreactive neurons in the laterodorsal tegmental nucleus and pedunculopontine tegmental nucleus send axons bilaterally to the pontine nuclei. Thus, it was assumed that mesopontine cholinergic neurons regulate neocortico-ponto-cerebellar projections at the level of the pontine nuclei. Muscarinic receptors (mAChRs) are widely expressed in the central and peripheral nervous systems and play an important role in modulating the cell activity and function. In mammals, five distinct subtypes (m1-5) have been identified on the basis of their primary sequences and pharmacological properties. The C57BL6J mice (male, ranging from 8 to 10 weeks old) were used in this study. We designed digoxigenin-labeled cRNAs based on the specific nucleotide sequence for m1-5. Using these riboprobes, we performed in situ hybridization and investigated the distribution of m1-5 mRNAs in the brainstem of the mouse. Since m2 and m3 mRNAs were preferentially expressed in the brainstem, we analyzed their distributions and measured the regional density of the distribution. We found a moderate to high level of accumulation of VAChT-immunoreactive fibers and of neurons expressing m2 and m3 mRNAs in several regions of the auditory brainstem. The results suggest the sites being under notable cholinergic regulations in the auditory brainstem presumably involved in the mechanisms of arousal and memory.

642 Duration Selectivity Underlies Responses to Social Vocalizations in the Inferior Colliculus

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Many animals use a variety of vocalizations to convey information to conspecifics. A fundamental function of the auditory system is to detect, discriminate and categorize these vocalizations so that appropriate motor responses can be produced. The means by which auditory neurons perform this function is not well understood. One way that individual neurons may discriminate among different vocalizations is by selectively responding to particular temporal or spectral features within the vocalizations. One temporal feature of vocalizations that neurons may selectively respond to is duration. Neurons in the inferior colliculus (IC) of many species are selective to the duration of pure tones, and the duration tuning characteristics of the neurons typically match the duration of the species' vocalizations. In the IC of mice, a high proportion of neurons are selective to pure tones longer than several tens of milliseconds. This selectivity qualitatively matches the range of vocalizations that are emitted by mice. However, duration selectivity has previously only been tested with pure tones and how these responses relate to encoding natural vocalizations has only been speculated. In this study, we systematically modified the duration of mouse ultrasonic vocalizations and recorded responses of single units in the IC of awake mice. We found that across the population of IC neurons, responses to vocalizations were heterogeneous and rarely predicted by the neuronal responses to pure tones. Altering the duration of vocalizations affected the response patterns of many neurons. Compressing vocalizations to half their original duration often reduced the neural responses and stretching vocalizations to two times their original duration often increased neural responses. Thus, the neurons selectively responded to vocalizations of particular durations. These findings suggest that duration selectivity is an important feature of IC neurons that enhances selectivity to vocalizations.

643 Spectrotemporal Feature Selectivity for Conspecific Vocalizations in the Auditory Midbrain

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Previous studies have shown that response selectivity for conspecific communication signals can be observed as early as the inferior colliculus (IC) in the auditory midbrain. Receiving a convergence of excitatory and inhibitory inputs from the brainstem, it is not surprising that emergent response properties like feature selectivity can arise in the IC. While it has been previously shown that blocking inhibitory inputs greatly reduced response selectivity, it is still unclear which spectral and temporal features of conspecific vocalizations are encoded by an IC neuron and how excitation and inhibition interact nonlinearly with the intrinsic membrane properties of IC cells to produce a feature selective output.

In this study, we recorded the extracellular response to a large repertoire of bat communication signals, extracted the average stimulus preceding each spike and analyzed its covariance. This allowed us to derive the relevant stimulus features (dimensions) that excite or inhibit the response of IC neurons. Furthermore, we were able to derive the nonlinearity associated with each feature by convolving that feature with all stimuli played and also with the stimuli that evoked a response. Most IC neurons we observed had at least three relevant features, with symmetric or non-symmetric nonlinearities. We show that the relevant stimulus features and their respective nonlinearities together define the overall receptive field of the neuron and provide better response predictions than using the spike-triggered average alone.

644 Non-Linear Mechanisms Underlying FM Direction Selectivity in the IC Revealed by Whole-Cell Patch-Clamp Recordings *in-Vivo*

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Frequency modulations (FMs) are critical components of auditory scene analysis and animal communication, including speech. Auditory neurons encode information about FM directionality by firing more to one FM direction than the other. The generally accepted mechanism for creating FM directionality assumes input linearity, where downward and upward FMs evoke synaptic conductance of identical magnitude, but different timing: this input "temporal-asymmetry" creates FM directionality. Studies suggest FM directionality is created by this linear mechanism in the inferior colliculus (IC), but evidence for input linearity has been inferred from spike rates measured from extra-cellular recordings. We tested the linear hypothesis directly using whole-cell patch-clamp recordings *in-vivo*.

We found that the mechanisms underlying FM directionality in the IC were predominantly non-linear, and that input temporal-asymmetry played at most a minor role. Contradicting the assumption of input linearity, downward and upward FMs evoked a "magnitude asymmetry": the total magnitude of the synaptic conductance evoked by downward FM sweeps *differed* from that evoked by upward FMs of identical frequency composition. This input *non*-linearity indicates that the pre-synaptic cells were themselves directionally selective. Modeling shows that the magnitude-asymmetries were more important for generating directionality in the post-synaptic potentials (PSPs) than temporal-asymmetries measured in the same cells. The second non-linearity was spike threshold. Directional selectivity in the spike counts was on average ~30% greater than selectivity in the PSPs, indicating that the spike-threshold non-linearity amplified FM directionality. Together, these results indicate that the major mechanisms underlying FM directional selectivity in the IC are non-linear, and that directionality is imposed on the IC by directionally selective neurons in the brainstem.

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645 Facilitation Shapes FM Sweep Selectivity in the Inferior Colliculus

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Recent studies of the mechanisms that shape the neuronal selectivity for the rate and direction of downward FM sweeps in the inferior colliculus of the pallid bat have revealed that inhibitory sidebands and short-pass duration tuning play major roles. Here we describe an additional mechanism, asymmetrical facilitation. Most of the neurons studied were "FM specialists" that responded to downward FM sweeps, but gave little or no response to single tones within these sweeps. However, pairs of tones presented at delays approximating their occurrence in sweeps did evoke responses greater than the sum of responses to individual tones, hence the term facilitation. Downward direction-selective neurons responded maximally to tone pairs in which the higher frequency preceded the lower. The delay producing a maximum response for a given tone pair predicted sweep rate selectivity (frequency difference/delay). Maximum facilitation could occur at stimulus offset as well as onset of the first tone, suggesting that a rebound from inhibition coinciding with excitation may contribute to facilitation. To delineate the excitatory and inhibitory regions of the receptive fields of these FM specialists, narrowband upward and downward FM sweeps were used, creating "FM tuning curves". Particularly remarkable was the finding that these neurons were direction-selective for sweeps as narrow as 2 kHz that remained within these receptive fields, suggesting that they have a precise spectrotemporal tuning for sweep direction over a frequency range that is narrower than the expected tuning of their excitatory and inhibitory inputs. One implication is that neuronal selectivity for complex vocalizations can arise through spectrotemporal tuning to a small fraction of the entire signal. We have reported similar properties in auditory cortex. Their presence in the inferior colliculus suggests that precise spectrotemporal tuning for FM sweeps first occurs at the midbrain level or lower.

646 Modulation of AMPA and GABA^{A} Receptor-Mediated Responses in Neurons of the Rat's Inferior Colliculus by Metabotropic Glutamate Receptors

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Many studies have shown that fast synaptic transmission in the inferior colliculus (IC) is mediated by ionotropic glutamate and GABA receptors, i.e., AMPA and $GABA_A$ receptors. However, few studies have examined metabotropic glutamate receptors (mGluRs) in IC. Immunocytochemical and *in situ* hybridization studies showed the distribution of different groups of mGluRs and their mRNA in the IC. But, the physiological role of mGluRs in synaptic transmission in IC is not well understood. The purpose of the present study was to investigate the contribution of mGluRs in modulation of synaptic responses mediated by AMPA and $GABA_A$ receptors in IC neurons. We also wanted to determine which group of mGluRs is involved in regulation of synaptic transmission in IC. Whole cell patch clamp recordings were made from IC neurons in brain slices of P8-12 rats. Excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) mediated by AMPA and $GABA_A$ receptors were induced by stimulation of the lateral lemniscus and isolated pharmacologically. The non-selective agonist of group I/II mGluR, ACPD (50 μM), reduced the amplitude of AMPA EPSCs (n=12) and $GABA_A$ IPSCs (n=10) by 41% and 66% respectively. The suppressive effects of ACPD were dose-dependent. ACPD did not have any effect on the neuron's resting potential, input resistance, action potential properties or firing characteristics. The specific group II mGluR agonist,

LY379268 (20 nM), also greatly suppressed the AMPA EPSCs and $GABA_A$ IPSCs. The ratio of the amplitude of 2nd to 1st AMPA EPSCs and $GABA_A$ IPSCs evoked by paired-pulse stimulation was significantly increased by ACPD and LY379268. In contrast, the group I and III mGluR agonists did not affect AMPA and $GABA_A$ synaptic responses or intrinsic membrane properties of IC neurons. All of these results suggest that fast glutamatergic excitation and GABAergic inhibition in IC can be modulated by group II mGluRs, likely through presynaptic mechanisms.
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647 Shut Up and Listen: Suppression of the Spontaneous Firing in Inferior Colliculus Neurons During Sound Processing

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Spontaneous activity is a widespread phenomenon in the central nervous system. Neurons in the central auditory system also show spontaneous firing. Little is known about its role in sound processing. The goal of this study was to determine whether spontaneous firing in inferior colliculus (IC) neurons is altered during sound processing. We conducted extracellular and intracellular recordings from IC neurons exhibiting spontaneous firing in awake little brown bats. Stimuli were frequency modulated (FM) downward sweeps (80-20 kHz, 4 ms duration) presented at different sound levels. The vast majority of IC neurons (86%) showed spontaneous firing ranging from 0.5 to 60 spikes/second (sp/s) with median of 6 sp/s. The majority of these neurons (65%) exhibited suppression of the spontaneous firing in response to an FM sweep. The duration of this suppression lasted hundreds of milliseconds and increased with sound level. The suppression was largely independent whether IC neurons exhibited firing in response to a sound. The neurons exhibiting lower rate of spontaneous firing showed longer duration of the suppression. To determine a cellular mechanism of the suppression we conducted intracellular recordings. Despite of our expectations, we failed to observe membrane hyperpolarization during the suppression. At the beginning of the suppression, however, many IC neurons exhibited a short (< 50 ms) membrane hyperpolarization. To check for a possibility of shunting inhibition, we hyperpolarized IC neurons during the suppression by applying a long (300 ms.) current pulse via recording microelectrode. We also measured and compared neurons' input resistance before, during and after the suppression. These two methods did not demonstrate any evidence of shunting inhibition during the suppression. Our data suggest that spontaneous firing in IC neurons is greatly suppressed during sound processing and this suppression is likely to be presynaptic. Supported by NIH R01 DC00537.

648 Effects of Cortical Cooling on Adaptation to Sound Level Statistics in the Inferior Colliculus

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Neurons in the inferior colliculus (IC) are known to adapt rapidly to the statistical distribution of levels in an ongoing sound; such adaptation is useful, improving coding around the mean sound level. Mechanisms contributing to this phenomenon remain to be determined. The IC, in addition to its ascending inputs, receives direct and indirect descending input from auditory cortex as part of the corticofugal system. The function of these pathways is a matter of debate. We examined the influence of the corticofugal system on adaptation to sound level statistics in the IC by reversibly inactivating auditory cortex, and therefore its descending projections. To do this, we used a cryoloop to cool the auditory cortex of anaesthetised guinea pigs, whilst simultaneously recording responses of IC neurons to continuous broadband noise which switched repeatedly between two sound level distributions. This stimulus allowed us to assess the rapidity of adaptation to a given distribution. Preliminary data suggests that the rapidity and extent of adaptation to sound level statistics is in some neurons affected by the descending pathways. Cooling the cortex appears to modulate adaptation time constants, usually slowing the adaptation process, raising the possibility that the descending pathways make adaptation faster. In addition, whilst adaptation leads to the modification of rate-level functions both with and without descending inputs, only with these pathways active do the resulting rate-level functions appear well-suited to the representation of sound levels around the mean level. These data, if supported by further work, suggest that the utility of adaptation, though not its existence, may in part rely on the descending pathways of the auditory system.

649 Enhancement of Neural Responses in the Awake Marmoset Inferior Colliculus to Stimuli That Induce Perceptual Enhancement

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Under certain conditions, an individual tone within a broadband sound stands out perceptually from others, even if each component is presented at the same SPL. Two factors that contribute to the perceptual enhancement of a probe are its onset time relative to the remainder of the complex and the bandwidth of a protected frequency region surrounding it. Specific physiological underpinnings of these effects have not been identified, but previous studies have essentially ruled out a peripheral origin, at least in the responses of single auditory nerve fibers (Palmer et al. 1995). The goal of this study was to determine whether neurons in the awake marmoset inferior colliculus exhibit enhanced probe responses that are consistent with the observed behavioral phenomenon. A best-frequency (BF) tone was introduced within a band-reject signal with a spectral notch positioned around BF.

In roughly half of the single units in the population, the response to the complex including the BF component was larger when preceded by the band-reject conditioner than when preceded by silence, in qualitative agreement with the human psychophysics. Parametric variations were made in the protected region notch width, conditioner component spacing, and overall SPL. Several fundamental response properties emerged from the diversity of the neural population. Enhancement depended on both notch width and overall SPL, with medium-sized notches (between 0.25 and 0.5 octaves protected above and below BF) and higher SPLs (more than 20 dB above threshold) usually eliciting stronger effects. Most neurons responded similarly using logarithmically spaced (0.1-octave) and harmonically related (200-Hz fundamental) conditioner components. These findings further delineate the role of sub-cortical auditory processing in the generation of a context-dependent representation of ongoing acoustic scenes. Supported by NIDCD grants DC00115 and DC009164.

650 Stimulus-Specific Adaptation and Novelty Detection in the Inferior Colliculus of the Big Brown Bat, *Eptesicus Fuscus*

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Novel sounds are likely to be behaviorally important. To assess novel sounds, the brain must retain a record of frequently occurring sounds. Neural responses to repetitive sounds often decrease over time, an event termed stimulus-specific adaptation (SSA). SSA is known to occur in the auditory cortex of cats and in the inferior colliculus (IC) of rats (both anesthetized). IC neuron responses were recorded in awake bats, using an oddball paradigm in which stimulus repetition rate, frequency contrast, and probability were varied. The amount of SSA and degree of novelty responses was quantified using an index (NSSI) with a scale from -1 (no novelty response) to +1 (full novelty response).

The neurons sampled covered nearly the entire audible range (10 kHz to 73 kHz) and 96% were located in the central nucleus of the IC. Strong SSA/novelty response (NSSI >0.50) was seen under all conditions in 3% of neurons and under one or more conditions (partial SSA/novelty response) in 6%. A moderate level of SSA/novelty response (NSSI >0.25 <0.50) was seen in 35% of neurons under one or more conditions. The highest NSSI values were found at a repetition rate of 4/s. Higher NSSI neurons had greater latency differences between the oddball and standard. Partially adapting neurons had the largest average latency decrease (26.9ms), followed by strongly adapting neurons (17.5ms) and non-adapting neurons (6.0ms). Neurons broadly tuned to frequency had higher NSSI values than those more narrowly tuned. For strongly and partially adapting neurons, Q10 values (repetition rate 4/s) were all less than 5.0 (2.2 +/- 1.1) while non-adapting neurons ranged from 0.750 to 30 (7.9 +/- 7.6).

The bat resembles the rat in that the amount of SSA/novelty response in the IC is distributed over a broad range in which neurons with the greatest SSA are most broadly tuned to frequency and exhibit the largest latency difference between the standard and oddball stimuli.
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651 Electrical Stimulation of the Human Midbrain Reveals Tonotopy, Plasticity, and Novelty Detection Within the Central Auditory System

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A new auditory prosthesis designed for stimulation of the human midbrain is in clinical trials and has been implanted in five patients. The overall results in terms of safety and speech perception have been encouraging though patients still have not achieved performance levels comparable to cochlear implant patients. Therefore we need to better understand the functional organization of the central auditory system and how essential features important for sound perception can be transmitted to higher centers through midbrain stimulation. We have begun to achieve this goal by stimulating within different midbrain regions across our patients and characterizing the perceptual effects to various types of stimuli. Consistent with animal models and human anatomical studies of the inferior colliculus (IC), we have confirmed a clear pitch organization aligned along the hypothesized tonotopic gradient of the IC in one patient. However, this pitch organization was not present until after 6 months of stimulation before which only low pitch percepts were perceived. Considering that the patient had only low frequency residual hearing prior to complete deafness, it appears that daily stimulation across the tonotopic gradient of the IC reversed the dominant representation of low frequency information within the central auditory system. There were also dramatic improvements in temporal coding properties (e.g., 100 ms down to 10 ms for gap detection) and loudness adaptation to repetitive pulse stimulation that can not be explained solely by learning effects. Across patients, we further observed that stimulation of spatially distinct midbrain regions elicits different loudness adaptation effects. Stimulation within the lateral lemniscus elicits no loudness decay to continuous pulse stimulation even with rates up to 1800 pps. Stimulation within the dorsal cortex of the IC exhibits complete loudness decay within seconds that can recover by stimulating other sites within the same region. Stimulation of the central IC exhibits partial loudness adaptation over tens of seconds that recovers by stimulating regions further away along the tonotopic gradient. These adaptation findings are consistent with one notion of novelty detection in that activation of unadapted neural pathways is involved with eliciting salient sound percepts. The fact that middle latency responses to midbrain stimulation maintained constant amplitudes while the loudness percept could disappear over time suggests that regions beyond the primary auditory cortex are coding

for the salience of sound, at least for the slow pulse rates required for the recordings, and processing adaptation information transmitted through segregated functional pathways.

652 Metabolic Changes in the Inferior Colliculus, Thalamus, and Auditory Cortex in Response to High Intensity Unilateral Noise Trauma Using a Rat Animal Model of Tinnitus and Hearing Loss

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Physiological studies have shown that the auditory cortex has the ability to reorganize itself as a function of acoustic experience or peripheral injury to the inner ear. Here we explore the neural correlates of auditory brain plasticity using a high resolution Positron Emission Tomography camera, the Focus 120, dedicated for small animal use. We have established an animal model of both hearing loss and tinnitus using noise burst prepulse inhibition of the acoustic startle (NBPIAS) and gap prepulse inhibition of the acoustic startle (GPIAS). The brains of six animals were scanned under two conditions, quiet baseline and sound evoked activity following one hour absorption of radio-labeled glucose analog F-18 Fluorodeoxyglucose injected via i.p. injection. Baseline scans in quiet showed bilateral low level activation of inferior colliculus (IC) and auditory cortex (AC). Following acoustic trauma asymmetries were observed under both quiet and sound evoked conditions with the contralateral IC showing lower levels of activation. In contrast smaller differences were observed at the level of the AC. Subsequent scans were performed at 2, 3, and 4 weeks post trauma. Early analysis shows that the asymmetries observed under quiet conditions decreased as a function of time. Additionally, two animals exposed to noise showed evidence of persistent tinnitus. Further analysis is underway to determine if these two animals differ from the rest of the group. Preliminary data suggests noise-induced unilateral hearing loss leads to larger asymmetries in metabolic activity in the midbrain than the auditory cortex. These differences in metabolic activity decrease with time suggesting that plastic changes are occurring to compensate for the peripheral hearing loss. Supported in part by the American Tinnitus Association and NIH grant R01DC00909101,

653 Development and Refinement of Excitatory-Inhibitory Balance in the Developing Auditory Cortex

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Early in life, neural circuits are highly susceptible to outside influences. The organization of the primary auditory cortex (AI) in particular is driven by acoustic experience primarily during the 'critical period', a period of time early in postnatal development during which AI is especially plastic. This neonatal sensitivity to the structure of sensory inputs is believed to be essential for constructing stable representations of the auditory world and for the acquisition of language skills by children.

Previous studies have shown that the critical period for development of rodent AI occurs during the second and third weeks of postnatal life. During this time, the tonotopic organization of AI can be altered by passive exposure to auditory stimuli such as pure tones (de Villers-Sidani et al. 2007). Additionally, the synaptic properties of cortical neurons reach maturity at the end of this period (Oswald and Reyes 2008). However, it is unclear how these processes at the cellular and network levels are coordinated to control the organization and plasticity of AI receptive fields over development.

Here we use in vivo whole-cell recording to measure AI synaptic receptive fields in the intact brains of anesthetized young rats. We first asked how excitatory and inhibitory frequency tuning curves are organized from P12 to P30. While tone-evoked synaptic currents could be evoked at all ages, excitatory and inhibitory frequency tuning profiles were uncorrelated early in life (P12-P16), indicating that excitation and inhibition are locally imbalanced at the onset of the critical period. By P21, however, excitatory and inhibitory inputs were highly correlated and indistinguishable from tuning curves measured in older animals (Wehr and Zador 2003; Froemke et al. 2007). Finally, we found that repetitive sensory stimulation increased the strength of tone-evoked excitation and inhibition together, leading to an increase in excitatory-inhibitory coupling across the receptive field.

654 Aural Specificity of Cortical Propagating Waves in Hearing and Congenitally Deaf Cats

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Congenital deafness affects developmental processes in the auditory cortex. In this study, local field potentials (LFPs) were mapped at the cortical surface with microelectrodes in response to cochlear implant stimulation. From amplitude-normalized LFPs evoked cortical activation profiles were determined for each ms post stimulus and were compared between four hearing controls and four congenitally deaf cats (CDCs). Pulsatile monopolar electrical stimulation (biphasic, 200 μs/phase) initially evoked cortical activity in the rostral parts of A1. This progressed both in the approximate dorsoventral direction (along the isofrequency stripe) and in the rostrocaudal direction. The dorsal branch of the wavefront split into a caudal branch (propagating in A1) and another smaller one propagating rostrally (into AAF). After the front reached the caudal border of A1, a "reflection wave" appeared in three hearing controls, propagating back rostrally. The waves required ~13-15 ms to propagate along A1 and return back. In CDCs, the propagation pattern was significantly disturbed, with a more

synchronous activation of distant cortical regions. The maps obtained from contra- and ipsilateral stimulation overlapped in both groups of animals. Differences in the latency - amplitude spatial patterns between contra- and ipsilateral stimulation appeared only in controls. Cortical waves evoked by contralateral and ipsilateral stimulation were more similar in CDCs. Additionally, in controls LFPs evoked with contra- and ipsilateral stimulation were more similar in caudal A1 than in rostral A1. This dichotomy was lost in deaf animals. Thus, propagating cortical waves are specific for the contralateral ear, they are affected by auditory deprivation and the specificity of the cortex for the stimulation of the contralateral ear is reduced by deprivation.

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655 Effects of and Recovery from Long-Term Exposure to Moderate-Level, Band-Limited Acoustic Environments on Primary Auditory Cortex of Adult Cats

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Noreña et al. [Nat Neurosci 9:932-9 (2006)] showed that a 20-week, uninterrupted exposure of adult cats to an 80 dB SPL, 4-20 kHz tone pip ensemble drastically reduced neural responsiveness to 4-20 kHz sound frequencies in AI, and increased responsiveness to adjacent frequencies. The concurrent reorganization of the AI tonotopic map, whereby neurons originally tuned to 4-20 kHz become tuned to adjacent frequencies, is reminiscent of that following local damage to the sensory epithelium, though in this case no cochlear hearing loss occurred. Here we expand on this result by lowering the duration and SPL, and varying the bandwidth and center frequency of the exposure, and by investigating the extent of recovery from exposure-induced changes in quiet laboratory housing conditions. Reduction of the exposure level to 68 dB SPL, and the exposure duration to 6 weeks, produced qualitatively the same reorganization in AI described by Noreña et al. Changing the exposure bandwidth to 2-4 kHz, thus masking the heart of the cat meow spectrum, also had the same effect. Upon cessation of the exposure, responsiveness to frequencies in the exposure band began to increase, and the distribution of neuronal CFs returned to normal. However, the restored neural population lacked clear tonotopic organization, even after 12 weeks of recovery. In addition, responses to tone pips remained longer in latency and duration, and the synchrony of spontaneous activity remained elevated relative to unexposed controls. We hypothesize that this new form of adult AI plasticity is the result of a bottom-up decrease in synaptic gain in the frequency range of the exposure. This would lead to a reduction in lateral inhibition to frequencies outside that range, and subsequently to an unmasking and Hebbian strengthening of excitatory cortico-cortical connections back into the range, producing map reorganization. Strong lateral inhibition from the strengthened outside regions could then delay map recovery.

656 c-Fos Immunoreactivity at the Auditory and Visual Cortex in Experimentally Induced Deafness in Guinea Pigs

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Background and Objectives: Recently, functional studies for auditory cortex are being watched with interest in accordance with development of many radiologic equipments and surgical devices for sensorineural hearing loss. And It is well known that the function of central auditory pathway is essential for hearing rehabilitation. There are some papers about the functional or metabolic changes of auditory cortex in deafness and in patients with cochlear implantation. The aim of this study was to investigate indirectly the metabolic changes of primary auditory cortex and visual cortex by c-fos immunoreactivities in experimentally induced permanent threshold shift animal model. Materials and Method: Ototoxic drugs (kanamycin and furosemide) and noise were used for induction of permanent threshold shift. Cochlear damages were evaluated with auditory brainstem responses (ABR) and morphologic studies. And c-fos immunoreactivities were observed with the lapse of time after deafening. Results: After administration of ototoxic drugs and noise exposure, ABR threshold shifts were not recovered until three months. And cochlear damages were observed in broad areas of cochlea. c-fos immunoreactivities in the primary auditory cortex were increased in acute period but it was decreased after one month. And it was recovered again with the level of control in three months later. In the visual cortex, increased and sustained immunoreactivities were observed after drugs and noise exposure. Conclusion: This result shows the plasticity of auditory cortex and possibility of some kinds of auditory-visual cross modal plasticity.

657 Collicular Tone-Specific and Nonspecific Plasticity Elicited by Pseudo-Conditioning: Role of the Auditory and Somatosensory Cortices and Acetylcholine Receptors

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Experience-dependent plastic changes in the central auditory system are due to activation of the sensory and neuromodulatory systems. Pseudo-conditioning elicits non-specific plasticity, whereas auditory fear conditioning elicits tone-specific plasticity. Suga (2008) hypothesized that the nonlemniscal and noncholinergic systems play an essential role in eliciting the nonspecific plasticity, whereas the lemniscal and cholinergic systems play an essential role in eliciting the tone-specific plasticity and that the somatosensory cortex is not involved in eliciting the nonspecific plasticity, whereas it plays an important role in eliciting the tone-specific plasticity. In our current experiment with the big brown bat, we examined changes in the response properties of the neurons in the central nucleus of the inferior colliculus (ICc) elicited by pseudo-conditioning: unpaired tonal and electric leg stimuli (CS_u –

US_u) and found that ICc neurons showed nonspecific plasticity: an increase in auditory response, a broadening of frequency tuning and a decrease in threshold. In addition, it showed a small, short-lasting tone-specific plasticity (BF shift) only when the best frequency (BF) of a recorded ICc neuron was 5 kHz higher than the CS_u frequency. We also found that unlike tone-specific collicular plasticity elicited by the conditioning, the nonspecific plasticity elicited by pseudo-conditioning was neither blocked by atropine (a muscarinic cholinergic receptor antagonist) applied to the inferior colliculus nor by muscimol (a GABA-A receptor agonist) applied to the auditory cortex or somatosensory cortex, but the small tone-specific plasticity was completely abolished by these drugs as were the tone-specific plasticity elicited by the conditioning. Our current results indicate that nonspecific collicular plasticity depends on neither the cholinergic neuromodulator nor the somatosensory and auditory cortices.

658 Cortical Delay-Tuning in Young Mustached Bats

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The auditory cortex of mustached bats contains a dorsal region where combination-sensitive neurons are located that selectively respond to pairs of stimuli representing FM components of echolocation call and echo at specific echodelays (FM-FM region, O'Neill and Suga 1979, Suga and O'Neill 1979). The delay-tuned cortical neurons are organized in a chronotopic order with short best delays represented rostrally and are thought to code target range distance during echolocation. The aim of the present study was to assess the postnatal maturation of delay-tuning at the cortical level.

Young mustached bats are highly dependent on their mothers for at least five postnatal weeks which are spend in maternity colonies in caves. They start to spontaneously emit echolocation calls in the second postnatal week and achieve active flight at an age of about 3-4 postnatal weeks. Prior to the first foraging flights, at an age of 3-4 weeks, the auditory cortex contains a functional FM-FM region where combination-sensitive neurons are organized chronotopically in form of bands responding to different echo FM harmonics, similar to the adult. They represent echo delays of up to about 20 ms. Before emergence of active echolocation, within the first postnatal week, certain dorsally located cortical neurons show either long lasting facilitation or inhibition (up to 60 ms) Significantly, already in newborn bats of less than 3 days of age, subgroups of cortical neurons are tuned to short pulse-echo delays of less than 10 ms with a tuning bandwidth that is comparable to adults. This implies that even before onset of echolocation or flight, the FM-FM region contains a hardwired machinery to compute target-distance.

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659 Onset of Spontaneous Cochlear Activity May Signal the Termination of Endogenous Oscillations in Neonatal Auditory Cortex

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Low levels of spontaneous activity affects the establishment and specificity of synaptic connections and sensory maps during development (Shatz,1990; Poo, 1994; Pallas, 2001; Sanes et al. 2006). In the developing neocortex including the thalamorecipient auditory cortex, endogenous activity propagates caudorostrally as oscillaltions and calcium waves (OWs), that gradually decline after the 1st postnatal week (Garashuk et al. 2000, Kotak et al. 2007). Similar OWs exist in the developing brains of intact mammals (Hirase et al. 2004; Adelsberger et al. 2005; Garashuk et al. 2006; Minlebaev et al. 2007; Hanganu et al. 2008). Since the age when OWs diminish may overlap with the onset of spontaneous cochlear activity (P6-7), a specific hypothesis is proposed: *Before hearing onset, cochlea is necessary for the termination of OWs in the auditory cortex.* To test this, both cochleae were removed at P2 and endogeneous activity was monitored in the supragranular layers with cell-attached or whole-cell recordings in slices from P8-16 gerbils. Control ACx neurons after hearing onset from P13-16 slices neither display spontaneous spikes nor OW-like patterns; only sub-threshold synaptic activity is detected (Kotak et al. 2007). By contrast, P13-16 post-hearing neurons from ablated animals displayed tonic discharge patterns (mean ± SEM Hz, 2.79 ± 0.5, N = 6). Further, neurons from ablated animals before hearing onset (P8-10) also displayed tonic discharge (mean ± SEM Hz, 2.77 ± 0.9, N = 6). Comparable age-matched control neurons exhibit only sporadic spiking activity (Kotak et al. 2007). Thus, prior to acoustic experience, spontaneous cochlear activity is essential for the decline in endogenously generated oscillatory activity in the thalamorecipient auditory cortex after the first postnatal week.

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660 Developmental Change of NMDA Receptors in Central Auditory System Affected by Environmental Noise

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The processing of complex sounds such as language requires rapid temporal information processing at multiple stages along the auditory pathway. Recent studies suggest that impoverished auditory experiences can lead to profound and potentially long lasting deficits in function and structure in the auditory cortex (AC). Raising new born rats in a constant, moderate-level noise environment during the critical developmental period could delay the development of AC tonotopic maps and the tuning property of AC neurons. Our studies showed that noise environment during the critical developmental period could also delay the maturation of temporal processing of rats. However, the molecular level changes induced by the constant environmental noise on the central nervous

system are not clear. Our previous studies suggest that constant moderate-level noise exposure can down-regulate mRNA expression of NMDA2A (NR2A) and 2B receptors (NR2B) in the AC. NR2A and NR2B play an important role in processes of neurodevelopment, including cell proliferation and synaptogenesis. To further test the effects of constant noise exposure on the development of the central auditory system at the protein level, we measured the developmental changes in NR2A and NR2B protein expression in the AC, the inferior colliculus (IC) and the hippocampus using Western blotting technique. Two groups of neonatal rats (postnatal 7 days) were separated into two groups: the Noise Group was raised in a constant noise (70 dB SPL, white noise); the Control Group was raised in a normal vivarium environment. Our data show that the expression of NR2B, but not NR2A, was significantly higher in the AC, IC and hippocampus in the Noise Group (n = 6) compared to the Control Group (n = 3) at P70. This suggests that constant, moderate-level noise during the critical period leads to an up-regulation of NR2B. These results are the first to identify a change in protein expression level of NMDA receptors in central auditory system and hippocampus associated with the noise-induced temporal processing developmental delay. Supported in parts by grants from NIH (R03DC008685, R01DC009219) and the National Organization for Hearing Research Foundation

661 Role of the Cholinergic Pedunculopontine Tegmental Nucleus in Cortical Learning-Induced Plasticity

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The process of learning continuously impacts and changes the way in which the brain functions (neural plasticity). Large-scale plasticity in the auditory cortex and thalamus can be induced in both human and animal subjects through auditory learning. This plasticity is highly specific to the frequency content of the learned sound (frequency-specific). Importantly, auditory plasticity is largely dependent on cholinergic regulation; disruption of cholinergic regulation abolishes learning-induced auditory plasticity.

In the past 10 years, the importance of the cholinergic nucleus basalis (NB) of the basal forebrain in learning-induced auditory plasticity has been confirmed. Electrical activation of the NB paired with a tone induces frequency-specific plasticity in the auditory cortex. However, learning-induced auditory plasticity cannot completely blocked by cortical application of atropine, a muscarinic acetylcholine receptors. Anatomic studies indicate that the NB directs cholinergic projections mostly to the cortex. It is therefore very unlikely that the NB directly regulates subcortical nuclei. The pedunculopotine tegmental nucleus (PPTg) in the brainstem is another cholinergic nucleus of interest. It directs cholinergic projections mostly to the thalamus. As with the NB, the PPTg shows increased activity during learning but its exact contributions are vague and poorly understood. Therefore, we investigated cortical plasticity by PPTg activation paired with various frequencies in mice. Our results shown that electrical stimulation of PPTg paired with a tone led to a shift towards the frequecny of the tone in the receptive field of the cortical neurons. Cortical application of atropine, the acetylcholine muscarinic receptor antagonist, did not eliminate the cortical plasticity evoked by PPTg-tone paired stimulation. Our data suggest that the cholinergic PPTg projection to the auditory thalamus is another important source for learning-induced auditory plasticity.

662 Functional Microcircuits in Neonatal Auditory Cortex Before and During the Onset of Hearing

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The functional connectivity of the primary auditory cortex (A1) is shaped by experience during development, especially during a critical period early in life. During early times in development, additional neuronal circuits, formed by subplate neurons (SPNs), are present in the prenatal and neonatal cortex that are absent in the adult cortex. Despite the demonstrated importance of SPNs in functional maturation of cortical circuits, little is known about how these neurons are integrated in the developing thalamocortical circuit. We addressed this question physiologically by studying SPN in vitro in thalamocortical slices of A1 and medial geniculate nucleus (MGN) in mouse from postnatal day (P) 3 to P15. Using electrical stimulation of the thalamocortical projections we find that SPNs receive functional excitatory inputs from MGN as early as P3. MGN inputs to SPN were capable of inducing action potentials in SPNs suggesting that SPNs are tightly integrated into the developing thalamocortical circuit and that they can receive early spontaneous and later sound driven activity. The projection targets of SPNs are unknown in any cortical area. We used laser-scanning photostimulation of SPNs to investigate which neurons receive functional SPN inputs. We find that layer 4 neurons receive functional inputs from SPNs. Thus, SPNs are tightly integrated into the developing thalamocortical circuit providing input to the eventual targets of MGN innervation. These results suggest that SPNs are a reliable relay of early spontaneous and sound evoked activity to the developing auditory cortex. Feed-forward SPN activity might regulate the functional maturation and plasticity of A1 and contribute to the development of normal cortical organization.

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663 MAP Kinase in Auditory Cortex vs. Thalamus Regulates Different Features of Tone-Evoked Cortical Responses

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Auditory learning can alter cortical responsiveness via modulatory neurotransmitters and activation of intracellular

signaling pathways. Here, we determine whether the effect of systemic nicotine, which acts via acetylcholine receptors to alter cortical responses to tones, involves mitogen activated protein kinase (MAPK). In other cortical regions, nicotine activates MAPK, likely due to nicotinic receptor-mediated increases in intracellular Ca2+ that can trigger signaling cascades. MAPK may also contribute to the cognitive enhancing effects of nicotine, and endogenous acetylcholine, given its involvement in synaptic plasticity.

We placed a 16-channel silicon multiprobe in primary auditory cortex of adult, urethane-anesthetized mice, and derived current-source density (CSD) profiles evoked by characteristic frequency (CF) stimuli. CF tones evoked a middle layer current sink at short latency, followed by longer-latency and longer-duration sinks in middle and upper layers. Systemic nicotine (2.1 mg/kg, s.c.) decreased the initial sink onset latency and enhanced both initial and longer-latency sink amplitudes. We determined the effects of microinjecting the specific MAPK inhibitor U0126 or its inactive analog (each 20 uM, 50-100 nl) on nicotine-induced changes in CSD profiles. Thalamic microinjection of U0126, centered near the auditory thalamocortical pathway, blocked the effect of nicotine on the middle layer sink latency and amplitude, whereas cortical injections adjacent to the multiprobe blocked enhancement of longer-latency sink components, but not enhancement of the initial sink. Microinjection of the inactive analogue into the thalamus or cortex did not block nicotine's effects.

Our results suggest that MAPK signaling in thalamus and cortex may mediate nicotine-induced changes in cortical responsiveness.

664 Comparison of Rapid Task Related Plasticity in Primary and Secondary Auditory Cortex

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Rapid task-related receptive field plasticity alters the tuning properties of neurons in primary auditory cortex in a task-specific fashion that enhances their ability to encode the salient parameters of the current task (Fritz et al., 2003, 2005, 2007, Atiani et al., 2008 submitted). Rapid receptive field plasticity has been suggested to contribute to our ability to perform natural acoustic tasks such as navigating a complex acoustic environment, and extracting relevant acoustic information from the irrelevant. In this study we investigated rapid task-related receptive field plasticity in two secondary auditory cortical areas in the ferret, and compared it to plasticity in the primary auditory cortex. We trained ferrets on an acoustic task that required them to distinguish a set of band pass noise stimuli from tones. We recorded single unit activity from primary auditory cortex and posterior fields of the auditory cortex, which are functionally and anatomically linked to primary auditory cortex (Bizley 2005, 2006). We recorded in two behavioral states: while the animals were in a passive, quiescent state, and while they were in an active state during task performance. We observed robust task-related changes in firing rates and response dynamics in secondary areas. Understanding differential types and magnitude of task-related plasticity in auditory cortex may clarify the functional contributions of these auditory areas to auditory attention.

665 Bi-Directional Effects of Reversible Developmental Hearing Loss on the Accuracy and Reliability of Stimulus Encoding in Auditory Cortex and Inferior Colliculus

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Many children experience prolonged bouts of degraded auditory experience due to otitis media and other forms of conductive hearing loss. In particularly severe cases, performance in listening tasks is subnormal for years following the resolution of peripheral hearing loss. To better understand the origin of these effects, we have developed a translational rodent model to study reversible unilateral conductive hearing loss (UCHL) on the single unit response properties and functional maps in the primary auditory cortex (A1) and central nucleus of the inferior colliculus (ICc). UCHL was initiated through ear canal ligation in P14 rat pups and then reversed 60 days later. Receptive fields (RFs) and tonotopic maps were characterized in A1 and ICc using tone bursts presented independently to the developmentally deprived contralateral ear or non-deprived ipsilateral ear. We observed a bi-directional effect wherein A1 RFs and functional maps derived from the deprived contralateral ear were degraded relative to sham controls yet the same A1 sites yielded significantly enhanced ipsilateral stimulus representations in terms of response latency, threshold, peak firing rate, RF continuity and tonotopic map integrity. Such bi-directional effects were not observed in ICc neurons. How might this bi-directional plasticity affect the ability to correctly classify auditory stimuli? We have begun to "train" computer models to classify tones of varying level and frequency presented to the deprived and non-deprived ears using trial-by-trial spiking activity across populations of A1 and ICc single units recorded in UCHL and sham rats (n = 90 per group). The neurophysiological plasticity described above translated into pronounced decrements in the accurate and reliable classification of contralateral stimuli – and improvements in the ability to classify ipsilateral stimuli – based on A1, but not ICc, responses. Supported by: National Organization of Hearing Research Foundation.

666 Auditory Learning Involving Complex Sounds Affects Nonlinear Integration Within Cortical Responses

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To examine how primary auditory cortex is affected by learning involving complex sounds, we trained rats to discriminate sequences of frequency sweeps and then analysed neuronal responses in both trained and naive animals. Extracellular recordings were obtained in anaesthetized animals during presentations of spectrotemporally rich dynamic random chord (DRC) stimuli. We fit linear spectrotemporal receptive field (STRF) models to the DRC-driven responses, as well as multilinear context models (Ahrens, Linden and Sahani, J Neurosci 28:1929, 2008) that capture nonlinear local interactions related to forward suppression and combination sensitivity. STRFs appeared to be modified in a subset of animals with very extensive exposure to upward moving frequency sweep sequences, showing both greater spectrotemporal inseparability and greater selectivity for upward moving sweeps (as assessed by power asymmetry in the modulation transfer function), relative to STRFs in naive controls. In a second group of rats, trained to the same level of behavioural performance but with considerably less exposure to the stimuli, these STRF effects were not seen. However, in both groups of animals, the contextual modulation fields in the multilinear models were profoundly altered relative to those in naive controls, showing significantly greater sensitivity to tones within the DRC that were preceded by tones at lower frequencies, but less sensitivity when such tones were presented near-simultaneously. These contextual effects are consistent with enhanced sensitivity to upward moving sweeps. Our results suggest that persistent effects of plasticity may first be manifest in nonlinear response properties of cortical neurons, and only later become evident in linear estimates of the response function such as the STRF.

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667 Neurometric Analyses of SAM Processing in Auditory Cortex Across Development: Correlates to Behavior

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The neural bases for developmental changes in auditory perception are poorly understood. Percepts such as sinusoidal amplitude modulation (sAM) detection thresholds take more than a decade to mature in humans, suggesting that central coding properties might limit behavioral performance. We have studied the development of sAM processing by auditory cortex neurons in awake animals, and compared the neural representation with behavioral performance in juveniles and adults. Single unit extracellular recordings were obtained in young (~P30) or mature (>P60) gerbils in response to sAM stimuli of varying modulation depths (MDs; carrier at each cell's CF, 2-5 Hz modulation frequency). Population period histograms indicated several maturational differences: better detection threshold, overall higher firing rate, and a phase shift with increasing MD. These differences were quantified for the purpose of comparison with psychometric functions. A spike-distance metric demonstrated that sAM detection is made up of temporal features with 100-1000ms resolution. This suggests that both firing rate and slow temporal variations are more important than fine temporal structure. Using this metric, juvenile neurons were found to have a higher MD detection threshold, and the magnitude of this difference was well-correlated to the behavioral difference in performance. A second set of metrics was calculated based on firing rate or vector strength (Nelson & Carney '07), and used to construct neural tuning curves to compare with behavior. These analyses confirm that developmental changes in both rate- and temporal-coding were present and paralleled behavioral performance. Therefore, we conclude that the sensory representation of sAM tracks the changes in behavioral performance during juvenile development.

668 Blast-Induced Traumatic Brain Injury in the Auditory and Non-Auditory Structures: MRI Diffusion Tensor Imaging and Spectroscopy Assays

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Traumatic brain injury (TBI) may be caused by concussion or secondary damage to the brain from exposure to explosive noise, which is a common occurrence among soldiers in active duty. TBI is often associated with auditory and non-auditory neurological disorders. We used a rat model to examine the effects of blast, a high-energy impulse noise, on the anatomical and chemical changes in auditory brain structures including the cochlear nucleus, inferior colliculus, medial geniculate body, and auditory cortex, as well as non-auditory structures including the amygdala and corpus callosum. The corpus callosum was included to control for any sound-induced changes. Nine adult Long Evans rats were used in this study. Prior to blast induction, each animal underwent MRI diffusion

tensor imaging (DTI) and magnetic resonance spectroscopy (MRS). All the MRI measurements were performed on a 4.7-T horizontal-bore magnetic resonance spectrometer (Bruker AVANCE). The animals were then placed in a shock tube and subjected to a single 14 psi, 194 dB SPL blast with pulse duration of approximately 8 ms. The same MRI procedures were conducted 2 and 4 weeks after blast induction. Animals were also tested for tinnitus and hearing loss at these time points (see presentation by Mao et al.). Our results showed that blast induced significant changes in the DTI measurements in several brain structures. However, such changes were not accompanied by significant chemical changes as revealed by MRS measurements. The results suggest that blast exposure causes complex changes to the brain, which may be an underlying factor for the manifestations of tinnitus and hearing loss in these animals. Nevertheless, further studies are needed to investigate the dose effects of blast on both anatomical and chemical changes in brain structures, and the relation of these changes to behavioral data of tinnitus and hearing loss.

669 Characterising the Neural Activity Associated with Attention-Demanding Tasks of Spatial Listening for Speech

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The ability to focus, switch, and divide attention contributes to the successful perception of speech in noisy environments. The nature and time-course of the neural activity associated with these attentional processes during complex listening tasks is not well understood. The current study examined this activity with magneto-encephalography (MEG) using a multi-talker spatial listening task.

Participants listened to overlapping phrase sequences, recorded using a mannequin and presented over tubephones. A new phrase started every 800 ms. Each phrase had the form “Ready CALL-SIGN go to COLOUR NUMBER now”, with 8 possible call-signs, 4 colours, and 4 numbers. Participants identified the phrase containing a target call-sign and reported the colour-number co-ordinate.

Two key processes were analysed: the discrimination of target and non-target call-signs; the resistance of distraction from a new talker when attention was focussed on the target phrase. Differences in the power and oscillatory nature of neural activity were analysed at the cortical level using minimum-norm and spatial filtering techniques.

Call-sign discrimination was associated with increased power in the left superior temporal plane, left inferior parietal lobe, and the right frontal lobe. Resisting distraction was associated with increased power in posterior temporal, inferior parietal, and pre-frontal regions bilaterally. When distraction had to be resisted, power increases in several regions correlated with performance, and decreases in power were observed in β and γ frequency bands, localised to right posterior parietal lobe and cingulate cortex.

The pattern of cortical regions suggests the recruitment of auditory, language, and attentional processes. The decreased oscillatory activity may reflect the volitional supression of processing to maintain the focus of attention. The results suggest that attention is integral to speech perception in a multi-talker environment.

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670 Spatial Release from Speech-On-Speech Masking as a Function of Temporal Overlap in Listeners with Sensorineural Hearing Loss

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Listeners with sensorineural hearing loss show less release from masking than listeners with normal hearing when competing talkers are spatially separated. We hypothesized that this problem does not reflect an essential deficit in the ability of these listeners to use spatial cues, but rather is a consequence of their reduced ability to segregate portions of competing speech signals that overlap spectrotemporally.

A corpus of monosyllabic words was temporally compressed so that each word was 200 ms long. Nonsense target sentences were created by concatenating five words from the same talker, with 200-ms intervening silent gaps. Masker sentences were created in the same way, using five different words and a different same-sex talker. Target and masker sentences were presented at equal intensities with three levels of temporal overlap: 0% (completely interleaved in time), 100% (completely simultaneous), or 50% (partially overlapping). Sentence pairs were presented either diotically, or dichotically with the masker sentence lateralized to the side via an interaural time difference of 0.6 ms.

For listeners with normal hearing, lateralization of the masker provided a large benefit in all overlap conditions. For listeners with hearing loss, the benefits were reduced in all conditions. Absolute performance in both diotic and dichotic conditions, however, was substantially poorer in the 100% overlap condition for listeners with hearing loss. The results support the idea that the primary factor limiting overall performance and spatial release from masking in speech-on-speech tasks for listeners with hearing loss is a reduced ability to segregate spectrotemporally overlapping signals. This result will be discussed in terms of relative amounts of energetic and informational masking.

671 Release from Perceptual Masking in Children and Adults: Benefit of a Carrier Phrase

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This study examined whether a carrier phrase improves children’s performance for recognizing monosyllable words in a competing background. The hypothesis was that the

carrier phrase improves performance of both children and adults by providing an auditory grouping cue based on spectral coherence. Thus, the cue assists listeners in the perceptual segregation of the signal from the masker. To test this hypothesis, 14 children (5-10 years) and 7 adults with normal hearing repeated monosyllabic words in the presence of a continuous speech-shaped noise or two-talker masker. The speech-shaped noise was expected to produce primarily energy-based masking. In contrast, the two-talker masker was expected to produce both energy-based and perceptual (or informational) masking. Performance was compared for two conditions: (1) with the carrier phrase, "say the word," before each target word and (2) the target word alone. The signal was presented at 65 dB HL. Masker level was 55 dB HL and 60 dB HL for the two-talker masker and speech-shaped noise, respectively. Adults performed near ceiling for these masker levels, so 7 additional adults were tested at lower signal-to-noise ratios to equate performance with that of children. For the two-talker masker, average performance of older children (8-10 years) improved 22% with the carrier phrase, comparable to improvements shown by performance-equated adults. In contrast, younger children (5-7 years) only improved by 7% with the carrier phrase. No systematic benefit was observed for the carrier phrase in the speech-shaped noise for any age group. Improved performance with the carrier phrase for the two-talker masker, but not for the speech-shaped noise masker, suggests that the carrier phrase provided an effective auditory grouping cue for children and adults. However, younger children appear to benefit less from the carrier phrase than older children or adults.

672 Exploring How Auditory Spatial Continuity Enhances Speech Perception

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Continuity of spatial location was recently shown to improve the ability to identify and recall a sequence of target digits presented in a mixture of confusable maskers (Best et al 2008). Here we present three follow-up experiments that explored the basis of this improvement. First, we trained listeners with the spatial trajectory of a moving sequence of digits to examine whether advance knowledge of upcoming target locations enhanced performance. In the second experiment, we tested whether refinement of spatial selectivity would arise if the target sequence moved but had no discontinuous jumps in location (i.e., the spatial trajectory only included transitions to an adjacent loudspeaker location). Lastly, we examined whether the benefit of spatial continuity was limited to the challenging case in which maskers were all potential targets. The results suggest that improvements in selectivity of spatial attention that arise when the target location is fixed from digit to digit cannot be attributed to a) the ability to plan where to direct attention well in advance; b) a freedom from having to redirect attention across large separations in location; or c) the challenge of filtering out nearby signals that are confusable with the target. [Work supported by ONR and NIDCD]

Best V, Ozmeral EJ, Kopco N, and Shinn-Cunningham BG (2008). Object continuity enhances selective auditory attention. PNAS 105(35):13173-13177.

673 The Auditory Attentional Blink: Target Identity and Order Influence Performance

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The ability to perceive meaningful novel sounds within a sequence of background sounds is critical to acoustic communication. Recognition can be influenced by the position of the target sounds within the sequence and their characteristics. We investigated how auditory discrimination of one target sound (T1) influences subsequent discrimination of a second target sound (T2) embedded in a sequence of distracter sounds. The attentional blink (AB) paradigm was used in which positioning T2 closer in time to T1 reduces the accuracy (blink) with which T2 is identified.

Targets and distracters were time-compressed (80 ms) spoken monosyllables. Target CVs were composed of stop consonants (/p/, /b/, /d/, /t/, /g/, and /k/) and /a/. Distracters were numbers (one, three, four, five, and nine; containing no stop consonant sounds). Interstimulus interval was 90 ms. T1 occurred after 5 distracters and T2 occurred with stimulus onset asynchrony (SOA) of 90 ms to 450 ms in 90 ms increments followed by 4 distracters. Percent correct (Pc) for T1 and T2, error patterns, and T1/T2 interactions were evaluated. Reduced recognition of both T1 and T2 was evident for the shortest SOA. The proportion of errors for a given CV differed at T1 and T2. At T1 /pa/ and /ba/ showed the highest Pc and were most frequently selected in error for other CVs. At T1 /ta/ showed low Pc. In contrast, for T2, /pa/ and /ba/ showed the lowest Pc and /ta/ showed the highest Pc. Specific interactions were also evaluated. For example, the lowest T2 Pc occurred when T1 was /ta/ and the highest T2 Pc occurred when T1 was /pa/ or /ba/. This suggests that ease of recognition of T1 (/pa/ or /ba/) places little load on attentional or processing capacities resulting in better recognition of T2. Conversely, low Pc on T1 (/ta/) reflects a high attentional or processing load for T1 resulting in poorer recognition of T2. This particular pattern of demands imposed by T1 on T2 was not identical for effects of T2 on T1.

674 Auditory Attention: Effects of Temporal and Spatial Expectation

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A naturalistic behavioral task was used to investigate mechanisms of spatial and temporal attention in audition. Attention was directed to a specific moment in time, respective to a specific location. Expectations were set up implicitly, using the information inherent in the movement of a sound. There were four conditions of expectation: temporal plus spatial expectation; temporal expectation

only; spatial expectation only; and no expectation. Event-related brain potentials were recorded while participants performed a go/no-go task, set up by anticipation of the reappearance of a target tone through a white noise band. Results showed that 1) temporal and spatial expectations independently enhanced target detection at both early (indexed by N1) and late (indexed by N2) processing stages; 2) later task-related processing (indexed by P3) was modulated only by both spatial and temporal expectations together; 3) temporal expectation alone speeded reaction time and increased response accuracy compared to the other conditions. Thus, the results indicate an important role for temporal attention in audition. There appear to be distinct mechanisms of spatial and temporal attention, which act independently on early processing stages. However, late synergistic effects demonstrate that these mechanisms are not completely independent, but interact according to task characteristics. Our results are consistent with the view from vision research that spatial and temporal attentional control is based on the activity of partly overlapping, and partly functionally specialized neural networks.

675 Visual Cues for When to Listen Improve Signal Identification Most When Target and Masker Are Perceptually Confusable

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If a target and competing maskers are perceived from different directions, knowing when the target will occur improves the ability to identify it. Here, we varied the properties of the masker and the spatial layout of target and masker to test the hypothesis that knowing "when" to listen improves performance most when it is difficult to focus attention on the target. Specifically, we expected the "when" benefit to be large if target and masker were co-located and easily confusable with one another.

Listeners first completed a training session in which they learned to identify five Zebra Finch song motifs (roughly 1 s long). We then tested their ability to identify the motifs in the presence of two different maskers (5 s in duration). The target onset time was random on each trial, occurring between 0.5 and 3.5 s after the masker onset, creating temporal uncertainty about when the target would occur. Maskers were either broadband noise with the same long-term spectral content as the motifs, or a random chorus of overlapping, unfamiliar motifs. To generate the chorus, unfamiliar motifs were added with random start times and then time windowed so that on average 4-5 motifs were present at any given time. Targets were simulated from in front of the listener (0deg), while maskers were either from 0deg or 90deg. In half of the sessions, a visual cue on a computer screen appeared for the duration of the target, informing the subjects of when to listen. Subjects completed six experimental sessions (blocked by spatial condition and masker type).

The "when" cue benefit was greatest for a random-motif chorus that was perceived as coming from the same location as the target. When the chorus masker was perceived in a different direction than the target or was noise rather than a bird-song chorus, the "when" cue had little or no effect on performance. Results suggest that knowing when to listen is most helpful if there is no perceptual feature that distinguishes target from masker.

676 Visual Priming of Filtered Speech in a 2IFC Task

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The effect of priming is demonstrated when the content of a distorted, filtered, or masked auditory message is provided prior to the acoustic presentation of the target. Priming causes distorted speech to appear much clearer than it would have been were the content not known. The phenomenon is striking, but it is difficult to quantify because the ability to repeat the content could obviously be attributed to remembering the prime rather than understanding the auditory message. The current experiment attempted to quantify aspects of priming by determining its effects on performance and bias in a lowpass-filter-cutoff frequency discrimination task. Nonsense sentences recorded by a female talker were sharply lowpass filtered at a nominal cutoff frequency (F) of 1 kHz with a +/- 100 Hz rove, or at a higher cutoff frequency (F + ΔF). The listeners' task was to determine which interval of a 2IFC trial contained the nonsense sentence filtered with F + ΔF. A different nonsense sentence was used in each interval of the trial. On half the trials the interval 1 sentence was displayed on a computer screen prior to the auditory portion of the trial (the priming condition). The prime markedly affected bias, increasing the number of correct and incorrect interval 1 responses, but did not affect overall discrimination performance substantially. It appears that on trials where cutoff frequency F was in interval 1, priming often caused the sentences to sound even clearer than those filtered with F + ΔF in interval 2. Listeners were apparently unable, or did not find it to their advantage, to ignore the prime or make compensating adjustments for its effects. The paradigm has the potential to help quantify the limits of speech perception when uncertainty about the auditory message is removed. [Work supported by NIH DC01625].

677 Subjective and Objective Measures of Auditory Stream Segregation and Integration

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The perceptual organization of sound sequences into auditory "streams" is an essential aspect of auditory scene analysis. Although streaming is often studied using subjective reports ("one stream", "two streams"), these are potentially unreliable, and in some contexts (e.g., animal studies), impractical. Therefore, performance measures of auditory streaming are called for. While tasks have been devised in which performance is either facilitated or hampered by stream segregation, studies combining

subjective reports with such performance measures remain rare. Here we compare streaming reports with thresholds in two temporal tasks. In the first task, listeners had to discriminate a temporal shift of the B tone relative to the two adjacent A tones in an ABA tone triplet, where A and B denote different frequencies. In the second task, listeners had to discriminate a temporal shift of the B tone relative to the B tone in the preceding triplet. To force listeners to use the relevant cue, the time interval between consecutive B tones was randomized in the first task, and the timing of the B tone relative to the two adjacent A tones was randomized in the second task. We hypothesized that thresholds in the first task would worsen with increasing perceived segregation, and so would worsen with: a) increasing A-B frequency separation, b) increasing inter-tone interval, and c) increasing sequence duration, as longer sequences allow for more build-up of segregation. We hypothesized that thresholds in the second task would improve with segregation, and predicted that they would show trends in the opposite direction. The results were generally consistent with these predictions, and with subjective measures of streaming, indicating that the two described tasks may be used to obtain performance measures of auditory streaming in future psychoacoustical and behavioral studies. [NIDCD R01DC07657]

678 Auditory Streaming in the Perception of Sinusoidally Amplitude-Modulated Signals

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Amplitude fluctuations and the temporal structure of signals provide important cues for auditory scene analysis (ASA) in the context of animal communication and human speech. The perceptual integration or segregation of successive sounds in auditory streaming can be used as a tool for analysing the effect of sinusoidal amplitude modulation (SAM) in ASA.

The perception of six human subjects was tested using the ABA_-stimulus condition (van Noorden 1975; A and B being SAM tones and _ indicating a silent interval of the same duration as the tone duration TD). The subjects' report of a galloping rhythm indicated the perception of one stream whereas the report of isochronous rhythms indicated the perception of two streams. Compared to prior studies, a wide range of stimulus parameters were tested in each subject. Three different ABA-stimulus types were presented: (1) 125ms TD and 100% tone repetition time (TRT, i.e., the time period from the onset of one stimulus component to the next), (2) 375ms TD and 100% TRT and (3) 125ms TD and 300% TRT. Carrier frequencies were 1000 or 4000Hz. The SAM frequency of the A-tone was 30, 100 or 300Hz, whereas the SAM frequency of the B-tone was up to four octaves higher. The depth of the SAM was either 30% or 100%. The ABA_-stimulus was repeated either for 5 or 15s.

Auditory streaming is elicited by the SAM stimuli varying in modulation frequency. Stimulus type 1 resulted in the largest stream segregation followed by stimulus types 2 and 3. At a carrier frequency of 4000Hz stream segregation was increased compared to a carrier frequency of 1000Hz. A higher modulation depth and a longer presentation time resulted in an increased perception of two streams. The results are discussed with reference to models of auditory stream segregation.

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679 The Perception of Sound Sequences by Normal-Hearing and Cochlear-Implant Listeners

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The perception of pitch or loudness by normal-hearing (NH) and cochlear-implant (CI) listeners is usually investigated with single sounds. Here we studied the perception of tone sequences containing pitch and loudness changes by NH and CI listeners. To be able to compare performance across auditory attributes and populations, we used a method that uncouples the discriminability of individual sounds and sequence processing itself. Our subjects had to compare random sequences in which a given auditory attribute, pitch (fundamental frequency) or loudness (intensity) could take only two different values. The values were selected individually so as to produce equal discriminability (d′=2) on single sounds. A Same-Different paradigm was then used to measure sequence discriminability. For NH listeners, we used band-pass filtered harmonic complex tones embedded in pink noise. There were two pitch conditions (resolved and unresolved harmonics pitch) and a loudness condition. Pitch sequences were found to be efficiently processed, performance being similar for sequences of 1, 2 or 4 sounds. In contrast, performance fell to chance for loudness sequences of 4 sounds. This advantage of pitch over loudness sequence processing , however, was only observed for stimuli that contained resolved harmonics. For CI listeners, we used a similar procedure but with broadband harmonic sounds for the pitch sequences, and pink noise for the loudness sequences. Performance was identical for pitch and loudness and it decreased rapidly with the length of the sequence. Therefore, contrary to NH listeners, CI listeners showed no advantage of sequence processing for pitch. This demonstrates the existence of a specific pitch processing impairment in CI listeners, independent of their elevated frequency discrimination thresholds.

680 Influence of Onset Asynchrony on the Detection of a Mistuned Component in a Harmonic Complex in Mongolian Gerbils

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Harmonicity is a strong cue to group frequencies belonging to the same sound source. Another grouping cue is the synchronous starting time of signal components. The interaction of both cues is currently discussed and the

topic of the present study. To evaluate grouping in Mongolian gerbils (*Meriones unguiculatus*), the influence of onset asynchrony on detecting mistuning of a component in a harmonic complex was measured and compared to the frequency difference limens (FDLs) of the same tone presented alone. For pure tones gerbils have a large FDL whereas for tones that are part of a harmonic complex gerbils show a small FDL.
Gerbils were trained in an operant Go/NoGo procedure with food rewards to report a frequency shift. We measured FDLs for the mistuned 2nd and 32nd harmonic of a 200Hz-complex comprised of the first 48 harmonics. The mistuned harmonic had an onset asynchrony of 0, 30, 70, 100, 200, 300, 400, or 500 ms. It had the same duration as the other components of the complex (400ms), i.e., started and ended earlier. In a control experiment we elongated the mistuned harmonic to induce an onset asynchrony but no offset asynchrony.
An onset asynchrony between 30 ms and 200 ms had almost no effect on the FDL of the 2nd and the 32nd harmonic. In these conditions, the FDLs did not differ significantly from the FDLs of mistuned harmonics when all harmonics started synchronously. The FDLs of the gerbils increased considerably at an onset asynchrony of 300 ms or more which suggests a perceptual segregation of the component from the rest of the complex. In the control experiments the FDLs for the 2nd and 32nd harmonic with 100 and 300 ms onset asynchrony were similar to FDLs for these harmonics in a complex with all harmonics in synchrony. The results suggest that a temporal overlap of more than 100 ms promoted grouping of harmonics in a complex.
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681 Energetic and Informational Masking of a Tonal Signal in a 4-Tone Masker Complex

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Studies of simultaneous masking have shown large detrimental effects of masker-spectral variability, created by varying the frequency content of the masker each time it is presented. Multi-tonal masker samples can differ widely in masking effectiveness, however, even when frequency components centered on the signal frequency are excluded. This study examined the degree to which differences in peripheral excitation contribute to this variability. Detection thresholds were measured for a 300-ms, 1000-Hz pure-tone signal presented simultaneously with 4-tone maskers. Masker frequencies were drawn from 300-3000 Hz, excluding 920-1080 Hz. Twenty-five masker samples were randomly generated and stored. The first set of "fixed" conditions, using a 2IFC adaptive task, measured thresholds with each sample fixed throughout trial blocks. Average thresholds differed by as much as 20 dB across masker samples. To examine whether this variability reflects differences in energetic masking, signal thresholds were predicted for each sample using an excitation-based model of loudness [Moore et al., J. Audio Eng. Soc. 45, 224-237 (1997)]. These predictions accounted for significant portion of the variance in observed thresholds. However, there were also marked individual differences, on the order of 20 dB, particularly for masker samples predicted to produce only minimal excitation-based masking. The second set of "random" conditions presented one of 5 maskers, randomly selected on each interval. In some conditions those maskers were associated with low fixed-masker thresholds and in others with high fixed-masker thresholds. Results were similar across random masker conditions regardless of associated fixed-masker thresholds or estimated energetic masking. These findings suggest that effects of informational masking are largest for conditions in which excitation-based masking is low.

682 Reaction Times for Natural Sound Identification

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Anecdotal evidence indicates that human listeners can identify natural sounds with remarkable ease. There is, however, little behavioral data that constrains the possible mechanisms involved. Here we report identification times for different types of natural sounds. Stimuli were 250-ms samples of 13 recorded musical instruments, including the singing voice. Sounds were presented one by one, drawn randomly from the same one-octave pitch range, so that pitch was not a cue for identification. Listeners had to react as fast as possible when they identified a target sound category and withhold their response when the sound was from a different category (the go–no-go task). On different experimental blocks, the category could be the human voice (vowels "A" and "I"); percussive instruments (marimba and vibraphone); or bowed strings (violin and cello). Simple detection times were also collected with the same sounds. The identification time was defined as the difference between categorization and detection times. Median identification time was as fast as 122 ms for the voice, shorter than the sound samples. An ANOVA on the mean log-transformed reaction times showed that there were no differences in detection times across categories. There were, however, significant differences in identification times between category. The voice was identified the fastest, followed by the percussions and the strings. Results were compared to subjective dissimilarity ratings on the same set of sounds, and with a model based on the distance between spectrotemporal excitation patterns. The ordering of identification times was consistent with the subjective dissimilarity ratings, but not with the spectrotemporal excitation pattern model. This suggest that sound source identification is facilitated by both salient acoustic features and familiarity with the source. In addition, spectrotemporal patterns may not be used in full to perform fast identification.

683 Spectrotemporal Modulation Sensitivity in Hearing-Impaired Listeners

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The most obvious features of a speech spectrogram are energy modulations in time and frequency. It has been shown that speech intelligibility in noise and reverberation can be predicted by a model of spectrotemporal modulation (STM) strength in the auditory periphery. Hearing-impaired (HI) listeners may have an impaired ability to process complex STMs, which could affect their ability to understand speech. STM sensitivity may also vary as a function of absolute frequency. This study examined effects of hearing loss and absolute frequency on STM sensitivity. Threshold modulation depths for the detection of STM applied to broadband (four-octave) or narrowband (one-octave) noise carriers presented at 80 dB SPL/oct. were measured in eight normal-hearing (NH) and 10 HI listeners as a function of spectral modulation density (0.5-4 cycles/oct.), temporal modulation rate (4-32 Hz) and absolute frequency region (500-4000 Hz). Consistent with previous results, performance declined with increasing density and rate. Furthermore, performance improved with increasing absolute frequency, with performance for the 4000-Hz narrowband condition roughly equal to wideband performance, suggesting that wideband measurements may not sufficiently characterize STM sensitivity. An existing model of STM sensitivity, consisting of a peripheral filterbank and STM-tuned cortical filters, accounted for the density and rate effects, while the incorporation of an increasing-Q gammatone filterbank captured the absolute frequency effect. The HI data showed similar qualitative trends, but with reduced performance at higher densities, which was accounted for by a reduction in the frequency selectivity of the peripheral model. Relations between STM and speech intelligibility measures are discussed. [Supported by the Oticon Foundation]

684 Behavioral and Physiological Studies of Amplitude-Modulation Detection

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Amplitude modulations (AM) of complex sounds represent a rich form of temporal information available to the auditory system. AM in the stimulus is altered by sharp tuning in the periphery, encoded by phase-locked responses of auditory-nerve fibers, enhanced by neural circuitry in the brainstem, and filtered by AM-tuning mechanisms in the midbrain. This study focused on the relationship between behavioral thresholds for AM detection in rabbit or human and physiological responses in the midbrain of the awake rabbit. Behavioral thresholds were estimated using a single-interval two-alternative choice task with a Bayes procedure. The Bayes procedure avoids the influence on threshold of the experimenter's choice of the starting stimulus, which is a problem for tracking procedures, and also avoids the influence on threshold of the range of stimuli used in constant-stimulus procedures. However, the Bayes procedure introduces more uncertainty into the task. Similar paradigms were used to estimate thresholds in rabbit and human, although rabbits were tested in free-field and reinforced with food, and humans were tested with headphones and reinforced with correct-response feedback. Behavioral modulation transfer functions for rabbits and humans were roughly similar in shape, but human thresholds were approximately 10 dB lower at most frequencies tested. For AM noise stimuli matched to those used in the behavioral experiments, neural synchrony (but not rate) thresholds explained the most sensitive behavioral thresholds of the rabbits. For AM tones at the neurons' best frequencies (BF), rate or synchrony thresholds may explain rabbit behavioral thresholds. Human thresholds were much lower than could be explained by either rate or synchrony for noise or tones at BF for stimuli at the levels used in psychophysical tests. [Supported by NIDCD-R01-01641]

685 Perception of Amplitude Modulations Near Threshold in Quiet: Temporal or Spectral?

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Many normal-hearing people show a fine structure, i.e. a ripple effect in their threshold in quiet of up to 15 dB over a frequency range of typically one-tenth of an octave. The present study investigated if this fine structure affects the perception of temporal amplitude modulation near threshold in quiet. Detection thresholds of a sinusoidal amplitude modulation were measured in normal–hearing listeners for a sinusoidal carrier with a low intensity. The frequency of the carrier was chosen either to be equal to the frequency of a minimum or to that of a maximum in the fine structure of the threshold in quiet. Modulation detection thresholds for a carrier level of 15 dB SL were higher for a carrier at a minimum than for a carrier at a maximum of the fine structure. The difference in modulation detection threshold was up to 14 dB for a modulation frequency that was equal to the frequency separation between maximum and adjacent minimum. In general, the modulation frequency was smaller than 150 Hz, i.e. a modulation frequency which is commonly associated with temporal perception of envelope fluctuations, at least at these low carrier levels. The lower modulation detection threshold for the carrier in the maximum may result from a higher sensitivity to the sidebands, since the sidebands fall within the adjacent minima due to the special choice of the modulation frequency. Given that the sidebands at modulation detection threshold are often below absolute thresholds, modulation detection can, however, not be interpreted as a purely spectral cue. This effect of carrier position relative to the fine structure tended to decrease for modulation frequencies that were higher or lower than the frequency separation between maximum and adjacent minimum. The

effect of carrier frequency was slightly smaller (up to 9 dB) when the same sound pressure level was used for the two carrier positions. Higher modulation detection thresholds for a carrier at a fine-structure minimum than for a carrier at a fine-structure maximum were found for carrier levels as high as 30 dB SL. The data indicate that the sensitivity to temporal amplitude modulation can already vary substantially for small variation of the carrier frequency due to the spectral shape of the hearing threshold.

686 Behaviorally Measured Gap Detection Thresholds in CBA/CaJ Mice

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Change is a fundamental characteristic of environmental sounds, transmitted through rapid amplitude and frequency fluctuations and providing vital information to a listener. Temporal resolution is the auditory system's ability to detect these changes. A common paradigm for assessing temporal resolution is gap detection. This simple task requires subjects to detect silent gaps within noise. Gap detection thresholds have been obtained from a number of mammals and several bird species.

Mice (*Mus musculus*) have become important models in the study of hearing, and the number and variety of genetically engineered mouse strains makes them ideal models for studies of deafness. In order to fully understand the effects of genetic mutations on hearing, it is necessary to determine the abilities of these mice. Unfortunately, auditory studies using awake and behaving mice are scarce. To date, most have used physiological or unconditioned reflex measurements. For instance, Walton et al. (1997) determined gap thresholds in CBA mice using behavioral reflex modification and neurophysiological recordings in the inferior colliculus. In both cases, they found gap thresholds between 1-2 ms. One of the drawbacks of reflex experiments is that they require stimuli to be extremely intense to get a response, and that makes them impractical for later use in studies of hearing loss. In the present experiment, gap detection thresholds for 55 dB (SPL) broadband noise bursts were obtained for CBA/CaJ mice using operant conditioning methods and the psychophysical Method of Constant Stimuli. Our results indicate thresholds near those of Walton et al., but for stimuli presented at much lower sensation levels. Psychophysical methods utilizing trained animal observers have been used in other species to obtain accurate and reliable measures of temporal resolution. The results here suggest that similar procedures can be used to assess hearing in normal and aging mice.

687 Tone Detection in the Presence of Continuous vs. Pulsed Maskers

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The detectability of a tone added to a noise masker is somewhat improved when the noise masker is turned on before the signal begins and turned off after the signal ends [e.g., Weir et al., J. Acoust. Soc. Am., 61, 1298-1300 (1977)]. The current study asked whether this finding held when an informational masker was tested. The expectation was that for an informational masker there would be no advantage of a continuous masker because the variation in energy across time and frequency would deter the formation of a useful reference/template against which the signal frequency . The two informational maskers tested were sequences of 60- or 30-ms bursts comprised of, on average, 20 tone pips drawn at random on a logarithmic scale ranging from 200 to 5000 Hz. The signal to be detected was a sequence of either three 60-ms or six 30-ms equal-frequency tone pips, depending on whether the masker was composed of 60- or 30-ms bursts, respectively. When present, the signal was temporally aligned with the masker bursts. Pink-noise maskers were also tested. The masker was either presented continuously, or was pulsed on and off with the sequence of signal pips. Although there were substantial individual differences, subjects' thresholds were at least as good when the masker was continuous as when the masker was pulsed, and typically superior when the masker was presented continuously, for all masker types. [Supported by NIH DC002012]

688 Infants' and Adults' Detection and Discrimination in Modulated and Unmodulated Noise

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Sounds in natural environments fluctuate in amplitude over time. When adult listeners process one sound among others, they can improve their perception of the target sound by listening at times when the competing sound amplitude is low. This study addressed whether infants are able to use fluctuations in the amplitude of a competing sound to improve their perception of a target sound. Infants aged 7-9 months and adults aged 20-30 years participated. All had normal hearing and passed screening tympanometry. In one experiment, listeners detected a 1-kHz tone in speech-spectrum noise. Listeners learned to respond when they heard the tone. In a second experiment, listeners discriminated the vowels /a/ and /i/ in a background of speech-spectrum noise. Listeners learned to respond when a repeating vowel changed from /a/ to /i/ or from /i/ to /a/. In both experiments, the background noise was modulated with the envelope of single-talker speech in one condition, while the noise was unmodulated in the other condition. Sensitivity, described as *d′*, was estimated using an observer-based procedure. The signal-to-noise ratio in both tasks was set to a level at which each listener could achieve *d′* between .8 and 1.5 (65-75% correct) in the unmodulated noise condition. The same signal-to-noise ratio was used in the modulated noise condition. Average *d′* in the unmodulated noise condition was just over 1 in both tone detection and vowel discrimination for both infants and adults. Average *d′* in both tasks improved to about 2.5 in the modulated noise condition for adults, but did not improve for infants. This result suggests that infants cannot take advantage of the temporal properties of competing sounds to improve their perception of target sounds. [Work supported by NIDCD, R01 DC00396 and P30 DC04661.]

689 Spectral and Temporal Masking Release in the Low-Frequency Range for Normal-Hearing and Hearing-Impaired Listeners

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For normal-hearing (NH) listeners, intelligibility is better for speech in fluctuating than in steady noise; the difference is called masking release (MR). MR is typically small for hearing-impaired (HI) listeners, and this may be partly due to a reduced ability to use temporal fine structure (TFS) information. Recent results suggest that the ability to use TFS may be reduced for HI listeners, even when the speech spectrum is restricted to the low-frequency region where absolute thresholds are normal or near normal. The goal of the present research was to examine MR for lowpass-filtered speech and noise using NH listeners and HI listeners with normal hearing or mild hearing loss (absolute thresholds < 15-20 dB HL or between 20-30 dB HL) below 1.5 kHz and moderate-to-severe cochlear hearing loss (> 40 dB HL) at higher frequencies. Consonant identification in noise was measured at various signal-to-noise ratios (SNR). The speech-shaped noise masker was: (1) unmodulated; (2) rectangular-wave amplitude modulated at 8 Hz with a 100% depth and duty cycles of 25 and 50%; (3) spectrally modulated, by passing the noise through an auditory filterbank, and setting to zero the outputs of filters 2, 4, 6... etc, or filters 3, 4, 7, 8, 11, 12... etc. This produced a series of spectral dips. Speech and noise stimuli were lowpass filtered at 1.5 kHz. A highpass filtered (>1.5 kHz) noise masker was added to each stimulus at +12-dB SNR to prevent off-frequency listening. For each listener, temporal MR was estimated as the difference in consonant identification between temporally modulated and unmodulated noise (condition 2 versus 1), and spectral MR was estimated as the difference in consonant identification between spectrally modulated and unmodulated noise (condition 3 versus 1). Spectral and temporal MR data were compared across groups of listeners. Preliminary data suggest that temporal MR is smaller for HI than for NH listeners, but the difference is less clear for spectral MR.

690 Masking Period Patterns of Exponentially Ramped and Damped Noises

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Noises that are modulated with either an exponentially rising, repeating modulator or a falling, repeating modulator may evoke strong perceptual differences. Pairs of stimuli with modulation half lives less than 32 ms sound different to most normal-hearing listeners when the modulation period is 25 ms, and the ramped noises tend to sound more “noise-like” than the damped noises. This study was designed to determine whether the perceptual differences between the ramped and damped noises are due to the internal envelope of the ramped stimulus being shallower than the internal envelope of the damped stimulus. Masking period patterns (MPPs) were measured for broadband noises with ramped and damped exponential modulators that varied in half life from 1 to 16 ms and had a modulation period of 25 ms. The signal to be detected was a 5-ms, 2000-Hz pure tone burst added in random phase to the modulated noise masker. The tone was presented in the middle of the masker at times which sampled the modulation period of the masker: 125, 130, 135, 140, and 145 ms. The ramped MPPs tended to show a slower change in threshold with changing signal delay than the damped MPPs. By time-reversing the ramped MPP, comparisons could be made between thresholds for which the masker power was the same between ramped and damped stimuli. Differences between the damped and the time-reversed ramped MPPs were the largest for the short half lives (1, 2, and 4 ms; often exceeding 15 dB) and were effectively absent at the 8- and 16-ms half lives. Although the results at the shorter half lives were consistent with the hypothesis that the internal representation of the ramped stimulus has a shallower envelope than the damped stimulus, the results at the longer half lives were not. As result, the perceptual processes that underlie the discrimination data are likely to be different than those that underlie the masking period patterns, at least for the longer half lives. Psychophysical modeling will be used to explore the possible auditory mechanisms responsible for the differences in the ramped and damped MPPs.

691 Probing the Cochlea with Partial-Tripolar Cochlear Implant Stimulation: The Feasibility of Electrically-Evoked Auditory Brainstem Measures

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The partial-tripolar configuration allows for systematic variation of electrical field size for cochlear implant stimulation while maintaining a constant locus of activation centered at the active electrode. Focal configurations such as partial-tripolar exhibit greater variations in behavioral measures of threshold and loudness, possibly due to local irregularities in neural responsiveness. Electrically-evoked auditory brainstem responses (EABRs) may also reflect these local irregularities. The slope of the EABR input/output functions are believed to reflect auditory neuron recruitment of a synchronized response; steep slopes indicate quick recruitment while shallow slopes indicate slow recruitment. EABRs were measured in cochlear implant subjects using both a broad and a restricted partial-tripolar stimulus configuration. EABRs were measured for two channels in each subject, those with the highest and lowest behavioral thresholds obtained with focal stimuli. As with behavioral thresholds, EABR thresholds were higher for partial-tripolar stimulation than monopolar. The slopes of the input/output functions were steeper for broad stimulus configurations for all subjects. However, there was no consistent difference between the slopes of the input/output functions for the highest and lowest threshold channels. These results will be discussed in the context of existing psychophysical tuning curve data.

692 Automated Detection of Evoked Compound Action Potentials in Intracochlear Telemetry Recordings

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Several cochlear implant models are equipped to measure the neural response to electrical stimulation using intracochlear electrodes. These measurements have the potential to provide complimentary information to psychophysics. However, measurable evoked compound action potentials (ECAP's) may not occur at all stimulus levels, even those within the subject's dynamic range. Therefore, it is of interest to determine an efficient and robust method for detecting the presence of an ECAP in response to a stimulus. ECAP's can often be detected visually after stimulus artifact removal; however, visual inspection can be time-consuming and can potentially introduce bias and inconsistencies. As an alternative, this study considers the performance of several classifiers, including a Fisher Linear Discriminant (FLD) and Distance Likelihood Ratio Test (DLRT), for automated ECAP detection. The features used by these classifiers to detect the presence of an ECAP include signal energy, the latency and amplitude of the initial negative and positive (N1 and P1) peaks that define the typical shape of an ECAP recording, and the latency and amplitude of global minimum and maximum. The performance of these classifiers is compared to a tree-based classifier, replicating the clinically-implemented AutoNRT system described in Botros et al. (2007), as a baseline. A dataset of 623 recordings was used, and these recordings were classified through visual inspection into four categories based on the certainty of the presence of an ECAP: full ECAP, ECAP with partial N1, ECAP with only P1 visible, and null response. The effect of incomplete ECAP measurements on detection performance is compared across classifiers. Initial results suggest the DLRT may provide a robust classifier for automated ECAP detection.

693 The Recovery Function of the Late Auditory Evoked Potential in CI Users and Normal-Hearing Listeners

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This study characterized the recovery function of the late auditory evoked potential (LAEP) elicited by acoustic stimulus pairs in postlingually-deafened adult cochlear implant (CI) users and young listeners with normal hearing (NH). Five tone-burst pairs with intervals varied at 0.7, 1, 2, 4, and 8 s were presented in a random order. The first stimuli in the pairs were referred to as the maskers and the second stimuli as the probes. The inter-pair interval (IPI) was fixed at 15 s. Stimuli were presented at each CI user's most comfortable listening level via a loudspeaker on the implantation side. In NH listeners, stimuli were presented via an insert earphone at 80 dB SPL monaurally to the left or right ear. Averages of the electroencephalographic data were derived independently for each masker and probe in each of the 5 tone-burst pairs. The probe responses normalized to the masker responses within pairs were plotted as a function of the masker-probe interval (MPI) to form a recovery function for each participant. The recovery functions in CI group and NH group displayed some similarities and differences. There was a greater inter-subject variability in the recovery functions across CI users compared to NH listeners. The correlation between the slope of the recovery function and speech perception capability in CI users was examined. The underlying neural mechanisms of the LAEP recovery from masker responses will be discussed. Findings of this study are meaningful in the context of our future research in restoring the natural recovery functions of the LAEP in CI users to improve CI benefits.

694 The Adaptive Patterns of the Late Auditory Evoked Potential

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Previous studies have investigated the late auditory evoked potential (LAEP) in cochlear implant (CI) users after averaging the responses to all stimuli presented, overlooking the LAEP change pattern occurring during stimulus repetition. This project provides a detailed description of the adaptive patterns of the LAEP elicited by acoustic stimulus trains in postlingually-deafened adult CI users. Stimulus trains consisting of 10 tone bursts in each train, with inter-stimulus intervals of 0.7 s and inter-train intervals of 15 s, were presented at each CI user's most comfortable listening level. Averages of the electroencephalographic data were derived independently for each tone burst within the train across the total number of train presentations. The N1-P2 amplitude to each tone burst was normalized to the first response in the train. Current data were compared with those in our previous study, in which normal-hearing (NH) young human subjects were presented with the same stimulus paradigm at 80 dB SPL. Results showed that, similar to that in NH listeners, the N1-P2 amplitude was maximal for the first tone burst and rapidly decreased over the next several tone bursts and reached a relatively stable level for the remainder of the stimuli of the train in CI users. However, the normalized amplitude reduction of the LAEP in CI users was significantly less than the reduction in NH listeners. The variation of LAEP measures across subjects was greater in CI users than in NH listeners. It was proposed that less amplitude reduction during the presentation of the tone-burst trains in CI users was due to the absence of the contribution of normal hair cells and hair cell-nerve fiber synapses. This study has the implication of restoring the natural pattern of the LAEP to repeated stimuli in CI users to improve their performance.

695 Developing a Novel Method for Evaluation of Cochlear Implant Signal Processing Strategies

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Evaluation of user-independent efficacy of novel cochlear implant (CI) signal processing strategies is difficult. Current practice relies on customization based on user performance in the clinical setting. As a result, it is impractical to achieve theoretical optimality, and improvements to stimulation strategies are achieved using trial-and-error. A computational framework that approximates the upper bound on CI performance is desirable to reduce the number of novel strategies that require evaluation on patients. Our goal is to develop a physiologically-based framework, using accepted models of the acoustically- and electrically-induced nerve activation patterns (NAPs). An initial framework has been developed using NAPs generated from the Zilany-Bruce auditory-periphery model to predict behavioral performance of normal hearing (NH) listeners. Following refinement, this framework will serve as a core element in optimizing novel CI electrical-stimulation. Initially implemented with a mean-square error cost function, the framework exhibits promising performance in predicting psychophysical performance of NH listeners undertaking a closed-set vowel identification task. Extension to NH listeners performing closed-set consonant identification is underway, where the goal is optimizing the cost function to extend to evaluation of the same tasks performed by CI users.

696 The Representation of Sinusoidally Modulated Electric Pulse Trains in the Spike Output of Auditory Nerve Fibers

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We previously examined the changes in auditory nerve fiber (ANF) responses caused by constant-amplitude electric pulse-trains (Zhang et al., 2007, JARO; Miller et al., 2008, JARO). Ongoing work is aimed at describing dynamic changes in ANF responses to more clinically relevant stimuli, i.e., sinusoidally amplitude-modulated (SAM) pulse trains. We hypothesized that adaptation and refractory effects will modify SAM pulse-train responses over intervals similar to the duration of speech tokens and may alter the information content of those responses. Acutely deafened cats were used and stimuli were delivered via an intracochlear electrode. The stimuli were high-rate (5000 pulse/s), 400 ms long, trains modulated by 20, 100, and 500 Hz modulation frequencies (MFs) at 0, 5, 10, 20, and 50% modulation depths (MDs). Levels were also varied to assess spike-rate effects. Initial data (Hu et al., 2008, ARO) indicated that adaptation to SAM pulse trains resulted in changes to the vector strength (VS) based on the MF period. VS increased over time, indicating a narrowing of the period histogram. While VS was sensitive to MF and MD changes, it also reached its maximal value for some stimulus conditions, limiting its usefulness. We have collected additional data and adopted a spectrally (FFT) based analysis of PST histograms, with the notion that it will provide a better assessment of ANF transmission of modulation information. As the histogram can contain a significant harmonic distortion, we focused on the spectral component at the modulation frequency (F1). As with our VS-based measure, F1 changed over the pulse-train duration, but in ways different from the VS trends. At lower MFs (20, 100 Hz) and MDs (5%, 10%, 20%), F1 generally increased over time for higher stimulus levels (or spike rates), while it tended to decrease over time for lower-level stimuli. At high MFs (i.e., 500 Hz) or MDs (i.e., 50%), F1 generally decreased over time. The trends were also related to the observed degree of rate adaptation. The data are discussed in the context of a model of nonlinear spike-rate growth and the influence of spike-rate adaptation.

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697 A Computational Model of an Electrically Stimulated Auditory Nerve Fiber with Realistic Anatomical Features and Rate Adaptation

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Efforts to describe auditory-nerve-fiber (ANF) responses to electrical stimuli (such as those used by prosthetic devices) have been aided by computational simulations. The utility of computational models is typically limited by the extent of available ANF data from animal studies. Our group has been collecting feline ANF data to describe adaptation to electric pulse trains as well as subsequent recovery from such stimuli. In a parallel effort, we are developing a biophysical model of a feline ANF that incorporates an ionic mechanism to simulate spike-rate adaptation, a property typically missing from computational models. Our long-term goal is to use this model to investigate ANF responses from a wider range of stimuli than is feasible from animal experiments.

A preliminary version of this model, based on a single node of Ranvier, was recently developed (Woo et al., IEEE Trans. Biomed. Eng., in press). Ongoing efforts are aimed at developing a model of a feline ANF that includes a cell body, the partially myelinated peripheral process, and the central, myelinated axon, with nodes that simulate refractoriness and longer-term rate adaptation.

This presentation describes model responses to single pulses, two pulses, and pulse trains presented at rates of 250 and 5000 pulse/s. The model generates a plausible conduction velocity and time delay presumed to be due to cell body. Responses to single pulses and pairs of pulses show dynamic range, spike latency, jitter and refractory properties consistent with ANF responses. Responses to pulse trains show adaptation properties characterized by

decreases in discharge rate over time as well as changes in inter-spike intervals. This report also demonstrates that site of spike initiation can be either be peripheral or central to the cell body, dependent on electrode-to-fiber distance electrode and different curvatures of the neuron.
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698 Does Electrical Stimulation of the Dorsal Cochlear Nucleus Relay Its Tonotopic Organization to the Inferior Colliculus?

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Auditory brainstem implants (ABIs) recover hearing through electrical stimulation of the cochlear nucleus (CN) of patients who do not benefit from cochlear implants. Depending on the physiological condition, duration of the pre-existing deafness, or the effectiveness of implantation and stimulation in the CN, speech performance from using ABIs varies greatly across patients. To enhance targeted implantation and stimulation, there is a need to better understand how each part of the CN contributes to electrical stimulation-induced hearing perception. We initially investigated whether electrical stimulation of the dorsal cochlear nucleus (DCN) relays the tonotopic organization of the DCN to the inferior colliculus (IC) and produces corresponding frequency-specific activation. Frequency tuning curves in both the DCN and IC were first obtained to determine the characteristic frequencies (CFs) of both stimulation and recording loci in the DCN and IC. Different loci in the DCN were electrically stimulated while both single- and multi-unit activities in the IC were recorded. The electrical stimuli were charge-balanced biphasic electrical pulses delivered to different stimulating channels in the DCN. Electrical spatial tuning curves (eSTCs) were constructed to determine the best channels in the IC based on the lowest thresholds to electrical stimulation of the DCN. Correlation analysis was performed between the CFs of the stimulation loci in the DCN and the CFs of the IC that matched the best channels from eSTC. Our preliminary results demonstrated that there is a strong correlation between the CFs of the stimulation loci in the DCN and those of the best channels in the IC. Other types of activation to DCN electrical stimulation were also observed. Our findings suggest that electrical stimulation of the DCN may participate in hearing process, in addition to stimulation of the ventral cochlear nucleus.

699 Speech Intelligibility and Spatial Release from Masking in Children with Unilateral Sensorineural Hearing Loss: Effect of Age and Amplification

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Spatial release from masking (SRM) is the improvement in speech intelligibility obtained when interfering speech is spatially separated from the target speech. SRM has been measured in children with normal hearing, bilaterally. It is unclear how UHL affects SRM. This study measured SRM in the aided and unaided condition in children with UHL who use a hearing aid in the impaired ear. The goal of the study was to determine whether children with UHL can take advantage of spatial cues.

Fourteen children with UHL from 2 age groups, 7 older children (10y-14y) and 7 younger children (6y – 9y), participated. Testing was done in a sound treated booth with 4 loudspeakers, 2 at 0º (front), and 1 each at 90ºand -90º, located at ear level 1 m from the listener's head. The CRISP (Litovsky, 2005) was used to obtain speech reception thresholds. A target spondee word(male voice) was randomly selected from a list of 25 and presented at 0º in each of 4 interferer (2 female voices) conditions: quiet (no interferers), front (0º), near impaired ear (90º right or left), and near normal ear (90ºright or left). Target level started at 60dB SPL and followed a 3-down-1-up adaptive procedure. Interferer level was fixed at 60dB SPL. SRT was measured in the aided and unaided conditions.

SRM was significantly greater when the interferers were near the impaired ear than when near the normal ear, regardless of amplification condition. No significant difference between aided and unaided SRMs was found. A significant age effect was found for SRT only (but not SRM). Degree of hearing loss had no effect on any measure.

Children with UHL use spatial cues to improve speech intelligibility if the interfering speech is near the impaired ear. Use of spatial cues is limited when the interferer is near the normal ear. The best listening strategy for children with UHL is to keep the impaired ear (aided or unaided) toward the interfering speech.

700 Psychophysical Evidence for Reduced Channel Interactions in Cochlear Implants Using Focused Electric Stimulation

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The multichannel cochlear implant is a neural prosthesis designed to convey perceptually important information in an acoustic signal to a deaf listener via direct electric stimulation of the auditory nerve. A commonly implicated performance-limiting factor, which occurs during stimulation with conventional techniques, is the effect of

current spread in the ionic fluids of the cochlea. Current spread can result in the excitation of large populations of auditory nerve fibers along the length of the spiral ganglion, and channel interactions occur where input currents from multiple electrodes influence the response of overlapping populations of nerve fibers.
We implemented a recently proposed method [van den Honert and Kelsall, J Acoust Soc Am 2007;121:3703-3716] that may improve performance by providing better control over the spatial patterns of elicited neural activity. By superimposing electric currents from all electrodes in the array, with appropriate stimulus magnitudes on each electrode, they showed that stimulating voltages can be canceled at the locations of electrodes where excitation is not desired. By "focusing" excitation in this way, higher spectral resolution should be possible.
We conducted preliminary testing of this hypothesis in two subjects using a psychophysical measure of electrode interaction: the degree to which subthreshold stimuli on one electrode influence the detection threshold for stimuli on a neighboring electrode for simultaneous and nonsimultaneous stimuli. In both subjects, significant interaction was measured using conventional stimulation techniques. "Focused" stimulation significantly reduced interaction in both subjects, suggesting that by using focused stimulation techniques, it should be possible to improve the spectral resolution of sound processors and ultimately the performance of cochlear implant listeners on speech recognition tasks.
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701 Psychophysical Versus Physiological Spread of Excitation with Tripolar Configurations

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In the research on focused electrode configurations in Cochlear Implant systems (CI), Tripolar configurations have increasingly gained interest as a means to reduce channel interaction compared to the standard monopolar stimulation. However, tripolar configurations require higher current levels to achieve sufficient loudness growth. In this study, we will compare Spread of Excitation (SOE) profiles generated by monopolar and (partial) tripolar electrode configurations at equal loudness levels in adult Advanced Bionics Cochlear Implant users, in the hopes of finding whether there is a subject specific optimum for tripolar or other focused electrode configurations.
Psychophysical SOE profiles from monopolar and tripolar electrode configurations were measured using an adaptive forward masking paradigm. In addition, physiological SOE profiles were collected, using a masker-probe protocol with fixed probe position and variable masker position. In both experiments, the monopolar probe was masked with a monopolar masker only. The (partial) tripolar probes were masked with a monopolar masker in order to compare differences in interaction between a monopolar probe on the one hand, and a tripolar probe on the other. In addition, the (partial) tripolar probes were also masked with equally configured (partial) tripolar maskers to obtain 'pure' (partial) tripolar psychophysical and physiological SOE profiles.
Earlier, we have established loudness balanced monopolar and tripolar threshold and supra-threshold levels and measured intracochlear electrical fields with the same electrode configurations, loudness levels and subjects as in this study. Although the results suggested a relation with the location of the electrode array in the cochlea, it was not clear whether the deviations were influenced by the amount of individual neural survival.
Preliminary results of this study showed no clear difference between physiological monopolar and tripolar SOE profiles, and no clear relation of the physiological SOE profiles with the loudness levels and the intracochlear electrical fields. However, we saw an individual optimum in the width and the slope of the excitation profile per subject and electrode tested so far, suggesting the possibility of a subject specific optimal electrode configuration.

702 Virtual Channels with Sequential Stimulation in Cochlear Implant Users

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In cochlear implants (CIs), virtual channels can be elicited by sequential stimulation (e.g., Kwon and van den Honert, 2006) of adjacent electrodes. In the present study, pitch discrimination was measured for sequentially interleaved pulse trains in six users of the Nucleus CI device. The stimulation rate for each pulse train was fixed at 250 pulses per second; the stimulation mode was fixed at BP+1. Pulses were interleaved with a 500, 1000, 1500 or 2000 microsecond offset. All stimuli were loudness-balanced, and stimulation levels were jittered over a small range. In the first experiment, the first pulse train was delivered to an apical electrode, and the second pulse train was delivered to either the same electrode or to an adjacent basal electrode, allowing independent measures of within- and across-channel effects of the interleave rate. In the second experiment, single- and dual-electrode stimulation was compared within each interleave rate. Results showed that, for interleave offsets <1000 microseconds, sequential stimulation of adjacent electrodes often produced changes in place-pitch between the single component electrodes. Interleave offsets >1000 microseconds often involved changes in rate pitch for both single- and dual-electrode stimulation. These results suggest that when interleaving multiple pulse trains, offsets less than 1000 microseconds may produce virtual channels. While sequentially stimulated virtual channels with high rates may increase the spectral resolution beyond the number of physical electrodes, such stimulation may also result in "jittering" of the spectral envelope, depending on the relative amplitudes delivered to adjacent electrodes. The results imply that the rate and sequence of stimulation should be well-considered to maximize the spectral resolution.

703 Improving Virtual Channel Discrimination Using Current Focusing in Cochlear Implant Users

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Introduction: Recently, much research has investigated using simultaneous stimulation on multiple electrodes along a cochlear implant electrode array. Stimulating two adjacent electrodes in phase (current steering) creates a current peak (and corresponding virtual channel (VC)) between the two electrodes. Tripolar stimulation (current focusing), where two flanking electrodes stimulate in opposite phase of a center electrode, reduces current spread relative to monopolar stimulation. We hypothesized that if current steering and current focusing are combined, a cochlear implant patient will have better discrimination of VCs when compared to VCs created by monopolar stimulation (MPVCs).

Methods: Stimuli consisted of VCs created with monopolar stimulation (MPVCs) or focused quadripolar stimulation (QPVCs). QPVCs consist of MPVCs with additional stimulation on the flanking electrodes in opposite phase. The flanking electrodes are used to focus the VC similarly to the way the flanking electrodes focus tripolar stimulation.

Seven Advanced Bionics Clarion II or HiRes 90K users were tested using a 3 interval forced-choice task. VC discrimination using either MPVCs or QPVCs was measured. Discrimination between 6 loudness-balanced VCs was compared for each VC type. The procedure was repeated 30 times for each subject for an apical, medial, and basal pair of electrodes.

Results and Discussion: The MPVC and QPVC cumulative d' was calculated for each electrode pair. Six of the seven subjects showed better VC discrimination for the QPVCs than for the MPVCs for the apical, medial and basal locations. One subject showed better QPVC discrimination for only the apical and medial electrodes. A two-way repeated-measures ANOVA revealed a main effect of VC type ($p < 0.005$) but no effect of electrode place or interaction was detected. Although there was great variability across subjects in both absolute performance and size of improvement, all subjects demonstrated some benefit from using QPVCs.

704 Spread of Excitation and Pitch Percepts in Cochlear Implants Revealed by Auditory Stream Segregation

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There are several electrode configurations to construct a stimulation channel in a cochlear implant (CI), such as spatially restricted bipolar (BP) or wide monopolar (MP) mode. While either BP or MP is typically chosen for speech processing based on the factors such as power consumption, subjective preferences and the availability of the mode in patient's device, it is unclear whether the choice of mode in clinical applications is made properly to maximize the benefit. Particularly 1) perceptual consequences of the differences in spatial excitation between MP and BP and 2) pitch relations of MP and BP channels are poorly understood. In a pilot experiment it was observed that pitch judgment across MP and BP is often difficult and unreliable in pair-wise pitch comparison, due to widely different sound percepts. Meanwhile, CI listeners are able to perform auditory stream segregation, as demonstrated in a rhythm discrimination task using a two-alternating tone sequence (ABAB…; Hong and Turner, 2006), where detection of a temporal irregularity between A and B becomes poorer as the frequencies of tones are farther apart; indicating that automatic stream segregation occurs in CI users in a similar fashion to normal hearing listeners where the dissimilarity of excitation pattern leads to segregation. In the present study, the same experimental procedure was taken for various pairs of stimulation channels (within and across MP and BP channels) as stimulation electrodes of A and B were systematically varied. Results indicate 1) a narrower spread of excitation in BP than MP mode in some subjects, and 2) a diverse degree of pitch saliency of BP channels—from highly salient to ambiguous, revealing possible local irregularities in the survival of neural population. In conclusion, a stream segregation test can provide an objective measurement of the excitation spread and pitch percepts of various stimulation configurations in electric hearing [Work supported by NOHR].

705 Current Spread in Monopolar and Partial Tripolar Stimulation Modes

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Reducing current spread in the cochlea has the potential to increase the number of distinct effective channels for cochlear implant users. It has been shown in physiological studies and computation models that bipolar, tripolar or partial tripolar stimulation could produce a narrower spread of current than monopolar stimulation within the cochlea. However, with bipolar or tripolar stimulation, absolute current levels must be increased in order to achieve equal loudness compared with a monopolar stimulus. Because an increase in stimulation level results in an increase in current spread, it is unknown if the current spreads from bipolar or tripolar stimuli are actually narrower than from monopolar stimuli when the stimuli are all of equal loudness.

An experiment examining the effects of focusing in partial tripolar stimuli using electrically evoked compound action potentials (ECAPs) was conducted, using a masker-probe subtraction technique. Stimuli consisted of probes with different amounts of focusing ( = 0.0 to 0.75) on a fixed electrode, and monopolar maskers presented on even numbered electrodes. All stimuli were loudness balanced at a comfortable level. The neural response for each masker position was calculated by subtracting the response of the probe alone from the masked response to the probe. Neural responses for each focusing condition were normalized to their peak amplitude. The current spread was quantified by calculating the width in

millimeters at the level of 70% dropoff from maximum masking level.
Preliminary results indicate that focusing in partial tripolar stimulation does reduce the current spread, even in equivalently loud stimuli. This decrease in current spread could lead to more distinct effective channels in cochlear implants.

706 Subjective Estimates of Multi-Channel Loudness Growth in Cochlear-Implant Listeners

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The aim of the current investigation is to quantify the subjective growth of the loudness of multi-channel stimuli in cochlear implant listeners. Subjective estimates of the loudness of 300ms-long multi-channel stimuli were obtained in cochlear implant listeners. Results indicate that, for a fixed per-channel pulse rate of 1000 pulses/sec and fixed per-channel stimulus level (in % dynamic range), loudness increases substantially and monotonically with increasing numbers of channels stimulated (up to at least seven channels). For a fixed number of channels stimulated, the increase of loudness with increasing per-channel stimulus level (in % dynamic range) followed an expansive nonlinearity, with some exceptions. The relation between the subjective loudness estimate, the number of channels stimulated, and the stimulus level could be described by relatively simple equation. The relationship of these results to previous findings, and their implications for cochlear implant speech processor design, will be discussed. [Work supported by NIH/NIDCD grant no. R01 DC004786]

707 Improved Analysis of Hearing with Cochlear Implants

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In order to measure the extent to which new cochlear implant (CI) technologies improve hearing, reliable outcome measures are required. Typically, speech recognition tasks have been used as a clinical outcome measure in CI research, but they are often not ideal for comparing performance among different sound processing strategies, because speech perception ability changes over time, and speech measures often do not reflect the underlying advantage or disadvantage of the signal processing. To address those problems, psychoacoustic measures are used to evaluate users' sensitivities to specific acoustic elements that are important for clinical success. The work is based on the hypothesis that psychoacoustic measures are more sensitive to signal processing changes than speech or music measures. The "Fidelity120" and "HiResolution" strategies from Advanced Bionics were evaluated using three psychophysical measures including a spectral-ripple discrimination test, an acoustic modulation detection test, and a Schroeder-phase discrimination test as well as three different clinical outcome measures including a monosyllabic word recognition test in quiet, word recognition in steady background noise, and a music perception test. Seven subjects were tested acutely. No difference in the speech and music was observed. Average spectral ripple discrimination ability was better with Fidelity 120. Average Schroeder-phase discrimination ability was better with HiResolution. No difference was observed in amplitude modulation sensitivity. The results demonstrate that Fidelity 120 appears to improve spectral resolution, but decreases Schroeder-phase sensitivity and shows no clinical advantage for either speech or music. The results support the hypothesis that psychoacoustic measures are more sensitive to changes in sound processing than traditional speech measures. [Supported by NIH grants R01-DC007525, P30-DC04661, P50-DC00242, and Advanced Bionics Corporation.]
Key words: spectral and temporal resolution, cochlear implant

708 Brainstem Encoding of Speech in Noise and Its Relationship to Reading and Listening in Noise

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Children with reading disabilities have been reported to have significant difficulty perceiving speech in challenging listening environments. We hypothesized that the effect of noise on the brainstem processing of speech is related to reading and listening in noise performance. Our subjects included 35 children, ages 8 to 14 years, who had a wide range of reading abilities. They were evaluated using standardized reading measures, speech-in-noise perceptual measures, and brainstem recordings to a speech syllable presented in quiet and in noise (competing multi-talker speech babble). For the neurophysiologic responses, we assessed the extent to which responses changed from quiet to noise conditions in the time and frequency domains. Specifically, we calculated the degree to which the response peaks increased in latency with the addition of background noise. We also measured the decrease in FFT amplitude resulting from noise, particularly the frequency region corresponding to the first formant of the stimulus. We found a significant relationship between poor reading scores and greater latency increases, as well as FFT amplitude decreases. We also found that these neurophysiologic changes significantly correlated with speech-in-noise scores, such that greater latency increases and amplitude decreases were associated with poorer perception. These results imply that the degrading effect of noise on subcortical auditory processing is one of the factors underlying poorer speech perception in noise in children with reading disabilities. The brainstem response in noise, therefore, has the potential to guide treatment efforts with these children, including environmental modifications to achieve a favorable signal-to-noise ratio and auditory training programs designed to improve listening-in-noise performance.
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709 Multi-Microphone Adaptive Noise Reduction Strategies in Unilateral and Bilateral Cochlear Implants

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Bilateral cochlear implants (BI-CIs) seek to partially restore the advantages of binaural hearing to the profoundly deaf by providing them with ample access to binaural cues. Although current clinical data with BI-CIs attest to substantial speech intelligibility gains over unilateral stimulation, many CI users still experience difficulties while communicating in background noise.

In this pilot study, we evaluate two computationally inexpensive multi-microphone adaptive noise reduction strategies suitable for behind-the-ear (BTE) cochlear implant processors. The performance benefits are assessed for two different cochlear implant configurations (1) unilateral where two microphones are mounted on a single speech processor at a distance of about 1.5 cm and (2) bilateral where each of the two microphones is located at each side of the head. For each of the aforementioned configurations, we employ (1) the BEAM strategy which is already implemented in the latest commercially available cochlear implant processors and (2) the spatial enhancement via source separation (SESS) processing strategy. The latter is a novel strategy based on the premise that the target and masker source signatures are spatially separated and thus by minimizing the statistical dependence between them, we can retrieve their individual (and original) form.

In our experiment, the target is placed directly in front of the listener (0 degrees), whereas noise is on the right (90 degrees). The benefits with the two processing strategies in both configurations are evaluated at 0 dB signal-to-noise ratio with speech-weighted noise and multi-talker babble. Speech intelligibility is assessed in five postlingually deafened adults. We conclude that the use of multi-microphone strategies in both unilateral and bilateral CIs can dramatically boost speech understanding in background noise.

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710 Effects of Basal Spectral Shift on Consonant Confusion: Implications for Cochlear Implants

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The effects of shallow insertion of cochlear implant on consonant recognition and its confusion matrices were examined. Insertion depths of cochlear implant were simulated using a noise-excited vocoder with 4 to 16 channels in which the location of the most apical carrier band varied from a full insertion (i.e., 28 mm from the base) to a basal shift (i.e., 22 mm from the base) in a step size of 1 mm. Ten normal-hearing subjects participated in the 20-alternative forced-choice test, where the consonants were presented in a /Ca/ context. Shift of 3 mm or more caused the consonant recognition scores to decrease significantly. There was an significant interaction between spectral shift and spectral resolution (i.e., number of channels). The effects of spectral resolution disappeared when the amount of shift reached 3 mm or more. Articulation features of voicing, manner, and place were all affected by basal shift, with place of articulation being transmitted particularly poorly. Spectral shift has shown to have specific effects on the confusion patterns of the consonants. The error patterns as a function of insertion depth might be accounted for by the change in acoustic features of the signals resulting from the spectral shifts. Articulation features transmitted with pure temporal cues may contribute to the release of the effects of shallow insertion.

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711 Perceptual Restoration of Interrupted Speech: Effects of Spectral Degradation

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We investigated the ability of listeners to recognize periodically interrupted sentences under conditions of varying spectral degradation. The sentences were noise-band-vocoded and interrupted by multiplication with a sequence of Tukey windows alternating with silence (e.g., 100-ms-on and 100-ms-off). Our results suggest that spectral degradation of the interrupted speech from unprocessed to 24, 16, and 8 bands of noise has a strong effect on performance. For instance, using the 100-ms-on, 100-ms-off sentences, the average performance of four listeners dropped from an average of 85.5% with unprocessed speech to 61%, 48%, and 25% with 24, 16, and 8 bands of noise. It was hypothesized that the F0-variations naturally present as speech intonation may help in the perceptual reconstruction of the sentences when some spectral cues to F0 are still present. Preliminary results suggest that flattening the natural F0 contour in the sentences may reduce the intelligibility of the interrupted speech considerably under conditions of moderate spectral degradation (24-channel condition). These results may help explain why cochlear-implant listeners, who listen to no more than 8 channels of spectral information on average, experience particular difficulty in such tasks.

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712 Factors Influencing the Sequential Organization of Speech

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Van Noorden (1975) demonstrated that the frequency separation required for listeners to hear a sequence of alternating tones as two different auditory streams decreases when the rate of alternation increases. This experiment investigated whether a similar relationship

might hold between the fundamental frequency (F0) and inter-onset interval (IOI) for speech. Intelligibility was measured for five-word target phrases that were interleaved word by word with two five-word masking phrases. The IOIs of the individual words varied from 100 ms to 300 ms and the F0 differences between the target and masking phrases varied from 0 – 12 semitones. As expected, intelligibility improved with increasing F0 differences at all presentation rates. However, there was no indication of the predicted increase in intelligibility when the IOI decreased at a fixed F0 separation. A second experiment investigated the impact of providing a preview of the initial target and/or masker words in the sequence. At slow IOI rates and small F0 differences, cueing the target word alone provided the greatest benefit, suggesting a strategy based on temporal expectation. At fast IOIs and wider F0 separations, masker cueing resulted in better performance, consistent with an effect of obligatory streaming.

713 Audiovisual Synchrony Detection and Benefits of Lipreading with Hearing-Impaired Listeners

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Previous studies have shown that normal-hearing (NH) listeners have a synchrony window of a few hundred milliseconds where the asynchrony between the audio (A) and video of audiovisual (AV) speech cannot be detected. Hearing-impaired (HI) listeners, especially of moderate and severe levels of impairment, have to rely on lipreading to supplement poorer speech intelligibility in every-day life. Therefore, these listeners could be expected to be more sensitive to AV asynchrony. The preliminary results, however, showed that the asynchrony sensitivity was similar between NH and HI listeners. For both subject groups, longer window duration seemed to be correlated with lower A-only and AV scores. There was no clear trend in correlation between the window duration and lipreading benefit, calculated as the difference between the AV and A-only scores. The lipreading benefit was observed to depend on the A-only score for NH listeners. When the A-only scores were high, the AV score reached the ceiling level of 100%, setting the limit for benefit. For lower A-only scores, the lipreading benefit asymptoted to around 50-60% regardless of the A-only score. There was more variability in the lipreading benefit observed with HI listeners, and in general this benefit was smaller compared to NH listeners.

714 Informational Masking in Children with Cochlear Implants

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For normally hearing children, correctly responding to a speech target message presented in the presence of a speech distracter is a significantly more challenging task than for adults. The negative impact of a speech distracter, called “informational masking”, is as great as 15 dB in some 6 year olds but only about 2 dB in 12 year olds. Typically, children receive some release from informational masking when the similarity of the target and distracter speaker is reduced, as when the target is male and the distracter is female. The informational masking release can be as great as 10 dB in young children. In children with cochlear implants (CIs), however, the pattern of performance is quite different. For example, in conditions with a non-speech distracter, children (age 6-10 years) with CIs require about a 7 dB greater target-to-distracter ratio (T/D) than their normal hearing peers to achieve 50% correct performance. In contrast, when the target and distracter are male speakers, children with CIs perform comparably to children with normal hearing. Thus, children with CIs demonstrate much less informational masking than their normal hearing counterparts. In addition, children with CIs demonstrate no difference in performance when the target and distracter speakers are of the same or different gender.

715 Connecting Hair Cells with Brain Cells: Afferent Responses and Efferent Feedback in Hearing and Deafness

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Cochlear inner hair cells (IHCs) and outer hair cells (OHCs) relay ascending signals to the auditory brainstem via two kinds of sensory neurons and receive descending control signals via two efferent neuronal pathways. The aims of our research are to understand the functional significance of these four major neuronal types, and the roles each plays in cochlear function and/or auditory processing, in both normal and traumatic acoustic environments. This talk will summarize recent studies on each of the four fiber types, suggesting 1) that degeneration of IHC afferents is widespread after acoustic overexposures that otherwise appear reversible, 2) that terminals of OHC afferents make reciprocal synapses comprising a neural network that could mediate local communication among OHCs independent of the CNS, 3) the mechanisms underlying the protective effects of OHC efferents , and 4) that efferent projections to the IHC area balance the excitability of IHC afferents from the two ears to ensure accuracy in sound localization.

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716 Stimulus-Specific Adaptation and Enhancement of Responses to Novel Sounds in the Inferior Colliculus

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There is mounting evidence that neural adaptation to common sounds, accompanied by enhanced responses to novel sounds, is a process that begins at subcortical levels, and is similar across vertebrate species. Here we compare

stimulus specific adaptation (SSA) in the inferior colliculus (IC) of the anesthetized rat with that in the IC of awake big brown bats. In the rat, approximately two-thirds of all neurons in the inferior colliculus (IC) exhibit SSA; in the bat, the proportion is lower, a little less than half of all neurons. In both preparations, the neurons that show SSA are distributed throughout all regions of the IC, including the central nucleus, with the amount of SSA differing across neurons. In both the rat and bat, the amount of SSA depends on several interactive factors, including stimulus repetition rate, relative probabilities of standard and novel stimuli, and the amount of contrast between the standard and novel stimuli. In both species, the neurons that exhibit SSA have shorter latencies in response to a novel sound than in response to a repeated sound, and are more broadly tuned to frequency than are non-adapting neurons. The basic process of SSA and novelty detection appears to be the same in a hearing specialist, the echolocating bat, and a hearing generalist, the rat, and is fundamentally the same in an awake animal as it is in the urethane-anesthetized preparation.

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717 Functional Mechanisms That Mediate Stimulus-Specific Adaptation in the Inferior Colliculus

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Many previous studies assume that adaptation to a repetitive sound, yet an enhanced response to the same sound when it is novel, is a unique feature of the auditory cortex (AC). This phenomenon is termed stimulus-specific adaptation (SSA) (Ulanovsky et al., 2003), and can be elicited using an oddball stimulus paradigm similar to that used to evoke mismatch negativity (MMN) in humans. Using such a stimulus, SSA is considered to not be present subcortically (Ulanovsky et al. 2003). However, more recently, SSA has been observed in the main auditory midbrain nucleus, the inferior colliculus (IC), of the rat (Perez-Gonzalez et al., 2005, Malmierca et al, in prep.). This raises some important questions, namely, is the presence of SSA in the IC due to a bottom-up or a top-down process? It may be that SSA is created de novo in the IC, or that it results from the dense cortico-collicular projection which exists in the rat. To address this question we recorded responses of single IC neurons to oddball stimuli using two different experimental protocols. Firstly, we recorded before, during and after microiontophoretic injections of the GABA antagonist, Bicuculline. Secondly, we recorded before, during and after reversibly inactivating the AC. Our data suggest that while the AC modulates SSA in the IC, it is not essential for its creation.

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718 Stimulus Specific Adaptation, Novelty Detection, and the Coding of Surprise in the Auditory System

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Neural responses in the auditory system depend not only on the current stimulus but also on the history of past stimulation. One form of this history-dependence is stimulus-specific adaptation (SSA), the reduction in the responses to a common sound relative to the same sound when rare, which has been described both in inferior colliculus and in auditory cortex. In humans, in addition to the SSA, there is a specific response component due to the novelty (rather than to the rarity) of the deviant sound. Here I show that neural responses in rat auditory cortex show true novelty sensitivity, responding more strongly to a sound when appearing over a background consisting of a single tone than to the same rare tone when appearing over a background consisting of tones of multiple frequencies. Furthermore, I show that the responses are well described by a model in which responses depend on the surprise (the logarithm of the estimated probability of the stimulus), where stimulus probability is estimated from an internal representation of the past stimulation sequence. Initial results suggest that the duration of the past sequence that is necessary to account for the responses of single neurons is typically long (>10 stimulus presentations), but that the internal representations of the past are typically coarse.

719 Stimulus Specific Adaptation in the Gaze Control System of the Barn Owl

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[1]*The Technion*

Gaze control circuitry is believed to be intimately linked with the control of spatial attention to salient stimuli. We therefore characterized a neurocorrelate of novelty detection, stimulus-specific adaptation (SSA), in gaze control centers in the forebrain and midbrain of the barn owl. We have found SSA to be highly ubiquitous. Neurons tended to respond stronger to rare sound features such as frequencies, interaural time differences, interaural level differences and sound intensity. In addition we report that neurons in the same centers tended to respond stronger to rare visual features such as movement direction and spatial location. Finally we examined SSA in bimodal scenes and show that the novelty response is enhanced when a rare visual is presented synchronously with a rare auditory stimulus. The manifestation of SSA in such a variety of independent acoustic and visual features, in both the forebrain and the midbrain, supports the notion that SSA is involved in sensory memory and novelty detection.

720 Top-Down Modulation of Novelty Processing in Human Auditory Cortex

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Unexpected auditory events in an otherwise repetitive stimulation background elicit a pattern of event-related

brain responses (ERP) with two prominent components: mismatch negativity (MMN) and the novelty-P3. These events also increase response time in concurrent task performance, i.e., induce behavioral distraction, all in all reflecting the mechanisms of stimulus-driven attention. Current theoretical accounts suggest that these ERP components reflect involuntary attention that is contingent upon the attentional set, i.e., modulated by top-down mechanisms. This talk will present ERP, magneto-encephalographic and neuroimaging (fMRI) data showing that stimulus-driven, involuntary attention is not as automatic as previously thought, since novelty-related responses in human auditory cortex and their behavioral concomitants (distraction) can be modulated in opposite directions by either loading components of executive attention, i.e., working memory, or inducing a negative emotional activation.

721 ERP Repetition Effects and Mismatch Negativity: A Predictive Coding Perspective

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Neuronal adaptation is a ubiquitous property of the cortex. I will review evidence from MMN studies which show ERP components with similar adaptive properties. Specifically, I consider the empirical evidence from the perspective of predictive coding models of perceptual learning and inference. Within this framework, ERP and neuronal repetition effects (repetition suppression) are seen as reduction in prediction error, a process which requires synaptic modifications. Repetition positivity is a human auditory ERP component which shows similar properties to stimulus-specific adaptation of auditory cortex neurons; a candidate mechanism for auditory trace formation.

722 Phoenix is Required for Mechanosensory Hair Cell Regeneration in the Zebrafish Lateral Line

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In humans, the absence or irreversible loss of hair cells, the sensory mechanoreceptors in the cochlea, accounts for a large majority of acquired and congenital hearing disorders. In the auditory and vestibular neuroepithelia of the inner ear, hair cells are accompanied by another cell type called supporting cells. This second cell population has been described as having stem cell-like properties, allowing efficient hair cell replacement during embryonic and larval/fetal development of all vertebrates. However, mammals loose their regenerative capacity in most inner ear neuroepithelia in postnatal life. Remarkably, reptiles, birds, amphibians, and fish are different in that, they can regenerate hair cells throughout their lifespan. The lateral line in amphibians and in fish is an additional sensory organ, which is used to detect water movements and it is comprised of neuroepithelial patches, called neuromasts. These are similar in ultra-structure to the inner ear's neuroepithelia and they share the expression of various molecular markers. We examined the regeneration process in hair cells of the lateral line of zebrafish larvae carrying a retroviral integration in a previously uncharacterized gene, phoenix (pho). Phoenix mutant larvae develop normally and display a morphologically intact and functional lateral line. However, after destroying hair cells with copper or neomycin, their regeneration in pho mutants is severely impaired. We show that the number of mitoses in the supporting cells is strongly reduced after damage to hair cells and can account for the reduction of newly formed hair cells in the regenerating phoenix mutant neuromasts. The retroviral integration linked to the phenotype is in a novel gene with no known homologs showing high expression in neuromast supporting cells. Thus, phoenix defines a new class of proteins with a hair cell regeneration-specific function.

723 Regeneration of the Inner Ear by Induced Pluripotent Stem (IPS) Cells

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We have been using several kinds of stem cells as a source for inner ear regeneration. Among stem cells, embryonic stem (ES) cells are the most potent cells because of its pluripotency and potential for self-renewal. However, use of ES cells involves several ethical debates. Recently, Yamanaka and his colleagues have generated ES cell-like pluripotent cells from fibroblasts by introducing a limited numbers of transcriptional factors, which are named induced pluripotent stem (iPS) cells (Takahashi et al., 2006). Germline-competent iPS cells have also been generated (Okita et al., 2007). The use of iPS cells enables us to generate personalized pluripotent stem cells without ethical arguments and immunerejection. We then examined the potential of iPS cells as a source of transplants for cell therapy for inner ears. In this study, we examined the efficacy of neural induction by stromal cell-derived inducing activity (SDIA) on mouse iPS cells, and fates of undifferentiated iPS cells after transplantation into the cochlea as the basis for future transplantation studies. Undifferentiated iPS cells were co-cultured to form neuronal colonies on a feeder layer of PA6 stromal cells, which is known as the SDIA method (Kawasaki et al., 2000). The results demonstrated that the SDIA efficiently induce neural differentiation of mouse iPS cells. Cell suspensions including undifferentiated mouse iPS cells were injected into cochleae of P3 mice. On day 7 after engraftments, histological analyses of the cochleae revealed the survival of transplants in mouse cochleae without tumor formation. These findings indicate that mouse iPS cells have similar characteristics to mouse ES cells as transplants for inner ears. Transplantation experiments using mouse iPS cell-derived neural progenitors are in progress.

724 Jagged1-Dependent Notch Signaling Regulates Cochlear Stem/Progenitor Cells Maintenance and Sensory Cell-Fate Determination

Withdrawn

725 Distinct Sphere Types from Cochlear Sensory Epithelium-Derived Stem/Progenitor Cells

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Despite the fact that hair cell regeneration does not occur in the mammalian cochlea, cells with proliferative capacity exist in the neonatal organ of Corti. The progeny of these cochlear stem/progenitor cells can differentiate into hair cell-like cells suggesting that these cells are a promising tool for experiments aimed to replace lost hair cells in the damaged organ of Corti. In serum-free, non-adherent culture conditions, organ of Corti-derived stem/progenitor cells display the capacity to form clonal floating colonies, so-called spheres. We noticed a considerable diversity in the reported morphology of spheres derived from the cochlear sensory epithelium. Here, we provide an in-depth characterization of different sphere morphologies that we classified as solid, transitional, and hollow. We show that these sphere types are derived from proliferating progenitor cells that initially grow into solid spheres, which gradually convert into buoyant hollow spheres, via a transitional morphology. This process is associated with differentiation of sphere cells and a decline in stem cell features, including the capacity for self-renewal. Comparative analysis of the features of the distinct sphere types revealed that solid spheres contained significantly more rapidly-cycling, Pax-2-expressing otic progenitors than hollow spheres. Pax-2-expressing cells could be enriched by specific culture conditions. Likewise, hair cell-like cells were found in significantly higher numbers in differentiated cell populations derived from solid spheres. Collectively, our results explain the different sphere types that can be observed in cultures of cochlear sensory epithelial cells. They also suggest that solid spheres represent the most suitable sphere type for development of stem cell-based assays or cell transplantation experiments aimed to regenerate or replace lost inner ear hair cells.

726 Activin Potentiates Proliferation in Mature Avian Auditory Sensory Epithelia

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The type II activin receptors, Acvr2a and Acvr2b, are members of the TGFb superfamily. Signaling through these receptors regulates cellular proliferation, differentiation, and/or apoptosis in many vertebrate systems, however little is known about their role in inner ear sensory epithelia (SE). In organotypic cultures of post-hatch avian basilar papilla (auditory SE), treatment with activin A, an Acvr2a/b ligand, for 3 days potentiated proliferation of support cells compared to untreated controls, as assayed by BrdU incorporation. Organotypic cultures of avian auditory SE incubated in the presence of Acvr2a and Acvr2b inhibitors showed decreased proliferation of support cells compared to control cultures. Neither treatment (with ligand or inhibitors) affected HC survival, suggesting a direct effect of Acvr2b signaling on support cell mitogenicity. Blocking TGFbR2, another receptor of the TGFb type II family, did not affect proliferation suggesting the effect is specific to signaling through the Acvr2a/b pathway. Immunocytochemistry (ICC) of cryosections from normal post-hatch chicken basilar papilla show Acvr2a and Acvr2b expression as well as expression of the downstream effector proteins phosphorylated Smad 2 and phosphorylated Smad 1,5,8 in auditory support cells. Together, these data suggest that signaling through Acvr2a/b promotes progenitor cell proliferation in mature avian auditory SE. The absence of Acvr2b in mammalian (mouse) auditory SE, where proliferation is completely quiescent, coupled with the localization of Acvr2b in the proliferative avian auditory SE, suggests this receptor may be involved in differences between the regenerative potential of these tissues.

727 Regulation of Supporting Cell Sox2 Expression During Direct Transdifferentiation and Proliferation in the Regenerating Avian Cochlea

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Sox2, a member of the SoxB1 family of transcription factors, is involved in neurogenesis and neural stem cell proliferation and maintenance. In the embryonic chicken and mouse cochlea, Sox2 identifies a sensory progenitor region of proliferating cells that will give rise to both hair cells and supporting cells (Keirnan et al., 2005; Neves et al., 2007). In the adult cochlea of both chickens and mice, Sox2 labels only supporting cells in the basilar papilla and the organ of Corti (Oesterle et al, 2007). Supporting cells in the avian cochlea utilize two distinct methods to produce new hair cells during hair cell regeneration: Direct Transdifferentiation (DT) and Mitotic Proliferation (MP). DT occurs early and involves a change of gene expression in supporting cells, so that they differentiate directly into hair cells without dividing. Thus, these cells should down-regulate Sox2 expression as they increase Math1 expression and begin to become new hair cells. We show a loss of Sox2 labeling in some supporting cells at 24h and 72h after gentamicin injection, as some nuclei in the damaged region label only with DAPI and not Sox2. In contrast to DT, later appearing hair cells are generated by supporting cell MP. Proliferating prosensory cells in the

embryonic ear express Sox2 and only down-regulate this as the newly divided cells begin to differentiate into hair cells. Our results show that at 76h when the first supporting cells that entered S phase begin to divide, many cells are double-labeled with EdU (a thymidine analog) and Sox2. This suggests that in regeneration, the supporting cells re-entering the cell cycle continue to express Sox2 while undergoing proliferation and at the earliest stages of differentiation into hair cells and supporting cells. Only later, once a subset of these postmitotic cells express Math1 and commit to hair cell differentiation, will they down-regulate their expression of Sox2.

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728 Expression of Math1 Positive Cells During Mitotic Proliferation in the Regenerating Chick Cochlea

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Previous studies in our lab have shown that the early upregulation of Math1, a bHLH transcription factor critical to hair cell differentiation, occurs in more supporting cells than will eventually give rise to new hair cells by Direct Transdifferentiation (DT). In addition, Math1 expression progresses well beyond the damaged region of the cochlea between 12 and 15 hours after gentamicin injection (AI). However, by 24 and 48 hours AI, the number of Math1 positive cells is reduced and the extent of labeled nuclei regresses back towards the damaged portion of the ear. Based on these results, we concluded that not all Math1 positive cells seen 12h-24h AI go on to become new hair cells through DT. However, this previous study did not examine Math1 expression at later time points, when new hair cells are produced by supporting cell mitotic proliferation (MP). Supporting cells in MP first enter S phase around 65h AI and begin to divide into daughter cells by 72h AI. To examine Math1 expression during supporting cell MP, 1-2 week old chicks were given a single gentamicin injection and their cochleae were dissected out at 72h, 96h, and 120h AI. These cochleae were labeled for Math1, Myosin VIIa, and phalloidin in order to identify the extent of cochlear damage and visualize the dying and regenerating hair cells. Cochlear montages show strong Math1 expression in nuclei of cells throughout, but not beyond, the damaged region at these later time points. Preliminary qualitative data indicate a stronger positive label at 96h and 120h than at 72h AI. These strongly-labeled nuclei are also larger and more rounded than the small, punctate nuclei from earlier time points. Ongoing studies will colocalize Math1 and Myosin VIIa labeling in order to determine the point at which these Math1 labeled nuclei begin to express definitive hair cell markers.

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729 Effect of P16Ink4a Deletion on Cochlear Hair Cells

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Exposure to harmful noise or ototoxic drugs can induce hearing loss which is primarily caused by damage to sensory hair cells (HCs) of the inner ear. Chicken, fish and amphibians can replace damaged HCs; however humans and other mammals cannot. Recently, many researchers have studied HC regeneration by manipulating the retinoblastoma protein as well as cyclin-dependent kinase inhibitors. These studies have obtained mixed results depending on the timing and the cell-type specificity of the gene deletion. The cyclin-dependant kinase inhibitor, $p16^{Ink4a}$, is not expressed until animals are aged or have experienced an insult; thus, deletion of this protein is attractive as it may allow supporting cells (SCs) to respond to signals naturally released by damaged HCs. In addition, $p16^{Ink4a}$ plays a critical role in the regenerative ability of adult cells in many tissue types. Here, we hypothesize that induction of $p16^{Ink4a}$ after HC damage is the physiologically relevant event that prevents HC regeneration in mammals. We have recently begun studies with $p16^{Ink4a}$-null mice and found that these mice have normal hearing and normal HC structure and morphology at 1 month of age; however, at 2 months old, $p16^{Ink4a}$-null mice have significant hearing loss at high frequencies (32 and 44 kHz). This functional change correlates with the expression of $p16^{Ink4a}$ in the cochlea of wildtype mice as $p16^{Ink4a}$ is below the level of detection at P21, becomes detectable at 2 months of age and is increased by 10 fold at 1 year of age. We are currently conducting studies with ototoxic drugs in $p16^{Ink4a}$-null mice and will examine cell cycle re-entry in SCs as well as possible regeneration of HCs after damage.

The $p16^{Ink4a}$ knockout mice were kind gifts from Dr. Ronald DePinho of the Dana-Farber Cancer Institute (Boston, MA). Supported by: ALSAC, Hartwell individual biomedical research award and NIH grants DC006471, DC008800, 1F31DC009393, and CA21765.

730 In Vivo Proliferation of Postmitotic Supporting Cells by Conditional Inactivation of P27Kip1 in Neonatal Mouse Cochleae

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Unlike non-mammalian vertebrates, mammals cannot generate new hair cells (HCs) to replace damaged cochlear HCs. Recently, *in vitro* cultured neonatal mouse cochlear supporting cells (SCs) have been shown to regenerate HCs either by down-regulating $p27^{Kip1}$ and proliferating or by direct transdifferention without cell division. Therefore, it is important to demonstrate whether proliferation and transdifferentiation of SCs occurs in neonatal mouse cochleae *in vivo*. Here, we acutely deleted the $p27^{Kip1}$ gene in neonatal mouse cochlear SCs using an

inducible CreER line (Prox1-CreER), thus bypassing embryonic defects in germline $p27^{Kip1}$ knockout mice. When induced at postnatal days 0 and 1 (P0-P1), we observed an apical to basal gradient of Cre activity along the cochlea with approximately 15% of apical and 5% of basal Deiters and Pillar cells displaying Cre activity. Within days after inactivation, $p27^{Kip1}$-null SCs reenter and complete the cell cycle, and successfully generate new daughter cells. Deiter and Pillar cell numbers are increased at P2, P4 and P6. No immediate cell death was observed between P5 and P10 within the organ of Corti, however TUNEL positive SCs were observed at P15. Interestingly, myosin7a (a specific HC marker) has not been detected in these SC daughter cells at P2, P4 or P6. The newly generated daughter cells invade the HC layer, leading to sporadic HC death at P15 and hearing loss at 6 weeks of age. Our results demonstrate that postmitotic, neonatal mouse cochlear SCs still have intrinsic proliferative capacity *in vivo*. However, to further transdifferentiate into HCs, we propose that a combination of $p27^{Kip1}$ deletion with HC damage or overexpression of HC differentiation factors (i.e., Atoh1) is necessary. In addition, our studies clarify the assumptive role of $p27^{Kip1}$ in maintaining the quiescent state of postnatal SCs.

The $p27^{Kip1}$ floxed mice were a gift from Dr. Matthew Fero at Fred Hutchinson Cancer Research Center. The Prox1-CreER mice were a gift from Dr. Guillermo Oliver at St. Jude Children's Research Hospital. These studies were supported in part by the Hartwell Individual Biomedical Research Award, ALSAC, and NIH grants DC006471, DC008800, and CA21765.

731 Inducible Expression of Atoh1 in Organ of Corti Explant Cultures

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We have developed an inducible model that allows for the conditional expression of Atoh1 in the organ of Corti. We have placed the Atoh1 gene in a construct that contains a C-terminal fusion to the estrogen receptor (ER) and a reporter (DS-Red) so that Atoh1 can be up-regulated in a dose-dependent manner by the addition of tamoxifen to cultured cells or to the cochlear environment. HEK cells transfected with this construct exhibited constitutive expression of the Atoh1-ER-DsRed fusion protein in the cytoplasm, where it is rendered quiescent. The addition of tamoxifen to the transfected cells resulted in a dose-dependent localization of the Atoh1-ER-DsRed fusion protein to the nucleus. The minimum effective dose and time for nuclear translocation was 1 nM tamoxifen for 2 days. The highest dose tested (100 [micro]M) produced cytotoxic effects after 2 days in culture. Removal of tamoxifen from the culture media resulted in a cytoplasmic localization of the fusion protein within 2 weeks. Since Atoh1 acts as an autoregulatory transcription factor that positively regulates its own transcription, the effects of tamoxifen on cellular Atoh1 gene expression were examined. We found that increasing concentrations of tamoxifen induced a dose-dependent increase in binding to the enhancer/promoter region of the Atoh1 gene as measured by a luciferase assay. Additionally, both RT-PCR and qPCR indicated that tamoxifen upregulated the expression of Atoh1 in a dose-dependent manner. Organs of Corti electroporated with this construct expressed supernumerary hair cells when exposed to 1 [micro]M tamoxifen. These data indicate that the Atoh1-ER-DsRed fusion protein may be used for time and dose-dependent regulation of Atoh1 expression.

732 Interaction of B-Catenin with an Atoh1 3' Enhancer Upregulates Atoh1 Expression and Increases Differentiation of Progenitors to Hair Cells

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Because of its role in hair cell differentiation, we would like to better understand upstream regulation of basic helix-loop-helix transcription factor *Atoh1*. We searched for genes that affected *Atoh1* expression by screening an adenoviral library that allowed us to express the genes in various cell types. We found that transcription of the *Atoh1* gene in mouse neuroblastoma cells and neural progenitor cells was regulated by *β-catenin*, the mediator of the canonical Wnt pathway. Direct interaction of β-catenin with the *Atoh1* enhancer and Tcf-Lef co-activators was demonstrated by chromatin immunoprecipitation. *Atoh1* expression was increased by transfection of *β-catenin* into mouse neuroblastoma cells and neural progenitor cells, and baseline *Atoh1* expression was decreased by siRNA directed at β-catenin and by dominant negative Tcf4. We found that two putative Tcf-Lef sites in the 3'enhancer of the *Atoh1* gene displayed an affinity for β-catenin and were critical for the activation of *Atoh1* transcription since mutation of either site decreased expression of a reporter gene downstream of the enhancer. Tcf-Lef co-activators were found in the complex that bound to these sites in the DNA together with β-catenin. Delivery of *β-catenin* in an adenovirus increased the level of GFP in inner ear stem cells isolated as neurospheres from an *Atoh1-nGFP* mouse. The number of hair cells was increased based on myosin VIIa staining of the *Atoh1* positive cells. Our data indicate that overexpression of *β-catenin* in progenitors for hair cells increases expression of *Atoh1* and other hair cell markers, indicative of a role for this pathway in the differentiation of hair cells.

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733 Wnt1 Promotes Engraftment and Differentiation of Somatic Stem Cells in the Murine Cochlea

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Cochlear implantation is currently the "standard of care" for treating profound sensorineural hearing loss. Some degree of preservation of spiral ganglion neurons is considered to be of primary importance for speech recognition and oral language development. To further improve outcome of cochlear implant, a technology to restore spiral ganglion neurons is crucial. We previously demonstrated that the canonical Wnt ligands, Wnt1 and Wnt3a, up-regulate expression of an array of proneural and gultamatergic genes in mouse mesenchymal stem cells (MSCs) in vitro. Based on these results, we hypothesized that engraftment and neural differentiation is enhanced if MSCs are implanted in the modiolus of animals in the presence of neuronal induction medium and Wnt1 (NIM+W1). GFP-positive MSCs isolated from 4-6 week old TgN(ACTbEGFP) mice were implanted into eight Mongolian gerbils, deafened by ouabain. A modified ALZET osmotic pump was used to continuously infuse NIM+W1 (experimental group) or physiological saline (control group) into the implanted cochlea for 28 days. Numerous GFP-positive MSCs were found in the modiolus of animals receiving NIM+W1. In contrast, a very small number of MSC were found in the modiolus of control animals. Strikingly, GFP-positive MSCs that had been injected into the basal cochlear turn were found throughout all cochlear turns and many were found in areas surrounding the spiral ganglion at 28 days post-implantation, thus indicating a migration of the MSC throughout the sensorineural tissues of the cochlea. Some of the GFP-positive cells exhibited spherical cell bodies with long neurites and expressed several neural markers, including neurofilament, TUJ1 and synaptophysin. Our results indicate that a co-application of neuronal induction medium and Wnt1 significantly promotes survival and neural differentiation of MSCs in vivo, and may lead to a strategy to enhance the auditory nerve in the deaf patient and improve the benefits of cochlear implantation.

734 Adenovirus Vector Mediated BDNF Expression Induces Neuronal Growth Into the Flat Cochlear Epithelium

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Following elimination of hair cells in severely traumatized cochleae, differentiated supporting cells are replaced by a flat epithelium, and the radial neurons are typically absent in the 1st and 2nd turns. The flat epithelium is a target for stem cell therapy for hair cell replacement. For new hair cells to become functional, it is necessary for them to be innervated. Therefore, attracting neurons into this tissue is an important step towards such therapy. Our goal was to determine whether neurons can be attracted into the flat epithelium by elevated levels of BDNF. We deafened the left ears of pigmented guinea pigs with an injection of 10% neomycin (10µl) into the scala tympani via a cochleostomy in the basal turn. Seven days later, we inoculated the cochleae with 5µl Ad.BDNF into the scala media via a cochleostomy in the 2nd turn. Contralateral ears and ears inoculated with Ad.empty served as controls. Fourteen days after inoculation, the ears were assessed histologically using whole mounts or plastic sections. Tissues were labeled with antibodies to neurofilament, parvalbumin, or SV-2 to determine the extent and type of neuronal process in the flat epithelium. Epi-fluorescence of Ad.BDNF treated ears revealed an increase of neurofilament-positive neurites in the flat epithelium. Positive staining of some neurites for parvalbumin and others for SV-2 suggests that both afferents and efferents were present. Ad.empty inoculated ears contained no radially-extending neurons in the flat epithelium. The results show that Ad.BDNF inoculation into the flat epithelium can induce innervation. The data demonstrate the ability of neurons to grow in large numbers into the flat epithelium of the deaf cochlea. Innervation of the flat epithelium has implications for feasibility of several therapies including cochlear implantation and stem cells.

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735 Neurog1, BDNF, and GDNF: The Cues Involved in SGN Development Can Drive Embryonic Stem Cells Toward a Glutamatergic, SGN-Like Phenotype in Vitro and in Vivo

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The proneural transcription factor Neurog1 is necessary for SGN development, playing a critical role in promoting glutamatergic identity in developing neurons in the CNS and PNS. The capacity of Neurog1 to drive such differentiation in pluripotent embryonic stem cells was examined, toward the goal of a stem cell-based therapy to replace auditory nerve. Expression of the Neurog1 transgene was controlled via activation of the tet-operon, which initiates Ngn1 transcription upon interaction with Doxycycline (Dox). Additional treatment with BDNF and GDNF was used to replicate some of the endogenous cues that guide SGN development. The efficacy of Dox to induce Neurog1 expression and drive neuronal differentiation was assessed both in vitro and in vivo. 5.0 x 10^4 cells were plated in each well of 6-well dishes under different NTF conditions and phenotype was assessed with TUJ1, VGLUT1/2, and GFAP immunocytochemistry

performed at 24h, 72h, and 5D. Expression of SGN-specific genes in the differentiating ES cells was also assessed with qRT-PCR at the different times. For in vivo studies, 2.5 x 10^5 ES cells were implanted into the scala tympani/modiolar region of five-week deafened guinea pig cochleae and 48h intrascalar dox was followed by GDNF and BDNF delivered for 4-5 weeks by a miniosmotic pump with a cannula in scala tympani. Transient expression of Neurog1 in vitro and in vivo, followed by application of BDNF and GDNF, induced over 50% of ES cells to adopt a neuronal phenotype, 75% of which expressed markers for glutamatergic identity. Results show that replication of SGN developmental cues using Neurog1 induces a phenotype suitable for replacement of lost auditory nerve. Auditory nerve replacement could qualify more patients to benefit from cochlear prostheses and improve performance.

736 Merlin Phosphorylation on Serine 518 by Protein Kinase a Contributes to Spiral Ganglion and Sciatic Nerve Schwann Cell Proliferation Following Denervation

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Deafening due to hair cell loss results in degeneration of the peripheral processes of spiral ganglion (SG) neurons and denervation of associated Schwann cells (SCs). Axon regeneration following injury depends on support from SCs. The molecular mechanisms regulating the response of SCs to loss of axonal contact are unclear. Merlin is a tumor suppressor that mediates cell contact information to regulate cell proliferation and survival. Loss of functional merlin contributes to schwannoma formation. Phosphorylation of serine 518 (S518) inactivates the growth-suppressive function of merlin, facilitating proliferation. Protein kinase A (PKA) phosphorylates merlin S518 and is required for SC proliferation *in vitro*. We hypothesize that phosphorylation of S518 by PKA is necessary for SC proliferation following denervation. In cultured SCs, forskolin (FSK), a PKA activator, increased BrdU uptake and S518 phosphorylation as assessed by Western Blot with phospho-specific antibodies verifying that PKA promotes SC proliferation and phosphorylates S518 *in vitro*. Further, we find that merlin is phosphorylated at S518 in SGSCs in response to deafening and in SNSCs following axotomy. S518 phosphorylation correlates with an increase in proliferation in these denervated SCs *in vivo*, consistent with the hypothesis that merlin inactivation facilitates proliferation. To verify that PKA is required for S518 phosphorylation and SC proliferation *in vivo*, osmotic minipumps were implanted adjacent to cut sciatic nerves and eluted a PKA-specific inhibitor, KT5720. Protein lysates were extracted from the proximal, distal, and contralateral nerve segments and evaluated by Western Blot with S518 phospho-specific antibodies. KT5720 reduced merlin phosphorylation in denervated SC's *in vivo*. These findings support the hypothesis that PKA phosphorylates merlin S518 in SCs following denervation and raise the possibility that PKA mediates critical responses in SGSCs following deafening.

737 Regulation of Tgf-β Signalling by Fbxo11, the Gene Mutated in the Jeff Otitis Media Mouse Mutant

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Otitis media (OM) inflammation of the middle ear, is the most common cause of hearing impairment in children. There is a very strong genetic component predisposing to recurrent and chronic forms of OM but we know little of the genes involved. *Jeff* is a dominant mouse mutant displaying chronic otitis media (Hardisty-Hughes et al. *Hum. Mol. Genet.* 15: 3272, 2006). The gene underlying *Jeff* is *Fbxo11*, a member of the large Fbox family, which are specificity factors for the SCF E3 ubiquitin ligase complex. *Jeff* homozygotes die shortly after birth displaying a number of developmental abnormalities including cleft palate and eyes open at birth. Tgf-β signalling is involved in a number of epithelial developmental processes and we have investigated the impact of the *Jeff* mutation on the expression of this pathway. Phospho-Smad2 (pSmad2) is significantly upregulated in epithelia of *Jeff* homozygotes. Moreover, there was a significant increase in nuclear localisation of pSmad2 in contrast to wild type. Mice heterozygous for both *Jeff* and *Smad2* mutations recapitulate many of the features of the *Jeff* homozygous phenotype. However, tissue immunoprecipitations failed to detect any direct interaction between *Fbxo11* and *Smad2*. Fbxo11 is known to neddylate p53, a co-factor of pSmad2, but we did not find any evidence of genetic interactions between *Jeff* and *p53* mutants. Nevertheless, p53 levels are substantially reduced in *Jeff* mice suggesting that Fbxo11 plays a role in stabilizing p53. Overall, our findings support a model whereby *Fbxo11*, possibly via stabilization of p53, is required to limit the accumulation of pSmad2 in the nucleus of epithelial cells of palatal shelves, eyelids and airways of the lungs. The finding that *Fbxo11* impacts upon Tgf-β signalling has important implications for our understanding of the underlying disease mechanisms of middle ear inflammatory disease.

738 Cytokine Polymorphisms Predict the Frequency of Otitis Media as a Complication of Rhinovirus and RSV Infections in Children

Withdrawn

739 Conditional Knockout of TGFß Signaling in Otic Mesenchyme Phenocopies Aspects of the Brn4/Pou3f4 Mutant Phenotype

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Targeted mutagenesis of the mouse *Brn4/Pou3f4* gene generates an animal model for X-linked deafness type III

(DFN3) that accurately recapitulates much of the phenotype observed in DFN3 patients, including enlarged internal auditory meatus, hypoplastic cochlea and dysmorphic superior semicircular canal (sSSC; Phippard et al., 1999). Further characterization of the mouse mutant demonstrates that much of the *Brn4* phenotype results from dysmorphogenesis during development. Interestingly, however, the sSSC is formed properly at P0, and then the canal later becomes constricted (Sobol et al., 2005). Postnatal changes in the sSSC result from increased proliferation of cells that appear histologically like interwoven bone, suggesting that the bone is poorly differentiated. To assess the molecular changes occurring in the bone surrounding the sSSC, we undertook expression profiling using microarray technology. We observed that the expression of a network of genes associated with TGFß signaling were altered in the mutant. To test the hypothesis that TGFß signaling plays a role in the *Brn4* phenotype, we conditionally inactivated the TGFß type II receptor gene (*Tgfbr2* ConKO) in otic mesenchyme. Our conditional knockouts utilized a pedigree of mice that directs expression of Cre recombinase to the otic mesenchyme using the *Brn4* otic enhancer (Ahn et al., Genesis, in press). The *Tgfbr2* ConKO mutants demonstrated enlarged internal auditory meatus that phenocopies the *Brn4* mutant. However, other phenotypic characteristics, such as foreshortened cochlea, are not observed. In toto, these data implicate changes of TGFß signaling in aspects of the *Brn4* phenotype, but indicate that other aspects of the phenotype are regulated by different signaling pathways. Additional analyses of the *Brn4* and *Tgfbr2* conditional knockout mutants are being undertaken to further characterize the molecular mechanisms that regulate the morphogenesis of the temporal bone.

740 Clinical and Molecular Characterizations of Novel *POU3F4* Mutations Identified in Families Carrying X-Linked Hereditary Deafness DFN3

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X-linked deafness type 3 (DFN3) is the most prevalent X-linked form of hereditary deafness. Clinical features of DFN3 include temporal bone abnormalities and stapes fixation, resulting in a mixed type of deafness. DFN3 is caused by mutations in the *POU3F4* locus, encoding a member of the POU family transcription factors. Despite numerous reports on clinical evaluations and genetic analyses describing novel *POU3F4* mutations, little is known about how such mutations affect normal functions of the POU3F4 protein, which lead to inner ear malformations and deafness.

Here we describe three novel mutations of the *POU3F4* gene and their clinical characterizations in Korean families carrying DFN3. In addition, as an attempt to better understand the molecular mechanisms underlying their inner ear defects, we examined the behavior of the normal and mutated forms of POU3F4 proteins in C3H/10T1/2 mesodermal cells. All three mutations were expected to affect DNA binding, since the mutations either cause a substitution (p.Arg329Pro) or deletion (p.Ser310del) of a highly conserved amino acid residue in the POU homeodomain, or cause a truncation of the carboxyl DNA-binding domains. Molecular modeling also suggested that the mutations are detrimental to the tertiary structure of the POU3F4 proteins, affecting the DNA-protein interaction. Indeed, we observed that the mutated POU3F4 proteins were unable to form DNA-protein complexes, and mislocalized to the cytoplasm. Consistently, each mutated POU3F4 protein failed to transactivate a reporter gene expression or inhibit the transcriptional activity of wild type proteins when both wild type and mutant proteins were co-expressed. Since most of the mutations reported for DFN3 thus far are associated with regions that encode the DNA binding domain or POU homeodomain of POU3F4, our results strongly suggest that deafness in DFN3 patients is largely due to the null function of POU3F4 protein.

741 Segregation Analyses of Enlarged Vestibular Aqueducts in Families with Nondiagnostic SLC26A4 Genotypes

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Hearing loss with enlarged vestibular aqueducts (EVA) may be isolated or associated with goiter in Pendred syndrome. In some cases, EVA is inherited as an autosomal recessive trait caused by bi-allelic mutations of *SLC26A4.* However, only one or zero *SLC26A4* mutations are detected in many patients. In these patients, the etiology of EVA and recurrence risk in siblings remain unknown. We thus sought to characterize the transmission of EVA in 37 families by classical segregation analysis and haplotype analysis of *SLC26A4*-linked short tandem repeat (STR) markers. These families had 36 unaffected siblings and 50 previously reported individuals with EVA. We assumed single ascertainment and grouped families according to number of mutant alleles of *SLC26A4*. The segregation ratio of EVA in the 1-mutation group (M1) was 0.44 (95%CI=0.23-0.67). This is similar to the ratio (0.45; 95%CI=0.21-0.72) in the 2-mutation group (M2) but significantly higher than the ratio (0.045; 95%CI=0.011-0.219) in the 0-mutation group (M0) (*P*=0.005). Since EVA is inherited as an autosomal recessive (AR) trait in the M2 group, these results suggest it is also inherited as an AR trait in the M1 group. The observed ratios greater than 0.25 for the M1 and M2 groups are likely due to an ascertainment bias for familial cases. Haplotype analyses of 30 families with one or zero mutant alleles revealed

discordant segregation of *SLC26A4*-linked STR markers with EVA in seven families: 5 families in group M0 and 2 families segregating p.L597S and c.IVS1-2A>G, respectively. If these two hypofunctional alleles are considered to be nonpathogenic, the lack of discordant segregation in M1 families supports the hypothesis of a second, undetected *SLC26A4* mutation rather than digenic inheritance. In contrast, the discordant *SLC26A4* segregation and low segregation ratio in the M0 group implies the contribution of non-genetic factors, complex inheritance, etiologic heterogeneity, or a combination of these mechanisms.

742 Digenic Inheritance of a Vestibular Phenotype in *Tmc1$^{\delta}$;Tmc2$^{\delta}$* Knockout Mice

Withdrawn

743 Differential Expression of Cadherin 23 Alternate Transcripts and Protein Isoforms in the Mouse and Primate Inner Ear and Retina

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In humans, mutations of *CDH23* cause Usher syndrome type 1 D (USH1D), characterized by profound congenital deafness, vestibular areflexia and retinitis pigmentosa (RP) or nonsyndromic deafness DFNB12. The *waltzer* (*v*) mouse phenotype is due to mutations in *Cdh23* and is characterized by deafness and vestibular dysfunction, but not RP. We performed an analysis of cadherin 23 mRNA and protein variants expressed in the inner ears and retinae of wild type and mutant mice. Semiquantitative real-time PCR of *Cdh23* alternative splice isoforms in mRNA from wild type and homozygous v^{6J} mice revealed that *Cdh23* splice variants have different expression patterns in the inner ear and retina. Some, but not all, of these patterns are affected by the v^{6J} nonsense mutation. For example, immunohistochemistry at the light and electron microscopy levels show that isoform cadherin 23_3, which lacks the extracellular and transmembrane domains, localizes exclusively to the basal body in the outer plexiform layer of the retina and to the synaptic region of auditory and vestibular hair cells in both mutant and wild-type mice. Western blot analyses of mouse inner ear and retina revealed that the longest CDH23 isoform (cadherin 23_1) is the only one affected by the v^{6J} mutation. Cadherin 23_1 is transiently expressed from embryonic age E13.5 to approximately postnatal day 12 in the wild-type mouse inner ear, but is not detected in the retinae of embryonic, juvenile or adult wild-type mice. Western blots of monkey and human retinae, however, do show expression of the cadherin 23_1 isoform. We hypothesize that the cadherin 23_1 isoform is not necessary for normal development of the mouse retina, and thus mutations that primarily affect the longest *Cdh23* isoform do not cause RP in mice.

744 Combining Genetic and Genomic Approaches to Identify Novel Deafness Genes

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While 50+ dominant genes for nonsyndromic deafness have been mapped, about half are yet unidentified. Critical intervals are often large containing dozens of genes. We have previously used a combined genetics/genomics approach to identify the *AUNA1* gene. This method uses expression information to prioritize positional candidate genes defined by linkage analysis. In Family 106, DNA samples from 9 deaf and 3 unaffected individuals were genotyped for 12,000 SNPs (Human Linkage12 chips, Illumina). MERLIN analysis identified a 15 Mb, 21 cM region on chromosome 19q13 (multipoint LOD >3.0), partially overlapping DFNA4 but excluding *MYH14*. Next, RNA from lymphoblastoid cell lines (LCLs) from affected individuals and controls was hybridized to whole genome expression arrays (Illumina Human-6 v2 Expression BeadChip). 95% of 48,000 probes could be annotated. Significant up-regulation was observed for *SLC45A2*, *FGFR2,* and *DIAPH1;* α-actin was significantly down-regulated, as also seen for *AUNA1*. None of the genes with significant expression changes mapped to 19q13; thus, we are focusing on positional candidates that are likely downstream of *DIAPH1* in the SAPK/JNK signaling pathway. Surprisingly, at least 50-60% of known deafness genes are expressed in LCLs (SymAtlas). Separately, in Family 95, 2 individuals were genotyped by high-density whole genome SNP genotyping (HumanHap-550 Genotyping Beadchip, Illumina). A linkage disequilibrium approach is possible using only 2 samples because the two individuals are separated by 18 meioses. In summary, powerful new genomic tools will allow mapping and cloning of genes in smaller families than was previously possible. Supported by NIDCD DC007380 and the University of Michigan Biomedical Research Council.

745 Overlapping Chromosomal Regions Contribute to Noise Resistance in S6 and MOLF

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The inbred mouse strains 129S6/SvEvTac (S6) and MOLF/Ei (MOLF) show strong noise resistance (NR) when compared to C57BL/6J (B6) and CBA/CaJ (CB). When compared to each other, S6 and MOLF share some properties of NR, but differ in others. For example, S6 and MOLF both show smaller noise induced permanent

threshold shifts than CB or B6 after identical exposure (8-16 kHz OBN, 97-103 dB SPL, 2 hr) when evaluated by ABR or DPOAE two weeks post-exposure. In addition, both S6 and MOLF show smaller threshold shifts 1 day post-exposure and recover more quickly and more completely than CB or B6. These results suggest that NR in S6 and MOLF arises from molecular mechanisms active at the time of the initial exposure to noise as well as those active during the subsequent period of recovery from damage. In contrast to these shared mechanisms, S6 displays NR that has a very slow growth function with increasing levels of noise exposure while MOLF shows a "critical level" above which noise-induced hearing loss (NIHL) grows very rapidly, like CB. These findings suggest that S6 and MOLF may share some genes that contribute to NR, but will use different genetic components as well.
We have developed quantitative trait locus (QTL) maps for the trait of NR in S6 and MOLF. We generated F1 animals by intercrossing S6 x CB and MOLF x CB. In each cross, the NR trait was recessive. N2 animals were generated by backcrossing the F1s to their respective recessive parental line (S6 or MOLF). Approximately 250 N2 animals from each backcross were tested for NR and evaluated by ABR and DPOAE. Chromosomal regions contributing significantly to NR were identified using the Rqtl software. In S6, five QTL peaks were identified ($p<0.05$); *nr1* on mChr 17 had a LOD >7.0 ($p<0.001$). In MOLF, three peaks were identified ($p<0.05$). QTLs on Chr 4 and Chr 17 were detected in both crosses. The implications of the overlapping QTLs in S6 and MOLF, as well as candidate genes in the QTL areas will be discussed.

746 Mild Hearing Deficit Despite Congenital Hypothyroidism in *Prop1* Mutant Mice

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Congenital hypothyroidism is associated with permanent hearing deficits in humans and mice unless corrected by thyroid hormone (TH) replacement. The *Prop1*^{df} and *Pit1*^{dw} mutant mice carry mutations in different pituitary transcription factors, each of which result in the inability to produce pituitary thyrotropin (TSH) and secondary to that, lack of detectable TH in the serum. Despite the indistinguishable TH deficiencies, *Prop1*^{df} and *Pit1*^{dw} mice have very different hearing abilities on their standard genetic backgrounds: DF/B-*Prop1*^{df} mutants exhibit modestly elevated ABR thresholds, while DW/J-*Pit1*^{dw} mutants are completely deaf. We showed that genetic background contributes to the severity of hearing impairment, and we uncovered multiple, permanent abnormalities that likely contribute to the deafness of *Pit1*^{dw} mutants. In this study, we analyze the etiology of the mild hearing deficit in *Prop1*^{df} mutants to identify processes that are not protected by the DF/B genetic background, i.e. those that are strictly dependent on serum TH. DPOAE develop more slowly in *Prop1*^{df} mutant mice compared to wild type littermates. Prestin and KCNQ4 appear normal by immunohistochemistry. The expression of KCNJ10 in the stria vascularis is moderately reduced in *Prop1*^{df} mice at 3 wks and appears normal by 6 wks. Consistent with this the EP of wild type and mutants are indistinguishable at 6 wks. Neurofilament and synaptophysin staining reveal no obvious defects in innervation. Immunostaining for the synaptic vesicle protein otoferlin normally shifts from OHC to IHC as temporary afferent fibers beneath the OHC regress in the first postnatal week. *Prop1*^{df} mutants exhibit persistent expression of otoferlin in apical OHC, possibly indicating incomplete regression of afferent fibers. In addition, phalloidin staining reveals abnormalities in actin organization in supporting cells at the apex of *Prop1*^{df} mutant cochlea. Thus, the genetic background of *Prop1*^{df} mutants is remarkably protective for most functions assessed except otoferlin expression and actin organization in the cochlear apex. The *Prop1*^{df} mutant is an attractive model for identifying the genes that protect against deafness due to hypothyroidism.
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747 Prevalence and Spectrum of Mutations in Mitochondrial 12S RRNA Gene in 1642 Han Chinese Subjects with Nonsyndromic and Aminoglycoside Induced Hearing Loss

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Mutations in mitochondrial 12S rRNA gene are one of the most important causes of most important causes of sensorineural hearing loss. We have investigated the prevalence and spectrum of mutations in mitochondrial 12S rRNA gene in a cohort of 1642 Han Chinese pediatric subjects with aminoglycoside-induced and nonsyndromic hearing loss. Aminoglycoside ototoxicity accounted for 28% cases of hearing loss in this cohort. Mutational analysis identified 68 (50 known and 14 novel) nucleotide changes in this gene. Here, the incidences of the known deafness-associated A1555G mutation were 1.43% and 10.41% in this cohort with nonsyndromic and aminoglycoside-induced hearing loss, respectively. Furthermore, the prevalence of mutations at positions 1494T, 1095 and 961 were 0.18%, 0.61% and 1.7% in this cohort, respectively. In addition, the A745G, C792T, A801G, A839G, A856G, A1027G, A1116G, C1310T, A1331G, A1374G and T1452C variants were absent in 449 Chinese controls and localized at the highly conserved nucleotides of this rRNA. Potential structural and functional alterations by these variants may lead to mitochondrial dysfunction, thereby causing or contributing to hearing loss phenotype. Moreover, 65 Chinese subjects carrying the A1555G mutation shared common features: being bilateral, symmetric, sensorineural, and the loss of hearing at the high frequencies. However, a wide range of severity, age-of-onset and audiometric configuration was observed among these subjects. In particular, the sloping and flat shaped patterns were the most common audiograms in individuals carrying the A1555G mutation. This phenotypic variability indicated the involvement of nuclear and mitochondrial genetic modifiers in the phenotypic manifestation of the A1555G mutation.

748 KCNQ Channels in Spiral Ganglion Neurons of the Mouse Cochlea

Withdrawn

749 Ototoxicity of Topical Azithromycin Solutions in the Guinea Pig

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Azithromycin is a macrolide antibiotic that has been widely used for systemic treatment of otitis media. Given its broad spectrum of effectiveness against both bacterial and non-bacterial organisms, it would seem to be a good candidate for use in formulations designed for topical treatment of otitis externa and otitis media. To our knowledge however, no animal studies on the safety of ototopically applied azithromycin have been reported.

We investigated the potential ototoxic effects of azithromycin formulations after a single bolus middle ear application in pigmented guinea pigs. Topical otic formulations containing 3%, 2%, 1% and 0.5% azithromycin were tested. Mild nystagmus was seen in the animals given the 3% formulation that resolved within 2 days. Histological examination performed 2 weeks after application revealed middle ear and inner ear changes in all concentrations tested. Changes include mucosal thickening and adhesions, outer and inner hair cell loss and strial atrophy. The severity of pathology increased with increasing concentrations of azithromycin. The results show azithromycin is ototoxic to the guinea pig inner ear, suggesting it is not a good candidate for topical otic application.

750 Ototoxicity of Cesium in Guinea Pigs

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To advance work on hair cell regeneration, it is necessary to develop lesions that result in thorough and reliable loss of hair cells. Here we report on experiments using elemental non-radioactive cesium (Cs), a monovalent alkali metal. In the inner ear, high concentrations of Cs^+ interfere with potassium ion (K^+) cycling between perilymph and endolymph, mimicking several mutations of K^+ channels. Those mutations are associated with progressive hair cell loss and deafness. No previous studies have examined whether Cs^+ exposure results in permanent damage or loss of hair cells, however we observed extensive hair cell loss after injection of 6M CsCl into perilymph. To quantitatively assess effects of Cs^+ on the inner ear, we injected CsCl (50 µL: 6M, 3M or 1M) into the middle ear space of adult male pigmented guinea pigs, allowed it to diffuse into the inner ear, and examined the sensory epithelia after 1 week. At 1M, CsCl did not induce a consistent lesion: some animals experienced complete loss of hair cells from the organ of Corti, but others suffered little or no loss. In contrast, 3M and 6M solutions always led to complete loss of inner and outer hair cells and supporting cells, leaving a "naked" basilar membrane. Although this surface may not be a viable target for therapies that aim to transduce surviving cells, it may be more conducive to repopulation by stem cells, which typically do not integrate into an intact epithelium. For therapies that do aim to transduce surviving cells, further studies will be needed to determine what doses between 1M and 3M consistently: a) kill hair cells but not Deiters cells and b) result in formation of a flat epithelium.

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751 Severity of Cesium Chloride Induced Inner Ear Lesion Depends on Concentration and Mouse Strain

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Achieving a hair cell lesion in the organ of Corti of mice has been challenging due to mouse strain differences in tolerating systemic aminoglycosides, and to date no consistent, non-invasive lesion of inner and vestibular hair cells has been achieved. Here we report a consistent lesion of the inner ear in mice which can be induced by application of cesium chloride (CsCl) to the middle ear. CsCl is known to be an inorganic potassium channel blocker that interferes with the proper cycling of potassium between perilymph and endolymph. As mutations in potassium channels mimicking this disruption lead to hair cell loss, we hypothesized CsCl application also would disrupt hair cell function and lead to hair cell death. To test this, we administered 10 µl CsCl solution (6 M, 3 M, 1.5 M, 1 M, 0.75 M, 0.5 M, 0.25 M or 0.1 M) to the middle ear of C57BL/6J and CD1 mice, and sacrificed the animals either 7 or 14 days later. Organ of Corti and vestibular sensory organs were stained with antibodies to myosin VIIa and/or neurofilament to detect hair cells and neurites, and fluorescent phalloidin to label actin. Depending on the concentration of CsCl used, all hair cells were absent from the apical turn of the organ of Corti and from the saccule, with higher concentrations yielding a more severe pathology reflecting a flattened epithelium. The ideal concentration for inducing a lesion while maintaining a very high animal survival rate differs greatly between CD1 and C57BL/6J mice, with C57BL/6J mice being much more sensitive to the effects of the CsCl. Whereas CD1 mice tolerated doses over 6 M, C57BL/6J mice suffered fatal seizures when given doses above 1.5 M. These results provide a new, minimally invasive approach to induce a hair cell lesion in the organ of Corti and saccule of mice, as well as guidelines to tailor the CsCl concentration to the strain of mouse used and the extent of damage desired.

752 Screening for Ototoxic Drugs Using the Zebrafish Lateral Line: In Vivo Mammalian Validation

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No standard screen for ototoxicity has been developed. For most FDA-approved drugs, ototoxic potential remains unknown. We hypothesize that a portion of idiopathic sensorineural hearing loss may be due to damaging effects of drugs or chemicals that are not known to be ototoxic. Previously, we identified 14 novel compounds that caused significant hair cell loss in the zebrafish lateral line using a screen of a library of FDA-approved drugs and bioactives (Chiu et al., 2008). Two of the identified drugs, pentamidine isethionate and propantheline bromide, were found to cause dose dependent hair cell loss when tested in an in vitro preparation from mature mouse utricle. In this current study, we tested whether these drugs cause physiologic hearing loss in rats in vivo as measured by changes in ABR (auditory brainstem evoked response) thresholds. Pentamidine isethionate and propantheline bromide were administered to 6-week old rats by daily intraperitoneal injection for a period of 6 weeks. Weekly ABR testing was used to measure hearing thresholds over a defined frequency range (1, 2, 4, 8, 12, 16, 32 kHz). After 6 weeks of drug injections, animals that received pentamidine injections demonstrated a mild (5-10 dB) increase in ABR thresholds compared to saline injected controls. These findings suggest that our rapid zebrafish screen for hair cell damage can identify drugs that have ototoxic effects in mammals in vivo.

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753 Bax Inhibition Prevents Neomycin- But Not Gentamicin-Induced Hair Cell Death in the Zebrafish Lateral Line

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Hair cell death is the primary pathology underlying most sensorineural hearing loss, yet the biochemical pathways that trigger hair cell death are not fully understood. Furthermore, different ototoxic stimuli such as different drugs or noise may not activate the same death and survival cascades. A complete picture of hair cell death and survival pathways, including identification of pathways involved in death due to different stimuli, offers the best opportunity for development of therapeutic intervention. We study hair cell death in the lateral line of larval zebrafish, a unique model system that offers advantages for *in vivo* imaging and quantitative analysis. Recent research by our group suggests that hair cell death following exposure to neomycin or gentamicin is not equivalent, but that different aminoglycoside antibiotics kill hair cells via multiple partially overlapping pathways. The present study begins to examine the role of one protein family, the Bcl-2 proteins, in lateral line hair cell death following aminoglycoside toxicity. Inhibition of Bax, a pro-cell death member of the Bcl-2 family, prevents up to 70% of neomycin-induced hair cell loss but does not protect against gentamicin damage. In other cell types, conformational changes in Bax trigger cytochrome c release from damaged mitochondria. The present data therefore strengthen our previous findings that mitochondrial changes are early events in hair cells damaged by neomycin. Further studies will examine mitochondrial changes following exposure to a variety of aminoglycosides and other ototoxins, with the goal of characterizing several of the major checkpoints in cell death cascades that are differentially activated by ototoxic agents.

754 Neomycin Mediates Changes in Cytosolic and Mitochondrial Calcium in Rat Hair Cells in Vitro

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Aminoglycosides preferentially target hair cells presumably due to their entry through transduction channels (Gale et al 2001, Marcotti et al 2005). Aminoglycosides are known to trigger changes in cytosolic calcium ($[Ca^{2+}]_c$) in avian hair cells but such signals have not been studied in mammals. Fura2 was used to measure changes in $[Ca^{2+}]_c$ at the focal plane of outer hair cell (OHC) bodies for 4 hours in cochlear cultures prepared from postnatal day 3 rats. Neomycin (1mM) caused a complicated but consistent response in OHCs. Within minutes of application onset, OHC $[Ca^{2+}]_c$ decreased to a new lower resting level then subsequently increased, superseding the original resting $[Ca^{2+}]_c$ reaching a plateau in ~60 minutes. In nominally Ca-free conditions the initial reduction in $[Ca^{2+}]_c$ persisted but in most cells the subsequent slow increase was prevented. In normal Ca^{2+} 2-APB (100 μM) significantly reduced the slow increase in $[Ca^{2+}]_c$ suggesting at least a partial dependence on store-operated Ca^{2+} entry. Consistent with this, patch clamp recordings revealed a neomycin-activated OHC conductance that could be inhibited by 2-APB. Subsequent to and typically between 10 and 90 minutes after,OHC $[Ca^{2+}]_c$ reached a plateau, OHCs exhibited a third and final increase in $[Ca^{2+}]_c$ and then appeared to lyse, indicated by a simultaneous decrease in the 340 and 380 nm Fura2 signals. Cell lysis triggered *inter*cellular Ca^{2+} waves that spread from the OHCs to adjacent Deiters' cells and in some cases Claudius cells. Spread of the Ca^{2+} waves was reduced by apyrase indicating a dependence upon extracellular ATP as described in other damage paradigms (Gale et al, 2004). Neomycin also caused changes in OHC mitochondrial calcium and mitochondrial membrane potential coincident with the later increases in $[Ca^{2+}]_c$. Were are currently assessing the protective effect of inhibiting store-operated Ca^{2+} entry and loss of mitochondrial potential in aminoglycoside toxicity assays.

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755 Response of Spiral Ganglion Cell Manganese Superoxide Dismutase (Mn SOD2) Expression in Kanamycin Challenge

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Previous work in our laboratory demonstrated a basal to apical gradient in Manganese Superoxide Dismutase (Mn SOD2) expression in type I spiral ganglion cells of control rodent and primate cochleae. This corresponds to clinical finding of high frequency hearing loss seen in presbycusis and ototoxin-induced hearing loss. The variation in expression level of Mn SOD2, a key metabolic anti-oxidant enzyme of the superoxide dismutase family for detoxifying free radical cascade inside the mitochondria of the cochlea, suggests that spiral ganglion cellular responses to reactive oxygen species (ROS) may vary along the cochlear spiral, with a lower response capacity in the basal turn. This raises the general hypothesis that a lower Mn SOD2 anti-oxidative capacity at the cochlear base could contribute to the high frequency hearing loss seen in clinical settings. To further evaluate the hypothesis that Mn SOD2 expression is regulated by ROS in spiral ganglion cells, we used a 2x2 factorial experimental protocal of young adult (4 weeks) wild type C57BL/6 mice treated with subcutaneous injections of kanamycin (700 mg/kg) as the ROS challenge and dihydroxybenzoate (300 mg/kg) as the antioxidant separately, and in combination for fifteen consecutive days. A subset from each animal group underwent microdissection of cochlear modiolus for quantitative-PCR assay of Mn SOD2. Another subset animal group underwent temporal bone sectioning for immunohistochemical study on Mn SOD2. Compared to the control animal group, Mn SOD2 expression is responsive to kanamyin-induced ROS load by a modest upregulation of gene transcription, (1.6 fold, $p < 0.05$). Similar cellular expression pattern was observed throughout spiral ganglion cells, independent of cochlear location, after kanamycin treatment, suggestive of an upregulation in protein translation. In this study, we conclude that lower Mn SOD2 expression in the basal turn of control animals may indicate lower baseline constant ROS exposure, which makes basal turn vulnerable to sudden oxidative challenges. Hence, enhancement of this dynamic Mn SOD2 response capacity is a potential otoprotective strategy in the face of dynamic ROS challenges.

756 Cimetidine, a Copper Transporter Inhibitor, Reduces Cisplatin Ototoxicity in Vitro

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Cisplatin is a widely used in anticancer drug that is both ototoxic and nephrotoxic. Cisplatin is taken up into cells and then forms inter and intrastrand cross links with DNA leading to programmed cell death. An important regulatory step in this process is the influx of cisplatin from the extracellular space into the cytoplasm. Recent studies in yeast have suggested that the copper transporter, Ctr1, plays an important role in regulating the uptake of cisplatin. Cimetidine inhibits Ctr1 and suppresses the uptake of cisplatin into cells. To determine if the copper transporter is involved with cisplatin ototoxicity, we treated cochlear organotypic cultures with cisplatin alone (10 μM or 50 μM), cimetidine alone (10, 100, 2000 or 4000 μM) or cisplatin plus cimetidine for 48 hours. Cimetidine alone did not cause any damage at concentrations between 10-2000 μM; however, 4000 μM caused stereocilia distortion. Thus lower doses of cimetidine have no obvious adverse effects on hair cells. Treatment of cultures with 10 μM of cisplatin alone damaged about 20% of the hair cells. When the cultures were treated with cisplatin plus cimetidine (10, 100 or 2000 μM), nearly 100% of the hair cells survived. Treatment of cochlear cultures with 50 μM cisplatin alone resulted in approximately 80% hair cells loss. However, when the cultures were treated with cisplatin plus 100 μM of cimetidine, hair cell loss decreased to 50%. When the dose of cimetidine was increased to 2000 μM, hair cell loss decreased to 20%. When cochlear cultures were pretreated overnight and later cultured with cimetidine plus cisplatin, cimetidine pretreatment failed to protect against cisplatin damage. Western blots showed that cisplatin increased the expression of Ctr1 and ATP7B (copper-transporting P-type adenosine triphosphatase) whereas cimetidine significantly reduced the expression of CTR1 and ATP7B. Cisplatin caused a slight decrease in the expression of ATP7A, a copper efflux transporter. However, ATP7A expression was greatly reduced with co-administration of cisplatin and cimetidine. These results suggest that cimetidine protects against cisplatin toxicity by modulating the influx of cisplatin through Ctr1 and the efflux of cisplatin by ATP7A and ATP7B. Research supported by NIH (5R01DC006630-05)

757 Protective Effect of Ginseng Saponin on Cisplatin Ototoxicity in Auditory Cell Line

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Various ginsenosides and compound K is the representative constituents of ginseng saponin, which show many beneficial effects including reversing pathological and physiological changes induced by stress and aging. Cisplatin, a very effective antineoplastic drug that is widely used to treat various cancers, promotes ototoxicity at higher doses by inducing apoptosis of hair cells in the cochlea.The purpose of this study was to investigate the effect of ginseng saponin on cisplatin-induced ototoxicity in auditory cell line. To investigate the protective effect of ginseng saponin, HEI-OC1 was pretreated with 2500μg of saponin for 30 min prior to treatment with 20 μM of cisplatin. After 48 hrs incubation, Cell viability was measured by the MTT assay. Evaluation of apoptosis is using Hoechst 33258 staining, nuclear fragmentation, and Caspase-3. Reactive oxygen species production was also measured. Protective effect of ginseng saponin on cisplatin induced cell death was 96%

using MTT assay, which is compared with 55% in HEI-OC1 treated with only cisplatin. Anti-apoptotic effect of ginseng saponin was evaluated using Hoechst 33258 staining, nuclear fragmentation, and Caspase-3. An effect of ginseng saponin on reactive oxygen species was meaningful. In conclusion, The ginseng saponin plays an anti-apoptotic role during cisplatin induced cell death in the auditory cell line HEI-OC1.

758 Hes5 Expression in the Postnatal and Adult Mouse Inner Ear and the Drug-Damaged Cochlea

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The Notch signaling pathway is a potent regulator of progenitors and stem cells and is known to have multiple roles during development of the inner ear. Notch signaling activates transcription of Hes5, a mammalian homologue of Drosophila hairy and Enhancer of split, which encodes a basic helix-loop-helix (bHLH) transcriptional repressor. Previous studies have shown that Hes5 is expressed in a subset of cochlear supporting cells during embryonic development, and loss of Hes5 leads to overproduction of auditory and vestibular hair cells. However, due to technical limitations and inconsistency between previous reports, the precise spatial and temporal pattern of Hes5 expression in the developing and adult inner ear has remained unclear. Here, we use transgenic mice in which GFP is expressed under the control of Hes5 cis regulatory elements (Hes5-GFP), and complementary in situ hybridization to report the expression pattern of Hes5 in the inner ear. We find that Hes5-GFP and mRNA are expressed in the developing auditory epithelium of the cochlea. During embryonic cochlear development, Hes5-GFP and mRNA become restricted to a particular subset of cochlear supporting cells: Dieter's cells, pillar cells, border cells, inner phalangeal cells, and a population of cells in the greater epithelial ridge. Hes5-GFP and mRNA are down-regulated in the cochlea during postnatal development and Hes5-GFP is undetectable in adults. In the vestibular system, we detect Hes5-GFP and mRNA in developing supporting cells as early as E12.5 and find that Hes5-GFP is maintained in some adult vestibular supporting cells. In order to determine the effect of hair cell damage on Notch signaling in the cochlea we damaged cochlear hair cells of adult Hes5-GFP mice in vivo, using injection of kanamycin and furosamide. Results of these experiments will be presented.

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759 A Novel Inner Ear Specific Sox21 Enhancer Directs Gene Expression to Supporting Cells in the Organ of Corti

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Molecular techniques now allow the design of precise genetic modifications in the mouse, including conditional gene targeting. To apply these techniques for *in vivo* research, spatiotemporally controlled loci or enhancers are indispensable genetic tools. Supporting cells in the organ of Corti include several distinct sub-populations that are morphologically different, and only three cell types, inner phalangeal cells, inner border cells and Deiter's cells, directly contact hair cells. They may, in addition to structurally supporting hair cells, also provide functional support by, for example, secreting neurotrophins that promote survival; however, cell-specific genetic elements for this domain have not been identified.

Here, we present our recent attempt to manipulate gene expression specifically in the subset of supporting cells that contact hair cells in adult organ of Corti by using a novel inner ear specific element associated with SOX21. We found endogenous SOX21 expression throughout the sensory epithelium in the developing cochlea in mice, followed by restriction into inner phalangeal, inner border and Deiter's cells in adult. Based on a previously reported highly conserved non-coding sequence database, we cloned an evolutionarily conserved sequence 13.5 kb upstream of the SOX21 coding region in mice. This element successfully induced reporter activity in an organ of Corti derived cell line, in inner ear-derived spheres, and within organ of Corti and GER of neonatal mouse explants.

This short control element was sufficient to reproduce endogenous expression patterns of Sox21 in the cochlea and provides a tool for inner ear specific expression whereas endogenous Sox21 expression is widespread in CNS stem cells. Also, unlike some other supporting cell markers, Sox21 expression persists into adulthood. This short enhancer may prove to be useful for future experiments involving viral gene transfer and Cre/lox or flippase/FRT mediated gene targeting.

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760 Expression of CTL2/SLC44A2 During Development of the Inner Ear

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CTL2/SLC44A2 is a membrane glycoprotein that belongs to the solute carrier family. It was discovered as a supporting cell antigen that appears to be the target of antibody-induced loss of outer hair cells and hearing deficits. It is therefore likely that CTL2 has an essential role in the development and maintenance of the cochlea. We examined the expression of CTL2 in the developing

murine inner ear using immunofluorescence with rabbit anti CTL2-NT antibody raised to an antigenic peptide located near the N-terminus of the CTL2 molecule. Ears of mice at developmental days e17, e18, p1, p7 and p20 were examined. CTL2 expression was present in the cochlea from e17 through p20. At e17 and e18, CTL2 staining was most intense in the pillar cells separating the inner hair cells from the outer hair cells, and lower intensity expression could be observed in supporting cells surrounding the outer hair cells as well as in other epithelial cells flanking the developing organ of Corti. An expression intensity gradient was observed with strongest signal next to the pillars and lesser intensity in Deiters cells near rows two and three outer hair cells. By p20 CTL2 expression was more extensive, resembling the adult distribution, which is restricted to supporting cells. Expression in the developing pillar cells was not as prominent as in adult animals. The results indicate that CTL2 is expressed early in the developing inner ear sensory epithelium, suggesting that it plays an important role in supporting cell development. Future experiments will assess the effect of knocking out CTL2 expression on inner ear development.

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761 The Expression Patterns of Phosphorylated Akt and PTEN in the Developing Cochlea

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The PI3K/Akt pathway is known to play a pivotal role in the survival of many tissues including the inner ear (Nagy et al., 2005; Jiang et al., 2006). PTEN (phosphatase and tension homologue of deleted on chromosome 10) is a tumor suppressor gene, and normally acts to inhibit the PI3K/Akt pathway by dephosphorylating PIP3. In the zebrafish, ptena, one of the two PTEN homologues, was revealed to be expressed in the developing inner ear, and ptena-deficiency caused the irregularities in the inner ear (Croushore et al., 2005). However, very little is known about the substantial function of PI3K/Akt pathway during the development of the mammalian primordial cochlea and vestibule. As the first step to clarify that, we tried to examine the expression patterns of pAkt (phosophorylated Akt) and PTEN in the developing cochlea of mouse. At E12.5, pAkt-IR (immunoreactivity) was diffuse throughout the cochlear epithelium. It gradually became stronger in the LER (lesser epithelial ridge) and decreased in the prosensory region. PTEN-IR, which was faintly detected in the prosensory region at E15.5, rapidly became robust in HC (hair cell) and SC (supporting cell) progenitors including Hensen cell progenitors at E17.5. The results imply that the PI3K/Akt pathway would be essential for the cell proliferation during the inner ear development, and PTEN might have some specific roles in the regulation of the cell proliferation and the maintenance of the quiescent states of HCs and SCs.

762 The Atoh1-Expressing Cell Lineage Develops Into Both Hair Cells and Supporting Cells

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The organ of Corti (OC) consists of a highly ordered mosaic of hair cells (HC) and supporting cells (SC). During development, the formation of the prosensory domain first distinguishes HC and SC progenitors from other epithelial cells in the cochlear duct. Expression of the transcription factor *Atoh1* within the prosensory domain is thought to be the earliest determinant of HC fate. In mice, absence of *Atoh1* results in a complete loss of both HCs and SCs, while misexpression of *Atoh1* in the adjacent nonsensory cells of Kölliker's Organ induces the formation of ectopic HCs. Early cochlear expression of an *Atoh1-LacZ* knock-in reporter is broad and diffuse, but later expression of *Atoh1-LacZ* is more restricted, and is found only in HCs by birth. Because of its broad early expression, it is not clear whether *Atoh1* is expressed in all sensory cell progenitors, or exclusively in HCs. To determine whether *Atoh1* is ever expressed in SC progenitors, we have used an inducible Cre-PR, expressed from the endogenous *Atoh1* locus, and the reporter strain R26R-EYFP. RU486-dependent activation of Cre recombinase permanently marks cells with EYFP. We find that a percentage of cells from the *Atoh1*-expressing lineage do develop as SCs. When Cre is activated early in cochlear development, at embryonic day 13 (E13), 13% of marked cells develop as SCs, whereas when Cre is induced at E15, only 5% of labeled cells develop as SCs. We are currently investigating whether earlier activation of *Atoh1-Cre* results in a higher percentage of labeled SCs. Our results suggest that the *Atoh1*/HC lineage is established early in cochlear development, but also that expression of *Atoh1* in prosensory cells does not absolutely result in commitment to a HC fate. Surprisingly, our data indicate that *Atoh1* is neither expressed in all prosensory cells, nor is restricted exclusively to HCs, suggesting that the pattern of *Atoh1* expression in the developing OC may be more complex than has previously been supposed.

763 Different Function of Atoh1 in the Postmitotic Hair Cells of the Ear and Premitotic Granule Cell Precursors of the Cerebellum Revealed by Studying Conditional NeuroD1 Mutants

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Basic helix-loop-helix (bHLH) proteins determine neuronal lineages and mediate differentiation of neurons through expression and interaction of different bHLH gene(s) before and after the cell cycle excit. In the inner ear, the bHLH gene Atoh1 is a crucial hair cell differentiation factor that is upregulated after hair cells have been specified and

exited the cell cycle. Another bHLH gene, Neurog1, contribute to hair cell fate decision and all neurons and certain hair cells are lost in Neurog1 null mice. Downstream of both Neurog1 and Atoh1 is NeuroD1 which is essential for neuronal differentiation but has no clear function in hair cells. In contrast to the ear, Atoh1 functions in the cerebellum in continuously proliferating granule cells (GCs) precursors in the external granule layer (EGL). Atoh1 is expressed in premitotic EGL neurons, post-mitotic cells of the EGL express NeuroD1 to initiate differentiation. Both Atoh1 and NeuroD1 null mice lose all or most GCs. We used an Atoh1-cre line (Matei et al., 2005) to eliminate a loxP flanked NeuroD1 in the inner ear and cerebellar neurons to sidestep the diabetic condition in the NeuroD1 null mice that leads to their early death. Our data do not show any ear specific phenotype apparently because the delayed elimination of NeuroD1 through Atoh1-Cre does not affect NeuroD1 in the ear. In the cerebellum, NeuroD1 is eliminated with delay in the posterior lobes, which retain a near normal morphology. Lobes in which Atoh1-Cre is upregulated earlier lose all granule cells. In lobes with severe loss of granule cells we find that Atoh1 is in a negative feedback loop with NeuroD1. These lobes show a transient expansion of Atoh1 in the inner EGL followed be complete and rapid loss of all granule cells. The retention of NeuroD1 expression in the hair cells and part of the cerebellar granule cells suggests an unprecedented dependence of effective LoxP recombination on the level of transcriptional activation of the NeuroD1 gene.

764 Sonic Hedgehog (SHH) Promotes the Differentiation of Mouse Cochlear Neural Progenitors (CNPs) Via the Math1 –Brn3.1 Signaling Pathway in Vitro

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Sonic hedgehog (SHH) is known as a mitogen for the proliferation of neuroepithelial cells and inner ear progenitors. However, little is known about the effect of SHH on the differentiation of cochlear neural progenitors (CNPs) which are isolated from the mouse postnatal day 1 organ of Corti. Here we demonstrated that SHH in vitro exerted its effects on the differentiation of CNPs. Addition of SHH to CNPs increased the formation of epithelial cell islands, simultaneously enhanced the promoter activity of the Math1 gene and increased the expression of Math1 protein that is a transcription factor for the initial differentiation of auditory hair cells. The increased expression of Math1 then increased the promoter activity of Brn3.1 that is a transcription factor for the further differentiation and maturation of auditory hair cells. More important, SHH increased the row numbers of outer hair cells in the postnatal day 1 organ of Corti explants compared with its control, verifying the importance of SHH toward the differentiation of auditory hair cells. Taken together, we concluded that SHH plays an important role in the differentiation of cochlear hair cells via the Math1-Brn3.1 signaling pathway.

765 Analysis of Stereocilia Development in Genotypically Math1-Null Hair Cells in the Inner Ears of Chimeric Mice

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The transcription factor, Math1, has been shown to play a critical role in the generation of hair cells of the inner ear. However, we previously demonstrated that the role of Math 1 is more complex by showing that genotypically Math1-null hair cells are able to survive and express hair cell markers in the presence of wild-type cells in a chimeric environment, although whether these cells become fully functional or fully differentiated hair cells remains unknown. To begin to address this issue, the expression of stereocilia markers was examined in cochlear hair cells from neonatal Math1-null chimeric mice to assess whether sterocilia development is proceeding normally. Because of differences in expression due to location within the cochlea, control comparisons were made with neighboring wild-type cells within the same chimeric animal as well as with Math1-heterozygous or wild-type cells. Expression of several markers was examined immunocytochemically using antibodies against Myosin XVa and acetylated alpha tubulin. GFP was bred onto the Math1 background so that genotypically Math1-null cells could be identified by the presence of the GFP marker. In the genotypically Math1-null cells examined, normal expression of markers was observed, both in comparison with neighboring wild-type cells in the same chimeras as well as compared to cells from non-mutant mice. These results provide evidence that the development of the stereocilia in genotypically Math1-null cells is proceeding in a normal fashion. Further these results provide additional support for the hypothesis that these cells are able to become fully functional hair cells. Supported by the Deafness Research Foundation.

766 Genetic Analysis of PTK7 During PCP Signaling in the Cochlea

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Stereociliary bundles located on the apex of auditory hair cells in the cochlea are responsible for the mechanotransduction of sound. The precise structure and uniform orientation of these specialized structures of the sensory epithelium are required for the proper perception of sound. The evolutionarily conserved Wnt/Planar Cell Polarity (PCP) pathway has been implicated as a key regulator of cochlear morphogenesis and stereociliary bundle orientation. Protein tyrosine kinase 7 (PTK7), an atypical receptor tyrosine kinase, was recently identified as a novel component of the vertebrate PCP pathway based on phenotypic similarity to the “core” PCP mutants. To date, it has not been determined how PTK7 interacts with other PCP components, but Dishevelled proteins provide a potential link given their central role in the PCP signaling pathway. We therefore set out to examine the genetic interaction between PTK7 and Dishevelled in the patterning of the inner ear. Three mouse Dishevelled genes have partially overlapping functions in PCP

regulation, with Dishevelled-2 (Dvl2) playing a dominant role. In collaboration with the Wynshaw-Boris laboratory, we have analyzed hair cells with various allelic combinations of Dvl1, Dvl2 and PTK7. On a mixed genetic background, stereociliary bundle misorientation of the triple knockout mutants was similar to that observed in the PTK7 mutants, indicating that PTK7 and the Dishevelled genes act in the same pathway to regulate hair cell PCP. Interestingly, our analysis has also revealed a novel hair bundle morphology defect present in compound mutant hair cells, suggesting that these genes may have a previously unappreciated function during bundle formation. We are currently pursuing experiments to understand the cellular basis for the bundle malformation phenotype of the compound mutants.

767 A Role for Pax6 in Cochlear Nucleus Development

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The cochlear nucleus is the obligatory synaptic relay in the brain of inner ear afferents. A large body of data exists on features of mature cochlear nucleus neurons, but studies are only beginning to examine the role of genes during development. Data is presented here implicating the paired homeodomain transcription factor, Pax6, in cochlear nucleus development.

In wild type mice, at embryonic day 16.5, in situ hybridization for Pax6 reveals expression in the ventral cochlear nucleus. As development proceeds, additional expression becomes detectable in the molecular/granule cell layer and is evident at postnatal day 0. To assess the effect of Pax6 on cochlear nucleus development, cochlear nuclei in Pax6 null mutant mice and their wild type littermates have been compared. Histological assessment of the cochlear nucleus using a general cell stain, cresyl violet, shows that in Pax6 loss-of-function mice the cochlear nucleus forms with the normal complement of major subdomains, but is slightly smaller in volume, measuring approximately 90% of the wild type cochlear nucleus. A more detailed assessment of a cochlear nucleus subdomain that normally expresses Pax6, using an independent molecular marker for the ventral cochlear nucleus, the mouse atonal homologue, Math5 (ATOH7) (Saul et al., MCN, 2008), shows that in the absence of Pax6, the domain labeling the ventral cochlear nucleus is reduced to 40% of the wild type volume.

These findings suggest that loss of Pax6 function has a direct effect on the ventral cochlear nucleus. The disparity between the decrease in ventral cochlear nucleus size versus the slight decrease in overall volume of the cochlear nucleus raises the possibility that other non-Pax6-expressing sub-domains may undergo expansion in Pax6 null mutant mice. Future studies will investigate this further along with the fate of normally expressing-Pax6 molecular/granule cells under Pax6 loss-of-function conditions.

768 Fas/CD95-Mediated Cell Death in the Mouse Anteroventral Cochlear Nucleus

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Neurons of the developing mouse AVCN are susceptible to cell death following deafferentation. Cochlea ablation at postnatal day 5 (P5) results in an ipsilateral loss of 61% of AVCN neurons. By P14, neuron loss is reduced to <1% (Mostafapour, 2000). The cellular and molecular mechanisms underlying this differential response to deafferentation are unknown.

The Fas (CD95/Apo-1) receptor is one candidate protein that has been recently studied as an inductor of apoptosis in the CNS. To initiate apoptosis, a Fas trimer must bind to its ligand, FasL. The Fas/FasL complex leads to induction of apoptosis by ultimately causing the activation of caspase-8.

We have recently shown that FasL expression is upregulated in AVCN following cochlea ablation in mice younger than P14, but not older (Luoma and Zirpel, 2008). We hypothesize that increased expression of FasL within the critical period results in enhanced Fas activation and subsequent apoptosis.

To determine the sufficiency of Fas activation to induce apoptosis, mice underwent an intrathecal injection with a Fas-activating antibody (Jo2), were allowed to survive for 24 hours, and then labeled with a fluorescent TUNEL stain. Jo2 significantly increased the number of apoptotic AVCN neurons in a dose-dependent manner ipsilateral to the injection site. Interestingly, this result was observed not only in mice within the critical period, but also in those beyond the defined critical period for deafferentation-induced death (P14 – P28).

These results are consistent with the hypothesis that following deafferentation within the critical period, NFAT-mediated increases in FasL induce apoptosis through interaction with Fas. Continuing studies will investigate the necessity of Fas activation for deafferentation-induced apoptosis using the synthetic antagonist peptide YLGA 12-mer.

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769 Postnatal Development of Neurotrophin 3 (NT3) and Tyrosine Kinase Receptor C (TrkC) in Mouse Ventral Cochlear Nucleus

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Previous work in our lab suggested that NT3 and its receptor TrkC in the mouse CN may be involved in directing neuronal migration and initial targeting of inputs from cochlear nerve axons in the embryo. NT3 is hard to detect soon after birth, but TrkC lingers longer (Hossain et al, 2006). Here we report the detection of NT3 and TrkC around P8 and their gradual peaking around P30. They were most prominent in the ventral CN, in and around PV. There, they associated with globular bushy cells and stellate cells, but were localized to different subcellular sites. The TrkC immunostain was cytoplasmic, and that of NT3 was axonal and perisomatic.

Our observations suggest that TrkC may be made by neurons in the CN, while NT3 has a cochlear origin. Based upon the temporal pattern of their development, and the likelihood that NT3 may be released from cochlear axons in an activity-dependent mode, we propose that it may not be critical during early synaptogenesis, but may provide long-term trophic effects, including stabilization of the synapses once established.

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770 Spatiotemporal Pattern of Cholinergic Innervation in the Developing Rodent Brainstem and Cochlea

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Acetylcholine (ACh) and γ-aminobutyric acid (GABA) are primary neurotransmitters in efferent oilvocochlear (OC) neurons that innervate cochlear hair cells in mammals. Olivocochlear neurons are classically divided into medial and lateral populations within the superior olivary complex of the brainstem. Studies of OC neurotransmitters in the brainstem suggest that medial OC neurons found in periolivary regions are mostly cholinergic whereas lateral OC neurons found in the lateral superior olive (LSO) contain separate populations using GABA and ACh. Additionally, cholinergic lateral OC neurons co-contain the neuropeptide CGRP. In adult rodents, medial OC neurons innervate cochlear outer hair cells (OHCs) while lateral OC neurons innervate cochlear inner hair cells (IHCs). However, in both the rat and the mouse, studies of OC neurotransmitters in the cochlea suggest that CGRP and GABA can also be found in medial OC terminals. Our study was undertaken to understand the temporal patterns of cholinergic, GABA-ergic, and CGRP expression and differentiation in both brainstem and cochlea during development.

In rats and mice, we investigated choline acetyltransferase (ChAT), vesicular acetylcholine transporter (VAChT), high affinity choline transporter 1 (ChT1), CGRP and glutamic acid decarboxylase (GAD67) immunoreactivities. In the cochlea, ChAT was detected below IHCs followed by VAChT and then ChT1. Both GAD67 and CGRP were found in efferent terminals below IHCs after ChT1 and below OHCs during the second postnatal week. In the brainstem, ChAT and then VAChT immunoreactivity are detected in periolivary regions during the perinatal period. GAD67 and CGRP are expressed in the LSO toward the end of the first postnatal week. Although the LSO had robust GAD67 and CGRP labeling during the second postnatal week, periolivary regions showed little evidence of GAD67 and CGRP. Our data suggest that cholinergic enzymes and transporters are expressed sequentially in both the brainstem and cochlea, and that CGRP and GAD67 are not expressed until cholinergic differentiation is nearing completion. In the rat and mouse brainstem, there is little, if any, neurotransmitter heterogeneity found in periolivary regions. Therefore, we hypothesize that the neurotransmitter heterogeneity in efferent terminals below OHCs in the rat and mouse may be due to lateral OC terminals.

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771 Maturation of MNTB Neurons Begins Before the Growth of the Calyx of Held

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The goal of this study was to determine whether maturation of principal neurons of the medial nucleus of the trapezoid body (MNTB) is influenced by two developmental milestones, growth of the presynaptic calyx of Held between postnatal days two and four (P2-P4) and the onset of sensitivity to airborne sound (P8-P10). Whole cell recordings were made from MNTB neurons in mouse brain slices beginning at embryonic day 17 (E17), when the MNTB is first distinguishable, through P14. MNTB neurons were innervated by glutamatergic and GABAergic inputs as early as E17. Stimulation of the contralateral cochlear nucleus evoked only glutamatergic responses indicating that these inputs likely originate from the calyx-forming globular bushy cells. Most biophysical properties, including the resting membrane potential, input resistance, and most action potential parameters, matured independently of calyx growth and settled near adult values by P6, well before mice become sensitive to airborne sounds. However, using real-time PCR, we found increases of more than 2-fold in mRNA of low-threshold K+ channel subunits, Kv1.1 and Kv1.2, from P0 to P2 when calyces begin growing in size and synaptic strength. An additional 2-fold increase in Kv1.1 mRNA levels occurred between P8 and P14, correlating with the onset of hearing, although Kv1.2 mRNA levels remained stable during this period. The threshold current necessary to generate action potentials was the only measured biophysical property that matured in a coordinated fashion with the growth of the calyx; small current steps of approximately 30 pA were sufficient to generate action potentials from E17-P1, but the average thresholds increased exponentially beginning at P2 until reaching a plateau of 250 pA at P6. These data indicate that MNTB neurons are innervated when they first form a distinct nucleus at E17, and the majority of biophysical maturation progresses independently of calyx growth and the onset of sensitivity to airborne sound.

772 Development of the Endbulb of Held in Congenitally Deaf Cats

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The endbulbs of Held are formed by the ascending branches of myelinated auditory nerve fibers and represent one of the largest synaptic endings in the brain. Deafness is known to cause morphological changes in endbulb structure including reduced branching, hypertrophy of postsynaptic densities (PSDs), and changes in synaptic vesicle density. Using electron microscopy, we compared endbulb development in deaf

white cats to that previously reported for normal hearing cats (Ryugo et al., 2006). Because cats are essentially deaf at birth, we wanted to determine if the development of brain abnormalities coincided with the onset of hearing. The rationale was that the lack of sound-evoked activity would trigger pathologic change. Morphometric data on ultrastructural features such as mitochondrial volume fraction, endbulb profile area, PSD length and number, density of cisternae and puncta adherentia, apposition length, and synaptic vesicle density were collected. Endbulbs of deaf cats exhibit a smaller profile area, flattened and elongated PSDs, and increased synaptic vesicle density as compared to normal endbulbs. These lifelong differences are present beginning at birth in white cats destined to become deaf despite the fact that cochlear histology is intact until the collapse of the scala media between postnatal days 5 and 10. We propose that abnormalities in hearing are signaled by a perinatal loss of spontaneous bursting activity in auditory nerve fibers (Jones et al., 2007; Tritsch et al., 2007) or perhaps by some trophic factor released by auditory nerve synapses before obliteration of the organ of Corti (Rubel and Fritzsch, 2002).

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773 Cell Fate Specification of Medial and Lateral Olivocochlear Neurons Occurs in Early Embryonic Development

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Throughout the spinal cord and hindbrain, efferents (motoneurons) exist that reach somite derived muscle fibers or neural crest derived visceral ganglia. Only rhombomere 4 of the hindbrain contains of a unique set of (moto)neurons, the vestibulocochlear efferents, that project to the inner ear. In contrast to the equally r4 derived facial branchiomotor and all other motoneurons, these efferents contact placodally derived hair cells and sensory neurons bilaterally. We wish to understand the timing of segregation of efferents into medial (MOC) and lateral olivocochlear (LOC) neurons in order to establish molecular cues for their development. In mice, the MOC neurons project bilateral (65%:35%) and terminate on outer hair cells. The LOC neurons project ipsilateral (99%) and synapse on afferent dendrites near inner hair cells. We show here through lipophilic dye tracing that LOC and MOC neurons overlap at E12.5, begin to segregate at E14.5 and are nearly distinct by E18.5. Segregation of LOC and MOC as well as distinct laterality of projections is established before they innervate their target. The segregation of LOC and MOC must be governed by molecular cues within r4 and not by signals from synaptic targets. In an attempt to identify such cues we investigated the fate of OC neurons in Gata3 and Hoxb1 mutants, genes that are expressed at the time those decision making processes are likely to occur (E10.5). Gata3 is necessary for ear development but is also expressed in OC neurons and r4 (Karis et al., 2000). Gata3 null mutants retain as late as E16.5 some efferents that can be traced via the facial nerve with lipophilic dyes. We also show that absence of the r4 specific transcription factor Hoxb1 reduces the contralateral projection of efferents. These data suggest that molecular specification of the early segregation of MOC and LOC that manifests around E14.5 may occur as early as E10.5, the time MOC and LOC neurons exit the cell cycle.

774 Inner Hair Cell Calcium Action Potentials Pattern Neural Activity Before the Onset of Hearing

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Neurons involved in processing auditory information exhibit electrical activity before the cochlea is capable of responding to external sounds. This experience-independent activity promotes survival of auditory neurons, and may contribute to refinement of projections and maturation of target neurons in the brain. Neural activity during the pre-hearing period consists of discrete bursts of action potentials separated by long periods of silence. We recently reported that 'spontaneous' electrical activity in developing spiral ganglion neurons (SGNs) results from the periodic depolarization of inner hair cells (IHCs) in response to the release of ATP from neighboring supporting cells in Kölliker's organ. We now report that the firing patterns of SGNs in cultured and acutely-isolated cochleas before the onset of hearing are highly stereotyped. Each burst of activity is composed of small groups of action potentials (termed 'minibursts') separated by a relatively constant interval of 100-300 ms. Under conditions that preclude the involvement of cholinergic efferents, focal application of ATP to IHCs initiated bursts of action potentials in SGNs that exhibited inter-spike intervals similar to those that occurred during spontaneous bursts. To investigate the cellular mechanisms responsible for generating these distinct patterns of activity, we performed paired recordings from synaptically-connected IHCs (whole-cell) and SGNs (loose patch). Depolarization of IHCs elicited regenerative, calcium-dependent action potentials in IHCs every 100-300 ms, depending on the membrane potential, and each hair cell calcium spike was associated with a miniburst of up to five action potentials in the SGN. The activity patterns elicited by calcium spikes in paired IHC-SGN recordings were similar to those arising from spontaneous purinergic signaling in *vitro*, as well as activity recorded *in vivo* from neurons in the medial nucleus of the trapezoid body (MNTB) of prehearing rats (A. Rodriguez-Contreras and G. Borst personal communication). Together, these data indicate that calcium action potentials in IHCs are responsible for triggering patterned neural activity in auditory pathways of the brain before the onset of hearing. Supported by NIDCD grants DC008860 and DC009464.

775 In Vivo Proliferation of Postmitotic Cochlear Supporting Cells in Neonatal Mice by Acute Ablation of the Retinoblastoma Protein

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Mammalian cochlear hair cells (HCs) are mechanosensory receptors that transduce sound into electrical signals. HC damage in non-mammalian vertebrates induces the surrounding supporting cells (SCs) to divide, transdifferentiate and finally replace lost HCs. However, this spontaneous HC regeneration does not occur in mammalian cochleae. A key factor in the lack of cochlear HC regeneration in mammals appears to be the inability of postmitotic SCs to reenter the cell cycle and proliferate in vivo. Here we acutely ablated the Retinoblastoma protein (Rb) in cochlear SCs of neonatal mice using an inducible Cre line (Prox1-CreER; Srinivasan et al., Genes & Dev. 2007). This strategy allows for normal embryonic development before the deletion of Rb. Induced at postnatal days 0 and 1 (P0 and P1), we observed a gradient of Cre activity in Deiter and Pillar cells from apex (~15%) to base (~5%) as revealed by the ROSA26R-lacZ reporter. Inactivation of Rb in SCs at P0 and P1 initiated rapid cell cycle reentry, as shown by bromodeoxyuridine (BrdU) incorporation. Between P2 and P6, these BrdU-positive SCs further exhibited molecular and morphological characteristics of M- and G1-phase cells, indicating completion of the cell cycle. Moreover, the numbers of Deiter and Pillar cells were increased at P4, P6, and P9. In contrast to Rb inactivation in cochlear HCs at similar neonatal ages, Rb-null SCs could proliferate and survive for at least 6 days; however, sporadic HCs eventually degenerated by 7 weeks of age leading to deafness.

These results accomplished the first crucial step towards mammalian cochlear HC regeneration from SCs and further demonstrated the key roles of Rb in maintaining quiescence of postmitotic SCs. The lack of Myosin7a expression in these proliferating SCs between P4 to P9 suggests that additional factors for HC damage or HC differentiation are required for the next step, transdifferentiation of mammalian SCs into HCs.

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776 FM1-43 Uptake and Recovery of Evoked ABRs in the Gentamicin-Damaged Chicken Basilar Papilla

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Gentamicin triggers loss of hair cells (HCs) in the proximal (high-frequency) half of the chicken basilar papilla (BP). We examined morphological and physiological features of HC regeneration using MyosinVI labeling, FM1-43 uptake, and analysis of evoked auditory brainstem responses (ABRs) in chickens over a 5-week period after Gentamicin treatment. In control BPs, 20-second in vitro exposures to FM1-43 led to significant uptake in HCs and nerve terminals but not in supporting cells. Thirty minutes after exposure, FM1-43 was evenly distributed in the HC cytoplasm. By 24 hours (h) post-Gentamicin (pG), FM1-43 uptake into proximal HCs was significantly reduced, and any incorporated FM1-43 was unevenly distributed in the cytoplasm. Once HC extrusion had occurred in the proximal BP (48-72h pG), little or no FM1-43 incorporation was seen. Although MyosinVI antibodies labeled regenerated HCs in the proximal BP by 4-5 days pG, new HCs showed no evidence for FM1-43 uptake until 7-8 days (d) pG. Birthdating with BrdU showed it takes 7d for new HCs formed by mitosis to take up FM1-43. Thus, HCs formed by non-mitotic processes are the first to regain function. By 16-21d pG, most regenerated HCs showed FM1-43 uptake. Original HCs in middle and distal regions, which persist after Gentamicin, continued to take up FM1-43, but its cytoplasmic distribution was asymmetric and clumpy as early as 48 hours pG and as late as 21d pG. Measurement of ABRs showed that thresholds were significantly reduced compared to controls by 2d pG at 2000-4000 Hz and by 3d pG at 500 and 1000 Hz. At the higher frequencies, threshold recovery occurred by 21d pG, and at the lower frequencies, by 16d pG. These results demonstrate a loss of low-frequency sensitivity despite retention of apparently functional HCs in the middle and distal regions. Further, collective functional maturation of regenerated HCs proximally appears to correlate temporally with high-frequency ABR recovery.

777 Live Imaging of Lateral Line Hair Cell Regeneration in Zebrafish Reveal Hair Cell Origins and Dynamic Cell Movements

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Mechanosensory hair cells within the zebrafish lateral line spontaneously regenerate after aminoglycoside antibiotic-induced death. Exposure of 5-day-old larvae to a high dose of neomycin kills mature hair cells within the lateral line neuromasts. Regeneration is rapid, with near complete hair cell renewal by 72 hours. Our previous studies have shown that replacement hair cells primarily arise from proliferating cells during a defined period

between 12 and 24 hours after neomycin damage. Using sequential incorporation of two thymidine analogs, we show that new hair cells are mainly generated during the initial phase of this proliferation period, while new support cells are produced throughout the period of proliferation. To directly follow cell divisions and cell movements during regeneration, we performed live time-lapse imaging of transgenic fish containing histone-2az-GFP, which labels all cell nuclei, and alpha-tubulin-Tomato, which is expressed in hair cells. New differentiated hair cells primarily originate in pairs from symmetric divisions of internal support cells. Asymmetric divisions giving rise to a hair cell and a support cell also occur, but with much less frequency. Divisions were also observed within peripheral support cells and within cells surrounding the neuromast. Extensive cell movements were often seen around the neuromast periphery during regeneration, including macrophage translocations. Peripheral cells were observed to migrate centrally into the neuromast, supporting the hypothesis that they may be responsible for renewing some of the internal support cells that were used to generate hair cells.

778 Microarray Analysis and Quantitative Real-Time PCR Validation of Gene Expression During Auditory Hair Cell Regeneration in Zebrafish (*Danio Rerio*)

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Fishes are capable of regenerating sensory hair cells in the inner ear after exposure to excessive noise. Adult zebrafish were exposed to a 100 Hz pure tone at 179 dB re 1 µPa RMS for 36 hours to cause noise-induced hearing loss (NIHL) and inner ear RNA was isolated at 0, 2, and 4 days post-NIHL (dpNIHL). Immediately following noise exposure, zebrafish saccules exhibited significant hair cell loss in the caudal region. Cell proliferation peaked at two days post-noise exposure in the caudal region, and to a lesser extent in the rostral region, thus establishing that cell proliferation is associated with the regenerative process. Microarray analysis was used to examine expression levels of candidate genes known to regulate hair cell development. No significant change in expression of Atonal 1A, JunB, Rb, nor CDKN1A were observed. Microarray analysis however, revealed several distinctly highly regulated genes that were subsequently validated using quantitative real-time PCR (Q-RT-PCR). A greater than 30 fold increase in growth hormone was seen at 2dpNIHL in direct correlation with saccular cell proliferation. Meanwhile two myosin heavy chain genes (atrial and smooth muscle) were repressed by comparable magnitudes at 2 and 4dpNIHL. Putative biological processes for some of these genes have been identified, but more work will be needed to determine the importance of growth hormone in stimulating inner ear hair cell regeneration.

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779 Transcriptional Profiling of Genes Involved in Inner Ear Hair Cell Regeneration in the Adult Zebrafish (Danio Rerio)

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Sensory hair cells of the inner ear are the mechanotransductory units in the neuroepithelia. In mammals, lost hair cells are not replaced, resulting in various permanent deficiencies in vestibuloauditory sensation. In contrast, zebrafish can replace lost hair cells with new ones throughout adulthood. Our ultimate goal is to understand the molecular mechanism of the inner ear hair cell regeneration in adult vertebrates. As an initial step, we defined the genetic programs used for hair cell regeneration in zebrafish using emerging techniques for gene expression profiling.

We modified a previous noise-exposure protocol, which enabled us to induce constant hair cell loss in the saccular epithelium of the adult zebrafish and characterize the subsequent regeneration process. We determined the inner-ear gene expression profiles at different time points during the regeneration, focusing particularly on those genes with significant increase in expression at the various time points. Here we used a new technique, Sequencing-Based Transcription Profiling (SBTP), to generate the gene expression profiles in an in-depth and high-throughput manner. Similar to SAGE in nature, the technique generates 3 million or more gene “signatures” from the mRNA pool, giving us a very deep view of gene expression at each time point. We have developed and/or utilized various bioinformatic tools for gene assignment, ontological analysis, and pathway analysis. Our preliminary analysis of the SBTP data already yielded results in agreement with our previous profile comparisons using oligomicroarray.

We are now working on refining our data-mining strategy to identify the candidate genes critical for hair cell regeneration in zebrafish. The candidate genes will be further verified with other techniques (e.g. qRT-PCR, in situ hybridization, immunostaining, etc.) to give us the final list. We will also try to explore the interrelationship among the candidate genes, which will give us clues about the intercellular and intracellular pathways involved in the regeneration process.

780 Differences in Gene Expression Between Regenerating and Non-Regenerating Avian Auditory Epithelia

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Auditory hair cells spontaneously regenerate following injury in birds but not mammals. A better understanding of the molecular events subserving hair cell regeneration in birds may allow for identification and eventually manipulation of relevant pathways in mammals to stimulate regeneration and therefore lead to reversal of deafness in patients. We treated six auditory epithelia from different chicks with forskolin, a potent adenylate cyclase activator which increases intracellular cAMP levels and has been shown to cause a widespread proliferation in hair

cells without upregulation of markers of apoptosis. Using Affymetrix microarrays, overall gene expression of these six samples was compared to that in six untreated control samples. Primary component analysis revealed significant large-scale differences between the two conditions. In the forskolin-treated epithelia, we found significant ($p < 0.05$; > two-fold change) upregulation of many genes thought to be relevant to cellular proliferation such as frizzled homolog 10, wnt3a, as well as numerous cell cycle regulators. Gene set enrichment analysis was performed on the data and revealed numerous microRNAs that are likely to be upregulated in the regenerating tissue. We have therefore identified a number of regulatory molecules whose role in hair cell regeneration warrants further investigation.(Supported by R01 DC 007894 and R01 DC 008130)

781 Labeling of Dying and Regenerating Chick Cochlear Hair Cells with Both Monoclonal and Polyclonal Antibodies to Myosin VIIa

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We have previously shown that a polyclonal antibody to Myosin VIIa specifically labels hair cells in the avian basilar papilla and exhibits a subcellular relocalization and an increased labeling intensity in hair cells undergoing apoptosis (Duncan et al., 2006). Moreover, this antibody can identify regenerating hair cells by 90h after gentamicin treatment, an early point in their differentiation. However, this is a polyclonal antibody, so co-localization of Myosin VIIa with other proteins in apoptosis and regeneration has been limited to those labeled with monoclonal antibodies. While Myosin VIIa monoclonal antibodies are available, their labeling pattern in normal avian hair cells, in dying hair cells, and in regenerating ones was not known. Our results indicate that the monoclonal myosin VIIa antibody specifically recognizes hair cells in the normal cochlea, exhibits the same relocalization and intensity increase in apoptotic hair cells, and appears at the same time in regenerating hair cells as the polyclonal antibody we previously used. Thus, the monoclonal myosin VIIa antibody can be utilized in double-labeling studies with polyclonal antibodies to other proteins to expand our repertoire of co-localization experiments. Moreover, it provides mechanistic data on the increase in fluorescence intensity in the dying hair cells. We previously hypothesized that an apoptosis-related loss of proteins binding to the tail of the Myosin VIIa molecule would result in the availability of more sites for polyclonal antibody binding along the tail and a correlated increase in labeling intensity. However, since the Myosin VIIa monoclonal antibody shows the same increase in labeling intensity as the polyclonal, it suggests that the changes are due to alterations in the myosin protein distribution and not just increased binding of polyclonal antibodies to the myosin VIIa tail.

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782 Comparative Analysis of Two Culture Systems for in Vitro Studies of Avian Hair Cell Regeneration

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Supporting cell proliferation and differentiation in the proximal region of the mature avian cochlea takes place between 72 and 144h after gentamicin injection. In this study, we evaluated anchored-in endolymph organ cultures developed by our lab and traditional free-floating organ cultures, as *in vitro* model systems for hair cell regeneration studies. Hair cell maintenance in non-gentamicin damaged regions of the basilar papilla (BP) and the regenerative potential of the BP in gentamicin-damaged regions were assessed in these culture systems. A damaged proximal region was defined as lacking F-actin positive hair cell bundles 72h after gentamicin injection (AI). One to two week-old chicks were injected with gentamicin, placed into culture at 72h AI, and fixed at 144h AI. Organs were placed in either anchored-in endolymph or floating-in perilymph conditions in the presence of BrdU. BrdU, a thymidine analog, was used as a cell proliferation marker and Myosin VIIa, a hair-cell specific myosin, was used as a hair cell differentiation marker. Cell counts of the entire damaged regions were conducted. In 72h AI controls, 2.6 ± 0.6 Myosin VIIa+ cells were seen (n=5). In 144h AI controls, 435 ± 59 Myosin VIIa+ cells were observed (n=4). In 144h anchored cultures (n=3), 192 ± 35 Myosin VIIa+/BrdU+ cells and 11 ± 11 Myosin VIIa+/BrdU- cells were observed. In 144h floating cultures (n=3), 142 ± 28 Myosin VIIa+/BrdU+ and 0 ± 0 Myosin VIIa+/BrdU- cells were seen. Hair cells in the non-gentamicin damaged region of the BP were well preserved in anchored cultures. In floating cultures, however, hair cells in the non-gentamicin damaged region of the BP were very poorly preserved. These data suggest that the culture systems are equal in allowing for regeneration potential in the gentamicin-damaged region of the BP, but that the anchored cultures best maintain hair cell viability in the non-gentamicin damaged regions of the BP.

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783 Characterization of Supporting Cells in the Superior Region of Normal and Damaged Chicken Basilar Papilla

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Supporting cells (SCs) of the chicken auditory epithelium (basilar papilla, BP) are normally quiescent but can give rise to new hair cells (HCs) after damage using either mitotic or non-mitotic mechanisms. The majority of mitotic regeneration after aminoglycoside-induced HC loss occurs in the superior region of the chicken BP (Cafaro et al., 2007). We have used electroporation to deliver fluorescent dextran into superior SCs in control and regenerating BPs from 1-week-old chickens to visualize SCs in quiescence and at different stages of the cell cycle. We found that most quiescent SCs share a common

bipolar morphology, with one processes contacting the lumenal surface and one contacting the basal lamina, with the nucleus positioned mid-depth in the epithelium. A few quiescent SCs have a basally located nucleus and a limited apical projection. During regeneration, at least some SCs in M-phase possess a long, thin actin-rich process that retains contact with the basal lamina. Treatment of BP cultures with the anti-mitotic drug, AraC, inhibits re-entry of some SCs into the cell cycle and triggers those that escape this inhibition to undergo apoptosis. Despite continual AraC treatment throughout the peak phase of SC division, SCs in the superior region were able to enter S phase approximately 24 hours after AraC was withdrawn, suggesting the presence of stem cells in this region. We are currently examining molecular markers of superior SCs using immunofluorescence and in situ hybridization. Although several genes/proteins are selectively upregulated in the superior region after damage (e.g, lnfg, Ser1, hes5), only a few markers so far – bmp7 and Prox1 – have shown expression limited to SCs in the superior region during quiescence.

784 Impaired Regenerative Ability of the Chick Inner Ear Following Cisplatin Treatment

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Cisplatin is a widely used antineoplastic drug which also causes sensorineural hearing loss in 15-30% of treated patients. Cisplatin is highly toxic to both proliferating cells and to sensory hair cells. Since the avian ear normally regenerates lost hair cells through renewed proliferation of supporting cells, it was of interest to determine whether such regeneration could still occur after cisplatin injury. To address this issue, we have utilized cell and organ cultures of the avian inner ear to characterize the patterns of cell death and hair cell regeneration following cisplatin treatment. In initial experiments, we treated cultures of utricular supporting cells with various doses of cisplatin and labeled proliferating cells with BrdU. We observed a dose-dependent decrease in supporting cell proliferation as well as an increase in supporting cell apoptosis (e.g., pyknotic nuclei). We then examined cultures of the chick cochleae after treatment for 24 hr with 20 uM cisplatin and observed a unique pattern of stereocilia injury and hair cell loss. At 24 hr after cisplatin treatment, hair cell death was confined to the distal (low frequency) region of the cochlea. After 48 hr, hair cell injury had progressed to the proximal region of the sensory epithelium. Further studies examined supporting cell proliferation and hair cell regeneration after cisplatin injury. Chick utricles were cultured for 24 hr in 10 uM cisplatin, which caused a moderate loss of hair cells. Specimens were then allowed to recover for 7 days in cisplatin-free medium. Notably, no regeneration was observed. Instead, we observed continued hair cell death as well as very low levels of supporting cell proliferation. Comparable results were obtained from cultures of the chick cochlea. These data indicate that cisplatin is not only toxic to hair cells, but also has injurious effects on inner ear supporting cells, leading to diminished regenerative ability.

785 Effect of Low Level Laser on Hair Cell Regeneration Following Gentamicin Induced Ototoxicity in Postnatal Organotypic Culture of Rat Cochlea

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In normal postnatal mammalian inner ear sensory epithelium, regeneration of hair cells is a very rare event. Low level laser has the capability to alter cellular behavior in the absence of significant heating. In this study, the effects of low level laser on hair cell regeneration following gentamicin exposure in postnatal organotypic culture of rat cochlea were investigated.

An organotypic culture of 5 day old rat cochlea was established to study aminoglycoside-induced cochlear hair cell damage and renewal. The cochlea were exposed to 1 mM of gentamicin for 48 hr and allowed to recover in a culture medium only condition (G group) or in the culture medium with daily irradiation (GL group) of low level laser (808 nm, 8 mW/cm2, 90 min/day). Whereas the Control group (C group) was not exposed to gentamicin nor laser, and the Laser group (L group) was not exposed to gentamicin but irradiated daily. Whole-mount cochleae were stained with FM1-43, which are known to be an efficient marker, to identify live hair cells in cultured tissues. The number of hair cells was counted in each group serially for 17 days.

While the C group kept on losing hair cells after in vitro culture, the number of hair cells started to increase again in the L group after 6 days of laser irradiation (11 days of in vitro culture). The RM ANOVA for the number of hair cells revealed a significant effect of group ($p=0.015$). That is, the number of hair cells was significantly larger in the L group compared to the C group even after considering the effect of time. After gentamicin exposure, the initial number of hair cells decreased to 37.2% of that of the gentamicin non-exposed groups. While the G group kept on losing hair cells after in vitro culture, the number of hair cells started to increase again in the GL group after 3 days of laser irradiation (8 days of in vitro culture). The RM ANOVA for the number of hair cells revealed a marginally significant effect for group ($p=0.082$). But the group x time interaction was significant ($p=0.037$). That is, although the number of hair cells was not significantly different between groups, the G groups showed more rapid decrease (steeper inclination) in the number of hair cells compared to the GL group.

These results suggest that low level laser promotes hair cell regeneration in cochlear explants.

786 Characterization of in Vitro Regenerated Synapses Between Auditory Neurons and Hair Cells

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Auditory nerve loss can result from direct effects on the spiral ganglion (primary neural degeneration) or from loss of trophic support from degenerated hair cells and supporting cells (secondary neuronal degeneration). Using an in vitro culture system developed for examining the regrowth of spiral ganglion neuron (SGN) processes, we here report the characterization of synaptic connections to denervated hair cells (HC) from postnatal organ of Corti. Regeneration of fibers was evaluated using dissociated SGN from newborn mice in culture with either a "microisolate" (mechanically isolated HC rows) or with dissociated HC. In both approaches all native connections were successfully eliminated. Fibers from single SGNs placed with newborn hair cells for 8 days in culture (DIV) made multiple contacts (up to 10) with either inner (IHCs) or outer (OHCs) hair cells, but not both. No contacts were observed with other neurons or supporting cells in the cultures. Multiple synapses could be established by single neurons on single hair cells. Newly regenerated synapses were identified using antibodies for C-terminal binding protein 2 (CtBP2), a presynaptic marker of ribbon synapses, and postsynaptic-density 95 (PSD95). PSD95 puncta were juxtaposed to presynaptic CtBP2 positive ribbons within IHCs and OHCs. After several days in culture, confocal imaging showed persistent CtBP2 labeling at the basolateral membrane of both IHCs and OHCs, indicating intact ribbons even in the absence of neurons. PSD95 staining supports the glutamatergic nature of the newly formed synapses. Moreover, after extended periods, pruning of SGN synapses led to single connections to hair cells (18 DIV). Further investigations in this model will quantify synaptogenesis after silencing axonal guidance molecules that may block the regeneration of auditory synapses to identify strategies that will increase functional synapses between auditory neurons and hair cells.

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787 Macrophage Response to Ototoxic Injury: A Comparative Study of the Avian and Mammalian Inner Ears

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Macrophages are professional phagocytes that remove apoptotic cells from injured tissues, stimulate inflammation, and promote repair. Macrophages are recruited to injury sites in the cochleae of chicks (ME Warchol, 1997) and mice (K Hirose et al., 2005) but the precise role of these cells in the injured ear is not known. The aim of the present study was to compare macrophage activity in the ears of birds and mammals. Cochleae and utricles from chicks and mice were injured via aminoglycoside ototoxicity (in vitro and in vivo) and the resulting patterns of macrophage recruitment were characterized. Chick specimens were labeled with the KUL01 antibody which labels macrophages and monocytes. Experiments in mice utilized a transgenic strain (CX3CR1 GFP), which expresses GFP in all macrophages and monocytes. Utricles from chicks and mice were examined for macrophage recruitment after aminoglycoside exposure. These cells were mostly confined to the stromal tissue, but frequently extended pseudopodia into the sensory epithelium. A smaller number of macrophages were present within the sensory epithelium and appeared to engulf injured hair cells. Macrophages were also observed in the chick and mouse cochlea. In the undamaged chick cochlea, a large population of resident tissue macrophages was observed in the hyaline/cuboidal cell region. After ototoxic injury, these macrophages appeared to migrate into the sensory epithelium, depleting the supply of macrophages in the hyaline cell area. In the murine cochlea, resident macrophages were few. After systemic kanamycin/furosemide treatment, monocytes migrated from the vascular space into the membranous labyrinth occupying the spiral ligament in large numbers. The results indicate that the ears of both birds and mammals contain resident populations of macrophages. Differences in macrophage recruitment and effector function in the mouse vs. chick ear may contribute to different regenerative abilities of those species.

788 Sphere-Forming Cells Derived from Supporting Cells of the Chick Utricle

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The vestibular organs of birds regenerate hair cells throughout the life of the animal. An attractive mechanism for this ongoing regeneration has been put forward by previous studies and implies replacement of lost hair cells via asymmetric division of supporting cells. As a result, vestibular supporting cells are never depleted and replacement of hair cells can be generated whenever needed. Asymmetric cell division and long-term self-renewal are the hallmark features of adult stem cells. Here, we characterize utricular supporting cells for their ability to form free-floating colonies, so called spheres. These sphere-forming stem cells could be propagated and expanded as clonal floating colonies. The sphere formation assay is a widely used technique for the isolation of somatic stem cells from the nervous system. Our data include analysis of the propagation features of chicken utricular sphere-forming cells; sphere propagation is an indicator of self-renewal capacity. Furthermore, we present an analysis of the ability of sphere cells to differentiate into cells that express hair cell markers. Our ongoing experimentation suggests that the sphere-forming cells from the chicken utricle are supporting cells. Overall, we provide evidence that utricular supporting cells are *bona fide* stem cells.

789 Generation of Embryonic Stem Cell-Derived Ectoderm That Is Responsive to Otic Inducers

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Our goal is to generate otic progenitor cells from murine embryonic stem cells (ESCs). We have previously shown that otic progenitors generated from murine ESCs are able to differentiate into hair cell-like cells *in vitro* and *in ovo* after grafting into chick embryo otocysts. Here we present a refined embryonic stem cell guidance protocol for generation of otic progenitor cells. We show that blockade of primitive streak formation by inhibition of Wnt and TGF-β signaling suppresses the formation of endo- and mesoderm. This inhibition, which is done during embryoid body formation, leads to an expansion of ectoderm, which can be rostralized by addition of IGF-1. We show that rostralized ectoderm cultures, derived from mouse ESCs, are responsive to otic inducers, such as FGF2, or a combination of FGF3 and FGF10. This improved stepwise guidance protocol is capable of generating up-to 35% of Pax-2-expressing presumptive otic progenitors from ESCs in as little as 8 days. This revised stepwise guidance protocol makes use of permissive and inductive steps that reflect natural inner ear development. In comparison with older ESC guidance protocols, it is more reliable and much faster. We present data for two murine ESC lines (the established R1 line and a novel ESC line isolated from Math1/nGFP mice), as well as a comparison with our ongoing effort to guide human H9 ESCs toward the otic lineage.

790 Differentiation of Human Embryonic Stem Cells Into Otic Progenitor Cells

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Degeneration and death of cochlear hair cells and their associated spiral ganglion neurons are causal in >80% of individuals with hearing loss. In mammals, cochlear hair cells cannot be replaced by natural regeneration. Therefore, stem cells have been introduced as an approach for regenerating cochlear hair cells *in vitro* and *in vivo*. Previously, murine embryonic stem cells have been shown to differentiate into sensory hair cells by means of a selection/induction protocol. However, the efficiency of differentiation was low and the protocol took more than a month. We are currently developing a more efficient stepwise guidance protocol for differentiating human embryonic stem cells (hESCs) into human inner ear progenitor cell populations. This protocol is based on the principals of generating ectoderm from hESCs by inhibiting formation of the primitive streak, a structure that is essential for induction of endoderm and mesoderm during mammalian gastrulation. In particular, primitive streak formation was inhibited by blocking WNT and TGFβ signaling via Dkk1 and either Noggin or SIS3. Human ectoderm is then exposed to known otic inducers and tested for expression of otic marker genes. Expression of both Pax8 and Pax2 which are important otic progenitor markers were seen both with immunostaining and PCR.

791 Characterization of Electrophysiological Properties in Mouse Embryonic Stem Cells Driven to a Neuronal Phenotype *in Vitro*

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Hearing loss due to hair cell injury or genetic defect is often accompanied by the progressive degeneration of auditory neurons. Our goal is to differentiate embryonic stem (ES) cells into a neuronal phenotype *in vitro* with the potential of using them to replace damaged auditory nerve *in vivo*. An ES cell line containing an inducible Neurog1 transgene was used to generate glutamatergic neurons at a high efficiency. In this study, ES cells were assessed after 2-3 days of Neurog1 induction. Whole-cell electrophysiology was used to characterize the electrical properties of cells reaching a neuronal morphology (i.e. multiple processes extending from the cell soma). Two days after Neurog1 induction, voltage-clamp experiments revealed a large degree of heterogeneity in excitability. About 70% of the cells displayed outward voltage-sensitive K^+ (K_V) currents that activated above -40 mV. None exhibited voltage-gated Na^+ (Na_V) current. In contrast, all cells after three days of Neurog1 induction exhibited K_V currents and TTX-sensitive Na_V currents. The K_V currents were partially blocked (40–50%) by 1 mM 4-aminopyridine (4-AP). Action potentials could also be elicited in 3-day cells under current-clamp conditions. Following pre-hyperpolarizing voltage steps to -80 mV or below, the majority of the cells exhibited single action potentials upon depolarization. Less than 20% of the cells exhibited multiple spikes for large current injections. Real-time quantitative PCR showed that genes encoding several varieties of K_V and Na_V channels were significantly up-regulated after 3 days of Neurog1 induction. This study demonstrates that functional neurons capable of generating action potentials can be derived from ES cells as early as 3 days after Neurog1 induction. We are currently pursuing physiological and genetic profiles to determine how culture environment may interact with the maturation of ES cell-derived neurons. (Supported by NIH P32DC005356 and P30 DC0578188)

792 Functional Regeneration of the Spiral Ligament by Cell Transplantation in Mice

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The spiral ligament (SL) is a crucial component for auditory function, especially for maintenance of the endocochlear potential (EP). The degeneration of SL causes the loss of EP resulting in sensorineural hearing loss (SNHL). Therefore, SL is included in therapeutic targets for SNHL. Previously, we have established a mouse model for SL degeneration showing significant ABR threshold shifts and EP reduction by local application of 3-nitropropionic acid (3-NP), an inhibitor of succinate dehydrogenase in mitochondria. On the other hand, our

prior studies have demonstrated that bone marrow stromal cells (bMSCs) have the ability for the survival in the cochlea and the migration into SL. In the current study, we examined the potential of bMSC transplantation for SL regeneration. The function was assessed by measurements of EPs and ABRs. C57BL/6 mice (6-10 w) were injected with 3-NP (3 mM) into the left posterior semicircular canal (pscc). ABRs were recorded preoperatively and 1 and 7 days after 3-NP administration. Seven days after 3-NP administration, bMSCs obtained from CAG-EGFP transgenic mice were transplanted into the pscc (n = 11). In control mice (n = 5), the medium was injected. ABRs were recorded 1, 2 and 4 weeks after transplantation and EPs were measured prior to sacrifice (4 weeks after transplantation). Histological analyses of the SL were carried out. The results demonstrated a trend of ABR recovery and s significant recovery for EPs following bMSC transplantation. Although transplants were found in the SL, the number of transplants was not enough for functional recovery of the EP. However, morphometric analyses revealed significant recovery of the cell density in the SL, especially in the type II area. Immunohistochemistry demonstrated the recovery of immunoreactivity for Na,K-ATPase in the type II region. These findings indicate that bMSC transplantation contributes to functional regeneration of the SL via paracrine of trophic or growth factors.

793 Identification of Tissue Specific Stem/Progenitor Cells in Auditory Pathway

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OBJECTS

Recently, in the region of regenerative medicine, it focused on the tissue specific stem/progenitor cells such as neural stem cells in adult brain that has multipotency and identified these cells in various organs. The treatment for the deaf patients that have the auditory damages has been tried, however it was difficult to heal completely. In this research, in order to find out the keys of treatments for these patients by using regenerative medicine, we tried to isolate stem cells from auditory pathway and identify the cell characterization and gene expressions.

METHODS AND RESULTS

3 days age of mice were injected BrdU to the hypodermic at twice a day for 3 days continuously, and sacrificed after 8 weeks to identify the slow-cycling cells suggesting the possibility of stem cells. The results of immunohistochemistry showed that a few cells were identified as BrdU+ and ABCG2+ double positive cells in the section of cochlear nuclei (CN) and inferior colliculus (IC) in auditory pathway. After preparing of cell suspension from CN and IC, BrdU+ cells were found about 1% in both tissues by the analysis of flowcytometry.

Furthermore, we assumed that it was possible to purify stem cells as side population (SP) cells. CN and IC cells were isolated from 6 weeks age of mice and stained with Hoechst 33342. After staining, SP cells were sorted as a negative fraction. The frequency of SP cells was about 1% of total cells in both CN and IC.

Furthermore, we analyzed the gene expression of SP cells by RT-PCR. The results showed that some specific markers of stem/progenitor cells, such as Oct-3/4, Sox2 and ABCG2, were over-expressed in SP cells compared with main population (MP) cells, especially in IC. Cell suspension from CN and IC were cultured in the conditioned medium, sphere formation was also found in both tissues. Now we are going to study whether these cells have the multipotency as stem/progenitor cells, and analyze the specific gene function that identified by microarray analysis.

794 Calcium Clearance from the Hair Bundle During Transduction in Mammalian Cochlear Hair Cells

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Transduction of auditory stimuli in the mammalian cochlea occurs by vibration of the stereociliary (hair) bundles of inner and outer hair cells which opens mechanotransducer (MT) channels highly permeable to calcium. The clearance of calcium from the hair bundle, where the MT channels are located, was followed with the low-affinity fluorescent indicators Fluo-5F and Fluo-4FF and the fluorescence changes were imaged with a fast CCD camera at 500 frames per second. The dye was introduced via a patch pipette during recording from individual neonatal rat hair cells. Following hair bundle deflection there was a rapid rise in intracellular calcium that at the end of the stimulus decayed with a time constant of about 50 ms. The fluorescence change was abolished by hair cell depolarization or block of the MT channels. The calcium fluorescence was localized to the hair bundle, the largest changes, especially in inner hair cells, occurring in the shorter third row stereocilia. The time course of the calcium transient was prolonged by inhibition of the CaATPase by raising extracellular pH to 9.0 or by blocking calcium uptake into the mitochondria with Ruthenium Red. Imaging of calcium in the belt of mitochondria beneath the cuticular plate using Rhod2-AM revealed a fast calcium accumulation during bundle deflection. The hair bundle calcium transients were simulated with a model incorporating calcium buffering, uptake and extrusion, which predicted a much larger calcium increase in the smaller third row stereocilia. We suggest that the sub-cuticular plate mitochondria, besides supplying energy for the bundle CaATPase, also act as a barrier to calcium diffusing from the hair bundle into the cell body. Compromise of the mitochondria during aminoglycoside toxicity or aging may be a major cause of outer hair cell death especially in the high frequency cells that experience greater calcium loads due to larger MT currents and smaller stereocilia. Supported by NIDCD 01362

795 Structure and Function of Hair Cells in Protocadherin 15 Mouse Mutants 3J and 6J

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The Ames waltzer (av) mouse carries mutations in the gene encoding protocadherin15 (Pcdh15) and is a model for both syndromic (Usher1F) and nonsyndromic (DFNB23) deafness. Genetic evidence from various av alleles demonstrates that Pcdh15 mutations affect hair-bundle development and hair-bundle polarity, with the severity of the defect being commensurate with the nature of the mutation. Pcdh15 protein isoforms are associated with the tip-links and kinociliary links of hair bundles (Ahmed et al. 2006; Kazmierczak et al 2007). The present study focuses on a functional and structural analysis of hair cells from Pcdh15av-3J (3J) and Pcdh15av-6J (6J) mice. 3J mice carry a presumptive null allele of Pcdh15 and 6J mice carry an in-frame deletion predicted to remove most of the 9th cadherin ectodomain from Pcdh15. Cochlear cultures prepared from mutants (3J/3J or 6J/6J) and control siblings (+/3J or +/6J) at P1 were used to evaluate hair cell function. In 3J/3J mice, hair cells were unaffected by gentamicin exposure, transduction currents were severely reduced, and the uptake of [3H]-gentamicin and FM1-43 were abolished. In contrast, hair cells from 6J/6J mice transduced and were sensitive to aminoglycosides. Confocal microscopy of the early postnatal cochlea of 3J/3J showed severe disruption in bundle morphology throughout the cochlea; in contrast, mild to moderate disruption of the bundle was noticed in the mid-apical turn of 6J/6J mice. Scanning electron microscopic assessment of cochlear hair cells from 6J/6J and +/6J mice at P9 revealed apparent tip links of normal morphology in both. Quantitative analysis of tip links indicated a reduction in the number of unambiguously identified tip links but an increase in links of uncertain designation. Most of the 6J/6J hair bundles were, however, abnormal with anomalies in the organization of rows and stereociliary height, reducing the number of possible tip-link sites. A similar analysis of the 3J/3J mouse is underway. Results are consistent with Pcdh15 being part of the tip-link complex.

796 Presence of Interstereocilial Links in Waltzer Mutants Suggests Cdh23 Is Not Essential for Their Formation

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Cadherin 23 is a member of a transmembrane family of cadherins and localizes to hair cell stereocilia. Loss-of-function mutations in the *Cdh23* gene cause deafness and vestibular dysfunction in zebrafish, mice and humans due to progressive disorganization of the stereocilia bundles leading to hair cell degeneration. Cadherin 23 is thought to be involved in the formation of interstereocilial links, including tip links. Here we showed that in the disorganised hair bundles of adult $Cdh23^{2J}/Cdh23^{2J}$ mice there was little difference in height amongst remaining stereocilia, all stereocilia showed rounded tips and stereocilia fusion was observed in the basal turn of the cochlea. The stereocilia of waltzer mutants were tightly connected to each other by horizontal links, and tectorial membrane attachment crowns were easily recognizable. In younger $Cdh23^{2J}/Cdh23^{2J}$ and $Cdh23^{J}/Cdh23^{J}$ at P4 also links connecting tips of shorter stereocilium with side of neighbouring stereocilium from taller row were apparent. Our results suggest that cadherin 23 may not be essential for formation of interstereocilial links.

797 MAGI-1, a Candidate Stereociliary Scaffolding Protein Associates with the Tip Link Component Cadherin 23

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Inner ear hair cell mechanoelectrical transduction is mediated by a largely unidentified multi-protein complex associated with the stereociliary tips of hair bundles. One identified component of tip links, which are the extracellular filamentous connectors implicated in gating the mechanoelectrical transduction channels, is the transmembrane protein Cadherin 23 (Cdh23), more specifically, the hair cell-specific Cdh23(+68) splice variant. Using the intracellular domain of Cdh23(+68) as bait, we identified in a cochlear cDNA library MAGI-1, a membrane-associated guanylate kinase (MAGUK) protein. MAGI-1 binds via its PDZ4 domain to a carboxyl-terminal PDZ binding site on Cdh23. MAGI-1 immunoreactivity was detectable throughout neonatal stereocilia in a similar distribution as Cdh23, and as development proceeded, MAGI-1 occurred in a punctate staining pattern on stereocilia, which was maintained into adulthood. Previous reports suggest that Cdh23 interacts via an internal PDZ binding site with the PDZ1 domain of the stereociliary protein harmonin, and potentially via a weaker binding of its carboxyl-terminus with harmonin's PDZ2 domain. We propose that MAGI-1 has the ability to replace harmonin's PDZ2 binding at Cdh23's carboxyl-terminus. Moreover, the strong interaction between PDZ1 of harmonin and Cdh23 is interrupted by a 35-amino acid insertion in the hair cell-specific Cdh23(+68) splice variant, which puts forward MAGI-1 as an attractive candidate for an intracellular scaffolding partner of this tip link protein. Our results consequently support a role of MAGI-1 in the tip link complex where it could provide a sturdy connection with the cytoskeleton and with other components of the mechanoelectrical transduction complex.

798 Characterization of Novel Cytoskeletal Hair Bundle Proteins Identified by Mass Spectrometry

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Recent advances in mass spectrometry (MS) based protein identification have greatly widened its application in all areas of biomedical research. We previously described

a method to identify stereociliary proteins using MS based sequencing of purified hair bundle proteins (Shin et al., 2007). We have further developed the method to identify more than 1000 putative bundle proteins, among them many cytoskeletal proteins. Here we report the initial characterization of two novel cytoskeletal bundle proteins, XIRP2 and SPUNK. XIRP2 (Xin repeat protein 2) is an actin binding protein and was previously reported to be restricted to striated muscle. Using a rabbit polyclonal antibody, we showed XIRP2 immunoreactivity in stereocilia of chicken hair cells. We also identified a previously unknown protein and called it SPUNK (for Stereocilia Protein of UNKnown function). Both Immunofluorescence and gene gun transfection of a GFP-fusion construct of Spunk demonstrate specific localization in the stereocilia. SPUNK has no known domains, but when transfected into COS cells, it colocalizes with actin cables, suggesting an interaction with actin. Experiments are planned to identify the actin interacting domain of SPUNK.

799 Stereocilin-Deficient Mice Reveal the Origin of Cochlear Waveform Distortions

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Although the cochlea is an amplifier and a remarkably sensitive and finely tuned detector of sounds, it also produces conspicuous mechanical and electrical waveform distortions. These distortions reflect non-linear mechanical interactions within the cochlea. By allowing one tone to suppress another (masking effect), they contribute to speech intelligibility. Tones can also combine to produce sounds with frequencies not present in the acoustic stimulus. These sounds compose the otoacoustic emissions that are extensively used to screen hearing in newborns. As both cochlear amplification and distortion originate from the outer hair cells, one of the two types of sensory receptor cells, it has been speculated that they stem from a common mechanism. Here, the non-linearity underlying cochlear waveform distortions is shown to rely on the presence of stereocilin, a protein defective in a recessive form of human deafness. Stereocilin was detected in association with horizontal top connectors, lateral links that join adjacent stereocilia within the outer hair cell's hair bundle, and these links were absent in stereocilin-null mutant mice. These mice become progressively deaf. At the onset of hearing, however, their cochlear sensitivity and frequency tuning were almost normal, although masking was much reduced and both acoustic and electrical waveform distortions were completely lacking. From this unique functional situation, we conclude that the main source of cochlear waveform distortions is a deflection-dependent hair bundle stiffness resulting from constraints imposed by the horizontal top connectors, and not from the intrinsic non-linear behaviour of the mechanoelectrical transducer channel.

800 Repair of Tip Links and Stereocilia in Mammalian Cochlear Hair Cells

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The senses of hearing and balance depend upon hair cells, the sensory receptors of the inner ear. Hair cells transduce mechanical stimuli into electrical activity. The site of hair cell transduction is the hair bundle, an array of modified microvilli or stereocilia arranged in a staircase. Adjacent stereocilia are connected along their shafts by side links, whereas tip links, reported to be formed by cadherin 23 and protocadherin 15 interaction, extend from the vertex of each stereocilium to the side of its taller neighbor. The hair bundle and tip links of hair cells are susceptible to acoustic trauma and ototoxic drugs. There is some evidence that damaged hair cells may survive and undergo intracellular repair in mammalian vestibular hair cells and in birds and lower vertebrates. We attempted to determine whether mammalian cochlear hair cells could survive and undergo spontaneous repair after loss of tip links and/or hair bundle. The experiments were carried out in the cultured organ of Corti from neonatal gerbils. The hair bundles of outer hair cells in a designated area of the organ of Corti were removed by a small suction pipette. Hair-cell survival/development and stereocilium morphology/function were examined using immunohistochemistry (with confocal microscopy), scanning electron microscopy, and electrophysiology. Our results showed that majority of the hair cells could survive and develop up to 12 days after their bundles were completely removed. However, no spontaneous bundle repair/regeneration was observed. Interestly, if only tip links were ruptured by traumatic mechanical stimulations, repair of tip links and restoration of mechanotransduction were observed in less than 48 hours. Our study suggests that the dynamic nature of the hair cell's transduction apparatus is retained despite that self-repair of the hair bundle is lost in mammalian cochlear hair cells. Supported by NIH grant DC 004696 from the NIDCD.

801 Nanoscale Imaging of Cochlear Hair Cells Using Hopping Probe Ion Conductance Microscopy

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Currently available techniques for imaging the complex three dimensional cell surfaces at high resolution require fixation or freezing, which introduces artifacts and does not allow direct visualization of functionally important processes in a live cell. Here we present a major advance in scanning probe microscopy that allows the imaging of live complex cellular samples without ever touching them. We demonstrate the effectiveness and nanoscale resolution of the technique by imaging mechanosensory stereocilia of the cochlear hair cells and very complex networks of cultured hippocampal neurons. Our technique

can be straightforwardly combined with fluorescence detection to identify functionally important surface structures. Because the same nanoprobe can be used not only for imaging, but also for local stimulation of surface nanostructures and/or probing their electrical activity, our technique opens up a possibility for structure-function studies at a nanoscale.

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802 Stereocilium Flexoelectricity: Electromechanical Motors of the Inner Ear

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Hair cell stereocilia bundles are the primary sensory receptors mediating the sensation of hearing and balance in vertebrates. A bundle-based amplification process has been shown to contribute to the exquisite sensitivity and frequency selectivity demonstrated by these cells. A variety of motor mechanisms associated with the mechano-transduction apparatus in stereocilia have been identified. Additionally, changes in the membrane potential evoke bundle movements even in the absence of functioning mechano-transduction channels. These voltage-dependent movements show similarity to voltage-dependent forces in membrane tethers. Tethers are simple cylindrical membrane structures with nanoscale dimensions similar to stereocilia, and hence the membrane biophysics would be expected to translate from tethers to stereocilia. Based on these findings, we formulated a biophysical model to investigate the potential contribution of membrane flexoelectricity to stereocilia motility. We examined the electrical to mechanical energy conversion mediated by this mechanism and found that the most efficient conversion provides an explanation for the tonotopic gradation in stereocilia height observed within the cochlea. The same biophysics may be a factor contributing to self-excited oscillations underlying the hair-bundle Hopf bifurcation described in hair cells. To further investigate the flexoelectric effect, a finite element model was developed by modeling the stereocilia as a cylinder with a conductive core and a shell endowed with flexoelectric properties. Preliminary results will be presented showing flexoelectric associated forces, movements and power output for typical mechanotransduction currents and somatic impedances. [Supported by NIDCD R01 DC004928, R01 DC006685, and GSRP 56000135].

803 Efficiency of Hair Bundle Motility as the Cochlear Amplifier

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The hair bundle's motility affects its mechanosensitivity. Here we examine channel re-closure (CRC) model and Tinevez-Julicher-Martin (TJM) model for their efficiency as the cochlear amplifier by imposing the condition that the energy gain by the hair bundle motor is greater than the loss due to viscous drag in the subtectorial gap. This condition leads to an upper limit of frequency supported by the hair bundle motor.

The limiting frequency based on CRC model is proportional to $(k_g x_g)^2 F_m \Phi$, where k_g and x_g are gating spring stiffness and gating distance of the mechanoelectric transducer (MET) channel, respectively, Φ a phase factor that depends on channel kinetics, and F_m (= $Ns^2/(hA)$) the morphological factor with N being the number of the MET channel per hair cell, s the rootlet separation, h the height of he tallest cilia, and A the area of the gap per hair cell. The limiting frequency of TJM model has the form $a\sqrt{(bF_m - 1)}$ with both a and b dependent on transducer stiffness k_g $(1- k_g x_g^2 P_o (1-P_o)/(k_B T))$, which is required to be negative, and friction coefficient of the motor that provides phase delay.

The limiting frequency estimated for the mammalian ear under realistic conditions is up to 1.2 kHz based on CRC model and is up to 2.8 kHz based on TJM model, still lower than the mammalian auditory range. If we can assume that the mechanical characteristics of the MET channel and the adaptation motor are shared among vertebrates, differences between mammalian and avian limiting frequencies could be attributed to the morphological factor in CRC model. A similar simplification is possible with TJM model with a proviso that the equal operating point of the MET channel is significant. If those properties do not depend on the location within the cochlea, the tonotopic map of the avian ear, which depends on the hair bundle motor alone as the cochlear amplifier, can be described by the morphological factor.

804 Distribution of Frequencies of Spontaneous Oscillations in Hair Cells of the Bullfrog Sacculus

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Under *in vitro* conditions, free-standing hair bundles of the bullfrog (Rana catesbeiana) sacculus have been known to exhibit spontaneous oscillations. A high-speed Complementary Metal Oxide Semiconductor (CMOS) camera was used to track the active movements of multiple hair cells in a single field of view. The techniques we developed allowed us to probe for correlations between pairs of cells, and to acquire records on over 100 actively oscillating bundles per epithelium. We measured the statistical distribution of the oscillation periods of cells from different areas within the sacculus, and on different epithelia. Spontaneous oscillations exhibited a peak period of 33 ms (+29 ms, -14 ms) and showed a uniform spatial distribution across the sacculus. Latest data will be discussed.

805 Fast Adaptation in Entrained Spontaneous Oscillations of Vertebrate Hair Cells

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We study the mechanism for fast adaptation in the vertebrate hair cell. Spontaneous oscillations of hair cells from an in-vitro preparation of the bullfrog sacculus are found to have instantaneous frequencies that wander around some characteristic frequency. We entrain these oscillations to their characteristic frequency with the application of a very small external mechanical stimulus. This then allows for the displacement record to be averaged over hundreds of oscillation cycles, reducing the noise and making visible detailed temporal features of the spontaneous oscillation. We will also discuss optical techniques we are currently developing to incorporate fluorescent detection of calcium signals into this measurement.

806 Postnatal Development of the Cochlear Microphonic and Compound Action Potential in the Chinchilla

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Sound-evoked electrical responses were recorded from the round window of the cochlea in chinchilla (*Chinchilla laniger*) 'kits' at various postnatal ages from P0-1 (first 24 hours) to P70 or more (adult). Stimuli were delivered via insert earphones that were calibrated *in situ* for SPL at the tympanic membrane for each animal. The cochlear microphonic (CM) and the compound action potential (CAP), representing the ensemble activities of the hair cells and auditory nerve fibers, respectively, were recorded using standard techniques. Measureable CM and CAP were present at P0-1. The frequency-dependent range of CM thresholds (minimum to maximum) at P0-1 was -5 to 25 dB SPL, similar to adults at 5 to 23 dB SPL. CAP thresholds at P0-1 ranged from 40 to 70 dB SPL and decreased in adults to 5 to 25 dB SPL, particularly at higher frequencies. The maximum CM amplitude, dynamic range, and the slope of the CM amplitude-SPL functions (computed over the range of CM amplitudes that were linear with increases in SPL) all increased from P0-1 through adult. The frequency-dependent range of average maximum CM amplitudes computed across animals for adults was 445 (1 kHz) to 16 μV (20 kHz) and were approximately an order of magnitude smaller in P0-1 animals. These results suggest that considerable cochlear hair cell and auditory nerve fiber function is present already at birth in chinchillas with many characteristics of the CMs and CAPs falling near or even within the adult range, at least over some frequency ranges. However, other important characteristics (e.g., amplitudes, dynamic ranges) exhibited considerable development postnatally. Due to similar physiological, anatomical and behavioral characteristics as those of humans, particularly the precocious nature of the auditory periphery at birth, the chinchilla is a good model for developmental studies of the auditory system. Supported by Evie & Ron Krancer Grant in Auditory Science from the National Organization of Hearing Research and T32NS007083.

807 Pannexin as a New Gap Junction Gene Family Expresses in the Mammalian Inner Ear

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Gap junctions are known to play a critical role in hearing function. Pannexin (Panx) is a new gap junction gene family identified from mouse and human genomes. Like connexins, pannexins also encode gap junctional proteins in vertebrates. In this study, expression of pannexins in the mouse and rat cochlea was investigated. PCR and Western blot analysis showed that all three pannexin isoforms (Panx1, 2 and 3) were expressed in the cochlea. Panx1 is a predominant isoform. Panx1 labeling was found in supporting cells, including pillar cells, Hensen cells, Claudius cells and Boettcher cells. Both plaque-like punctate surface labeling and diffuse-cytoplasmic labeling were visible. However, the labeling was weak and rare in Deiters cells. No labeling was found in the hair cells. Intense labeling for Panx1 was also observed in the interdental cells in the spiral limbus, the inner and outer sulcus cells, and the type II fibrocytes in the spiral prominence and central region in the cochlear lateral wall. In addition, Panx1 labeling was detectable in the Reissner's membrane and strial blood vessel cells. Panx2 labeling was restricted to the basal cells in the stria vascularis and was also detectable in the spiral ganglion neurons. Finally, Panx3 labeling was exclusively observed in the cochlear bone. Thus, Panx1, 2 and 3 are abundantly expressed in the mammalian cochlea and demonstrate distinct cellular distributions. These data also indicate that pannexins may play an important role in hearing as connexin does in the cochlea.
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808 Relationship of Cx26, Cx30 and ZO1 Expressions in the Cochlear Sensory Epithelium

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The intercellular junctions, such as tight junctions and gap junctions play a critical role in hearing. The gap junctions form intercellular channels and mediate direct transfer of low molecular weight metabolites, ions, and small molecules between neighboring cells. The tight junction plays a distinct role in barrier function. The relationship between the tight junctions and gap junctions in the cochlear sensory epithelium remains unclear. Zonula occludens-1 (ZO-1) is a scaffold protein participating in forming of the tight junctions. In this study we performed immunofluorescent staining and confocal microscopy to examine the distribution and relationship of Cx26, Cx30,

and ZO-1 in the cochlea. Both Cx26 and Cx30 were expressed in Hensen cell, inner and outer pillar cell and Claudius cell. Cx26 labeling largely overlapped that of Cx30 in these regions. Cx26 and Cx30 were also co-expressed in the spiral limbus. Neither Cx26 nor Cx30 labeling was seen in the hair cells. The expressions of Cx26 and ZO1 demonstrated distinct expressions between the surface layer and the deep layer in the cochlear sensory epithelium. In the surface layer of cochlear sensory epithelium, ZO-1 positive labeling was mainly localized between sensory hair cells and supporting cells, there were fewer or no Cx26-postive spots. In the deep layer of cochlear sensory epithelium, Cx26 was widely expressed. However, ZO1 staining was undetectable. These results indicate that tight junctions maintain the ion barrier between the endolymph and perilymph and that gap junctions take part in ion transportation.
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809 Size Limitation in Inner Ear Gap Junction Permeability Measured by Fluorescence Recovery After Photobleaching (FRAP)

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Gap junction is an intercellular channel providing an intracellular conduit allowing permeation of ions and small molecules between cells. The diameter of gap junction channels is about 1-1.5 nm, corresponding to the estimated permeable size up to 1,000 Da. Recently, it has been found that gap junctional channels have size and charge selectivity in permeability. The permeability of gap junctional channels assembled by different connexins is different. Also, different channel configurations can result in different permeabilities. Previously, we have reported that inner ear gap junctions possess strong charge selectivity (Zhao, Eur. J Neurosci., 2005). In this experiment, the permeability of inner ear gap junctions and the gap junction between different cochlear supporting cells was studied by fluorescence recovery after photobleaching (FRAP). We found that gap junctions between different cochlear supporting cells have differing size selectivity in their permeability. Fluorescent dye Calcein (MW=995) could not pass through the gap junctional channels between any cochlear supporting cells. Little fluorescence recovery was visible after photobleaching. Dye BCECF (MW=622) was only permeable to gap junctions between Deiters cells but impermeable to gap junctions between Hensen cells, Claudius cells, and Boettcher cells. Inner ear gap junctions had good permeability to dye SNARF (MW=453). No significant difference was found in the permeability of gap junctions between different supporting cells to dye SNARF. In addition, as our previous report that hypotonic challenge can uncouple the gap junctional coupling between cochlear supporting cells, perfusion of low osmotic solution also dramatically reduced fluorescence recovery after photobleaching by 75%. Thus, the upper-size limit of gap junctional permeability between cochlear supporting cells is different; gap junctional coupling between Deiters cells has the largest size permeability in the cochlea.
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810 A Prediction for the Role of Gap Junction Mutations from a 3D Model of Ionic Circulation in the Cochlea

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We have recently developed a computational model of the 3D electrical current flow within the cochlea (Mistrik et al., J. R. Soc. Interface, 2008). The model splits the cochlea longitudinally into 300 cross-sections and divides each section into 6 compartments: scala media, inner and outer hair cells (IHC, OHC), extracellular space near the IHCs, and extracellular space near the OHCs and stria vascularis. The electrical properties of these compartments are represented in the model by standard elements (resistors, capacitors and batteries). The transducer conductances are driven by a basilar membrane displacement generated by a physiologically realistic model of cochlear mechanics. This approach allows us to analyse how the resistance of K^+ reabsorbing radial pathways within and longitudinal electrical coupling between the cross-sections would affect the frequency selectivity and sensitivity of the OHC transmembrane potentials and therefore cochlear amplification. The electrical resistances are dictated predominantly by gap junction networks in the organ of Corti and would be altered by mutations in the connexion genes. We have also investigated the role of extracellular space and intercellular capacitative coupling. The simulations suggest that reduced conductivity of gap junctions would result in decreased OHC receptor potentials with a roll-off of 6dB/octave. The effect is therefore most significant at high frequencies. The result argues for a compromised ionic recirculation within the cochlea, rather than decreased metabolic signalling between cells, as a possible mechanism for the high frequency hearing loss observed in patients carrying pathogenic mutations in GJB2.
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811 Claudin Expression During Early Postnatal Development of the Murine Cochlea

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Tight junctions between epithelial and endothelial cells form barriers that are essential for cochlear function. The barrier function of tight junctions is largely determined by the expression of claudins. Claudins are a family of tetraspan proteins that determine the anion and cation permeability of tight junctions and that contribute to cell signaling between epithelial and endothelial cells. Requirements for barriers change drastically during early postnatal development of the cochlea when steep electrochemical gradients between endolymph and

perilymph are established. Although a number of claudins have already been shown to be expressed in the cochlea (Florian et al. 2003, Kitajiri et al. 2004), the family of claudins has since grown and the question whether claudin expression changes during early postnatal development has been address only for very few claudins (Gow et al., 2004). Expression amounts were determined by quantitative RT-PCR using SYBR green for detection and 18S rRNA for calibration. Total RNA was isolated from whole cochleae of mice at postnatal (P) ages P2, which is before the onset of K^+ secretion and Na^+ reabsorption, P6, which is after establishing mature Na^+ and K^+ gradients but before the generation of the endocochlear potential, and P15, which is after establishing a mature endocochlear potential and nearly adult levels of hearing sensitivity. Developmental increases in expression were observed for *Cldn2*, *Cldn5*, *Cldn9*, *Cldn11*, *Cldn15*, and *Cldn19v1*. Developmental decreases in expression were observed for *Cldn6*. No significant change in expression during development was observed for *Cldn1*, *Cldn3* and *Cldn7*, and no expression was found for *Cldn16*. In conclusion, these data demonstrate that claudin expression changes in the cochlea during early postnatal development and that studies determining claudin expression in separate fractions of cochlear tissues are necessary. Supported by NIH-R01-DC01098, NIH-R01-DC00212, NIH-P60-RR017686.

812 Trafficking Pathways of Inner Ear Connexins

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Gap junctions consisting of connexins 26 (Cx26) and 30 (Cx30) are essential for hearing in mammals, and are the predominant connexin isoforms found in the supporting cell network of the cochlea. The majority of connexins, such as Cx43, have been shown to traffic to the plasma membrane (PM) via the Golgi apparatus. Others, such as Cx26, show conflicting data, with some literature suggesting Cx26 traffics via a novel Golgi-independent pathway. Some gap junctions in the cochlea have been shown to consist of Cx26/Cx30 heteromeric connexons, which are likely to oligomerize prior to insertion in the PM. We have investigated the trafficking of mouse Cx30 (mCx30) and mCx26 in vitro, with particular attention to potential Golgi interactions.

In HeLa cells stably expressing mCx43 (whose trafficking is well characterized), Cx43 immunofluorescence was localized to gap junction plaques at the PM, and co-localized with the cis-Golgi marker GM130. In comparison, cells stably expressing either mCx30 or mCx26 displayed distinct gap junction plaques, but displayed little discernible co-labeling with GM130. In dye transfer experiments, Brefeldin A (BFA), which disrupts the Golgi apparatus, caused a 59% reduction in the intercellular transfer of neurobiotin between mCx43 expressing cells, consistent with an observed decrease in the number of gap junction plaques. BFA did not significantly affect neurobiotin transfer between mCx30 expressing cells, but, interestingly, neurobiotin transfer between mCx26 expressing cells decreased by 86%.

These data suggest that mCx30 may traffic to the PM via a pathway that is unaffected by the disruption of the Golgi apparatus, in contrast to mCx26, which appears to be BFA sensitive. Current work is ongoing to determine the sub-cellular site of Cx26/Cx30 oligomerization, and how the heteromeric channels are trafficked subsequently to the membranes between cochlear supporting cells.

813 Gap Junctional Intercellular Coupling in the Cochlear Lateral Wall: Evidence for K^+ Re-Circulation

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Cochlear homeostasis relies on gap junctional intercellular coupling within the epithelial cell network, and within the connective tissue network of fibrocytes in the spiral ligament. It has been hypothesized that K^+ ions released from hair cells during auditory transduction are buffered by epithelial supporting cells, and re-circulated via gap junctions to the lateral wall. They would then be transferred to the spiral ligament fibrocytes by an as yet undetermined mechanism. K^+ ions would be returned via fibrocytes to strial intermediate cells for re-secretion into the endolymph. Dye-transfer experiments have been carried out to explore these K^+ re-circulation pathways.

Neurobiotin was injected into cells in whole cochlear slices (P10-P15 rats) or lateral wall slices (adult guinea pigs) via whole-cell patch electrodes (Jagger & Forge, J Neurosci, 2006). Following injection into individual fibrocytes (type I, type II, or type V), neurobiotin could be detected throughout the spiral ligament. Gap junction blockers prevented this spread. Closer inspection revealed that neurobiotin also transferred via type I fibrocytes into basal cells and intermediate cells (identified by Kir4.1 immunofluorescence). There was no evidence of dye-coupling between the connective tissue gap junction network and the epithelial gap junction network. Similarly, neurobiotin injected into outer sulcus cells or root cells spread to other epithelial cells, but did not transfer to fibrocytes.

These experiments confirm that there are two independent gap junctional networks that meet at the cochlear lateral wall. Also, there is a pathway in the spiral ligament that would allow return of recycled K^+ ions to the stria vascularis. The mechanism by which K^+ ions transfer from the epithelial cell network to the connective tissue network is currently under investigation.

814 Supporting Cells in the Organ of Corti Show Spontaneous Depolarizing Activities in the Developing Cochlea of Mice

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Developing inner hair cells and afferent fibres display spontaneous activities that are thought to be involved in refining the auditory nerve innervation in the auditory

centers. Recently, it has been shown that these activities are driven by the spontaneous depolarizing activities (SDAs) of the inner supporting cells located in the Köllicker's organ. Here, we investigated whether similar SDAs are present in the supporting cells of the organ of Corti.

Using both loose-patch extracellular and whole-cell patch clamp recording techniques performed on a novel flattened cochlear preparation obtained from P0-P10 mice, we observed that Hensen's cells, Deiters'cells, Claudius cells and outer sulcus cells all displayed SDAs. Duration of SDAs lasted 914±224 msec (n=639 spikes). The distribution of intervals between depolarizations showed two peaks of about 7 and 17 sec respectively. The frequency of SDAs was increased by the application of ATP (10 microM) and was decreased by suramin (200 microM), a broad spectrum antagonist of P2X and P2Y purinergic receptors. SDAs were reversibly inhibited by octanol (2 mM), a non-specific gap junction (GJ) and hemichannel blocker. Control application of butanol (2 mM) had no effect. Double electrode patch clamp recordings demonstrated synchronized SDAs between neighbouring supporting cells. These results are consistent with the contribution of gap junctions and/or hemichannels in the initiation/propagation of the SDAs among the supporting cells located in the organ of Corti, which are known to express high levels of connexin26 (Cx26) and Cx30. We are currently using both Cx30 and conditional Cx26 null mice to further investigate the contribution of GJ-mediated intercellular communication in the SDAs. Our data demonstrated that SDAs are a universal phenomenon displayed by all types of supporting cells in the developing spiral limbus and the organ of Corti.

815 2-Aminoethoxydiphenyl Borate (2-APB) Inhibits Gap Junction Coupling and Delayed Rectifier K^+-Channels in Guinea-Pig Arterioles in the Cochlea and Other Beds

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2-APB has been found directly inhibit gap junction channels of connexin26 and/or connexins32 expressed in cultured cells. A similar action and the other membrane effects in native tissues remain unknown. Using whole-cell and intracellular recording methods, we studied these actions on vascular smooth muscle cells (VSMC) *in situ* of acutely isolated arteriole segments from the cochlear spiral modiolar artery (SMA), brain artery (BA) and mesenteric artery (MA), and in dispersed VSMCs and endothelial cells (EC). We demonstrated that 1) 2-APB and its analog DPBA both reversibly suppressed the input conductance (G_{input}), or increased the input resistance, with an IC_{50} of 4 - 8 μM in the three vessels tested. A complete electrical isolation of the recorded VSMC was achieved at 100 μM. A similar gap junction blockade was observed in tubules of endothelial cells dissociated from the SMA. 2) 2-APB and DPBA (≥ 10 μM) reversibly depolarized the cells with larger amplitudes than that by 18β-glycyrrhetinic acid (18βGA). 3) On dispersed VSMCs, 2-APB and DPBA (100 μM) had no significant effect on G_{input} or I/V relation in a range between -140 and -40 mV but inhibited the delayed rectifier potassium current (K_{DR}). 4) The inhibition of K_{DR} was always more pronounced at potentials ≤20 mV than at 40 mV and was more pronounced for the fast than the slow component in step-activated current, similar to that by 4-AP rather than by TEA. 5) The inhibition of K_{DR} was concentration-dependent with a similar IC_{50} of ~120 μM for both 2-APB and DPBA in the three vessels compared to ~50 μM for 18βGA. We conclude that 2-APB and DPBA are about half as potent as 18βGA in blocking gap junctions in arterioles as well as in inhibiting K_{DR} channels in VSMCs; no significant difference was observed among the three arterioles tested for the gap junction blockade potency of any of the three agents, suggesting a homogeneous property of the gap junctions among these vessels but with coupling strength being the highest in the MA. Supported by NIH NIDCD DC 004716 (ZGJ)

816 The Role of the Cav1.2 Channel in the Cochlea

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Voltage-gated L-type Ca2+ channels (L-VDCCs) play an important role in synaptic transmission, secretion and gene expression. In the cochlea the Cav1.2 and Cav1.3 are expressed shown by RT-PCR (Green et al., 1996).

Within the auditory system the function of Cav1.3 is already known. Constitutive Cav1.3 KO mice are deaf due to the complete absence of L-type calcium currents in the cochlear inner hair cells and degeneration of outer and inner hair cells (Platzer et al., 2000).

For Cav1.2 protein was shown to be located under the hair cells (Waka et al., 2003) and in spiral ganglion neurons in adult mice, using an a1C calcium channel subunit specific antibody.

Preliminary results confirm the expression of Cav1.2 in the inner ear. We could show by In situ Hybridization mRNA expression in spiral ganglion neurons.

Until now the function of Cav1.2 for hearing has not been elucidated. Mouse models, in which Cav1.2 is deleted, are available but lethal with birth. Therefore, a conditional KO mouse model is required. To generate this model a mouse line with Cre expression under the promoter of spiral ganglion neuron specific genes would be essential. We will introduce two possible mouse models, as candidates in driving Cre expression.

A conditional Cav1.2 KO mouse model could help to understand the function and the role of Cav1.2 in physiological conditions and auditory pathologies as acoustic trauma and tinnitus.

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817 CTL2/SLC44A2 Binds Selectively to a Specific Subset of Phospholipids

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CTL2/SLC44A2 (choline transporter like protein 2/solute carrier protein 44A2)(CTL2) was discovered as a prominent antigen expressed on supporting cells in the organ of Corti that serves as a target of antibody-induced hearing loss. Recently, we have shown that this multi-transmembrane domain containing glycoprotein is widely expressed throughout the cochlea and vestibular system. Antibody to CTL2 can bind to supporting cells in vivo and result in loss of outer hair cells and hearing loss, indicating that the function of this protein is essential for inner ear homeostasis. The mechanism by which this occurs is unknown. As a member of the solute carrier family, CTL2 is suspected to be a transporter of organic osmolytes. To better understand the function of CTL2 we assessed the ability of rHuCTL2-His protein to interact with membrane lipids. rHuCTL2 was produced in Sf9 cells and purified using Nickel NTA affinity chromatography. Screening was performed using Membrane Lipid Strips (Echelon Biosciences, Inc, Salt Lake City, UT). Sphingostrips and PIP strips containing 5 sphingolipids, ceramide, sulfatide, myriosine, sialogangliosides, psychosine, cholesterol, lysophosphatidic acid, lysophosphorylcholine, triglycerides, cardiolipin, 8 phosphoinositides, phosphatidic acid, phosphatidylserine, phosphatidylcholine, and phosphatidylethanolamine. Initial screening identified phosphatidic acid (PA), phosphatidylserine (PS), and the phosphatidylinositol monophosphates PtdIns(3)P and PtdIns(4)P. Only weak binding was observed with PtdIns(5)P, PtdIns(3,4)P2, PtdIns(4,5)P2, and PtdIns(3,4,5)P3. The highest affinity was for PA, PS, PtdIns(3)P and PtdIns(4)P. Controls including solvent blanks, primary and secondary antibodies without CTL2 were all negative. Thus, we conclude that CTL2 binds specifically to these phospholipids. Whether CTL2 function involves specific transport of these molecules is currently under investigation.

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818 Investigating BK Channel Expression and Function in Outer Hair Cells of the Rat Cochlea

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In the mammalian cochlea, outer hair cells (OHCs) are innervated by inhibitory cholinergic neurons. Acetylcholine (ACh) activates α9α10-containing nicotinic ACh receptors (nAChRs), leading to K+ efflux through Ca2+-dependent SK2 channels. Recent immunohistochemical data have suggested that BK channels, like SK2 channels, are localized to the basolateral region of the OHC. To investigate the expression and subcellular localization of BK channels in OHCs and to test whether these channels are involved in the OHC efferent inhibitory response, we performed a variety of experiments using immunofluorescence and patch-clamp electrophysiology on OHCs from 19 to 21 day old rats. Confocal microscopy of rat organs of Corti immunolabeled with antibodies specific for the BK channel and for markers of either the OHC (prestin and CtBP2) or the efferent neurites (SV2 and synapsin) verified the localization of BK channels to the basolateral membrane of the OHCs, specifically postsynaptic to the sites of efferent contact. Immunolabeling experiments also quantified the tonotopic gradient of BK channel expression. BK immunoreactivity was observed in fewer than half of the efferent contacts on OHCs from apical cochlear turns. In contrast, the majority of OHC efferent terminals from middle and basal turns were associated with BK immunoreactivity. The BK-specific channel blocker iberiotoxin was applied during whole cell patch clamp recordings from rat OHCs to determine if BK channels contribute to voltage- or ligand-gated currents in these cells. Consistent with BK immunolabeling, iberiotoxin applied to apical OHCs had little or no effect on K+ currents activated by either membrane depolarization or local application of Ach. Preliminary data show that voltage-gated K+ currents in basal OHCs are IBTX-sensitive.

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819 The Morphology of Fibrocyte-Vascular Coupling in Microvessels of Guinea Pig Cochlea

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The dynamic regulation of oxygen and the supply of glucose to match changes in metabolic demand are critical to the maintenance of cochlear homeostasis. It is possible that increases in hair cell activity are accompanied by rapid, spatially localized elevations of oxygen and glucose delivery. Although spiral modiolar artery autoregulation is one local control mechanism, reactive to systemic pressure and energy changes, another local mechanism based within capillaries has a cellular basis of function that has remained elusive. In the present study, with transmission electron microscopy combined with fluorescence confocal imaging, we found that fibrocytes are uniquely positioned and spatially distributed near pre-capillaries and post-capillaries of the spiral ligament. Immunohistochemical techniques revealed that inter-connected fibrocytes were positive to Na+/K+ ATPase β-1 and connexin 40. With fluo-4, a fluorescent calcium indicator, we found that connected fibrocytes contained relatively high intracellular fluo-4 fluorescent signals in comparison with other cells in cochlear lateral wall. Elevation of Ca2+ within a few fibrocytes, induced through photolysis of the caged divalent ion chelator NP-EGTA, resulted in Ca2 signal propagation to neighbor cells, including the vascular cells: pericytes and endothelial cells. The connection between the fibrocytes and the vascular cells suggests a “fibro-vascular” coupling in the cochlear microvessel system in the spiral ligament. The unique

arrangement between fibrocytes and vascular cells provides morphological basis for fibrocytes to transduce and transfer the signals to the cochlear microcirculation. This signal pathway may be important for the local blood flow control to meet cellular metabolic needs.
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820 Endolymphatic Sac is Involved in the Regulation of Endolymphatic Pressure

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Background: To clarify the involvement of the endolymphatic sac (ES) in endolymphatic pressure regulation, we examined whether the hydrostatic pressure of cochlear endolymph *(P*e) could be affected by the agents changing the ES direct current potential (ESP). It has been reported that catecholemines (by beta-adrenergic action) and acetazolamide (ACTZ), a carbonic anhydrase inhibitor, decrease the ESP, and that these agents induce no remarkable change in the endocochlear potential (EP) in their dose producing near-maximum or maximum decrease of the ESP.

Materials & Method: Albino guinea pigs with a positive Preyer's reflex were used. The tympanic bulla was opened by the retroauricular approach to expose the round window membrane (RWM). The tip of a recording micropipette was then inserted from the RWM through the basilar membrane into the scala media, in which *P*e was measured. The EP at the pipette tip was simultaneously measured to verify the position. For the measurement of cochlear perilymph hydrostatic pressure (*P*p), the tip of a micropipette was inserted through the RWM into the scala tympani. The tip of a micropipette was inserted into the cistern magna to measure the hydrostatic pressure of the cerebrospinal fluid (*P*c). The pipette was connected to a servo-null system(900A Micropressure System; World Precision Instruments, FL, USA). Isoproterenol (ISO) diluted in 2.5 ml of saline was infused for 5 min at a concentration of 6.25 μg/kg/min through a catheter inserted into the jugular vein using an infusion pump. A dose of 10mg/kg ACTZ dissolved in 2ml of saline was infused for 1min.

Results: Iso and ACTZ induced no change in the EP. Iso reversibly increased *P*e and *P*p, whereas ACTZ produced no change in *P*e and *P*p. An increase in *P*p by Iso showed a time lag behind an increase in *P*e. Iso produced no change in *P*c. The action of Iso on *P*e and *P*p disappeared in ears with obstructed ES, suggesting the involvement of the ES in the endolymphatic pressure regulation.

821 11β-Hydroxysteroid Dehydrogenase (11β HSD) Expression in the Rat Endolymphatic Sac

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11β-Hydroxysteroid dehydrogenase-type2 (11βHSD-2) is an enzyme that modulates corticosteroid action by converting the active form to the inactive form, and it protects aldosterone selective binding to the mineralocorticoid receptor (MR). Although the endolymphatic sac (ES) is considered to be an aldosterone target organ, and it has been suggested aldosterone regulates ES homeostasis and functions, 11βHSD-2 expression has not been identified in the ES. In the present study, 11βHSD-2 expression in the rat ES was indicated by RT-PCR and was found on immune-histochemistry to be mainly localized in the intermediate portion of the ES, a central area for ion and water absorption; this is also where MR is known to be localized. In the other parts of the ES, faint immune reacts were observed. This is the first report confirming the expression of 11βHSD-2 in the rat ES. Our results strongly suggest that ES is one of a mineralcorticoid target organ, aldosterone directly affects the ES through binding to MR by the action of 11βHSD-2, and aldosterone and 11βHSD-2 have strong relationships with endolymph absorption mechanisms.

822 Presence of the FXYD6 in the Endolymphatic Sac Epithelia

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Objective: The FXYD proteins are a family of seven homologous single transmembrane segment proteins, expressed in a tissue-specific fashion. The FXYD proteins modulate the function of Na^+, K^+–ATPase, thus adapting kinetic properties of active Na^+ and K^+ transport to the specific needs of different cells. It has been reported that the FXYD6 (phosphohippolin) also modulates Na^+, K^+–ATPase activity in the cochlea, especially in the stria vascularis. The mammalian endolymphatic sac is a part of the membranous labyrinth, and may play importanrt roles to maintain the endolymph regulation. And we have reported that the endolymphatic sac epithelial cells had high Na^+, K^+–ATPase activity. In this study, we examined the presence of the FXYD6 in the endolymphatic sac epithelia.

Methods:

RT-PCR SD rats were deeply anaesthetized by inhalation of diethyl ether and decapitated. The cochlea, the ES and the liver were harvested. Total RNA was extracted from frozen tissues using a High Pure RNA tissue and reverse transcribed into cDNA. cDNA was amplified by 30 cycles of PCR.

Immunohistochemistry Albino guinea pigs were deeply anesthetized by the inhalation of diethyl ether and used in this experiment. The endolymphatic sac epithelial was

fixed in 4% paraformaldehyde and treated with 0.1% Triton X-100. The endolymphatic sac epithelia was stained by immuno-fluorescence method with FXYD6 specific antibody.
Results: FXYD6 was expressed in the ES, in the cochlea, and the brain. The intermediate portion epithelial cells of the endolymphatic sac were stained with FXYD6 specific antibody.
Conclusions: The study demonstrated the presence of FXYD6 in the endolymphatic sac epithelial cells. FXYD6 may regulate endolymph via modulation of Na^+, K^+–ATPase activity in the endolymphatic sac epithelia.

823 Effect of Extracellular Lactate on Cochlear Microvessels Diameters Through the Regulation Of

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Cochlear blood flow is required to be tightly coupled to local metabolic needs. Our previous results showed that pericytes are present on cochlear blood vessels at a high density. These pericytes may play a key role in regulation of local cochlear blood flow (CBF). In this study, we focused on the effect of lactate because this metabolic product is in perilymph at high concentrations. In freshly isolated lateral wall microvessels from guinea pig, pericytes were visualized under differential interference contrast (DIC) microscope and capillary diameters were measured from time-lapse images. We found that extracellular lactate has a bidirectional control of cochlear vessels. Upon exposure to lactate, capillaries and venules of spiral ligament were constricted at the location of pericyte soma. In contrast, the diameters of pre-capillaries and spiral modiolar artery were dilated. These data suggest that extracellular lactate may serve as a local vasomediator for regulation of CBF perfusion to meet with metabolic needs and the mechanism of these responses is to be investigated.
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824 Effects of a Combination of Antioxidant Drugs on Noise-Induced Hearing Loss with Varied Durations and Delays of Treatment

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Acute acoustic trauma (AAT) results in oxidative stress occurring shortly after noise exposure and extending to 7-10 days after noise exposure through overproduction of cellular reactive oxygen, nitrogen, and other free radical species. This study tested the effects of a combination of hydroxylated alpha-phenyl-tert-butylnitrone (4-OHPBN) and N-acetyl-L-cysteine (NAC) and acetyl-L-carnitine (ALCAR) on noise-induced hearing loss when the duration and delay of treatment were varied. Duration of treatment was ranged from 3 to 10 days after noise exposure. Treatment delays were from 24 to 48 hours after noise exposure.
Thirty-six chinchilla (six per group) were exposed to a 105 dB octave-band noise centered at 4 kHz for 6 hours and received the following treatments: 1) no noise exposure group 2) noise-exposed control group 3-6) 4-OHPBN (20mg/kg) + NAC (50mg/kg) + ALCAR (20mg/kg) intraperitoneally injected beginning 24 or 48 hours after noise exposure twice daily for the next two, eight, or nine days. Auditory brainstem response (ABR) threshold shifts, outer hair cell (OHC) loss and organ of Corti immunohistochemistry were analyzed with ANOVA.
Administration of the combination of agents decreased permanent threshold shifts, inhibited OHC loss, and reduced 4-hydroxynonenal (4-HNE) staining. The amount of protection was influenced by the duration of treatment as well as the time delay before treatment. Significant decreases in the threshold shifts and reduction in OHC loss were observed with longer duration (9 days) treatment and shorter delay (24 hours) before starting treatment. These results demonstrate that the administration of antioxidant drugs extended up to 10 days after noise exposure can effectively treat AAT even when given 24 hours after noise exposure. This provides a significant and potentially clinically important increase in the effective therapeutic window for treatment of AAT.
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825 Effectiveness of Antioxidant Treatment on Acute Acoustic Trauma: Reduction of 4-Hydroxynonenal and Nitrotyrosine Production in the Cochleae of Chinchilla

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Noise-induced oxidative stress plays a major role in noise-induced hearing loss (NIHL). Oxidative stress leads to the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which may result in hair cell death in the inner ear. Consequently, antioxidants have been used to prevent or treat NIHL through systemic or local application in animal models. Recently, we have successfully used a combination of antioxidants (4-hydroxy phenyl *N-tert*-butylnitrone (4-OHPBN) + *N*-acetyl-L-cysteine (NAC) + Acetyl-L-carnitine (ALCAR)) to treat acute acoustic trauma (AAT). However, the mechanisms of these antioxidants in treating AAT have not yet been confirmed at the molecular level.
In the present study, chinchilla were divided into 3 groups (6 in each group): 1) control; 2) noise exposure only (105 dB SPL octave-band noise centered at 4 kHz for 6 hrs); 3) noise exposure plus antioxidant treatment (4-OHPBN+NAC+ALCAR, began 4 hours after noise exposure and lasted for 3 days). Auditory brain-stem responses (ABR) were recorded before and 10 days after

noise exposure. Cochleae were collected after 2nd ABR measurement. Immunohistochemical labeling for biomarkers: 4-hydorxynonenal (4-HNE), nitrotyrosine and malondialdehyde was carried out on cochlear sections to evaluate the effectiveness of antioxidant treatment in AAT. Antioxidant treatment significantly reduced hearing threshold shifts, as well as 4-HNE and nitrotyrosine formation in the organ of Corti and spiral ligament, respectively. However, no malondialdehyde expression was found in the cochleae of all three groups of chinchilla. These results suggest that 4-HNE might be a major target of antioxidant treatment on AAT. The present study provides direct evidence of the effectiveness of antioxidant treatment on AAT and underlying molecular mechanisms.
Supported by the Office of Naval Research and INTEGRIS Health.

826 Reduction in Permanent Noise-Induced Threshold Deficits in Mice Fed a Combination of Dietary Agents

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In this study, protection against permanent noise-induced threshold shifts (PTS) was evaluated in CBA/J mice treated with beta-carotene, vitamins C and E, and magnesium, administered via dietary supplementation beginning 28-days prior to noise insult (8-16 kHz OBN, 113-dB SPL, 2 hrs). Experimental subjects received treatment with the active agents via one of two custom commercial diets; control subjects were fed an otherwise identical, nutritionally complete, diet with no nutrient additives. Custom diets were formulated to approximate either 5X or 10X increases in doses of beta-carotene, vitamins C and E, and magnesium established as effective in previous studies using guinea pig subjects treated using a combination of oral and injected routes of delivery for the dietary micronutrients. Subjects on the 10X diet had significantly less PTS than control animals. Decreasing the amount of vitamin content in the enhanced diet reduced protection against PTS. Anatomical protection was examined for the mice receiving the 10X dose. The noise exposure resulted in little loss of either hair cells or neurons in either the control or the treatment groups, except within the deep cochlear base. Compelling evidence for protection of the inner ear microstructure was observed in preservation of the lateral wall. While CBA/J mice typically experience significant loss of Type I and II fibrocytes and strial basal cells after noise exposure, the loss of Type II fibrocytes was significantly less in the treated subjects relative to the controls. Strial cell density was also protected, although the reliability was less robust ($p<0.10$). Pre-noise oral treatment with this combination of antioxidants and magnesium protected a variety of cells in the inner ear, and effectively preserved threshold sensitivity. These data support the potential for translation of these agents to human populations given the well-established safety profile for high-level supplementation of these micronutrients, as shown in multiple long-term human studies.
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827 Prevention of Temporary Noise-Induced Threshold Deficits Using Dietary Agents

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Shared histopathological correlates between temporary noise-induced threshold shift (TTS) and permanent shifts (PTS) have led to the suggestion that preventing repeat TTS may prevent later PTS. If TTS and PTS are linked by common pathological mechanisms, then agents that reduce that trauma should reduce both TTS and PTS. To date, a variety of antioxidant agents have been successful in preventing PTS in rodent models. However, most of these agents fail to reduce TTS. In this study, the potential for prevention of TTS was evaluated in guinea pigs treated with beta-carotene, vitamins C and E (supplied as Trolox), and magnesium, beginning one day prior to moderate noise insult (4-kHz OBN, 110-dB SPL, 4 hrs). Experimental subjects were treated with the 4 active agents either once daily, or in two half doses to better maintain serum levels of active agents. Control animals received saline injections once/day. There were no statistically reliable differences between the treatment groups on any threshold measure; thus, all treated animals were combined into a single group. Subjects in the treated group had smaller threshold shifts than control animals immediately post-exposure as well as 24 hours later, and neural response amplitude was consistently greater in treated animals across time points. All subjects in all groups showed significant recovery; subjects treated with micronutrients had better hearing outcomes at the final test time. Consistent with functional recovery data, there were no significant hair cell losses in any subject group. Previously it has been shown that treatment with these agents significantly reduced outer hair cell death and PTS after traumatic noise (4-kHz OBN, 120-dB SPL, 5 hrs). These agents are known to be safe in humans, when administered at levels below established upper limits. Use of these agents could thus be readily translated to human populations, if they are found to be effective in reducing TTS and/or PTS in human clinical studies.
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828 Role of Coenzyme Q10 in Preserving Hearing in Noise-Exposed Mouse - Permanent Threshold Shift Model

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Background and Objectives : Exposure to loud noise can induce temporary or permanent hearing loss, and acoustic trauma is the major cause of hearing impairment in industrial nations. In addition to its involvement in respiratory chain of adenosine triphosphate production in mitochondria, coenzyme Q10 (CoQ10) has a role as antioxidant. We evaluated the role of CoQ10 in preserving hearing in mice exposed to noise that can induce permanent hearing loss.

Materials and Method : BALB/C mice with normal hearing were used. Mice fed with sesame oil in control group and with CoQ10 in experimental group before 2 and during 3 consecutive days of noise exposure. All mice were exposed to noise (120 dB SPL broad band white noise) for 3 hours daily for 3 consecutive days. The hearing level was determined by auditory brainstem response measurement at 1, 3, 7, 14 days after noise exposure. Cochleas from each groups were obtained in order to obtained hair cell survival rates and evaluated the effect of CoQ10 for cell apoptosis.

Results: Experimental group had a more preserved hearing threshold than control group. Histological and TUNEL staining of the cochlea showed significantly enhanced preservation of the organ of Corti, including outer hair cells and relatively low apoptotic nuclei, in the experimental group than in the control group.

Conclusion : CoQ10 has an anti-apoptotic effect on cochlea exposed to noise.

829 Protection Against Noise Induced Hearing Loss by the Administration of Ferulic Acid in the Guinea Pig

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Ferulic Acid (FA) is known for its antioxidant properties and its free-radical scavenging activity towards peroxynitrite, hydroxyl radicals and oxidized low-density proteins. FA has been studied as a neuroprotector in neurodegenerative disorders and cerebral ischemia injury. In this study, we tested the hypothesis that FA might exert, by virtue of its antioxidant properties, beneficial protective effects against noise-induced hearing loss (NIHL). Loud noise induces hearing impairment by increasing the production of reactive oxygen species (ROS) and toxic free radicals which, in turn through a series of reactions, induce lipid peroxidation and cell damage in the organ of Corti. We evaluated the protective effect of FA against NIHL in guinea pigs (n=22). Noise-induced hearing loss was induced by exposing animals to a continuous pure tone of 6kHz, 120dB for 1 h; FA was injected intraperitoneally 1 h before acoustic trauma and once daily for 3 days. Auditory function was investigated by recording auditory brainstem responses (ABR) at 2-20 kHz; morphological studies were performed with scanning electron microscopy (SEM) and immunohistochemistry was performed for identification of missing and apoptotic cells and free radical activity. Guinea pigs in the FA group showed significantly smaller auditory threshold shifts than unprotected control animals, indicative of a lesser extent of both apoptotic activation and hair cell loss in the organ of Corti. Our preliminary data confirm the antioxidant properties of FA and show its protective function in NIHL. This might be because FA inhibits the process of lipid peroxidation as documented by immunohistochemistry. Our results suggest a therapeutic potential of FA in NIHL through its antioxidant property.

830 The Ingestion of Heat-Processed Red Ginseng Facilitates the Fast Recovery of Mice from Noise-Induced Temporary Hearing Threshold Shifts

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Introduction: It is well known the saponin of Korean red ginseng (KRG) had a anti-oxidant effect and could suppress the lipid peroxidation. The aim of the present study was to observe the inhibitory effect of KRG in mice with noise induced hearing loss and to determine the optimal dose.

Materiasl and methods: We used Balb/C mouse with normal hearing level and normal Preyer's reflex. We exposed the mice to noise (110 dBSPL, white noise band) for 3 hours in noise booth. Mice were grouped as experimental where mice were administerd heat-processed red ginseng powder (50mg/kg, 100mg/kg, 200mg/kg) and control where mice were administered with normal saline alone before noise exposure. Hearing level was measured by auditory brainstem response (ABR) at baseline, after administration of red ginseng and 3rd 5th, 7th, 2weeks, 3weeks, 4weeks after noise exposure under anesthesia by IM injection of ketamine hydrochloride (30mg/kg) and xylazine (2mg/kg). After the experiment, cochleae were removed from mouse and immunochemical staining was performed to observe the expression of 8-oxo-G in the cochlea.

Results: Hearing was recovered after noise exposure in all groups. The early recovery was observed in mice with experimental groups ingested with 100 mg/kg and 200 mg/kg of KRG. The expression of 8-oxo-G was observed in stria vascularis of control group, but no positive staining was observed in experimental group with 100 mg/kg and 200 mg/kg of KRG. Mice ingested with KRG of 50 mg/kg had no difference from control mice.

Conclusions: From these results, we could conclude that high concentration of KRG has a protective effect on the noise-induced threthold shift. Because KRG has been reported as safe compound even up to hundreds mg/kg, higher concentration of KRG will be effective on the protection of noise induced hearing loss.

831 Long-Term Administration of Magnesium After Acoustic Trauma Caused by Gunshot Noise in Guinea Pigs

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In a previous study we observed that a 7-day post-trauma magnesium treatment significantly reduced auditory threshold shifts measured seven days after gunshot noise exposure. However this improvement was only temporary, suggesting that it could be potentially beneficial to prolong this treatment. The aim of the present study was to evaluate the efficacy of a long-term (one month) magnesium treatment after an impulse noise trauma, in comparison with either a 7-day magnesium treatment, an administration of methylprednisolone (conventional treatment), or a placebo (NaCl). Guinea pigs were exposed to impulse noise (three blank gunshots, 170 dB SPL peak). They received one of the 4 treatments, one hour after the noise exposure. Auditory function was explored by recording the auditory brainstem response (ABR) and measuring the distortion product otoacoustic emissions (DPOAE) over a 3-month recovery period after the gunshot exposure. The functional hearing study was supplemented by a histological analysis. The results showed that a 1-month treatment with magnesium was the most effective treatment in terms of hair cell preservation. The DPOAE confirmed this effectiveness. Methylprednisolone accelerated recovery but its final efficacy remained moderate. It is probable that magnesium acts on the later metabolic processes that occur after noise exposure. Multiple mechanisms could be involved: calcium antagonism, anti-ischaemic effect or NMDA channel blockage. Regardless of the specific mechanism, a 1-month treatment with magnesium clearly attenuates NIHL, and presents the advantage of being safe for use in humans.

832 Differences Between Acute and Chronic Stress in the Auditory System

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It is well established that stress affects the auditory system. Many different acute stressors have been previously studied, such as acute restraint stress and sound conditioning. These stressors provide physiological and morphological protection against acoustic trauma by activating the hypothalamic pituitary adrenal (HPA) axis and subsequently glucocorticoid receptors (GR). However, there is at present little known about the effects of chronic stress on the auditory system. We have previously demonstrated that chronic stress does not protect against acoustic trauma. Our hypothesis is that differences in protection against acoustic trauma between acute and chronic stress is due to changes in the HPA axis. In order to test this hypothesis CBA mice were subjected to either chronic stress (4 hours of restraint stress for five consecutive days) or acute stress (4 hours of restraint stress for one day). Control mice were kept individually in ordinary cages during these 4-hour periods. All experiments were performed during the same time of the day to minimize circadian rhythm influences. Changes in GR was assessed in the spiral ganglion of the cochlea by immunohistochemical methods. Computer assisted stereology was used in the paraventricular nucleus of the hypothalamus. Nuclear translocation of GR in the cochlea, inferior colliculus and cochlear nucleus was assessed by western blot. Preliminary findings indicate differences in both total GR expression and nuclear translocation between acute and chronic stress treated animals. The implication of these findings and the differences between acute and chronic stress in the auditory system will be discussed.

833 Activation of Nuclear Factor Kappa B Pathway by Dexamethasone is Required for the Prevention of Tumor Necrosis Factor-Alpha Induced Apoptosis of Rat Auditory Hair Cells

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Background: At high doses, tumor necrosis factor-alpha (TNFα), a cytokine released following inner ear trauma, can initiate apoptosis of auditory hair cells (HC) *in vitro*. Dexamethasone base (DXMb) protects against TNFα ototoxicity by altering the pattern of gene expression of pro- and anti-apoptotic factors. Method: 168 organ of Corti explants (OC) were dissected from P-3 rat pups. Of these, 60 OC were cultured in either: (1) No treatment; (2) TNFα (2μg/ml); (3) TNFα (2μg/ml) + DXMb (70μg/ml); (4) TNFα (2μg/ml) + DXMb (70μg/ml) + NFκB Inhibitor (NFκBI); or (5) TNFα (2μg/ml) + DXMb (70μg/ml) + NFκB Control (NFκBC) for 96 hrs for hair cell (HC) studies. The remaining 108 OC were cultured in conditions 1 – 4 for 0, 24, and 48 hrs for gene expression studies. After *mRNA* isolation, two-step real-time PCR was performed using primers for *B-actin*, *Bax*, *Bcl-2*, *Bcl-xl*, and *TNFR1*. Mean fold changes were calculated using the $2^{-\Delta\Delta Ct}$ method. Results: TNFα exposed OC showed significant increases in gene expressions of *Bax* and *TNFR1* as well as the *Bax/Bcl-2* ratio at 24 and 48 hrs and also decreases in *Bcl-2* and *Bcl-xl* gene expressions at 48 hrs. In TNFα+DXMb treated OC, there were significant increases ($p<.05$) in *Bcl-2* and *Bcl-xl* and significant decreases ($p<.05$) in *Bax* and *TNFR1* gene expressions as well as the *Bax/Bcl-2* ratio. OC treated with TNFα+DXMb+NFκBI showed results paralleling findings from TNFα-only exposed explants, suggesting that downstream activation of the NFκB pathway by DXMb is an essential event for mediation of DXMb otoprotection. Gene expression results were confirmed by the results of the HC counts. Conclusion: DXMb protects HCs against TNFα ototoxicity by up regulation of anti-apoptotic *Bcl-2* and *Bcl-xl* gene expression in the explants through activation of the NFκB pathway. These findings support the use of dexamethasone in the treatment of trauma-induced inner ear diseases.

834 Dexamethasone Treatment Alters the Pattern of Apoptosis-Related Gene Expression in Unexposed, P-3 Organ of Corti Explants Compared to Untreated-Control Explants

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Background: Tumor necrosis factor alpha (TNFα) is a trauma-related cytokine that can initiate apoptosis in rat auditory hair cells. Recent studies have demonstrated that treatment of organ of Corti explants (OC) with dexamethasone base (DXMb) protects against TNFα ototoxicity.

Hypothesis: DXMb treatment modulates the pattern of expression of both pro- and anti-apoptotic factor genes in the absence of a challenge with TNFα.

Methods: 84 OC were dissected from P-3 rats. 24 OC were cultured in either: 1) Untreated-Control; or 2) Unexposed, DXMb (70 µg/ml) treated for 4 days and stained with FITC-phalloidin for hair cell (HC) counts. 60 OCs cultured under the above 2 conditions for 0, 24 and 48 hrs. were used for the gene expression studies. Two-step real-time RT-PCR was performed with the following primers: *β-actin* (housekeeping gene), *Bax*, *Bcl-xl, Bcl-2* and *TNFR1*. Mean fold changes in gene expression were determined with the $2^{-\Delta\Delta Ct}$ method. There were no significant differences (p>.05) in HC counts between these 2 culture conditions.

Results: Pro-apoptotic *Bax* expression was significantly decreased in DXMb treated control explants when compared to untreated-controls at 24 (p=.001) and 48 hrs. (p=.026). Receptor *TNFR1* gene expression was reduced in DXMb treated explants at 24 (p=.002) and 48 hrs. (p=.005). In DXMb treated cultures, both anti-apoptotic *Bcl-2* and *Bcl-xl* gene expression levels were greater than in controls at 48 hrs. (p<.001 and p<.001, respectively). Unexposed, DXMb treated OC also demonstrated a significant decrease in the *Bax/Bcl-2* ratio at 24 (p=.044) and 48 hrs. (p<.001).

Conclusion: DXMb pretreatment causes up regulation of anti-apoptotic *Bcl-2* and *Bcl-xl* gene expression while inhibiting pro-apoptotic *Bax* and receptor *TNFR1* gene expression in unexposed explants. These results suggest that pre-treating with DXMb prior to inner ear surgery (e.g. cochlear implant surgery) may provide protection against trauma-induced hearing loss.

835 Corticotrophin-Releasing Hormone Sets Auditory Thresholds and Protects Against Noise-Induced Hearing Loss

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Corticotrophin releasing factor (CRF) signaling typically coordinates response to stress via the hypothamalic-pituitary-adrenal axis. We have found that CRF is also expressed in the cochlea, along with its two receptors, CRF1 and CRF2. To date, the role of the cochlear CRF system was unknown. In order to understand its role in the cochlea, we examined the physiological and biochemical state of mice lacking CRF2. Our results demonstrate that cochlear CRF sets acoustic sensitivity, since CRF2 null mice exhibit a basal hyperacusis, and prevents noise-induced damage, since the null mice have a greater susceptibility to noise-induced trauma. To investigate whether the hyperacusis stems from altered K+ secretion into the endolymph, we examined expression levels of proteins regulating potassium secretion. KCNJ10 levels were not statistically different in the null mice despite a trend toward downregulation. Paradoxically, KCNQ1 levels are reduced by 40-50% in CRF2 null mice, suggesting lower endolymph K+ levels and a less sensitive system, contrary to our physiology data. To reconcile these conflicting results, we turned our focus from the mechanisms pumping K+ into the endolymph to the mechanisms that remove it. ATP is released from support cell hemichannels that open in response to mechanical stimulation. This release mechanism is disrupted in CRF2 null mice. Expression of the connexin hemichannels Cx26 and Cx30 is reduced by approximately 50%. ATP release reduces the endocochlear potential via P2Y4-mediated inhibition of K+ secretion through KCNQ1/ISK and via P2X2 mediated shunting of K+ from the endolymphatic compartment. Thus, insufficient release of ATP would lead to an accumulation of K+ in the endolymph. We therefore propose a model in which CRF2 signaling regulates connexin expression to control ATP release during stress, resulting in decreased acoustic sensitivity during intense stimulation, preventing noise-induced damage. Supported by DC006258.

836 An Unexpected Mechanism Offers Protection from Acoustic Trauma in Old Mice Lacking the Nicotinic Acetylcholine Receptor Subunit β2

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Acoustic trauma such as noise-induced hearing loss (NIHL) affects a large population but currently, there is no effective medication. The olivocochlear efferent system is implied in cochlear protection from NIHL. One particular synaptic connection in this system, between the lateral olivocochlear terminals and the afferent fibers of spiral ganglion neurons, is via nicotinic acetylcholine receptors (nAChRs) containing the β2 subunit. Unexpectedly, old β2-/- mice are dramatically protected from NIHL; however, the same protection is not observed in young β2-/- mice or after pharmacological modulations of cholinergic transmissions. This enigmatic finding is resolved after further studies clearly demonstrate that the protection from acoustic trauma offered in old β2-/- mice is due to age-related increases of corticosterone instead of disruption of the lateral olivocochlear efferent cholinergic transmissions. These results provide clues for pharmacological treatment of neural trauma such as NIHL.

837 The Transcription Factor PLZF, Present in Hair Cells and Spiral Ganglion Cells, is Upregulated with Stress and is a Candidate to Mediate Sound Conditioning-Related Protection Against Acoustic Trauma

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It has long been known that stress can provide protection against acoustic trauma, an action suggested to be mediated by corticosteroids (Wang and Liberman, 2002; Tahera et al, 2007). PLZF (Promyelocytic leukemia zinc finger protein), a transcriptional repressor (Chang et al, 1996) seen in the inner ear during development (Avantaggiato et al 1995), can be induced by steroids (Fahnenstich et al 2003). PLZF was recently identified in a yeast two hybrid screen as a prestin interaction partner localized to the cytoplasm of hair cells (Nagy et al, Hear Res 2005). We have re-examined the cochlear localization of PLZF after optimizing immunohistochemical procedures to label PLZF in nuclei. In addition to the cytoplasmic localization of PLZF in the organ of Corti reported by Nagy (2005), we also found PLZF in the nuclei of spiral ganglion cells. We looked for effects of noise stimulation on the cochlear expression of PLZF with real time quantitative PCR. Exposure to an 88 dB SPL broadband noise for 2 h increased levels of PLZF mRNA 6-7 fold, though the effect was not noise-dose responsive for exposures at levels of up to 102 dB SPL. Moreover, identical handling and placement of the mouse in the noise exposure chamber, but without exposure, also increased PLZF 6-7 fold. PLZF levels increased maximally at 2 h and recovered over 48. We suggest that stress increased levels of PLZF in the inner ear. The presence of PLZF in key cochlear locations (hair cells and spiral ganglion cells) and its potential induction by corticosteroids makes it a reasonable candidate to mediate stress-related gene expression with sound conditioning.

838 Blast-Induced Tinnitus and Hearing Loss in Rats: Behavioral Assay

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Tinnitus is a distressing symptom that has been estimated to afflict 9 million Americans and is often associated with hearing loss. Returning soldiers from Iraq and Afghanistan have experienced even higher prevalence of tinnitus and hearing loss. Recent study shows that 49 percent of all soldiers exposed to blasts in Iraq and Afghanistan had tinnitus, and 60 percent had hearing loss (Delaney et al. 2007). In this study, we investigated the effects of blast, a high-energy impulse noise, on tinnitus and hearing loss in rats. Tinnitus and hearing loss were evaluated using Gap detection testing (GAP) and prepulse inhibition (PPI) startle reflex paradigm. Nine adult Long Evans rats were used in this study. Before blast treatment, each animal underwent GAP and PPI testing to collect internal control data. Following anesthesia, each animal was placed in a shock tube and subjected to a single 14 PSI, 194 dB SPL blast with pulse duration of approximately 8 ms. Following blast treatment, GAP and PPI testing was performed again on post-blast day 1, 14, and 28. MRI diffusion tensor imaging was also conducted on these animals to detect evidence of traumatic brain injury resulting from blast (see presentation by Pace et al.). Our results showed that rats exhibited tinnitus and hearing loss one day after blast. The tinnitus was of a lower frequency range of 4 kHz to 12 kHz, and hearing loss was in the higher frequency range of 16 kHz to 30 kHz including broadband noise. However, behavioral evidence for both tinnitus and hearing loss were recovered by post-blast day 14, and further stabilized by post-blast day 28. Our results indicated that a single exposure to the blast wave did not induce the sustained tinnitus and persistent hearing loss in rats. Further studies are needed to investigate the effects of high dose blasts by increasing intensity, duration, and frequency on tinnitus and hearing loss.

839 Salicylate and Noise-Induced Tinnitus Findings in Rats Using the Acoustic Startle Reflex

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Sodium Salicylate has been shown to reliably induce short-term tinnitus when administered at high doses. Noise trauma has been instead reported to induce long-term tinnitus. The present study compared salicylate to noise induced tinnitus-like behavior in rats using the novel gap pre-pulse inhibition of acoustic startle (GPIAS). 24 rats were divided into 2 groups (n=12); one group was injected intraperitoneally with salicylate (250 mg/kg), the other was exposed unilaterally to a 12kHz, 126dB narrow-band noise (BW=100Hz) for 15 minutes. All rats were subsequentially tested for tinnitus and hearing loss at 6, 12, 16, 20 and 24 kHz; tinnitus was assessed using GPIAS, hearing function was measured with DPOAE, ABR and noise-burst pre-pulse inhibition of acoustic startle (NBPIAS). The results revealed transient tinnitus-like behavior in all rats following salicylate injection, starting 2h after treatment and resolving spontaneously 24h later; rats exposed to noise developed a transient tinnitus during the first week and stable long-term tinnitus with a pitch near 16kHz starting 21 days after exposure. Hearing function was tested in all animals: salicylate caused an average temporary threshold shift of less than 10 dB and essentially no permanent shift; animals in the noise-trauma group showed a temporary and permanent threshold shift at high frequencies (+/- 20dB,12-24Khz). The present study confirms the validity of the GPIAS protocol to rapidly assess tinnitus in rats with

no training, learning or food deprivation necessary and the ability to identify the pitch of tinnitus. Data obtained on salicylate and noise-induced tinnitus are consistent with those available in literature and contribute to clarify tinnitus induction and assessment in the animal model with potential applications in future tinnitus research.

840 Effects of the BK Agonists BMS-204352 and the Enantiomeric Compound ("*R*-Enantiomer") on Transient, Salicylate Induced Tinnitus in Rats

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The physiological mechanisms that give rise to tinnitus are largely unknown; however, the general consensus is that tinnitus results from spontaneous hyperactivity in the central or peripheral auditory system. Since potassium ion (K^+) channels play an important role in regulating the resting membrane potential, compounds that can modulate the flux of K^+ through specific K+ channels could be attractive tools for suppressing the spontaneous hyperactivity underlying the symptoms of tinnitus. To address this hypothesis, we evaluated two compounds that modulate K^+ channels. KCNQ and BK, a voltage- and intracellular Ca^{++}-gated K^+ channel, regulate cellular excitability by efflux of K^+ from cells thereby shifting the membrane potential to a more hyperpolarized and less excitable state. We looked at the effects of the compounds BMS-204352, a BK and KCNQ4/KCNQ5 channel activator and KCNQ1 inhibitor, and the *R*-enantiomer of BMS-204352 ("*R*-enantiomer"), a BK activator and pan-KCNQ inhibitor, on transient salicylate-induced tinnitus. Two behavioral paradigms, Schedule Induced Polydipsia Avoidance Conditioning (SIPAC) and Gap Prepulse Inhibition of the Acoustic Startle (GPIAS) were used to assess the presence of tinnitus. Treatment with BMS-204352 suppressed salicylate induced tinnitus in a dose dependent manner using the SIPAC; tinnitus-like behavior was completely abolished with 10 mg/kg of BMS-204352. The *R*-enantiomer significantly reduced the presence of tinnitus using SIPAC. Both BMS-204352 and the *R*-enantiomer reduced salicylate induced tinnitus-like GPIAS behavior in some animals. In preliminary experiments on noise induced tinnitus, BMS-204352 and the *R*-enantiomer partially reduced evidence of tinnitus-like behavior in some animals. These results suggest that K^+ channel modulators may be effective in reducing certain types of tinnitus. Research supported in part by NIH (R01DC00909101; 1R01DC009219-01) and Tinnitus Research Initiative.

841 Hair Cell Damage and Tinnitus After Noise- Induced Hearing Loss: An Animal Model for Therapeutic Intervention

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Noise- induced hearing loss (NIHL) may often be correlated to auditory phantom perceptions, i.e. tinnitus. In a patient, masking phenomena and subjective tinnitus loudness allow only uncertain estimates of the objective hearing disorder and impairment by the phantom percept.
A behavioral rat model offers the alternative of a defined induction of hearing loss and examination of tinnitus formation, validated by the examination of the expression of proteins indicating the viability of cochlear hair cells.
Our aim was to examine the correlation of NIHL, the occurrence of tinnitus and the topology of hair cell damage within the distinct cochlear turns. Animals were exposed to various sound pressure levels for 1-2 h. Hearing loss and altered OHCs phenotype were examined using immunostaining of KCNQ4 as marker protein. Although exposed to identical sound paradigms, only some animals developed tinnitus. They also showed differences in protein expression and a more pronounced hearing loss. Pharmacological substances will be tested for new therapeutical approaches and a better understanding of hearing loss and tinnitus origin.
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842 Tinnitus and Hyperacusis in Ears with Normal Hearing Thresholds: Towards a Diagnostic Evaluation Scheme Involving Measurements of Minor Cochlear Lesions

Withdrawn

843 Influence of PDE-Inhibitors on Noise and Salicylate Induced Hearing Loss

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The NO–cGMP–cGK pathway plays an important role in regulating vascular tone and cell destiny (see for review: Takumida & Anniko et al, 2002). NO is released from nerve endings and endothelial cells and stimulates the activity of soluble guanylate cyclase (sGC), leading to an increase in cyclic guanosine-3', 5'-monophosphate (cGMP) and activation of cGMP-dependent proteinkinases (cGKs). Cytosolic cGMP is catabolized by specific phosphodiesterases (PDE's).
The role of the NO / cGMP pathway in inner ear function and response to trauma (acoustic over stimulation / noise trauma) is still unclear. Questioning if NO via cytosolic cGMP levels protects or detoriates cell damage after trauma, we studied the vulnerability of rats to acoustic over stimulation or salicylate treatment in the presence or

absence of PDE-inhibitors. We furthermore analyzed PDE isoforms in the cochlea and isolated hair cells with RT-PCR.
The hearing function was investigated by measuring auditory brainstem response (ABR) and distortion products of the otoacoustic emissions (DPOAEs) before, 0-7d after and 21d after acoustic over stimulation (noise trauma 4-16 kHz band noise, 120 dB SPL, 1h).
Data are discussed in the context of a role of NO-cGMP-signaling cascades and presumptive novel therapeutic strategies.
Supported by Hahn Stiftung (Index AG) and Bayer-AG

844 Contribution of Across-Frequency Input from Auditory Nerve Fibers to the Octopus Cell Response Pattern: Implications of a Computational Model

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The octopus cells in the posteroventral cochlear nucleus respond to high-frequency tones with an onset response pattern that exhibits a well timed initial response followed by a low level of random activity. In contrast, for low – frequency tones, they can exhibit a sustained response, discharging at every cycle. Recent experimental studies have explored the effect of intrinsic membrane properties on the response patters of octopus cells (Bal R and Oertel D 2000, 2001), but little is known about how the synaptic patterns shape the response. In the present study we use computer simulation to study the contribution of across-frequency input from auditory nerve fibers to the octopus cell response to a variety of acoustic stimuli.
Our octopus cell model is based on the wildly used, conductance-based, Hodgkin Huxley model. Four ion channels are simulated in our model: fast sodium channels, low threshold potassium channels, high threshold potassium channels, and hyperpolarization-activated potassium channels. The parameters for the potassium channels were estimated using electrophysiological data from Bal and Oertel (2001). Other model parameters were adjusted to match experimental data that used intracellular current steps as stimuli (Oertel D et al 2000) .
A peripheral model developed for the EarLab Project (http://earlab.bu.edu) was then used to provide auditory nerve fiber input with a specified frequency range to the octopus cell model. The results from changing the frequency range of the fibers that project to the octopus cells and from changing their synaptic strength on response areas of octopus cells will be presented.
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Research Center

845 Spatio-Temporal Processing of Auditory-Nerve Activity in the Cochlear Nucleus

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The spatio-temporal pattern of activity across the tonotopic array of auditory nerve (AN) fibers encodes spectro-temporal information for acoustic stimuli. To evaluate whether cochlear nucleus (CN) neurons are sensitive to manipulations of the spatio-temporal pattern of AN activity, we recorded from single AN fibers and CN units in anesthetized cats and compared these responses to those of a peripheral auditory model (Zilaney & Bruce, J Acoust Soc Am 120:1446). We used Huffman sequences, which have a flat magnitude spectrum and a 2π phase transition around a frequency FT, to manipulate the relative timing between neighboring AN fibers (Carney J Neurophys 64:437).
Responses of a single AN fiber to a set of Huffman stimuli with varying FT resembled the responses of an array of model fibers with different CFs to a fixed FT stimulus. Thus we could infer the spatio-temporal pattern of AN activity from the response of a single fiber. Changing the steepness of the phase transition produced systematic changes in the spatio-temporal pattern of discharge. A broad phase transition excited fibers more coincidentally across CF at the response onset, while a sharp phase transition led to later coincident firings.
When FT was equal to the CF, AN fibers with low CF (<2 kHz) tended to fire at 1/CF intervals. In comparison, responses of CN units had fewer peaks and were shorter in duration. In particular, primary-like-with-notch responders typically showed one peak for broad phase transition stimuli and two peaks for stimuli with sharp transitions. The extra later peak for stimuli that excite AN fibers more coincidentally later in their responses is consistent with the notion that these CN neurons act as cross-frequency coincidence detectors. Overall, our results suggest that the firing patterns of some CN neurons reflect processing of the spatio-temporal patterns of AN activity.
Supported by an NDSEG fellowship and NIH grants RO1 DC002258 and P30 DC005209.

846 Lateral Inhibition in the DCN Molecular Layer

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Parallel fibers distribute somatosensory information throughout the molecular layer of the dorsal cochlear nucleus (DCN), synapsing onto the apical dendrites of fusiform cells, a major output neuron of the DCN, and onto the dendrites of cartwheel cells, the major inhibitory interneurons in the molecular layer. Cartwheel cells, in turn, possess local axons that synapse onto neighboring fusiform cells and other cartwheel cells. This pattern of connectivity suggests two possibilities for how parallel fibers might drive cartwheel cells to interact with

neighboring cells. If a given cartwheel cell and its neighbors receive largely overlapping parallel fiber input, then these cells will be excited synchronously, allowing the cartwheel cell to provide feedforward inhibition to its target neurons. Alternatively, if a cartwheel cell receives parallel fiber input that is substantially different from that which neighboring cells receive, then the cartwheel cell will provide lateral inhibition to its target neurons. We are using a functional approach to distinguish between these possibilities. Dual whole-cell recordings were made from pairs of nearby cartwheel cells and from pairs of nearby cartwheel and fusiform cells in acute brainstem slices. To examine EPSCs arising from single parallel fibers, we applied the K^+ channel blocker 4-AP to increase spontaneous firing. Recordings of spontaneous, unitary EPSCs revealed that pairs of cells shared few parallel fiber inputs. This was the case regardless of whether or not the pair of cells was synaptically coupled. In contrast, recordings of spontaneous IPSCs revealed that most pairs receive common inhibitory inputs. These data suggest that cartwheel cells provide lateral inhibition to neighboring cartwheel cells and fusiform cells.

847 Spectro-Temporal Receptive Fields of Dorsal Cochlear Nucleus Units in Awake Gerbils

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The dorsal cochlear nucleus (DCN) is the first-stage signal processing unit in auditory pathway. The DCN, which has a cerebellum-like circuit, produces complex responses to stimuli. Although DCN responses to pure tones and broadband noise have been well studied, responses to other types of stimuli are not well understood because the DCN is nonlinear. In this study, we investigate the spectro-temporal receptive fields (SRTFs) of DCN units in the awake gerbil using two wide-spectrum stimuli: dynamic ripple noise (DMR) and ripple noise (RN). The stimuli were chosen because they are rich in spectrum information. Moreover, the DMR consists of chirp-like temporal features that exist in gerbil pup's calls. The SRTFs were grouped according to the unit response maps (RMs). SRTFs were recorded from 28 DCN units. We found that the SRTF of type I/III and type II units had an excitatory area tuned to the best frequency (BF). The SRTF of some type III and type III-i units showed an excitatory area with side-band inhibition. The side-band inhibition could be at either or both sides of the BF. Because type IV-i units were inhibited by the given stimuli, the inhibitory and excitatory areas of the SRTFs appeared randomly distributed. SRTFs derived from DMR and RN appeared somewhat different in type III and type III-i units.

848 Single Neuron Recording in the Dorsal Cochlear Nucleus of Awake Gerbil

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Most dorsal cochlear nucleus (DCN) units to date have been recorded from either anesthetized or decerebrate preparations instead of awake preparations because of technical difficulties. Anesthesia, however, suppresses neural activity and decerebration cuts off feedback pathways from rostal auditory and possibly somatosensory centers to the DCN. Combining techniques of survival surgery, training, and restraining permitted us to record from neurons in awake gerbils. We investigated response maps (RMs), peri-stimulus time histograms (PSTHs) and responses to notch noise stimuli. Some aspects of the units' responses were compared to those from previous experiments in anesthetized and decerebrate gerbil preparations. Of 102 units, we found all of the RM types that have been observed in decerebrate gerbil, except type IV units. Type III units were still the most common recorded unit in the gerbil DCN. No significant changes were observed in the shapes of the RMs or in their responses to notch noise. We found, however, that the spontaneous activities of the units in the awake gerbil were lower compared with those in decerebrate gerbil. We speculate that DCN neurons are more inhibited in awake gerbils compared to those in decerebrate gerbils. [Work supported by Boston University's Hearing Research Center and Biomedical Engineering Department.]

849 Testing the Cochlear Nucleus Wideband Inhibitor Model of Comodulation Masking Release

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When trying to detect a signal in noise it has traditionally been assumed that behavioural performance is governed by the output of the single auditory filter giving the highest signal to noise ratio. This auditory filter concept has accounted for a vast amount of psychophysical data and has received support from physiological findings on frequency selectivity. However, it is now clear that the auditory system is able to make use of information from regions very disparate from the signal of interest. Here we examine one of these across-frequency processes, comodulation masking release. Comodulation masking release depends on coherent amplitude fluctuations across a wide region of auditory filters and enables the detection of an otherwise masked signal. While lateral suppression on the basilar membrane may account for some aspects of CMR it cannot account for all of them. We have produced a computational circuit that shows how lateral inhibition may explain the responses of single units in the ventral cochlear nucleus to stimuli that produce a comodulation masking release. This circuit receives input from a revised auditory nerve fibre model incorporating improved low-frequency characteristics which more accurately reflect single unit tuning and thresholds, as observed in the guinea pig. A simple two-cell circuit consisting of a wideband inhibitor (hypothesized to be an onset-chopper unit) and a narrowband multipolar cell (hypothesized to be a chopper unit) can replicate the CMR observed physiologically in the guinea pig. These models were, however, restricted to the use of deterministic maskers. Here we also explore the magnitude of the CMR using the same circuit with a variety of more complex, stochastic maskers.

850 Gap Coding of Fusiform Cells in the Dorsal Cochlear Nucleus

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Temporal features of sounds found in speech and other species-specific vocalizations include abrupt starts and stops which can be approximated by varying gaps (silent periods) embedded in ongoing signals. The present study examined how gaps were coded in extracellular recordings from output neurons (fusiform cells) in the dorsal cochlear nucleus (DCN) of rats. Recordings from 20 putative fusiform cells showed distinct differences in gap coding when gaps (1-50msec.) were embedded in pure-tone frequencies (60dB SPL) *at* (nearest), *above* or *below* the characteristic frequency (CF) of the cell being studied. Response to gaps could be classified into three basic response types or combinations of these types: 1, Tonic responses to the background tone which cease firing during the gap and resume firing when the signal resumes (classic response); 2, Suppressed (below spontaneous) or low level discharge rates with short firing bursts signaling the beginning and end of the gap (field-goal response); 3, Suppressed (below spontaneous) or low level discharge rate with increased firing within the gap (dysinhibited response). Responses from all cells were collapsed into difference plots for each gap duration. “Classic” responses to longer gap durations were observed *near* or *below* CF while “field-goal” responses were observed when gaps were embedded in frequencies *above* CF. Gap threshold were 1msec for all conditions, meaning that gaps were discernable in the 20 fusiform cell collapsed plots for all conditions tested.

These results suggest fusiform cells code gaps differentially depending upon the location of the background sound(s) within the frequency response area (FRA). Gap responses of signals with spectral energy near the upper edges of fusiform cell FRAs maybe shaped by complex excitatory to inhibitory transitions found for these neurons.

851 Dorsal Cochlear Nucleus Integrates Spinal Trigeminal Nucleus Inputs with Responses to Tone

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The dorsal cochlear nucleus (DCN) integrates acoustic and somatosensory input. In the guinea pig, the fusiform cells, principal output neurons of the DCN, receive somatosensory input indirectly via granule cells from the trigeminal ganglion (Shore et al., J. Comp. Neurol., 2000) and spinal trigeminal nucleus (Sp5; Zhou et al., J. Neurosci. Res., 2004). We investigated the effects of Sp5 stimulation on the firing rate and spike timing of sound-evoked responses in DCN units. The activity of DCN units was recorded in response to best frequency (BF) tones preceded by Sp5 stimulation and BF tones alone. Current passed through an electrode placed stereotaxically into the Sp5 activated neurons projecting to the CN. We measured changes in the firing rate (mean percent change = 30.4%, n=49), the coefficient of variation (CV, mean percent change = 10.9%, n=35), and first spike latency of responses to BF tones when preceded by Sp5 stimulation. An equal number of units show enhancement and supression of sound-evoked firing rate with Sp5 stimulation. Unit responses to Sp5 stimulation alone are typically excitatory or sub-threshold. These changes in spike rate and timing by Sp5 inputs have implications for mechanisms underlying multisensory integration in the CN and may play a role in the modulation of tinnitus by maneuvers of the jaw and other facial regions (Pinchoff et.al., Am. J. Otol.,1998; Biesinger et al., HNO, 2008).

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852 Influence of Dorsal Column and Spinal Trigeminal Nucleus Stimulation on Dorsal Cochlear Nucleus Responses

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Several lines of evidence suggest extensive crossmodal interactions between the somatosensory and auditory systems. In normal people, auditory sensations can be evoked by contractions of the jaw, head, and neck muscles. In patients these and other manipulations can modulate the pitch and loudness of their tinnitus (Pinchoff et.al., 1998; Sanchez et al., 2002; Biesinger et al., 2008). In addition, somatic disorders of the head and upper neck, such as whiplash, TMJD and dental trauma, are often associated with tinnitus. Somatosensory information from these sites is transmitted to the dorsal column nuclei (cuneate and gracilis) and spinal trigeminal brainstem nucleus (SP5). Dorsal column nuclei receive input from the trunk, limbs, neck, back of the head, whereas SP5 receives input from the face, vocal tract, intra oral structures. Both dorsal column nuclei and SP5 project to the cochlear nucleus (Wright and Ryugo, 1996; Zhou & Shore, 2004; Haenggeli et al., 2005). Here, we compare the effects of cuneate nucleus and SP5 stimulation on acoustically evoked discharges of units in the guinea pig dorsal cochlear nucleus. Multichannel silicon probes were used to record responses to pure tone stimuli of varying frequency and intensity and to tone stimuli preceded by cuneate nucleus or SP5 stimulation. Preliminary findings indicate that both Sp5 and cuneate nucleus stimulation can alter rate and timing of responses to acoustic stimulation, suggesting similar underlying mechanisms of action.

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853 Noise-Gated Detection of Slow Signals by a Model of Phasic Auditory Brainstem Neuron

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Phasic neurons fire only an onset spike to steady current injection. Biophysical analysis has implicated a low-threshold potassium current (I-KLT) as a contributing mechanism for the phasic firing of several types of auditory brainstem neurons. Phasic neurons behave as band-pass filters; they do not spike in response to slow-varying input. Surprisingly, we found using an I-KLT-based model of a phasic cochlear nucleus neuron (Rothman & Manis, J Neurophysiol 89: 3097-113, 2003) that, in the presence of noise, a normally undetectable slow-varying input, e.g., low-frequency sinusoids, can be encoded precisely. The detection mechanism shares some features with stochastic resonance, e.g., the highest signal-to-noise ratio was achieved at a moderate noise level, while other features were qualitatively different. For comparison, we created a tonic model by freezing the conductance of I-KLT at its resting level. This tonic model showed typical stochastic-resonance behavior. It responded for weak noise primarily at the subthreshold signal's peak and gradually lost phase preference with increasing noise levels. In contrast, the phasic model provided better phase information by precisely responding to the signal's rising and falling phases, which yielded less ambiguous temporal patterns and higher reliability. Detection by the phasic model was robust over a large range of noise levels even after the firing rate saturated, a feature tonic models lack. Further analysis using spike-triggered averaging provided insight on the spike-generating mechanisms, which could be different for the rising and falling phases of the signal. Our study provided a novel view of the signal processing by phasic neurons, that is, with noise their response can precisely encode slow-changing input, which alone causes no response. Corresponding in vitro experiments are underway. Supported by NIH/NIDCD-008543.

854 A Model of Brainstem Responses to Bilateral Electrical Stimulation with Amplitude Modulation

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A simplified, biophysical model is used to predict responses of neurons that are sensitive to interaural time difference (ITD) in response to peripheral bilateral electrical stimulation. Simulations focus on the effects of envelope modulation on input spike patterns, interactions between input patterns and membrane activity, and resulting ITD sensitivity. Several empirically observed aspects of ITD sensitivity in the inferior colliculus (IC) were simulated without evoking complex, inhibition-based neural mechanisms. When stimulating with unmodulated electrical pulses, ITD responses may be minimized (by shunting) or saturated to eliminate ITD dependence. If the pulse trains are modulated by low-frequency envelopes, sustained (periodic) responses return and ITD-dependent responses are achieved by adjusting input rate, synaptic strength, and modulation frequency, Furthermore, when fine-structure ITD and envelope ITD of simulated electrical innervations are not matched, the average rate and temporal pattern of model inputs change significantly with the fine-structure delay of low-frequency, electrical pulse inputs. These effects lead to asymmetries in the tuning to fine-structure ITD, even when the basic model structure is symmetric. Overall, we conclude that for electrical stimulation, amplitude modulation could affect ITD processing at the initial stage of bilateral interaction. [Supported by NIH grants R01 DC 05775 (B. Delgutte) and R01 DC00100 (H. S. Colburn)].

855 A Model of Brainstem Responses to Unmodulated Bilateral Electrical Stimulation

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A simplified, biophysical model for a medial superior olive (MSO) cell is used to predict responses of binaurally sensitive neurons to patterns of multiple excitatory input spike trains that represent stimulation by acoustic and electric waveforms. Specifically, the effects of changes in input parameters on model responses to interaural time difference (ITD) were studied for low-frequency unmodulated periodic stimuli. Input parameters explored include average firing rate, synchrony index, and latency differences between the input spike trains, as well as the conductance and time constant of the excitatory synapses. Results are compared to physiological recordings from the MSO and inferior colliculus, and discussed in terms of ITD-discrimination abilities of listeners with cochlear implants. In simulations, although the dynamic range of input spike rates that support ITD sensitivity is reduced as a result of highly synchronized electrical stimulation, the model MSO cell maintained reasonable ITD sensitivity for certain values of synaptic strength. More specifically, excitatory synaptic strengths must be strong enough to overcome the suppressive effect of low-threshold potassium-channel activation at preferred ITD, and weak enough to avoid "monaural coincidences" that produce responses at un-preferred ITD that result in saturated rate-ITD functions. Simulations also indicate that there is a favorable value range for the excitatory synaptic time constant to support ITD sensitivity at multiple frequencies. Together, our results suggest that for ITD sensitivity, the synaptic functions of MSO cells may need to be modified to accommodate the changes of input characteristics produced by peripheral electrical simulation. [Supported by NIH grants R01 DC 05775 (B. Delgutte) and R01 DC00100 (H. S. Colburn)].

856 Auditory Responses in the Owl's Nucleus Laminaris to Clicks: Impulse Response and Signal Analysis of Neurophonic Responses

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Stimulation with clicks offers the possibility to study the impulse response of a system. We have used clicks to characterize the neurophonic potential in the barn owl's nucleus laminaris and its dependence on stimulus level.

Clicks evoked an oscillatory response at the same frequency as measured with tonal stimuli. After separating the envelope and the waveform with the Hilbert transform, the response was quantified by fitting with auditory filters, like Gabor and Gammatone filters, with and without frequency glides. All filters provided reasonable fits, although the envelope was better fitted with a Gabor filter than with a Gammatone filter. A Gabor filter with frequency glide provided the best fit for the waveform. The frequency glide was similar to that observed in the auditory nerve of mammals. To our knowledge, Gabor filters have not been discussed in the auditory literature. The Gammatone and Gabor filters become more similar the higher the order of the Gammatone filter is. Although the Gabor filter is an acausal filter, we argue that for practical purposes it has advantages over a higher-order gammatone filter for modeling.

Response amplitude, group delay, frequency and phase depended in a systematic way on click level. Response amplitude decreased as stimulus level decreased. A linear fit provided the best fit of response amplitude versus level in about 60% of the cases, while a saturating function was better in 35% of the cases. Group delay and response phase increased as level decreased. The dependence of group delay and response phase on level was best represented by a straight line in almost all cases. Frequency typically increased as level decreased, and this relation was well fitted by a linear or quadratic function. In summary, the impulse response of the neurophonic in the nucleus laminaris of barn owls reflected many of characteristics observed in the responses of the basilar membrane and auditory nerve in both and mammals.

857 Response Characteristics of the Avian Superior Olivary Nucleus

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The processing of acoustic cues relies on the integration of both ascending and descending neural activity. One of the most well understood auditory circuits is the pathway devoted to processing interaural time disparities, the primary cue for low frequency sound localization. While the ascending ITD circuitry is well characterized both anatomically and physiologically, descending components of the circuit have received much less attention. The superior olivary nucleus in birds (SON) provides the major descending and inhibitory input to all major nuclei in the avian ITD pathway. Despite its central role in avian ITD processing, little is known about the SON's physiology. We have begun to investigate the cellular and acoustic properties of SON neurons. Our in vivo studies have confirmed previous reports that identified two general classes of SON neuron, phasic and sustained, that are distinguishable on the basis of responses to tones. In addition, we have identified two classes of SON neuron in response to current injection using whole cell current clamp. The first type fires one or two spikes at the beginning of depolarizing current steps, and shows a rectified membrane response for the remainder of the pulse. These properties are common among auditory neurons specialized to process timing information and may correspond to the phasic cells observed in vivo. The second and more common type fires multiple action potentials with depolarizing current steps. This type may correspond to the sustained neurons observed in vivo. These data represent the initial studies toward our goal to build a comprehensive understanding of the SON, and its role in ITD processing.

858 Responses from the Trapezoid Body in the Mongolian Gerbil

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The Mongolian gerbil (Meriones unguiculatus) has seen increasing use in auditory research. Unlike other small mammals, such as bats or mice, it possesses good low frequency hearing and physiological recordings in binaural nuclei show sensitivity to interaural time differences. To better understand the monaural inputs to the binaural nuclei in the superior olivary complex, we studied the responses of monaural axons in the trapezoid body.

We used a ventral approach in which the trapezoid body was exposed either at the midline, between the two bullas, or unilaterally using a transbulla exposure. Responses to short (25 ms) pure tones and broadband noise (1 s) were obtained via recordings with glass micropipettes followed by routine amplification, filtering, and discrimination of the neural signal. Fibers were classified according to the shape of the post-stimulus-time histograms (PSTHs) in response to pure tones at the characteristic frequency.

As expected, PSTHs that were frequently observed included phase-locked (PHL), primary-like with notch (PLN), and chopper. Surprisingly, few fibers with primary-like (PL) responses were observed, but a large group of fibers with onset responses. The latter showed mostly a pure onset response without sustained activity (Oi), but responses with low sustained activity (Ol) or chopping (Oc) were encountered as well. Although we recorded several instances of "high-sync" responses (maximal vector strength > 0.9), the occurrence of such responses was not as prominent as in the trapezoid body of the cat.

Because the onset responses observed occurred over a similar range of depths as the PHL and PLN responses, we hypothesize that these responses are also derived from axons of bushy cells.

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859 Discharge Patterns of Single Units in the Lateral Superior Olive of Decerebrate Cats

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Most studies of the lateral superior olive (LSO) have been conducted in anesthetized preparations. In such preparations, LSO units usually respond to ipsilateral best-frequency tone bursts with a chopper type discharge pattern, characterized by regularly spaced peaks of activity initially time-locked to the stimulus onset. Limited data from one decerebrate cat study suggest, however, that the chopper nature of these responses may be augmented by anesthesia. The goal of the present study was to classify objectively the temporal discharge patterns of single units in the LSO of decerebrate cats. Post-stimulus time histograms obtained at 20 dB re threshold were classified based on a decision tree. Tests in this tree quantify several features of a unit's response including reliability of spike activity in the first two peaks of the response, peak to sustained rate ratio, inter-peak gap and discharge regularity as a function of time. Of 32 units recorded, 13 units were classified as primary-like, three as primary-like with notch, six as onset-sustained and 10 as choppers. The first three response types (all associated with cochlear nucleus bushy cells which project to the LSO) fire irregularly. Thus, on the whole, LSO units in a decerebrate preparation are indeed much less regular than those observed in anesthetized preparations. These results suggest that LSO units can follow the temporal patterns of their inputs more faithfully than thought previously, and thereby can transmit monaural temporal information as well as compare effectively instantaneous amplitude fluctuations at the two ears. Supported by NIDCD grant R01 DC 05161.

860 A Possible Substrate of Frequency Integration in Nuclei of the Lateral Lemniscus

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In intermediate and ventral nuclei of the lateral lemniscus (INLL and VNLL, respectively), some neurons display an inhibitory spectral interaction in which responses to high best frequency (BF) sounds are inhibited by sounds at much lower frequency. This is termed *combination-sensitive inhibition*; it is especially prominent in INLL. Previous work suggests that this response property depends on low-frequency tuned glycinergic inhibition. To identify sources of low-frequency glycinergic input to INLL, we combined retrograde tracing with immunohistochemistry for glycine. We deposited retrograde tracer at recording sites displaying 1) high frequency BFs with combination-sensitive inhibition, or 2) low-frequency BFs (23-30 kHz). After transport time, the brain was fixed by perfusion and processed to mark glycinergic cells. There are three main results. First, after inhibitory combination-sensitive deposits, most retrogradely labeled cells were in ipsilateral medial nucleus of trapezoid body (MNTB) and contralateral anteroventral cochlear nucleus (AV). We observed labeling in other nuclei, but in smaller numbers: contralateral lateral division of posteroventral cochlear nucleus (PVl), ipsilateral lateral nucleus of trapezoid body (LNTB) and other ipsilateral periolivary nuclei. Second, when tracer deposits were combined with glycine immunohistochemistry, most double-labeled cells were observed in ipsilateral MNTB, with a few in LNTB. Third, labeling in MNTB occurs in two regions, which appear to correspond to high-and low-frequency representations. Moreover, there is an apparent overlap between the combination-sensitive and low-frequency labeling in the lateral MNTB. These results suggest that MNTB is the most likely source of low-frequency glycinergic input to high-BF, inhibitory combination-sensitive INLL neurons. The combination-sensitive inhibition contributes to selectivity in response to social and sonar vocalizations. Supported by NIDCD grant RO1 DC-00937 (J J W).

861 Paired-Pulse Evoked Responses in the Inferior Colliculus of Albino Rats Following Unilateral Kainic Acid Lesions of the Ventral Nucleus of the Lateral Lemniscus

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Important information about complex sounds is carried in the temporal properties of the stimulus. The study of temporal resolution, the ability to process rapid changes in sound over time, is critical for understanding how the auditory system preserves the temporal characteristics of an acoustic stimulus. Several nuclei within the central auditory system, such as the ventral nucleus of the lateral lemniscus (VNLL) and the inferior colliculus (IC), have been implicated in temporal processing and their mutual connections are hypothesized to play a significant role in timing. Previous work has indicated that the VNLL contains primarily glycinergic and GABAergic neurons, which provide inhibitory projections to the central nucleus of the IC. In the present study, an electrophysiological measure was used to investigate temporal resolution in a paired pulse paradigm. Evoked responses to paired clicks with 2, 5, 10, 25, 50 or 100 ms delays were recorded from normal rats as well as rats with unilateral lesions of the lateral lemniscus. Lesions were made by multiple injections of 0.9% kainic acid into the VNLL and animals were allowed 6 weeks of recovery from the surgical procedures before recordings were made. All recordings were made from the IC of rats under ketamine/isoflurane anesthesia. Responses recorded from rats with VNLL lesions were of higher amplitude than those obtained from animals with an intact VNLL, indicating a loss of inhibition in the IC. However, temporal resolution was conserved within the IC despite significant VNLL cell loss. These results indicate that substantial loss of inhibitory input to the IC following VNLL lesions does not necessarily degrade temporal resolution as reflected in evoked responses to paired clicks.

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862 Effects of Hearing Aid Gain on Slow Cortical Auditory Evoked Potentials

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The P1-N1-P2 Acoustic Change Complex (ACC), a cortical auditory evoked potential complex (CAEP), can be elicited by changes occurring within an ongoing stimulus, such as changes in periodicity, amplitude, and frequency content of the stimulus. The ACC has been successfully recorded in various clinical populations including hearing aid (HA) and cochlear implant (CI) recipients and therefore the ACC may be a useful clinical tool to evaluate the efficacy of amplification intervention methods. Tremblay and colleagues (2006) recorded the ACC to CV speech stimuli in both aided (HA) and unaided test conditions in normal hearing listeners. An unexpected finding in this study was that no significant differences occurred in peak amplitudes and/or latencies for ACC response peaks when comparing these two test conditions. The present study was designed to further investigate the effects of amplification on the ACC.

The purpose of this study was to compare the ACC in response to /si/ and /su/ presented via loudspeaker at 45, 60, and 70 dB peSPL, in 18 normal hearing adults in aided and unaided test conditions. A mild gain hearing aid with a linear input-output function was programmed to provide 15 dB of gain from 125-6000 Hz for use in the aided test condition.

Results revealed substantial latency decreases and amplitude increases in aided versus unaided conditions, particularly for 45 and 60 peSPL stimuli. Increasing stimulus intensity resulted in latency decreases and amplitude increases for both aided and unaided test conditions. Current findings indicate the effects of hearing aid gain are reflected in ACC responses to speech and are generally consistent with the response properties of slow cortical auditory evoked potentials reported in the ERP literature.

863 Intracranial Mapping of Human Auditory Association Cortex Using Simple and Complex Sounds

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The lateral posterior superior temporal gyrus is involved in processing complex sounds. However, the timing and functional significance of the contribution of auditory association cortex to sound processing remains poorly understood. To address this issue, we compared directly intracranial recordings and electrocortical stimulation mapping (ESM) of auditory association areas. Participants were six adult epilepsy patients, three male, with medically intractable seizures who had subdural electrode arrays (6x8, 8x8) implanted over lateral left temporal cortex. All were left-hemisphere language dominant (Wada test) and had normal hearing, cognitive function, and auditory processing. Intracranial recordings and ESM were performed using simple and complex sounds: steady-state tones, frequency-modulated (FM) tones, and speech syllables. Evoked N1 responses and induced spectral power changes were derived using time-domain averaging and time-frequency analysis.

For all patients, the largest auditory N1 responses and spectral power changes to speech occurred at sites on the posterior superior temporal gyrus. ESM disrupted perception of speech at the same sites. N1 speech responses were broadly distributed. Spectral power changes were predominately in the high gamma range (> 60 Hz) and occurred at a subset of the N1 sites. Event-related responses to steady-state tones were identified mainly in the inferior parietal lobe. ESM did not disrupt perception of steady-state tones. Conversely, FM tones elicited a pattern of evoked and induced responses similar to speech, and ESM disrupted perception of FM tones and speech at the same sites. Our results indicate that auditory association cortex plays an early and critical role in processing complex, frequency-varying sounds. These findings support a functional hierarchical organization of human auditory cortex.

864 Functional Neuroimaging of Evoked Potentials in Bilaterally Implanted Cochlear Implant Users

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Cochlear implants (CIs) provide access to sound via stimulation of the auditory nerve with electrical pulses and promote auditory development. CIs are typically only available for one ear. Because unilateral hearing is known to cause reorganization in the auditory system, we suspect that development of the auditory cortex in children using unilateral CIs is abnormal. It may be possible to correct this by fitting unilateral CI users with a second implant. It was hypothesized that the latency, amplitude and location of cortical activity would be abnormal in children using unilateral CIs relative to normal hearing peers and more similar to those of unilaterally deaf subjects. We further hypothesized that closer to normal cortical activity would be detected in CI users after bilateral cochlear implant use.

In this study, EEG measured from 64 scalp locations was obtained from (a) unilateral CI users, (b) CI users fitted with a second implant, (c) age-matched subjects with unilateral hearing loss, and (d) age-matched normal hearing controls. Combined with an anatomical image of the head, beamformer analysis of the multi-channel EEG data collected allowed the identification of locations of neural activity.

Data from the first set of 6 children suggest that (a) there are differences in response amplitudes across all groups, (b) that response latencies of CI users are different from those of children with normal hearing and unilateral hearing loss, and (c) that there may be differences in location of cortical activity between normal hearing peers and bilateral CI users. This study will allow us to better characterize how the auditory pathways in children using CIs differ from their normal hearing peers. This will permit us to assess the effectiveness of unilateral cochlear implantation and that of sequential bilateral cochlear implantation.

865 Cortical Measures of Selective Auditory Attention in Musicians and Non-Musicians: Effects of Musical Training

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In both normal and impaired systems, cortical functions like music and language pervasively influence early auditory processing. These enhancements are likely mediated by top-down influences that operate via an extensive corticofugal circuitry of descending efferent fibers synapsing at a wealth of points along the auditory pathway. By helping to reinforce top-down mechanisms, music could benefit neural mechanisms related to selective auditory attention.

A cortical response reflective of selective auditory attention, the N100, can be measured in children as young as 3 years of age and relates with deficits in child language impairments: it is decreased in language-impaired children relative to normals. Furthermore, the N100 of children with language deficits proves highly amenable to short-term auditory training[14]. In light of these observations, we investigated musical training's effects on neural measures of selective auditory attention.

In order to investigate influences of musical training on neural mechanisms of attentional filtering, we recorded cortical evoked potentials in adult musicians and non-musicians while attending to one of two simultaneously presented short stories (a paradigm established by Coch *et al.*[3]). Responses were recorded to the speech sound /da/, which was embedded within the stories and presented in an alternating fashion to the attended and ignored ears. Our data indicate that musicians have a more robust attentional effect on cortical responses. Furthermore, relationships between cortical and subcortical responses further our understanding of cortex-brainstem interactions that may drive cognitive capabilities related to language and reading.

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866 Spatiotemporal Reconstruction of the Auditory Steady-State Response to Frequency Modulation Using MEG Beamformers

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Natural sounds contain modulations in both amplitude and frequency. Modulated sounds can elicit the auditory-steady state response (ASSR). The aim of this study was to assess the success of magnetoencephalography (MEG) beamformer methods in reconstructing the ASSR to frequency-modulated stimuli.

The ASSR can be localised using beamforming analyses. However, when auditory stimuli are presented diotically, the ASSR is represented bilaterally with correlated sources in both hemispheres. Standard beamformers fail to localise correlated sources accurately [e.g., Van Veen *et al*., (1997). "Localisation of brain electrical activity via a linearly constrained minimum variance spatial filtering," *IEEE Trans. Biomed. Engineer.* 44, 867-880].

In the present study standard beamformers [vectorised linearly-constrained minimum-variance beamformers (VLCMV), e.g., Van Veen *et al*., (1997)] were used to localise the ASSR based on changes in power between experimental conditions. In addition a novel beamformer based on the Hotelling's T^2 test for the Fourier component of interest was used to localise the ASSR. Virtual electrode analyses were used to reconstruct the time series at each source. Fast Fourier Transforms of the virtual electrode time series were calculated to obtain the magnitude and phase characteristics of each source identified in the beamforming analyses.

For the standard VLCMV beamformer, significant ASSR sources ($p \leq 0.05$, non-parametric statistics) were found only in auditory cortical areas in the right hemisphere. ASSR sources were found in both left and right auditory cortical areas using the Hotelling's T^2 beamformer. Virtual electrode analyses confirmed that the Fourier component of interest was represented at each source. In summary, standard beamformers fail to reconstruct the bilateral ASSR. Beamformer analyses based specifically on the Fourier component of interest may offer improved spatiotemporal reconstruction of the ASSR.

867 A Detailed View at FMRI Activation Maps in Relation to Sound Intensity and Loudness

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BACKGROUND: Neuronal processing of sound intensity and loudness in the human auditory cortex is still discussed controversially. The present study investigated whether the neural activity in the auditory cortex is more related to physical measures like sound intensity or perceptual measures like loudness, and examined the functional dependence of activation size and activation magnitude, as measured by the number of activated voxels and voxel intensity in a functional MRI experiment.

METHODS: Auditory fMRI was conducted with 45 normal hearing listeners. Individual loudness sensitivity was assessed by a categorical loudness scaling procedure inside the MRI scanner. The dependence of neural activation magnitudes on sound intensity and loudness was analyzed in a detailed way, using continuous pink noise at sound pressure levels up to a value just below the individual categorical rating of "very loud".

RESULTS: We observed a strong nonlinear growth of activation size, both with increasing categorical loudness and sound intensity. In contrast to this, the BOLD signal strength grows almost linearly with sound intensity and linearly with categorical loudness in the auditory cortex over the whole range of presentation levels used. In addition BOLD signal strength discriminates significantly between different categorical loudness sensations even at

a fixed sound pressure level. However, it does not discriminate between different sound pressure levels at a fixed value of categorical loudness.
CONCLUSION: The neural activity in auditory cortex appears to be a direct linear reflection of subjective loudness sensation, rather than a display of measured sound pressure level as in a sound-level meter.
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868 Decreased Temporal Processing in the Patients with Temporal Lobe Epilepsy

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Purpose: Central auditory processing disorder (CAPD) has been defined as a disorder in the recognition, discrimination, ordering, grouping and localization of sounds. When central auditory lesions, such as deficits in cerebral cortex and brain stem, were present, it has been reported that CAPD tests generally yielded abnormal scores. Temporal lobe epilepsy (TLE) could induce excessive electrical discharges in the area of the auditory pathway resulting in the dysfunction of central auditory integrity. The aim of this study is to evaluate the CAPD tests in patients with temporal lobe epilepsy.
Methods: Thirty-two patients (24 male and 8 female) with TLE with normal hearing in pure tone audiometry participated in this study. Eighteen patients had right temporal lobe epilepsy, 12 left and two both temporal lobe epilepsy (mean duration of illness: 17.0 years; range: 1-41 years). Twelve patients with intractable epilepsy underwent temporal lobectomy. Frequency, duration pattern tests and dichotic test were measured in all the patients..We analyzed data according to the site of lesion (right TLE versus left TLE), operation (before and after surgery), and MR findings (hippocampal sclerosis verse normal).
Results: Frequency pattern test showed abnormal score in 78% of all patients, duration pattern test 57% and dichotic test 23%. Among these three tests, the score was worse than normal cut-off (mean - 2 SD) in duration test. The score of frequency test and dichotic test were in the normal cut-off value. There were no significantly differences between right TLE and left TLE in all the tests. In patients underwent temporal lobectomy, there was no significant difference before and after surgery. However, patients with hippocampal sclerosis performed significantly worse than patients with normal MR findings. (p=0.033, p=0.009)
Conclusions: In the patients with temporal lobe epilepsy, a risk of speech recognition may increase because of the defective central processing especially in temporal processing.

869 The Effect of Stimulus Context on Cortical Measures of Pitch Processing Using FMRI

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Many different paradigms and pitch-evoking stimuli have been used to study pitch. A growing body of neurophysiological evidence shows that cortical responses are sensitive to the context or 'background' from which stimuli are presented. FMRI studies typically present bursts of pitch-evoking stimuli from a silent background and compare the resulting activation to a sequence of spectrally matched noise bursts, also presented from a silent background. However, MEG studies have shown that when the stimulus is presented from a silent background, the large energy-onset response may obscure the smaller pitch-onset response. A sample of noise can be appended to the beginning and end of a pitch stimulus, keeping the overall energy constant, so that the cortical response becomes more sensitive to changes in the spectral and/or temporal regularity of the auditory signal.
We tested the hypothesis that the stimulus background influences the pattern of pitch-related fMRI activation in the human auditory cortex. Fifteen listeners participated in a blocked design experiment. A diotic complex tone and a dichotic Huggins pitch stimulus were presented from either a noise or silent background and activation was contrasted with matched noise control conditions. Results revealed significant ($p < .001$) main effects of both background (noise/silence) and pitch stimulus (diotic/dichotic) with no significant interaction. Measurements of pitch-related activation were greater for the noise than for the silent background conditions especially in bilateral medial Heschl's gyrus and planum temporale. The diotic pitch also evoked greater activity than the dichotic pitch.
These results indicate that the stimulus context significantly modifies the pattern and the location of pitch-related activation. If the feature specificity of the pitch-related response is to be inferred from the data, future studies should include careful controls for stimulus context.

870 Benefits of Active Noise Cancellation During Auditory FMRI: A Comparison Between Sparse and Continuous Imaging

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Auditory functional magnetic resonance imaging (fMRI) is complicated by the intense scanner sound. Its impact on stimulus-evoked activity can be reduced by size of the dataset. Our Institute has developed an active noise cancellation (ANC) system that attenuates scanner noise by up to 35 dB (from 93 to 58 dB SPL at 600 Hz). The ideal goal would be to remove the need for sparse sampling by using ANC, without compromising sensitivity to stimulus-related activity. ANC would also enable stimuli to be presented at much lower levels than has been possible hitherto and to address questions that can be

confounded by the excessive acoustic noise levels, such as the neural correlates of speech intelligibility. The current experiment investigates these specific issues.
A 2x2x3 repeated-measures design crossed the operation of the ANC system (cancelled and non-cancelled) with the scanning protocol (continuous and sparse sampling) and stimulus (speech, narrowband noise and silent baseline). Both speech and noise were presented at a level at which intelligibility and audibility depended on the ANC state and scanning protocol. Participants reported benefits of cancellation and sparse sampling for listening effort ($p<0.05$). Stimulus-evoked activity was analysed for eight participants using ANOVA statistics ($p<0.05$, FDR-corrected). Scanning protocol had the largest effect. Sparse sampling enhanced auditory cortical activity. ANC also exerted an effect, with non-cancelled conditions associated with activity in higher cortical sites. In right parietal and frontal cortex, the magnitude of activity was positively correlated with listening effort.
In summary, both sparse sampling and ANC benefit auditory fMRI. Sparse sampling improves the detection of auditory cortical responses by providing quiet intervals for stimulus presentation, whilst ANC decreases activity in higher cortical regions by reducing the effort required for listening.

871 Evaluation of White Matter Abnormalities in Patients with Hearing Loss and Tinnitus Using Diffusion Tensor Imaging

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Subjective tinnitus is the perception of sound in the absence of acoustic stimuli. About 15-20% of the population suffers from chronic tinnitus, the majority having some degree of hearing loss. Brain imaging techniques such as fMRI and PET have advanced our knowledge of functional changes in brain gray matter due to this disorder. However, little is known about the white matter changes due to hearing loss or tinnitus. Diffusion tensor imaging (DTI) reveals information about white matter integrity by quantifying the directionality of water diffusion via computation of the fractional anisotropy (FA) index.
We used DTI to evaluate white matter integrity in 22 subjects (8 normal-hearing controls, 7 age-matched subjects with tinnitus and no hearing loss, and 7 subjects with tinnitus and hearing loss). Subjects were scanned in a 3.0 Tesla GE Excite scanner using an 8-channel receive-only coil. Water diffusion was measured along 33 non-collinear directions.
DTI analysis using Tract-Based Spatial Statistics (Smith et al., Neuroimage, 2006) revealed that controls had significantly greater mean FA values in the left temporal region than the hearing loss groups ($p < 0.05$) with no significant differences seen in the right hemisphere. Further analysis revealed that the mean FA values were greatest in controls, followed by the tinnitus and then non-tinnitus hearing loss subjects. Finally, statistically significant differences were seen between controls and the non-tinnitus hearing loss group, but not between controls and the tinnitus group. This may indicate the existence of abnormal white matter in auditory cortical areas of the brain in patients who suffer from hearing loss. Having tinnitus in addition to hearing loss may reduce abnormal white matter changes.
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872 Development of the Auditory Cortex in Children with Bilateral Cochlear Implants

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In the auditory system, cochlear implants (CIs) provide a unique model for studying the effects of auditory deprivation in humans. In individuals with binaural deafness, bilateral CIs are provided. Some of these individuals are simultaneously implanted while others are sequentially implanted with a period of delay between the experienced and the naïve ears. In this study, we first questioned whether we can measure response differences between the experienced and naïve ear stimulations in sequentially implanted bilateral CI users by using a single recording electrode placed on the midline cephalic location (Cz) on the scalp. Second, we asked whether the cortical response change with ongoing implant use.
In the present study, we recorded unilateral cortical auditory evoked potential (CAEP) responses in 47 children who were sequentially implanted with at least 2 years of delay between each implantation. CAEP recordings were completed on 3 different days of bilateral CI use: first day, 6 months, and 12 months. The ages of these subjects varied between 3 to 14 years (μ =7.2 σ =2.9). Stimuli were biphasic pulse trains (36 ms) presented at 1 Hz from an apical electrode (E20) on the experienced and naïve sides separately.
There were 3 different types of CAEP responses that were identified on the first day of implant use. Type 1 was typical of previous reports with a dominant positive peak, Type 1 had a negative peak preceding a positive peak, and Type 3 responses were multi-peaked. Overall, 66% of the children showed different types of response patterns between the experienced and naïve ears. On day one, responses from the experienced side were most commonly Type 1 responses (24/31, 77%), followed by type 3 (4/31, 13%) and type 2 (3/31, 10%). Responses from the naïve ear were most commonly type 1 responses (15/31, 49%) followed by type 3 (8/31, 27%) and type 2 (7/31, 24%). Over time, the responses from the experienced ears showed continued prevalence of type 1 responses. On the other hand, the responses from the naïve ear showed only a small increase in type 1 responses after 12 months of implant use and a small decrease in type 2 responses.

Our results suggests that differences in CAEP responses between the experienced and naïve ears indeed be seen by recording at the midline cephalic location on the scalp. In general, the responses patterns from the experienced ear seem to stay the same, while the responses from the naïve ear indicate a limited change with ongoing implant use. This might be an indication of restricted cortical plasticity in these children due to a long period of unilateral deafness.

873 Neurophysiological Mechanisms of Bone-Conducted Ultrasonic Perception Assessed by Electrophysiological Measurements in Humans

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Several studies have reported that bone-conducted ultrasound (BCU) is perceived even by the profoundly sensorineural deaf; however, the mechanisms involved remain unclear. We have reported some unique characteristics of BCU perception; suggesting unique perception mechanisms. For example: (1) the pitch of BCU is ten-odd kHz and is independent of its frequency, (2) no beats occur between BCU and ten-odd-kHz air-conducted sinusoids, (3) BCU masks 10-14 kHz air-conducted sounds, and (4) the dynamic-range of loudness for BCU is less than 20 dB. In this study, several electrophysiological measurements were carried out to clarify the neural pathway involved in BCU perception. Electrocochleogram (EcochG), auditory brainstem (ABRs) and middle latency (MLRs) responses evoked by BCU were measured in humans. We also recorded the cortical magnetic activities evoked by air- and bone-conducted sounds with frequency variations in the audible to ultrasonic range.

In the EcochG measurements, the compound action potential (AP) was clearly elicited, while the cochlear microphonic (CM) and the summating potentials (SP) were not identified. Substantial MLRs and ABRs were evoked by BCU as well as by air-conducted sound (AC). In terms of the cortical activities, clear N1m responses were elicited by BCU with their equivalent current dipoles (ECDs) being localized in the auditory cortices. However, BCU showed larger latencies, smaller ECD moments, and more posterior and lateral locations than did all audible-frequency sounds; appearing not to follow the tonotopic organization at the cortical level.

These results indicate that: (1) the cochlea substantially contributes to the BCU perception, (2) BCU goes through the normal auditory pathway; that is, there is no special organ for the BCU perception, and (3) some differences in the inner ear mechanisms may exist between audible sounds and BCU.

874 Mismatch Negativity Responses to Short and Long ITDs

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Human listeners can lateralise sounds leading at one ear by many 1000's of microseconds, well beyond the maximum ±600-700μs created by the interaural distance. Although neural detectors that explicitly offset such delays have been considered necessary to account for this ability, recent studies suggest only a restricted range of such detectors, with an upper limit of approximately ½ the period of the centre-frequency within each auditory frequency channel - an inherent "π-limit". We tested this assertion by recording the mismatch negativity (MMN), a derived component of the auditory evoked potential thought to represent changes in the perceptual qualities of sounds, including their spatial attributes. A central tenet of the π-limit is that ITDs lying beyond the limit are encoded by the neurons within it. To test this, we examined the MMN for stimulus blocks comprising a standard 400-Hz band of noise centred at 500 Hz with one of four possible ITDs (-500, +500, -1500 and +1500 μs) and two deviants of opposite sign; e.g. a standard of +500 μs was presented with deviants of -1500 and -500 μs. Large MMN potentials were elicited bilaterally for standards of -500 and +500 μs when either was presented as standard or deviant; +500:-500 (standard:deviant) and -500:+500 configurations. The magnitude of the MMN was greater in both brain hemispheres, and more strongly lateralized, for +500:-500 compared with -500:+500, a pattern consistent with the greater responsiveness of right vs. left cortex reported in the EEG literature. No MMN was elicited in either cortical hemisphere for -1500:+500, consistent with the two stimuli, despite being lateralized to opposite sides, activating the same neural populations in left and right cortex. In contrast +1500:-500 elicited a significant MMN in the right hemisphere. For -1500:+1500 and +1500:-1500, configurations separated by 3000 μs, the MMN was greater in the hemisphere contralateral to the leading ear.

875 Functional Magnetic Resonance Imaging of Cortical Sensitivity to the Binaural-Level Characteristics of High-Frequency Gabor Click Trains

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We investigated the sensitivity of blood-oxygen-level-dependent (BOLD) responses in human auditory cortex (AC) to the binaural level characteristics of 4000-Hz Gabor click trains. Click trains were synthesized with an interclick interval (ICI) of 3 ms and presented repeatedly throughout each stimulation block of 12 seconds duration. The click-train presentation rate [40 trains of 4 clicks (fast) or 5 trains of 32 clicks (slow) per second] and binaural-level configuration [independently assigned levels at each ear ranged 55 to 85 dB SPL (peak equivalent) or "silent" (-10 dB SPL)] was varied pseudorandomly between blocks, with silent blocks (-10 dB SPL in both ears) occurring every 4th block. Sounds were delivered over piezoelectric

earphones (Sensimetrics, Malden MA) while listeners detected rare ICI changes ("targets"). Whole-head echoplanar images (32 slices, 3.0 x 3.0 x 4.5 mm resolution, 3 Tesla) were acquired at the end of each block (TR = 12 s). Relative to silent blocks, sound blocks revealed widespread bilateral activation of the superior temporal plane surrounding Heschl's gyrii and including nearby lateral portions of superior temporal gyrus. Slow (5/s) stimulus presentation produced significantly greater activation than fast (40/s) presentation throughout these regions, consistent with previous reports. Binaural levels affected BOLD responses in two key ways: First, responses to monotic stimulation were significantly greater contralateral to the stimulated ear. This overall contralateral advantage was consistent with numerous published studies, but varied across regions of AC in individual subjects. Second, compact subregions of AC responded parametrically to changes in average binaural level and interaural level difference, consistent with the view that AC fields differ in the level- and binaural sensitivity of their neural populations. [Supported by NSF IOB-0630338, NIH T32 DC005361, and University of Washington.]

876 The Effect of Attentional Load on Auditory Cortical Representation of Temporal Edges in Sound

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The emergence of an auditory object from a background is signalled by the existence of temporal edges, or transitions, in the properties of the ongoing input to the ears. Often, the background and the object possess substantial non-stationary statistics, and the task is therefore to detect a transition in the pattern of ongoing statistics. In certain cases, edges are manifest as a violation of a previously-acquired representation of the scene ('violation of regularity' - VR edges), e.g. when an auditory object, against some background, disappears or changes its properties. In other cases, the transition that the system must detect is the emergence of regularity, or 'order', from disorder ('emergence of regularity' - ER edges). We have recently demonstrated (Chait et al, 2008) that auditory cortical responses to VR and ER edges exhibit different temporal dynamics and that they recruit distinct neural substrates.

Sensitivity to temporal edges plays a key role in auditory scene analysis and an important issue is whether this sensitivity is affected by the attentional load of the listener. To address this question, we use MEG to measure early auditory cortical responses to transitions between constant-frequency and random-frequency tone-pip sequences (VR edges) and vice versa (ER edges) while manipulating listeners' attentional load. Simultaneously with the 'auditory edge' stimuli, listeners were presented with streams of rapidly-alternating auditory and visual signals and had to perform high- and low- attentional load tasks on these streams. The data reveal that auditory attentional load does not affect the cortical representation of VR edges, but significantly reduces responses to ER edges. Visual attentional load in the current experiment had no effect on either transition response. These results suggest that, under high attentional load, the representation of certain kinds of sound events in the environment is impaired while that of others is unaffected.

877 Self-Vocalization- Induced Auditory Cortical Attenuation in Humans Is Field Specific

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Self-vocalization is known to alter firing rates of auditory cortical neurons in non-human primates in a predominantly suppressive manner. Imaging and electrophysiology studies in humans show excitation of temporal cortices during vocalization but to a lesser degree than that seen during listening. The mechanisms responsible for these findings remain unclear, and no studies to date have shown differential responses between primary and secondary auditory cortices.

We have investigated 2 patients undergoing surgical treatment of epilepsy to examine the influence of self-vocalization on three different auditory cortical fields. Recordings were taken from intracranial electrode arrays positioned on the pial-surface over secondary auditory fields on lateral superior temporal gyrus (STG) and stereotactically implanted into primary auditory fields on medial and lateral Heschl's gyrus (HG). We recorded brain activity simultaneously from all fields while subjects self-vocalized and also when these same vocalizations were played back to the patient. Recordings were analyzed offline and average evoked potentials (AEPs) and time-frequency power analyses were examined for each condition.

On posterior STG, self-vocalization produced marked attenuation of AEPs compared to playback. Conversely, AEPs obtained on medial HG were preserved, and lateral HG responses showed intermediate attenuation of AEP amplitude. Gamma and high gamma band power analyses showed a slight increase from the self-vocalization condition compared to playback on HG, while STG band-specific power was severely reduced during self-vocalization.

These findings suggest that self-vocalization influences auditory cortex in a field-specific fashion, with higher-order auditory cortex showing greater attenuation than that seen in primary fields. Further study is warranted to elucidate the mechanisms involved.

878 Representation of Temporal Sound Features by High Frequency Gamma Activity in the Human Core Auditory Cortex

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Periodic non-speech acoustic stimuli, e.g. click trains and sinusoidally amplitude modulated (sAM) noise, can elicit different percepts depending on the repetition rate, and provide a convenient model to study representation of timing information in the auditory system. This study sought to investigate physiological correlates of temporal sound features over a range of repetition rates.

Experiments were carried out in patients undergoing invasive monitoring for refractory epilepsy. Stimuli were click trains, sAM white noise and speech sentences. Recordings were made from multi-contact depth electrodes implanted in Heschl's gyrus and analyzed as averaged auditory evoked potentials and event-related band power (ERBP), calculated using complex Morlet wavelet transform.

Responses from core auditory cortex within posteromedial Heschl's gyrus exhibited three distinct patterns of high gamma ERBP changes, roughly corresponding to the perceptual classes of discrete events, flutter and temporal pitch. At the lower end of studied repetition rates (4–16 Hz), responses to clicks were characterized by bursts of phase-locked ERBP; the envelope of sAM noise stimuli was reflected in amplitude modulation ERBP. Repetition rates of up to 64–128 Hz produced frequency-following responses, evident as augmentation of phase-locked ERBP at the stimulus repetition rate. At still higher stimulus rates (128–256 Hz), responses were characterized as increases in non phase-locked ERBP. Such patterns were also evident in responses to speech, where the temporal envelope was represented by amplitude modulation of ERBP, while transients were emphasized by bursts of phase-locked power.

The results of the study demonstrate differential neural coding of timing information within the human core auditory cortex and suggest a physiological counterpart of perceptual categorical boundaries for periodic acoustic signals.

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879 Competing Streams at the Cocktail Party - A Neural and Behavioral Study of Auditory Attention

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The mechanism by which a complex auditory scene is parsed into separate objects depends critically on attentional processes. We illuminate these mechanisms in a simultaneous behavioral-neurophysiological study, in which we manipulate subjects' attention to one of two competing rhythmic streams of an auditory scene. Our experimental results reveal that attention to the target stream correlates with a sustained increase in the neural target representation, as measured by magnetoencephalography (MEG), beyond auditory attention's well-known transient effects on onset responses. This enhancement, in both power and phase, occurs exclusively at the frequency of the target rhythm, for either stream, and is only revealed when contrasting two attentional states that direct subjects' focus to different features of the acoustic stimulus. The enhancement originates in auditory cortex and covaries with behavioral state, with a right-biased hemispheric asymmetry. Furthermore, for the slower stream, whose rhythmic rate is commensurate with that of speech prosody, the target's perceptual detectability improves over time, correlating strongly, within subjects, with the target representation's neural buildup. We put forward a computational model showing that the buildup of the neural representation is due to increasing temporal coherence, and not to increasing power.

880 A 3-T FMRI Study of Cortical Reorganisation in Unilateral Tinnitus

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INTRODUCTION

Although the brain mechanisms of tinnitus remain hypothetical, one main proposition based on phantom sensations in other modalities is the idea of a reorganisation of the auditory cortex. This reorganisation would be greater at the cutoff frequency (i.e., the frequency at the edge of hearing loss) than at any other frequency. We investigated this hypothesis using a 3-Tesla fMRI scanner. We postulated that tonotopic map reorganisation, as revealed by stronger and greater bold signal change, would be greater at the cutoff frequency than at other, control frequencies, at the side contralateral to tinnitus.

METHODS

Participants: Nine participants (mean age = 58.1, SD = 8.6) with unilateral subjective chronic tinnitus (6 left-sided, 3 right-sided) ten controls (mean age = 59.6, SD = 7.9) matched for age and socioeconomic status, participated in the study.

Tasks and stimuli: Tinnitus participants had medium-to-high frequency hearing loss in their tinnitus ear with a slope of at least 25dB/octave and underwent extensive psychophysical testing in order to find the cutoff frequency of their hearing loss. In the scanner, four frequencies (1kHz, 2kHz, 5.6kHz and each subject's cut-off frequency) were presented unilaterally to both ears at the lowest level (30dB Loudness Equivalent to 1kHz). Stimuli were presented with TDT system controlling filters and fMRI compatible Etymotics headphones.

Procedure: During image acquisition, a visual prompt on the screen instructed participants to judge the loudness of the presented sound. They had 2.5 seconds to provide a

response before the next trial began. The sparse sampling paradigm was used, so that stimuli were presented in relative silence. Scanning was performed on a 3-T Magnetom Trio (Siemens) magnetic resonance imaging scanner.
Image flips: Brain images for the three right-sided tinnitus subjects were flipped so that all subjects were aligned according to tinnitus side (left = ipsilateral, right = contralateral).
RESULTS
Brain activation of the auditory cortices was clearly visible both ipsi- and contra- laterally for stimulation of both ipsilateral and contralateral ear at all frequencies. Patterns of activation were, however, abnormal in tinnitus participants.
CONCLUSIONS
Our findings will be discussed further with respect to the controversy of side of brain activation in tinnitus, that is, whether activity is ipsilateral or contralateral to the side of tinnitus.

881 Influence of Instantaneous-Frequency Fluctuations on Frequency Discrimination: Implications for the Perception of Temporal Fine Structure

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In the context of the recent debate about the role of temporal fine structure (TFS) in pitch and speech perception, it may prove important to address basic questions regarding how the auditory system processes frequency information. It is widely believed that low-frequency (< 4 kHz) pure-tone frequency discrimination (FD) relies on temporal fine-structure (phase-locking) rather than spectral (tonotopic) cues. Consequently, it may be expected that FD performance should be adversely affected by relatively rapid fluctuations in instantaneous frequency (IF) over time. We tested this hypothesis by measuring thresholds for the discrimination of shifts in the carrier frequency of quasi-frequency-modulated (QFM) tones at rates ranging from 1 to 100 Hz, and for carrier frequencies of 500, 2000, and 6000 Hz. The results revealed no substantial effect of QFM on thresholds, regardless of rate, relative to unmodulated or sinusoidally amplitude-modulated tones. These results suggest that, if FD is based on TFS information, the underlying mechanism is surprisingly immune to IF fluctuations, over a wide range of rates. To determine the minimum depth at which FD starts to be significantly affected by IF fluctuations, a second experiment measured FD thresholds for frequency-modulated (FM) tones over a wide range of depths (1-100%) and rates (1-100 Hz). The results revealed that, for relatively slow FM rates (<10 Hz), thresholds increased as FM depth increased beyond about 10% (i.e., $+/f${5%) of the carrier frequency. For higher FM rates, however, thresholds were largely unaffected by FM, even at large depths (50%). This suggests that whatever mechanism is responsible for accurate FD performance, it is surprisingly insensitive to rapid fluctuations in TFS. [Work supported by NIDCD R01DC05216.]

882 Investigation of Synergistic Interactions for Temporal Envelope and Temporal Fine Structure Cues in Speech Sounds

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Temporal Fine Structure (TFS) and Temporal Envelope (E) cues alone provide sufficient information for accurate speech identification in quiet. However, the importance of such cues in each frequency band remains unclear, as well as the synergistic and redundancy effects between bands and across cues (E and TFS). These issues are addressed here by presenting for identification to normal-hearing listeners bandpass filtered intact, TFS- and E-vocoded speech stimuli. Intact and vocoded speech stimuli are split into 5 frequency bands, spanning the range 80-5000 Hz. The importance of each frequency band is assessed by measuring identification performance independently for each band. Synergistic and redundancy effects are assessed by measuring identification performance for two bands out of five, the two bands conveying either the same or different temporal information (E or TFS). All speech stimuli are presented in a complementary speech-shaped noise masker. Each listener is presented with all possible combinations of bands for each stimulus type (TFS or E speech) and between stimulus types. Pilot data suggest that synergistic interactions exist for E and TFS in the mid-frequency range (800 – 2000 Hz).

883 Single-Fiber Population Modeling of Schroeder Phase Discrimination: A Comparison to Psychophysical Results

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Although speech perception with cochlear implants has been steadily improving over time, we still lack a detailed understanding of how the auditory neurons capture the information carried in the stimulating waveform. Towards this goal, we have developed a stochastic, biophysically-validated model of the feline auditory nerve and matched its probabilistic and temporal response properties to those measured *in vivo*. In addition to replicating the behavior of singular fibers, we have also matched the response characteristics of diameter—distributed model fibers to the response characteristics of an *in vivo* population. This ability to realistically model firing behavior of a population of fibers allows us to examine how much of the information in the stimulus is captured by the neural responses.
Temporal fine structure (TFS) of a sound has been shown to be an important factor in sound source segregation and localization, as well as in perception of music and tonal languages. To gauge the sensitivity of auditory neurons to changes in TFS, as well as to validate our biophysical model, we examined the differences in neural responses of the model. As an input, we used pulsatile or analog

sound encodings of single-channel positive and negative Schroeder phase harmonic complexes. Our hypothesis is that the amount of information in the spiking behavior of a simulated neural population is related to cochlear implantees' performance on a psychophysical discrimination task described in Won, *et al.* (ARO 2009). Preliminary results support our hypothesis, with modeled spiking behavior exhibiting the same trends as those observed in psychophysical testing.

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Keywords: temporal fine structure, modeling, cochlear implant

884 Single-Channel Schroeder-Phase Discrimination as a Measure of Within-Channel Temporal Fine-Structure Sensitivity

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Cochlear implant (CI) sound processing strategies in clinical use are variations of temporal envelope-based, interleaved pulsatile strategies. A limited amount of low-frequency acoustic temporal fine-structure (TFS) information can be delivered through the electrical envelope modulation. The TFS is important for speech recognition in noise and music perception; and consequently, many attempts are being made to represent the TFS through sound processors. Positive and negative Schroeder-phase stimuli have similar envelopes but differ in their TFS. Previous work (Drennnan et al., JARO, 2008) has shown that CI listeners can use across-channel timing differences in the temporal envelopes to discriminate Schroeder-phase stimuli. The Schroeder-phase test might also be employed with one-channel maps to access within-channel TFS sensitivity. If a processing strategy improves within-channel TFS encoding, the single-channel Schroeder-phase test should show it. In order to test this hypothesis, the CIS (Continuous Interleaved Sampling) and SAS (Simultaneous Analog Stimulation) strategies were evaluated. Five subjects were tested using one-channel CIS and SAS maps taking broadband input on electrode 1 (apical) or 4. On both electrodes, the single-channel Schroeder-phase discrimination ability was significantly better with SAS. Subjects were also tested with their 16-channel clinical strategies (HiResolution). The single-channel Schroeder-phase test scores using SAS were comparable to the scores obtained with the multi-channel clinical strategies. The results suggest that the single-channel Schroeder-phase discrimination test can be used to evaluate future sound processing strategies which attempt to improve the within-channel TFS encoding. The results also suggest that with current clinical strategies similar to CIS, TFS cues are not accessible. [Supported by NIH grants R01-DC007525, P30-DC04661, and Advanced Bionics Corporation.]

885 Evidence Suggesting That Spectral Rather Than Time-Interval Information Underlies Pitch Perception in Low-Frequency Pure Tones

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It is commonly believed that pitch perception in low-frequency pure tones (below 4 kHz) is based on a time-interval analysis of the phase-locked temporal information in the auditory–nerve firing patterns, rather than on the distribution of activity along the tonotopic array (internal spectrum). This study aimed to test this hypothesis, by measuring frequency discrimination and frequency modulation (FM) detection for a 1-kHz pure tone, which was partially masked by a lowpass- or highpass-filtered noise to obscure the apical or basal flanks of the tone's internal spectrum, respectively. In the first experiment, the frequency discrimination threshold for the tone was measured as a function of its sensation level (SL). The maskers were presented at a level of 60 dB SPL per equivalent rectangular bandwidth (ERB) in this experiment. In the second experiment, the FM detection threshold was measured for two modulation rates (2 and 10 Hz). In this experiment, the tone was always presented at 10 dB SL and the masker level was either 40 or 70 dB SPL per ERB. At higher levels, the tone's internal spectrum becomes asymmetric, with a shallower basal and a steeper apical flank. In both experiments, frequency discrimination performance was consistent with this asymmetry and its dependency on level, in that, at high levels, frequency discrimination performance was weaker in the lowpass than the highpass condition. This strongly suggests that performance in all tasks was based on spectral information, because time interval-based information would be expected to be independent of the shape of the internal spectrum. To verify this conclusion, the 2-Hz FM detection threshold was re-measured in a dichotic condition, where the modulated tone was replaced by a static tone in one ear, so that FM detection would be based on interaural temporal cues. As expected, there was no difference in dichotic FM detection between the lowpass and highpass masking conditions, even at the highest levels.

886 Tuning Properties of the Auditory Frequency-Shift Detectors

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Demany and Ramos (J. Acoust. Soc. Am., 2005) found that it is possible to hear an upward or downward pitch change between two successive pure tones differing in frequency even when the first tone is informationally masked by other tones, preventing a conscious perception of its pitch. This phenomenon provides evidence for the existence of automatic frequency-shift detectors (FSDs) in the human auditory system. The essential function of the

FSDs may be to create perceptual links between successive sounds which, due to their spectral proximity, are likely to emanate from the same acoustic source. The aim of the present study was to measure the size of the frequency shifts optimally detected by the FSDs. In experiment 1, subjects were presented with sound sequences consisting of: (1) a 300-ms random "chord" of six synchronous pure tones, separated by intervals of 650 cents (for some chords) or 1000 cents (for other chords); (2) a 500-ms inter-stimulus interval (ISI); (3) a single pure tone, randomly positioned 50, 100, 150, 200, 250 or 300 cents above or below a randomly selected component of the chord. The task was to indicate if the final pure tone was higher or lower in pitch than the closest component of the chord. For the two types of chords, performance was optimal when the frequency shift amounted to 100-150 cents. Experiment 2 was similar except that here the chords had a duration of 100 ms, their components were always separated by 1000-cent intervals, and the ISI could be equal to 100, 250, or 900 ms. When the ISI was 100 or 250 ms, performance was again optimal for frequency shifts of 100-150 cents. When the ISI was 900 ms, performance was less dependent on the magnitude of the frequency shifts. Overall, these results suggest that the FSDs are optimally sensitive to frequency shifts of 100-150 cents.

887 Are Efferents Involved in Masking by Schroeder-Phase Complexes?

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The amount of masking produced by a harmonic complex depends, among other things, on its phase curvature. The effects of masker phase curvature have been attributed to the phase response and compression of the basilar membrane (BM) response. Comparisons of simultaneous on- and off-frequency harmonic maskers have produced results consistent with expectations based on the phase response of the BM at and below characteristic frequency (CF). This study tests the prediction that the effects of compression on forward masking should be limited to masker frequencies around the CF, and should be absent for maskers well below the signal frequency. Results with a 150-ms harmonic masker and signal frequencies of 1 and 2 kHz were broadly consistent with this prediction. However, for a 6-kHz signal, masker phase curvature continued to affect forward masked thresholds, even when all the masker components were well below the signal frequency. The result is unlikely to be due to the middle ear reflex, as that would be more likely to affect low, not high, frequencies. The possible role of the medial olivocochlear efferent system was tested using 20-ms maskers, which were less likely to stimulate the efferents. Under these conditions, the effect of the phase curvature diminished or disappeared for both on- and off-frequency maskers. A model of efferent activation, including time courses derived from physiological data was able to account for the trends in the data by assuming that masker waveforms with flatter internal envelopes lead to stronger efferent activation. The data and model predictions suggest that the results cannot be accounted for by "static" compression but may be consistent with the temporal dynamics of the efferent system. [Supported by NIH grant R01DC03909].

888 The Effect of Precursor Duration and Signal Delay on Behavioral Estimates of Cochlear Gain

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In a series of experiments, our laboratory has shown that the temporal effect in simultaneous masking is consistent with a decrease in gain, possibly mediated by the medial olivocochlear reflex (MOCR). In simultaneous masking, the temporal effect is a decrease in threshold signal-to-masker ratio for a signal at masker onset when a precursor is added. This work has been extended to a forward-masking paradigm. A growth of masking (GOM) function is measured with a short-duration off-frequency masker (which should not activate the MOCR), and a long-duration precursor which is intended to activate the MOCR. The estimated input-output function is compared for a precursor well below the signal frequency and a precursor at the signal frequency. In a recent study, we found that a precursor at the signal frequency decreased the gain of the input-output function. Short and long duration precursors caused the same reduction in gain, suggesting that delay from precursor onset rather than duration should be considered. In the present study, precursor duration and delay from precursor offset to signal onset were manipulated independently. Results will be interpreted in terms of physiological data on the time course of the MOCR, as well as previous data from simultaneous masking experiments.
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889 Forward Masking with Reproducible Noise Samples

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We utilized a molecular psychophysical approach to evaluate individual differences in performance on a forward masking task using reproducible noise samples. The task was to detect a 10-ms, 500-Hz sinusoid with 5-ms onset and offset ramps presented 10 ms after one of 25 reproducible broadband noise samples. The noise samples were presented alone or followed by the signal. Data were collected at a single presentation level with the S/N for each subject fixed to maintain P(C)=70%.
As expected, there was substantial within and across subject variability in the responses to the individual noise samples on both NA and S+N trials.
A two-parameter, single-channel electrical analog model (EAM) [Jeffress, 1967,1968; Gilkey & Robinson, 1986] explained only 19-40% of the variance in the individual subject responses. Predicted variance is substantially less than that found in model results to a simultaneous masking task. Model results demonstrate best-fitting bandwidths that are wider for the forward masking task than seen in

simultaneous masking tasks. Implications and results from other models will be discussed. Supported by the AAO-HNS/AHRF Wiley-Harrison award.

890 Modeling the Masking of Low Frequency Signals by High Frequency, High Level Narrow Bands of Noise

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Low frequency masking by intense high frequency noise bands has been referred to as remote masking. Remote masking cannot be explained by the traditional power spectrum model. Nor can it be explained by the partial loudness model (PLM) of masking (Moore, Glasberg, & Baer, 1997). Remote masking is thought to result from a cochlear saturating nonlinearity (Bilger, 1958; Karlovich & Osier, 1979). However, the mechanism is unclear. Masking was measured in five normal hearing young adults at 250, 350, 500, and 700 Hz using equal power Gaussian narrow band (GNBN) and low-noise noise (LNN) maskers. Masking was also measured using equal power two-tone (TC2) and eight-tone (TC8) maskers. Maskers were centered at 3000 Hz and bandwidths were 1 or 2 ERBs. Masker levels varied from 80 to 95 dB SPL in 5 dB steps. Results indicated that LNN produced negligible masking in all conditions. An increase in bandwidth in GNBN yielded significantly greater masking over a wider frequency region. The PLM cannot account for such differences since it ignores the masker's temporal properties. Masking for TC2 was limited to 350 and 700 Hz for 1ERB, but shifted to only 700 Hz for 2 ERB. A spread of masking to 500 and 700 Hz was observed for TC8 when the bandwidth was increased from 1 to 2 ERBs. The spread of excitation cannot account for such data. Results were modeled using two versions of a cochlear saturating nonlinearity model, the masker envelope detection model (MEDM) and the quadratic distortion products model (QDPM). Both assume that the cochlea introduces low frequency distortion to its input due to its saturating nonlinearity. The MDEM assumes that the nonlinearity is realized by extracting the masker envelope whereas the ODPM assumes that the nonlinearity is realized by a quadratic process. The advantages and limitations of the models will be discussed.

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891 Impact of Sloping High Frequency Hearing Loss on the Temporal Resolution in Low Frequency Region and Potential Association with Speech Perception Difficulty

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Deterioration in temporal resolution in hearing impaired (HI) subjects has been confirmed by many studies. However, it is not entirely clear if high-frequency hearing loss (HFHL) may result in an off-channel impact on temporal resolution in low-frequency region where the hearing threshold is virtually normal. In this study, we evaluated the temporal resolution in the low frequency region in subjects with sloping HFHL. They were grouped based upon the high-frequency cutoff of their hearing with normal threshold. The temporal resolution was evaluated in both gap- and amplitude-modulation (AM) detection-tasks. The impact of temporal resolution on speech perception in noise was also evaluated in this study using Chinese version of hearing-in-noise-test (HINT) program with different time compression ratios.

The deterioration of temporal resolution in normal threshold, low frequency channels in subjects with HFHL was demonstrated by the poorer performance in both gap- and AM-detection tasks comparing to normal hearing (NH) subjects with the matched age. These results suggest an off-channel impact of HFHL on temporal processing in the low frequency region in which the hearing threshold is normal. Both NH and HI subjects performed more poorly in HINT with increasing time-compressing ratio (speech sped-up), suggesting the role of temporal resolution deterioration and probably other factors for the difficulty of the speech perception in noise in these subjects. Potential correlation between the speech-perception performance and these of the two temporal processing tasks was evaluated and discussed.

892 Consonant Identification in Noise With and Without Spectro-Temporal Enhancement by Normal Hearing and Hearing Impaired Listeners

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Difficulty understanding speech in noisy environments is the most common complaint of listeners with sensorineural hearing loss despite appropriate amplification. Previous laboratory studies and commercial implementations of signal processing strategies designed to enhance speech in noise have met with limited success, while benchmark studies involving manual manipulation of individual phonemes demonstrate the potential for statistically and clinically significant improvement with speech enhancement. Our previous investigations using a novel spectro-temporal enhancement strategy have demonstrated significant improvement in vowel and monosyllabic word identification in noise by normal hearing listeners and consonant identification in noise by normal-hearing and a small group of hearing-impaired subjects. The present study demonstrates similar improvement in consonant identification in noise with larger groups of listeners with normal hearing and with moderate to moderately severe sensorineural hearing loss. These statistically and clinically significant changes in speech perception in noise are considered in the context of corresponding acoustic changes and alterations in phonemic error patterns. Supported by NIH/NIA AG09524 and the ICHSR.

893 Developmental Profile of Ionotropic Glutamate Receptors in Nucleus Laminaris of the Chick Brainstem

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Glutamatergic transmission from nucleus magnocellularis (NM) to nucleus laminaris (NL) is essential for binaural processing but it is unclear how ionotropic glutamate receptors (iGluRs) in NL develop and the functional role they play in synaptic refinement. We investigated the development of excitatory postsynaptic currents (EPSCs) mediated by iGluRs in chicken embryos. Using whole-cell voltage clamp recordings from NL while electrically stimulating excitatory inputs from NM, we show that EPSCs are mediated by both AMPA- and NMDA-type glutamate receptors during a period when hearing is first emerging and dendritic morphology is being established (embryonic days 13-17). Evoked activity contained dual-component EPSCs recorded at -60 mV in Mg^{2+} free artificial cerebral spinal fluid (ACSF) or while voltage-clamping cells at +40 mV in normal ACSF. Dual component EPSCs were pharmacologically separated into an early AMPA-R mediated response and a late component that was dominated by the NMDA-R. The amplitude of AMPA-R EPSCs and the amount of inward rectification significantly increased while its decay kinetics became more rapid with development. Similarly, the amplitude of NMDA-R EPSCs increased 2-fold with the time to half decay becoming significantly faster during the same developmental period. Our results show that as early as synaptic transmission could be recorded, EPSCs are mediated by both AMPA- and NMDA-Rs. Increases in the amplitude of evoked iGluRs EPSCs suggests possible changes in receptor density at a given NL synapse or a developmental increase in the number of functional synapses containing both receptors. The kinetic changes seen in both receptors and increased rectification of the AMPA-R EPSCs suggest maturational changes in subunit composition. How biophysical properties of developing iGluRs influence the structure and function in NL will be the focus of future investigations. Support by DC03829

894 Adaptation of Spike Timing Precision in the Chick Nucleus Magnocellularis Reduces ITD Sensitivity of Nucleus Laminaris Neurons

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At a cellular level, adaptation is commonly defined as a decrease in firing rate in response to a constant stimulus. However, in the interaural time difference (ITD) detection pathway information about the sound location is transmitted in the timing of individual spikes rather than in the firing rates. We previously observed slow adaptation (Ad_{slo}) *in vitro* in Nucleus Magnocellularis (NM), which contains first-order central neurons of the ITD detection circuit. Ad_{slo} manifests as a slow increase in firing rate accompanied by an increase in spike jitter that is mediated by slow inactivation of Kv1 channels. In this study we investigated the effect of Ad_{slo} on ITD processing *in vitro* using the chick brain slice preparation (E21- P1). Ad_{slo} was elicited by simulated 8th nerve synaptic inputs phase-locked to 1000 Hz in dynamic clamp. We found that the dominant effect of adaptation to such input was an ~10% increase in spike jitter. We hypothesized that Ad_{slo} reduces ITD sensitivity at the next level of processing in Nucleus Laminaris (NL). NL neurons modulate their firing rate as a function of ITD, by detecting simultaneous arrival of inputs from the ipsilateral and contralateral NM. A decrease in spike timing precision caused by Ad_{slo} could desynchronize NM inputs across NL and reduce its firing rate modulation by ITD. We tested this hypothesis by measuring the firing rate of NL neurons in response to simulated synaptic inputs with different ITDs. The inputs to NL were generated based on the statistics of the NM responses before and after adaptation. We found that the ~10% increase in the NM spike jitter caused a greater reduction of NL firing rate as ITD became shorter. This decreased the modulation of firing rate with ITD by ~25%. Thus, Ad_{slo} may reduce firing rate modulation by ITD in NL during a long lasting sound, tuning out background inputs. This process may allow for better localization of novel stimuli.

895 Analysis and Simulation of the Neurophonic Potential in the Laminar Nucleus of the Barn Owl

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It is a challenge to understand how the brain represents temporal events. One of the most intriguing questions is how sub-millisecond representations can be achieved despite the large temporal variations at all levels of processing. For example, the neurophonic potential, a frequency-following potential occurring in the network formed by nucleus magnocellularis and nucleus laminaris in the brainstem of the bird, has a temporal precision below 100 microseconds.

Here we address the question of how the neurophonic potential is generated and how its remarkable temporal precision is achieved, using a theoretical model. The neurophonic potential consists of at least three spectral components, and our studies aim at revealing their origin. Our hypothesis is that magnocellular axons are the origin of high-frequency component of the neurophonic, whereas action potentials in the laminar neurons are the origin of the 1-2 kHz component. We present an advanced analysis of in-vivo data, numerical simulations of the neurophonic, and analytical results to test this hypothesis. The analysis of the signal-to-noise ratio of the high frequency component of the neurophonic potential lets us estimate the number of independent sources to be at least 250, further implicating the magnocellular axons and indicating that the laminaris neurons alone can not be the source of neurophonic potential.

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896 Bilateral Matching of Frequency Tuning in Neural Cross-Correlators of the Owl

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Sound localization requires comparison between the inputs to the left and right ears. One important aspect of this comparison is the differences in arrival time to each side, also called interaural time difference (ITD). The process of detecting and encoding ITD has been compared to an effective cross-correlation between the input signals to the two ears. Because the cochlea performs a spectrotemporal decomposition of the input signal, this cross-correlation takes place over narrow frequency bands. Since the cochlear tonotopy is arranged in series, sounds of different frequencies will trigger neural activity at different times. Thus, the matching of the frequency tuning of the left and right inputs to the cross-correlator units becomes a 'timing' issue. Here we studied the owl's nucleus laminaris, the equivalent to the medial superior olive of mammals, which is the site where ITD is detected. We used reverse correlation analysis and stimulation with uncorrelated sounds to extract the effective monaural inputs to the cross-correlator neurons. We show that the spectra of left and right inputs are matched. This is consistent with predictions made by the classic model put forth by Jeffress (1948).

897 Phase Shifts in Monaural Field Potentials of the Medial Superior Olive

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Jeffress (1948) proposed that external interaural time differences (ITDs) are compensated by internal, axonal delays allowing ITD to be represented by a population of coincidence detectors in the medial superior olive (MSO). The MSO shows a strong extracellular field potential, the neurophonic (NP), consisting of a slow onset response superimposed on a sustained response locked to the stimulus. Studies in the barn owl reported a phase shift in the NP along the nc. laminaris and concluded that this phase shift is consistent with an axonal delay line. Our goal is to study whether the NP in mammals provides clues regarding the origin of internal delays.

We recorded the NP in the MSO of the cat at various locations along its dendritic axis, using glass-insulated tungsten electrodes in a ventral approach, while presenting monaural contra- or ipsilateral tones at different frequencies. The NP was tuned to low frequencies with maxima at different spatial locations for ipsi- and contralateral sounds, consistent with earlier reports. A phase shift of about 0.5 cycles was observed at depths close to the amplitude maxima, sometimes accompanied by localized amplitude minima. Current source density analysis for contralateral (ipsilateral) stimulation shows a current source close to the contralateral (ipsilateral) NP amplitude maximum and a sink 200 microns ventromedially (dorsolaterally).

These results indicate that the NP can be interpreted in terms of a dipole. Contralateral (ipsilateral) excitation causes a current sink at the ventromedial (dorsolateral) dendrites and a source at the soma and dorsolateral (ventromedial) dendrites. The difference in phase at the sink and source is 0.5 cycles. This interpretation raises the question whether the NP phase shift reported in the barn owl reflects axonal delays or simply a nc. laminaris dipole configuration.

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898 Best Interaural Time Delay Is Invariant to Best Frequency in the Gerbil Medial Superior Olive

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The two current competing models of the encoding of interaural time delay (ITD) of low-frequency sound in the medial superior olive (MSO) generate different predictions about the relationship between the ITD that elicits the strongest response (best delay: BD) and the best frequency (BF) of a cell. The place code model predicts that the distribution of BD is invariant to BF, yet largely confined to the physiological range of delays experienced by the animal. The slope code model states that BD increases as BF decreases, placing the slope of the ITD tuning function within the physiological range of delays. To test these hypotheses we recorded extracellularly from 50 single units in the MSO of Nembutal/ketamine-anesthetized adult gerbils in response to calibrated, dichotic pure tones. Pure tones generate periodic ITD tuning functions, therefore the resultant ambiguity in determining which tuning function peak corresponds to the BD was resolved by constructing a composite of responses to frequencies at and near the BF. The resulting distribution of BDs was invariant to BF, inconsistent with the slope code model. However, inconsistent with the place code model, the range of observed BDs was large with a majority occurring outside the physiological range of delays (mean BD = 223 μs, favoring contralateral-leading delays). This led to the surprising result that at higher frequencies, the BD was often not the peak closest to zero ITD. When all BDs were period-wrapped to identify the peaks closest to zero ITD, peaks below 1 kHz were overwhelmingly contralateral-leading, while those above 1 kHz were split between ipsilateral- and contralateral-leading. This is inconsistent with prior studies of the inferior colliculus where, in agreement with a slope code, peaks have been reported both to vary systematically with BF, as well as to be overwhelmingly contralateral-leading at all frequencies.

899 Establishing the Synaptic Origin of ITD Sensitivity in Two Species with Different Hearing Ranges

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Binaural cues for sound localization are first assessed in the Superior Olivary Complex (SOC) at the level of the

auditory brainstem in the Medial and Lateral Superior Olive (MSO and LSO, respectively). These auditory nuclei are required to process very fast and precisely-timed inputs; achieving this high fidelity requires specialization of both cell morphology and physiology. To this end, MSO and LSO neurons have extremely fast membrane time constants compared to other neurons in the central nervous system, and it is likely this characteristic, combined with a specialised subcellular distribution of their synaptic inputs, underpins sensitivity to the binaural cues of interaural time and level differences (ITDs and ILDs respectively). Whereas it is well known that MSO neurons are sensitive to ITDs, *in vivo* studies have shown that LSO neurons also display ITD sensitivity (in the envelope of amplitude modulated stimuli), suggesting a continuum across the SOC that caters for binaural localization. We suggest such a functional continuum is reflected in the specific synaptic organization across the SOC complex, such that the pattern of synaptic sites found in principal neurons from the low-frequency regions of the LSO may more closely resemble those in the MSO. Furthermore mammalian species with hearing extending into the high-frequency range may utlilize this region of the LSO more extensively for ITD discrimination than in lower frequency-hearing animals and this may be evident in their synaptic distributions. We have combined immunohistochemistry and electrophysiology on guinea pig and rat brainstem slice preparation to explore the subcellular localization of inhibitory synapses. In addition, to assess the distribution of ITD sensitivity across the SOC nuclei we are employing *in vitro* calcium imaging techniques which allow us to monitor the activity of multiple MSO and LSO neurons in response to electrical stimulation of the trapezoid body fibers.

900 A Frequency-Dependence in Both the Acoustic Interaural Time Difference Cues to Sound Location and Their Encoding by Neurons in the Inferior Colliculus

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Interaural time differences (ITDs) are a primary acoustical cue for the spatial location of low-frequency sounds in mammals. The chinchilla (*Chinchilla laniger*) is a good model to study the space- and frequency-dependence of ITDs as an acoustical cue and the neural mechanisms by which ITDs are encoded due to its human-like sensitivity to low frequencies and the similarities in the organization of the ascending auditory system relative to other species used for such research. Here we examined low frequency ITDs with sinusoidal stimuli from 250 Hz to 4 kHz. The ITD cue exhibited a systematic dependence on frequency being ~42% larger for the lowest frequencies tested (250 Hz) than for the highest (4 kHz). All ITDs were significantly larger than those predicted from a variety of common spherical head models. We also studied ITD sensitivity in 54 low characteristic frequency (CF) (< 5 kHz) neurons in the central nucleus of the inferior colliculus (ICC) in the adult chinchilla to both noise and tone stimuli. The best (maximum rate response) ITDs exhibited a significant dependence on neural CF favoring larger ipsilateral delays with decreasing CF. The best ITDs increased smoothly and systematically from ~190 μs for CFs > ~1500 Hz to ~850 μs for CFs of ~180 Hz. Even with the demonstrated frequency-dependence of the physical ITD cue in chinchilla, many low-CF ICC neurons still had their maximum response for ITDs that were well outside the "physiological range" of ITDs. This pattern of results – frequency-dependence of both ITDs and their encoding - agrees well with that observed in other species with good low-frequency hearing (cat, gerbil, guinea pig, etc). However, the absolute range of ITD cues and their encoding by neurons is not consistent across species and frequency. Across-species comparison suggests that the range of physical ITD cues an organism experiences must have a major influence on the mechanisms by which the ITD cues are centrally encoded.

901 Spatial Selectivity of Neurons in the Gerbil Inferior Colliculus: Effects of Natural and Unnatural Sound Reflections by the Ground

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In an ecologically realistic environment, sounds arriving at ears include sounds reflected from the ground as well as direct sound. The interference of the direct and reflected sounds has a comb-filtering effect, which imposes spectral notches on the spectral envelope of the sound in the ear that is addition to the head-related notches. Ground-related and head-related notches are both dependent on sound source direction. We investigated the effects of ground-related spectral notches on the auditory spatial selectivity of neurons in the external nucleus of the inferior colliculus (ICx) of anesthetized gerbils. Single-unit responses were recorded from the ICx at two stimulus levels (10-15 dB and 30-40 dB above the unit's threshold). The virtual acoustic space technique was used to simulate a white noise burst presented from various directions in the space. The following three conditions were tested. The *free-field* condition simulated stimuli presented in a free field, i.e., only the head-related notches were imposed. In the *normal-notch* condition, source direction dependent ground-related notches were added, which simulated a situation where the gerbil is standing on sandy ground. In the *random-notch* condition, the association between the sound direction and corresponding ground-related notch pattern was randomized. Most of ICx units exhibited spatial tuning to a specific direction in the contralateral hemisphere under all three conditions. The spatial selectivity of ICx units measured in the normal-notch condition was significantly higher than that in the free-field condition; the difference between the conditions was greater at the lower sound level. However, this sharpening effect was not observed for the random-notch condition. The results suggest that the ICx neurons are adapted to spectral notches that are naturally induced by reflected sounds from the ground, which might contribute to accurate sound localization by gerbils living in a natural environment.

902 Adaptive Coding for Auditory Spatial Cues in the Auditory Midbrain

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Adaptation of sensory neurons to the prevailing environment is a widely-accepted phenomenon thought to underlie improved sensory coding of relevant stimulus features. Previously, we reported that neurons in the inferior colliculus (IC) of anaesthetised guinea pigs adapt to statistical distributions of interaural time differences (ITDs), a binaural cue used for localization of sound sources in azimuth. ITDs are particularly suited to examining the site at which adaptive mechanisms arise in the central nervous system, as contributions from mechanisms below the level of binaural integration can be excluded. Here, we modelled IC responses to statistical distributions of ITDs containing a high probability (80%) range (HPR), with the remaining ITDs presented with 20% probability; all ITDs were restricted to the range ±330μs. As with the physiological experiments, either the centre or the width of the HPR was altered systematically. Rate responses of medial superior olive neurons (assumed to be instantaneous ITD detectors) were modelled with Gaussian-shaped ITD-functions. Assuming a population of similarly-tuned independent Poisson neurons inputting to an IC neuron, we used an established model of synaptic depression to compute the IC postsynaptic current. The model predicts the changes in neural gain and the shapes of rate-ITD functions observed physiologically, including the observation that IC neurons adapt their responses to changes in the mean, but not the variance, of the underlying statistical distribution of ITDs. Including a stochastic leaky integrate-and-fire mechanism generating IC spiking, the model can replicate shifts observed in discharge rates and Fisher Information, a measure of coding accuracy, in IC neurons.

903 Modeling the Responses of Inferior Colliculus Cells to Binaural Clicks

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A model was developed to simulate the responses of low-frequency inferior colliculus (IC) neurons to interaural time differences (ITDs) of broadband clicks. The model incorporates existing models for auditory-nerve fibers, cochlear nucleus bushy cells and cells in the medial superior olive (MSO). The IC model neurons receive excitatory inputs from the ipsilateral MSO, as well as late long-lasting inhibition from both ipsilateral and contralateral MSO. The IC model neurons display sensitivity to ITDs of single binaural clicks. The maximal firing rate occurs for ITDs (best ITD of that neuron) to the contralateral side, consistent with the fact that most IC neurons prefer sources in the contralateral field. For single binaural clicks with very large ITDs, the long-lasting inhibition evoked by the leading click on one side produces suppression of responses to the click on the other side, matching measured physiological behavior. The long-lasting inhibition also affects responses to a pair of binaural clicks separated by an inter-stimulus delay (ISD) between the lead and lag clicks. For some IC neurons, the lagging responses are suppressed most when the leading stimuli are located at the cell's best ITD, whereas other IC neurons show the greatest reduction of the lagging response when the lead ITD produces the lowest response on its own. The physiological responses of these two types of neurons were simulated by adjusting only the relative strength of ispilateral and contralateral inhibition. For both types of IC model neurons, the suppressive effect decreases as ISD grows. When the ISD equals 1 ms, the lagging response is totally suppressed. As ISD increases to 30 ms, the lagging response recovers completely. The time course of the inhibition matches that of perceptual effects seen in the phenomenon of the precedence effect, whereby a single fused sound is perceived from the location of the leading source when two sounds arrive at the ears in close temporal proximity.

904 Spatial Tuning in the Owl's Auditory Thalamus

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The barn owl's midbrain contains a map of auditory space. The forebrain also has neurons sensitive to spatial cues, although no topographic representation has been found in this area. The spatial receptive fields of these space-specific neurons result from sensitivity to combinations of interaural time (ITD) and intensity (ILD) differences over a broad frequency range. The neural pathways that lead to the forebrain and midbrain representations of auditory space separate before spatial receptive fields are fully formed. The first nuclei that belong exclusively to the forebrain and midbrain pathways are the thalamic nucleus ovoidalis (Ov) and the external nucleus of the inferior colliculus (ICx), respectively. Both receive projections from the same midbrain nucleus, the lateral shell subdivision of the inferior colliculus, but are not interconnected. To address the question of auditory space coding in the forebrain vs. the midbrain, we compared the spatial tuning of ICx and Ov neurons and the underlying tuning to ITD and ILD.

Single units (42 in Ov and 19 in ICx) were recorded with tungsten electrodes in anesthetized barn owls. We restricted our analyses to thalamic neurons that were broadly tuned to frequency. For each Ov and ICx neuron, we measured the ITD and ILD sensitivity using headphones. We then removed the headphones and evaluated the tuning to auditory space with a calibrated 144-speaker array.

We found that ITD and ILD tuning curves are significantly broader in Ov. In addition, the selectivity to ITD and ILD of Ov neurons varies more with frequency. Also, Ov neurons are tuned to a broader frequency range. However, some Ov neurons have spatial tuning as sharp as in ICx and their distribution is also restricted to the frontal and contralateral space. Our results are consistent with the forebrain using a similar mechanism for auditory spatial tuning as described in the midbrain, but integrating ITD and ILD over a broader frequency range.

905 Spatial Selectivity in Cortical Area PAF in Awake Behaving Cats

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Several lines of evidence suggest that cortical area PAF plays a special role in sound localization. Inactivation of PAF produces deficits in sound localization behavior in cats[1]. Electrophysiological studies in anesthetized cats show that PAF neurons have higher spatial sensitivity than neurons in A1, with greater location-dependent modulation of spike latency and spike rate[2]. In the current study we investigated the spatial sensitivity of PAF neurons while cats were engaged in various auditory tasks. Broadband noise bursts, 80 ms in duration, were presented from free field speakers in the horizontal plane, spaced in 20 degree increments of azimuth. Generally, awake PAF neurons were more spatially selective than awake A1 or anesthetized PAF neurons. Tonic responses for preferred locations are commonly seen, including, in some cases, tonic inhibition of spontaneous activity. Moreover, the best spatial areas of PAF neurons were distributed more uniformly throughout space than has been observed in A1 or other auditory areas. We compared neuronal responses under three behavioral conditions: 1) Idle: cats were exposed to probe stimuli without engaging in behavior tasks. 2) Discrimination: cats detected a change from the probe stimulus to a click train, regardless of the location of the sound. 3) Localization: cats distinguished a shift in stimulus elevation to 40 degree above horizontal plane, regardless of azimuth. Responses with excitatory or inhibitory spike patterns both were modulated by behavioral conditions. In general, spatial selectivity sharpened and response pattern became more tonic when the animal performed the localization task. Our results further support the hypothesis that area PAF is a key element in the brain mechanism for sound localization.

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1)Lomber & Malhotra, *Nat. Neurosci.* 11**,** 2008. 2)Stecker, Mickery, Macpherson, Middlebrooks, *J Neurophysiol*, 89, 2003

906 Effect of Manipulating Cortical Cholinergic Input on Sound Localization and Experience-Dependent Adaptive Plasticity in Adult Ferrets

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Cortical cholinergic modulation has been implicated in a number of processes, including working memory and adaptive plasticity. In primary auditory cortex (A1), stimulus-specific plasticity can be induced by pairing tone presentation with electrical stimulation of the nucleus basalis, its main source of cholinergic input. The aim of the present work was to investigate the role of the neuromodulator acetylcholine (ACh) in perception (sound localization in azimuth) and plasticity (adaptation to unilateral ear plugging). The auditory cortex was deprived of ACh either by intraventricular infusion of a neurotoxin (ME20.4-SAP) specific for basal forebrain cholinergic cells, or by bilateral implantation of slow-release polymer (Elvax) slices containing the muscarinic receptor antagonist scopolamine over A1. Ferrets were trained to localize broadband noise of differing durations (40-2000 ms) and levels (56-84 dB SPL). Their ability to adapt to the altered spatial cues produced by occluding one ear was also tested. Cholinergic lesions were assessed in brain sections using choline acetyltransferase and p75NTR immunocytochemistry.

Initial data showed sound localization performance to be unaffected by either neurotoxin infusions or muscarinic receptor antagonism, whilst the two approaches differentially affected adaptation to an earplug following daily training. Neurotoxin infusion impaired adaptive performance relative to controls, whilst the performance of scopolamine-implanted animals varied between animals. In the absence of an earplug, the initial, non-categorized head orienting movements appeared normal and, relative to controls, no significant differences in magnitude were found for animals with either neurotoxin infusion or scopolamine-implantation.

Cortical ACh may play a modulatory role in experience-induced auditory plasticity, but seems less important for spatial hearing accuracy, precision and initial head orienting responses.

907 Directionality of the Gecko Auditory Periphery

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Lizard ears are the ultimate pressure-gradient receivers. Their directionality is created by strong acoustical coupling of the eardrums with almost perfect transmission from the contralateral ear (Christensen-Dalsgaard and Manley, J Exp Biol 208:1209-1217, 2005; JARO 2008, in press).

To exploit the consequences for directional processing in lizards we have combined biophysical measurements of acoustical transmission in Tokay geckos with neurophysiological investigations. The biophysical measurements (laser vibrometry) show that the Tokay ear is a two-input system with approximately unity interaural transmission gain at the peak frequency (1.6 kHz). Interaural transmission is probably boosted by resonances in the large, open tympanic cavities. Because of these resonances, the interaural delay is approximately 200 µs, three times larger than the arrival-time differences at the gecko eardrums. At low frequencies, however (below 400 Hz), vibrometry measures of the eardrums show little sensitivity or directionality, because the phase difference between the external and internal side of the eardrum is small irrespective of sound direction.

In the auditory nerve, dichotic stimulation produces strongly ITD-dependent responses that reflect the

acoustical interactions of direct and indirect sound components at the eardrum. Best ITDs and click delays reflect the interaural transmission delay. Both interaural transmission and ITD sensitivity are blocked by inserting a mould in the mouth cavity.

The free-field response of the auditory nerve fibers resemble the strongly asymmetrical, ovoidal directional characteristics of the eardrum, but the directionality at low frequencies is stronger than expected from the eardrum directivity. We propose that the low-frequency directionality is produced by unknown, extratympanic pathways.

908 Acoustic Cues for Sound Localization Measured in a Rabbit and a Tennis Ball and Computed Using a Rigid Spherical Model

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Rabbit pinnae shape the spectra of signals with sound location that may help the rabbit to avoid predators. This is the first study of the acoustic cues for sound localization in the rabbit. With the rabbit placed in an anechoic chamber, we made acoustic measurements with miniature microphones placed deep in each ear canal to an acoustic point source at 270 different positions (10-160 cm distance, ±135º azimuth). The sound was a logarithmically swept broadband chirp producing a constant level within ±12 dB over 0.1 – 20 kHz. The transfer functions representing signal transformation between the source and each ear canal, i.e., the head-related transfer functions (HRTFs), were measured for each sound location. From the HRTFs we computed the following candidate acoustic cues: interaural time difference (ITD) and interaural level difference (ILD) as a function of frequency, distance and azimuth. For comparisons we also obtained the HRTFs from a tennis ball and a theoretical model of a rigid sphere (Duda & Martens, JASA, 1998). We found in the rabbit: 1) ITD changed with frequency and distance in a complex way. ITD decreased with decreasing distance. At distances ≥20 cm, ITD increased with decreasing frequency. At distances ≤14 cm, ITD remained constant or decreased with decreasing frequency. 2) ILD increased with decreasing distance. Although ILD was minimal at low frequencies for far distances, it increased substantially with decreasing distance. 3) Pinnae position substantially affected ITD and ILD. 4) The spectral patterns in each ear changed with sound source location, a potential monaural cue of sound location. The changes in ITD and ILD with frequency and distance seen in the rabbit were reproduced, in general, by the rigid sphere model. However, these changes could be many times greater in the tennis ball and greater still in the rabbit. The rabbit HRTFs can be used to determine the contribution of each candidate acoustic cue for sound localization.

909 The Acoustical Cues to Sound Location in the Pallid Bat (Antrozous Pallidus)

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Although some aspects of the space- and frequency-dependence of the acoustical cues to location in the pallid bat (*Antrozous pallidus)* have been studied, less is known about the role of their particularly large pinnae and tragus. Here, directional transfer functions (DTFs), the directional components of the head-related transfer functions, were measured for both ears of 4 adults from 325 locations in the frontal hemisphere and the monaural and binaural cues to location computed both before and after removal of the pinnae and tragus. Head and external ear dimensions were also measured. With tragus and pinnae intact, spectral notches were present in the frontal hemisphere at frequencies from ~16-40 kHz and the frequency corresponding to the first (lowest frequency) notch increased with source elevation and with azimuth towards the ipsilateral ear. With increasing frequency, the acoustic axis, the location of maximum acoustical gain, moved from medial to lateral with abrupt jumps back to medial and moved from lower to higher elevations with abrupt jumps back to lower elevations; these transitions occurred at ~27 kHz. The area of space encompassing -3 dB re: the maximum gain decreased with frequency. This pattern was well-predicted by a simple circular aperture diffraction model with an aperture diameter corresponding to the empirically-measured pinnae height. Maximum ILDs increased with frequency, from 5 dB or less up to 10 kHz and increasing to ~35 dB by 25 kHz, but decreased somewhat > 30 kHz. Maximum envelope-based ITDs were ~76 μs. Removal of the pinnae eliminated the spectral notches and reduced the maximum gain (from 22 to 12 dB), ILD (32 to 10 dB) and ITD (76 to 66 μs). The acoustic axes were also altered, particularly in azimuth, where the axes shifted towards the lateral poles. The -3 dB spatial areas increased substantially after pinnae removal, particularly above 15 kHz. Potential roles for the large tragus will be discussed. Support: NIDCD R01 DC05202 (ZMF) and R01-DC6865 (DJT).

910 Sound Localization Cues in the Marmoset Monkey

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The acoustic cues available to the brain for sound localization are produced by the interaction of sound with the animal's head and external ears. As a first step in understanding the interaction between these cues and their neural representation in a vocal new-world primate, we measured interaural time and level differences across frequency for a wide range of sound locations in two anesthetized marmoset monkeys. A probe microphone was surgically implanted ~2 mm in front of the tympanic membrane, and sound was presented from a speaker 1 meter away from the animal over 360^{o} of azimuth and at elevations from -30^{o} to 90^{o}. Interaural time differences varied as a function of azimuth between +/- 250 μs. In the frequency range from 0.2-10 kHz, interaural level

differences varied as a function of azimuth over a +/-20 dB range. At higher sound frequencies (15-40 kHz), level differences were complicated by spectral notches (SNs) in the head-related transfer function (HRTF). The HRTF had a broad resonance peak at 7-9 kHz. Interestingly, this amplified region falls within the frequency range of the major call types of this species. A prominent SN in the HRTF was observed for peripheral sound locations (from ~135 to 75 azimuth). The SN frequency increased monotonically from ~15-23 kHz with increases in elevation. At medial sound locations (from ~60 to -30 azimuth), a smaller SN was observed whose frequency increased monotonically from ~28-40 kHz with increases in elevation. Changes in SN frequency with azimuth also occurred but were nonmonotonic and irregular. The sound localization cues measured in this study will be used to construct virtual space stimuli. This will allow us to investigate the representation and interaction of sound localization cues in the auditory nervous system of this species. Supported by grant DC00115 from NIDCD.

911 Masked Detection Thresholds in the Barn Owl (Tyto Alba) Using the Pupillary Dilation Response

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Barn owls detect low level sounds in complex auditory environments in order to locate their prey. When a novel stimulus is detected, there is a rapid pupillary dilation response (PDR), which can be used as a detection measure. Threshold for PDR is determined to be the lowest sound level that elicits a statistically significant pupillary response. Previous experiments have established -9 db SPL_A to be the detection threshold for a 100 millisecond broadband noise presented directly in front of the bird in the absence of an added masker. We have further explored detection thresholds for broadband noises in the presence of a continuous co-localized masker. It was found that detection thresholds scale with the masker level, in accordance with Weber's Law, suggesting that the barn owl's internal auditory representation adapts to ambient noise. Higher masker levels required increasingly higher level target sounds to elicit a statistically significant response. It has been shown that this type of adaptation is present in the anesthetized mammalian inferior colliculus. Preliminary physiological data in awake behaving owls is consistent with the hypothesis that activity in the inferior colliculus correlates with this observed behavior.

912 Independence of Echo-Delay and Echo-Threshold in Humans

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Despite their prevalence in nature, echoes are not perceived as events separate from sounds arriving directly from an active source, until the echo's delay is long. While presenting a long duration noise-burst, and a simulated echo, human listeners were asked to localize both sound sources, when possible, (Experiment 1) or the source of the sound that was localized most accurately (Experiment 2). Under both tasks, there were two possible stimulus segments that could potentially signal the location of the echo. One was at the onset of the echo; the other, after the offset of the direct (lead) sound, when only the echo (lag) was present. Lead sounds were localized preferentially when the echo's delay was short. By lengthening the echo's duration, independently of its delay, listeners were able to localize the echo even when the delay was short. Echo-threshold was not therefore determined by the echo's delay, per se. The echo's location was instead signaled after the offset of the lead, when only the echo was present. These results are consistent with those observed recently in the barn owl (Nelson and Takahashi, ARO 2008) except that: When listeners reported hearing two sound sources (Experiment 1), and when the lag was present, alone, for a constant period of time, listeners were more likely to report the lead sound's location (Experiment 2) as the onset-delay was increased. Listeners thus experienced "anti-precedence" when the lag's influence was held constant. Vigorous neural responses, evoked by sounds at their onsets, may be sufficient to explain why lead sounds were always localized. In contrast, lag sounds may need to be present for longer periods of time since they are signaled nearer to the offsets of stimuli, when neurons have adapted. Supported by grants from the NIDCD F32-DC008267 and RO1-DC03925.

913 Orienting Towards Auditory Double Stimuli in Elevation

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The spatial percept evoked by auditory double stimuli in azimuth is well understood (Blauert 1997). For example, when two speakers are symmetrically arranged around the mid-sagittal plane subjects perceive a phantom source at straight-ahead. This phenomenon can be fully understood from peripheral physical interactions of sound waves that create the binaural timing and intensity differences for azimuth.

In elevation, spectral shape cues (so-called HRTFs) are used for localization. When two identical sounds are simultaneously presented in the mid-sagittal plane, subjects appear to perceive a single source (Litovsky *et al.* 1997; Best *et al.* 2004). Consistent with this, we show that goal-directed head-saccade endpoints are distributed between the two speaker positions. This behavior is reminiscent to what is known in the visuomotor literature as "the global effect", or averaging (Findlay 1982).

We demonstrate that elevation-averaging responses are systematically modulated by the intensity ratio of the top and bottom speakers. We further tested the influence of sound-source coherence on target averaging by varying the cross-correlation between the two white noise tokens, and we applied a task condition in which a white noise non-target was paired with a quasi-noise target. Interestingly, all responses could be described by a weighted average of sound pressures of the two sounds.

Yet, the amount of correlation as well as the task requirement had a small but systematic influence on response distributions.
To gain a deeper understanding of the potential mechanisms underlying averaging behavior, we confronted the data with two hypotheses: 1) responses are determined by acoustic interactions at the periphery (i.e. summed HRTFs), and 2) responses rely on computational processes in the auditory system. A preliminary analysis of HRTFs and a modeling study support the latter hypothesis, which suggests a fundamental difference between averaging in azimuth and elevation.

914 Temporal Weighting of Interaural Time and Level Differences in High-Rate Click Trains

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Lateralization of high-carrier-frequency amplitude-modulated stimuli is thought to be dominated by interaural information contained at stimulus onset when modulation rates exceed ~200 Hz. While this enhanced temporal weighting at onset has been well established for free-field and headphone ITD stimuli, the time course of ILD processing is less clear. Studies of binaural adaptation, for example, have demonstrated strong onset dominance for ILD, whereas some recent studies suggest maintenance of sensitivity to ongoing ILD beyond the 200-Hz modulation limit evidenced for sensitivity to ongoing envelope ITD. We measured temporal weighting functions (TWFs) for ITD and ILD discrimination in 6 subjects, using 4000-Hz Gabor click trains presented at 4 click rates from 100 to 800 Hz. ITDs and ILDs were randomly perturbed for each click in a train. ROC analysis was then used to generate TWFs describing the time course of ITD and ILD sensitivity for each subject across click rates. TWFs were compared to results from previous ITD and free-field studies and models of onset dominance. These results have implications for mechanisms of precedence in normal hearing listeners and for the binaural processing of high-rate pulsatile stimuli experienced by bilateral cochlear implant users.

915 Adaptive Plasticity of Sound Localization After Direct Modification of Auditory Spatial Cues by Pseudophones

Withdrawn

916 Optimal Integration of Rapidly-Changing Auditory and Visual Localization Signals

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Due to the lower spatial resolution of the auditory system, sound localization is commonly thought to be dominated by visual spatial perception; a phenomenon which is referred to as the ventriloquist effect. Recent studies have suggested that visual capture could result from optimal integration of the auditory and visual cues, yielding auditory dominance only for highly degraded vision. Here we demonstrate dynamic optimal integration under more natural conditions. Subjects generated saccades toward auditory, visual, or audiovisual targets amidst a distracting audiovisual background. Our data demonstrate that auditory reliability depended on the sound's signal-to-noise ratio, but was only weakly related to reaction time. In contrast, visual reliability was strongly affected by saccade latency, reflecting a clear speed-accuracy trade-off. Thus, audition was more reliable than vision for fast responses. The audiovisual responses were well predicted by a parameter-free dynamic optimal integration model without assuming visual capture. Furthermore, the strong influence of the auditory system was highlighted in an extended paradigm which included a large number of trials (80%) with a non-informative auditory distractor. Despite the subjects' task to saccade to the visual target, fast responses to the distractor could not be repressed.

917 Lateralization of Amplitude-Modulated Bone-Conducted Ultrasounds: Positions of Inner-Head Sound Images by Interaural Intensity Differences and Interaural Time Differences of Envelopes

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Some profoundly hearing-impaired people are able to "hear" an amplitude-modulated bone-conducted ultrasound (AM-BCU) and to recognize part of the information on the modulating signal of the AM-BCU. These perceptive characteristics were utilized in the development of a new hearing-aid system, Bone-Conducted Ultrasonic Hearing Aids (BCUHA), for the profoundly hearing-impaired. To verify whether it is possible to transmit spatial information of environmental sounds through bilaterally attached BCUHAs, we conducted two psychological experiments for lateralization of AM-BCUs with a 30-kHz carrier. In one experiment, subjects were instructed to indicate on a head-shaped diagram the perceived inner-head position of a sound image induced by interaural intensity differences (IID) or interaural time differences of envelopes (ITD_{env}). In

the other experiment, subjects were instructed to match their inner-head images of dichotic probe sounds (300-Hz tones, presented with earphones) to those of AM-BCUs induced by IID or ITD_{env} by manipulating IID or ITD of the probe sounds. The results showed that the inner-head position of a sound image of bilateral AM-BCUs changed continuously according to ITD_{env} or IID. Thus, it was clarified that it is possible to lateralize AM-BCU with ITD_{env} or IID.

918 Horizontal Sound Localization: Effects of Age at Implantation and Auditory Experience in Young Children with Bilateral Cochlear Implants

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The aim was to study the development of directional hearing in children implanted bilaterally with cochlear implants (CI) at a low age (median age at 1st and 2nd implantation was 23 months and 49.5 months, respectively). Effects of binaural CI listening experience, time interval between implantations, chronological age and age at implantation were examined.

Sixty-six children who were full-time users of two cochlear implants participated in the study. Subjects were engaged in a sound source identification task using 5 loudspeakers, separated by a 45 degree angle in the frontal horizontal arc. Stimulus was a pink noise pulse train presented at 65 dB SPL, randomly roved ±5 dB. Testing was performed in one condition (bilateral CI) and repeated for 34 of the subjects at one or several occasions, typically 6 months apart. The median age at test was 6 yrs 2 mos.

Children who received two CI were able to correctly identify presented azimuths in the frontal plane. Correctly perceived azimuths improved significantly with increased binaural CI experience. Multiple linear regression analysis revealed that binaural CI experience was the main explaining factor for directional hearing abilities ($p < 0.01$), reflecting the effect of binaural CI experience to be an important basis for sound localization abilities. Time interval between implantations, age at 1st implantation and chronological age did not reach statistical significance ($p > 0.05$). In conclusion, the ability to identify the azimuth of a presented stimulus developed gradually, and was attributed to the listening experience with bilateral CI.

919 Relationships Between Spatial Hearing Ability, Speech-In-Noise Intelligibility, Language, and Non-Verbal Intelligence in Children Who Use Bilateral Cochlear Implants

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In recent years there has been an increasing trend toward providing children with bilateral cochlear implants. A primary reason to implant both ears is driven by potential improvements in sound localization and understanding speech in environments where the signal-to-noise ratio (SNR) is poor. Although most children with bilateral implants appear to benefit from the second implant, their performance on behavioral tasks can be highly variable. This research is aimed at studying the emergence of auditory skills in a group of children who received bilateral cochlear implants prior to six years of age. Additionally, this work is directed at identifying several non-auditory factors that may account for the variability in performance across children. To date, 17 children (mean age 5.4 years; range 4.1 - 7.8) who received bilateral cochlear implants have participated. Children were placed into one of four groups determined by their amount of bilateral hearing at the time of testing: 3-6 months; 12-15 months; 24-27 months; or 36-39 months. Auditory measures included minimum audible angle (MAA), sound localization accuracy, and speech intelligibility in the presence of interferers. In the latter measure, speech reception thresholds were obtained in four conditions: quiet, front interferers, symmetrical interferers (+/- 45 deg) and asymmetrical interferers (+ or - 45 deg, presented on side of first implant). Non-auditory measures included non-verbal intelligence (NVIQ) and expressive and receptive language ability. Results to date indicate that MAA thresholds decline (improve) with increasing bilateral experience. Additionally, NVIQ and spoken language composite scores correlate significantly with some of the speech intelligibility conditions, as hypothesized.

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920 Sound Localization Acuity in Children with Unilateral Sensorineural Hearing Loss: The Effect of Age, Amplification, and Degree of Hearing Loss

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Accurate sound localization requires integration of interaural disparities in intensity and time. A child who has been deprived of interaural cues, due to a unilateral hearing loss, might be expected to have difficulty localizing sounds.

This study measured sound localization abilities in children with unilateral sensorineural hearing loss (UHL) who used a hearing aid in the impaired ear to determine the extent to which amplification, age, and degree of hearing loss affected localization acuity when level cues were minimized.

Two groups of children participated in this study, 12 children with UHL and 12 age-matched controls with normal hearing. Children were divided into two age groups: 12 older children (10y – 14y) and 12 younger children (6y – 9y). Children with UHL were divided into two groups: 7 children with mild-to-moderate loss and 5 children with moderate-to-severe loss. All testing was done in a sound treated booth with an array of 15 loudspeakers placed on a horizontal arc with a radius of 1 m. The stimuli consisted of a spondee word, “baseball,” digitally recorded with a male voice. The stimulus levels averaged 60 dB SPL and randomly roved from 52 to 68 dB SPL (± 8 dB). Each child was asked to identify the location of an azimuthal sound source. Sound localization acuity was measured in RMS error.

Children with UHL had significantly higher RMS error than age-matched controls. A significant age effect was found for

all children regardless of hearing status. Children with moderate-to-severe UHL showed significantly higher RMS error than children with mild-to-moderate UHL. Younger children with UHL showed some benefit from hearing aid use whereas older children with UHL showed a significant decrement in performance with a hearing aid.
Localization acuity in children with UHL depends primarily upon age and degree of hearing loss. Benefits of amplification may change as a child with UHL matures. Developmental factors may improve localization acuity in children.

921 Optimization of Round Window Drive with the Otologics MET-V Transducer with Different Interposed Fascia Materials

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Options for mechanical stimulation of the cochlea via the round window (RW) are being explored to treat patients with mixed hearing losses. The Otologics MET-V™ transducer can effectively drive the round window from a stable bone mounted position posterior to the RW. We investigated whether a particular autograft, allograft or xenograft would optimize the coupling of a MET-V™ mounted 1mm ball (Kurz) at RW interface.
An atticotomy plus extended facial recess approach was drilled in five fresh human temporal bones. The RW niche was saucerised and mucosa removed. A transjugular approach was also drilled for a perpendicular drive angle (control). After measurement of ball tip length and transducer mounting a variety of fascia materials (2mm discs) were compared at various loads; human temporalis fascia (thin), fibro-fatty fascia (thick), Permacol®, Tutoplast®, cartilage, and silicone. Loading in the round window was optimized by visualization (deflection of RW), physical measurement (calibrated micro adjustment), and changes in transducer load (impedance and inductance measures).
RW loading was visually guided by the degree of RW displacement achieved without bony contact. With a small bend in the ball probe a degree of 'side-loading' increased the RW loading Significant inductance changes where only seen with accidental loading onto the bony RW annulus. Both incus and RW drive efficiencies were assessed by the resultant stapes velocity measured by laser Doppler vibrometry (HLV-1000 Polytec). The load angle to the RW was estimated at 35-45 degrees.
Thick fascia, Permacol® and Tutoplast® provided optimal drive when a ball probe approached the RW at an angle. Because Permacol® and Tutoplast® may be more stable implants than fibrofatty autograft tissue these materials warrant further study. Processed connective tissue materials are available that improve RW drive at an oblique angle and may achieve a stable interface when used with a bone mounted transducer.

922 Biomechanics of the New Generation of Stapesplasty Pistons: Efficiency of the Self Crimped Incudo-Prosthesis Junction

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Background: Fixation of prostheses to the long process of the incus is a key determinate of early hearing results and long-term outcomes in otosclerosis surgery. The Kurz CliP® àWengen, the Kurz Soft CliP® and the Gyrus SMart™ stapesplasty pistons have been designed to avoid manual crimping and improve biomechanical performance.
Methods: Six fresh cadaver temporal bones and laser Doppler velocimetry (LDV) was used to compare ease of piston placement and immediate sound transfer function. Each prosthesis was placed via an extended posterior tympanotomy through a fixed stapes foot plate (0.8mm stapedotomy). The test pistons included: Kurz àWengen CliP® and Soft CliP® **(**0.4mm & 0.6mm), SMart™ (0.4mm, 0.5mm, 0.6mm), Fisch (0.4mm), and Schuknecht (0.6mm). The nitinol prostheses were heat activated with diathermy. After placement of the prosthesis the sound transfer efficiency at the incus-prosthesis junction was assessed by comparing the relative movement of reflectors on the incus and prosthesis shoulder. Calibrated pure tone stimuli were delivered at 940dB SPL from 0.1 to 10 kHz. All results considered were recorded at >10dB SNR.
Results: The àWengen CliP®, Soft CliP®, and SMart™ pistons showed losses of <5dB at the test sound intensity with consistent phase across the prosthesis-incus junction. Some variability was seen above 4 kHz without clear trend. The three newer piston designs could be removed and replaced without impairing their fixation. The older pistons were difficult to crimp via the posterior tympanotomy and the results were more variable. Calculated volume velocity for the test pistons indicated a 5dB increased drive to the inner ear for the 0.6mm pistons vs 0.4mm (across all test frequencies).
Conclusion: The àWengen CliP®, Soft CliP®, and SMart™ stapedotomy prostheses achieve an efficient sound couple to the incus. Our results appear to correlate well with the emerging clinical literature regarding the advantages of these pistons.

923 Tissue-Engineered Cochlear Implants: A Murine Stem Cell Model

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Introduction and Objective
Cochlear implants function by stimulating the tonotopically organized structures of the spiral ganglion. Electrical signals arrive at platinum-based electrodes and are carried through perilymph prior to reaching their neural targets. Perilymph has variable impedance, and serves to disperse current over

large areas within the scala tympani, reducing the specificity of channel stimulation. This purpose of this study is to develop tissue-engineered cochlear implants to provide excitatory neural bridges from cochlear electrodes to their stimulation targets, thereby augmenting the resolution of implants and decreasing the excitatory thresholds for stimulation.

Methods

Recent work in our laboratory produced novel techniques designed to drive murine embryonic stem cells (mESC) to glutaminergic neural phenotype in vitro and in guinea pig cochlea models. A mESC strain transduced with the proneural neurogenin1 gene, essential for normal spiral ganglion differentiation during development, was shown to guide cell differentiation into excitatory neural phenotype. Using this mESC line, our laboratory is studying different extracellular matrix proteins to affix cells onto cochlear prosthesis models and support neurite growth towards specific targets.

Results

These bio-scaffolds have demonstrated promising results in vitro, enhancing neurite density and organization. By addition of hyaluronate gel, cells grew in significantly greater density, with increased staining for TUJ1 (neuron-specific beta III tubulin marker) and similar GFAP (glial fibrillary acidic protein) staining. Current investigations are centered on laminin and alginate bio-gel substrates for affixing mESC/hyaluronate gel suspensions onto cochlear prosthesis models. The final arm of the project entails implantation and histological analysis of mESC-coated cochlear prostheses in guinea pig models, specifically evaluating the migratory properties of the cells, and the targets of neurite growth.

Conclusion

By incorporating these techniques, we will develop a cochlear prosthesis model, coated with neurogenin1 murine embryonic stem cells, for the purpose of inducing neural growth from cochlear implants towards recipient auditory nerve beds in guinea pig models.

924 Characterization of the Marmoset Monkey Temporal Bone: A Cochlear Implant Feasibility Study

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The marmoset monkey is a valuable non-human primate model for studying cortical neural processing of vocal communication sounds. The marmoset also poses to be well suited for investigating neural mechanisms related to cochlear implants (CI). The feasibility of implanting a multi-channel CI electrode into the marmoset cochlea was investigated in the present study. The anatomy of temporal bones from adult and infant marmosets was examined using micro computed tomography (microCT), a non-invasive high resolution imaging technique. Temporal bone structures including cochlear fluid spaces were reconstructed in three-dimensional space. To confirm that the scala tympani was appropriately identified, histology slices were made and compared to the microCT images. Like humans, the marmoset cochlea has 2.75 turns. The length of the marmoset cochlea is ~15mm. The cross-sectional area of the scala tympani is greatest at 1mm from the base of the scala, measuring ~0.8mm2 (1.3mm wide, 0.625 high), drops to ~0.4mm2 (0.75 wide, 0.6mm high) at 5mm from the base, and decreases at a constant rate for the remaining length. We identified a suitable CI electrode for the marmoset given these dimensions, the 10 channel half-band H12 electrode from Cochlear Co. The electrode was inserted 3/4 turn into the scala tympani through a cochleostomy at 1.5mm from the start of the scala. The depth of the most apical band was 8mm. According to a Greenwood function frequency-place calculation, the electrodes are estimated to cover a range of ~4-20 kHz, which includes the marmoset's primary vocal frequency range of ~5-9 kHz. Our results demonstrate the feasibility of implanting the marmoset with a multi-channel electrode for use in future studies of neural representations of cochlear stimulation, neural plasticity as a consequence of extended implant use, as well as auditory-vocal interaction mechanisms in a vocalizing non-human primate CI model.

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925 Lasers: A Tool for Atraumatic Cochleostomies

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Advancements in implantable auditory prostheses now demand preservation of residual auditory function following the surgery. Atraumatic cochleostomy formation is essential to this goal. Clinically reported hearing outcomes in human implantation are still quite variable in this regard. The objective of the study was to determine whether the CO2 laser operated with a handheld hollow waveguide could consistently produce cochleostomies without damaging the residual auditory function.

15 adult guinea pigs were used as an animal model. The basal turn of the guinea pig cochlea was accessed by surgical bullotomy. Baseline cochlear function was determined by recording compound action potential thresholds evoked by acoustic tone pips of 2 to 50 kHz. A silver ball electrode was placed in the round window niche to measure compound action potentials (CAPs). A reference electrode was placed under the skin of the animal. The electrodes were connected to a differential amplifier (ISO-80, WPI, Sarasota, FL) with a high input-impedance (>$10^{12}\Omega$), set at 1,000 times amplification. Measurements were conducted at 6 steps per octave and 5 octaves starting at 50 kHz. The sound level as attenuated from 0 to 80 dB in steps of 5 dB.

A cochleostomy was performed using the handheld hollow waveguide coupled to the CO2 laser. An opening of approximately 500 µm was fashioned utilizing multiple single pulses of 100 ms at 10W power settings. Cochlear function was reassessed after formation of the cochleostomy.

Monitoring cochlear function after cochleostomy using compound action potential thresholds did not show significant changes in threshold.

We would like to acknowledge funding support from the Omniguide® Corporation.

926 Insertion -Force and -Depth of Laser Fibers Into a Cochlea Model

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Optical stimulation of the cochlea for hearing restoration is a potential alternative to the present cochlear implant (CI). It has been hypothesized, and demonstrated in preliminary animal studies, that laser stimulation within the cochlea can achieve more frequency-specific activation, thus potentially improve hearing performance, compared to conventional electrical stimulation. While there are various insertion studies investigating CI arrays into the cochlea, to our knowledge, there is no existing insertion study with laser fibers. Therefore, we evaluated the insertion depth and force of different laser fibers into the cochlea and correlated those measurements to the core and cladding diameter as well as the design of the fibers. The fibers had a core diameter ranging from 20 to 105 µm with varying cladding diameters. Single or bundle of fibers with or without silicon coating were inserted into a scala tympani model made of Teflon (Cochlear Ltd.) and measurements were made using an Instron 5543 force device. Our results showed that insertion of a bare fiber requires more force than silicon coated fibers. Furthermore bare fibers are less resistent to bending stress and exert greater forces along the outer scalar wall. Silicon coated fibers (e.g., bundle of 5 fibers each with a 50 µm core and 5 µm cladding diameter) enabled insertion into the first turn with forces and force profiles comparable to what has been achieved with conventional CI electrode arrays. These findings are encouraging as to the safety of fiber implantation within the cochlea and the ability to reduce insertion forces through different types of coatings. Further studies are needed to identify the optimal type of fiber and coating to achieve the desired flexibility, insertion forces and biocompatibility for a non traumatic implantation of an optical cochlear implant.

927 Optical Stimulation of Cochlear Spiral Ganglion Cells Through Sections of Bone

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Recently, pulsed infrared radiation has been shown to be an effective method of nerve stimulation. We have used infrared nerve stimulation in the rodent cochlea as an alternative to electrical stimulation. When optical stimulation will be used in cochlear implants, the optical radiation must reach the target structures, the auditory neurons, in the modiolus. To reach the auditory neurons the radiation has to penetrate the bony modiolar wall. The effect of the bone is an important factor because the bony modiolar wall in gerbils less than 50 µm and the bony wall in the human cochlea is about 100 µm. Bone potentially attenuates and scatters the optical radiation. Therefore, to further optimize the laser parameters towards an application as a cochlear implant, the effect of bone on the neural stimulation needs to be tested. Here we report on the effects on neural stimulation in vivo of bone sections placed in the optical path.

In gerbils, different sections of cochlear wall and temporal bone harvested from pig and human were placed between the tip of an optical fiber (200µm diameter) and the spiral ganglion cells. The bone sections, ranging in thickness from 100-400µm, were placed in the round window and the optical fiber was approximated to the bone. Optically evoked compound action potential (CAP) amplitudes were compared for bone specimen in front of the fiber to amplitudes obtained without the bone. In all cases, presence of the bone section decreased the maximum CAP amplitude evoked and increased the radiant exposure needed to reach threshold CAP.

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928 Optical Stimulation of the Central Auditory System

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In neural prostheses, optical stimulation may offer more selectivity than electrical stimulation due to reduced spread of the stimulus. Results of peripheral auditory neural stimulation using low power infrared lasers have been previously described by Izzo et al. (2006, Lasers Surg Med 38: 745-53) and Richter et al. (2008, Hear Res 242: 42-51). We now demonstrate that similar techniques can be applied to central auditory neurons. In acute preparations of anesthetized rats, a 400 µm diameter optical fiber was placed on the surface of the cochlear nucleus and used to introduce radiant energy (wavelength=1849-1865 nm) produced by a mid-wavelength infrared laser source. An evoked response was recorded from vertex to ear-level electrode. This optically-evoked auditory brainstem response (oABR) is a multi-peaked waveform reminiscent of an ABR evoked by acoustic stimulation. The oABR waveform peaks had latencies between about 3 and 8 ms, longer than ABRs evoked by direct electrical stimulation of the same region. Reproducible oABRs were detected at radiant exposure thresholds as low as 169 mJ/cm^2, 50 µs pulse width, and 5 Hz repetition rate. The oABR was stable following continuous optical stimulation for thirty minutes. Control experiments in which the light path was blocked and in which the rat was euthanized eliminated the oABR. No thermal tissue damage was found on histology when pulse widths were less than 1 ms and radiant exposure levels were less than 2.05 J/cm^2. This study is the first description of optical stimulation of the CNS and our data suggest that mid-wavelength infrared lasers are capable of acutely stimulating neurons of the cochlear nucleus

without tissue damage. These findings may provide the basis for novel auditory brainstem implant stimulation paradigms in the future. *Supported by NIH-NIDCD K08 DC06285 and the Helene and Grant Wilson Auditory Brainstem Implant Program at MEEI.*

929 Selectivity of Optical Stimulation Determined in the Inferior Colliculus of Guinea Pigs

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Pulsed, mid-infrared radiation has been used to stimulate neurons as an alternative to electrical stimulation. Based on the theory of laser-tissue interaction, nerve stimulation with optical radiation should be more spatially selective than stimulation with electrical current. We have conducted experiments using optical radiation to stimulate the spiral ganglion cells of the guinea pig cochlea. Here, we hypothesize that stimulation with infrared radiation from a pulsed laser is as selective as stimulation with acoustic tone pips.

A cochleostomy was drilled into the basal cochlear turn of healthy adult guinea pigs to stimulate the spiral ganglion cells with optical radiation. At the same time neural responses from the inferior colliculus were recorded with a multichannel electrode. Response areas to optical stimuli were compared to response areas obtained with acoustical stimulation and electrical stimulation. Acoustical stimuli were tone pips and acoustic clicks.

Response areas obtained with optical stimulation were tuned. Spatial selectivity obtained by stimulation with optical radiation was similar to the spatial selectivity obtained by stimulation with tone pips. Electrical stimulation and stimulation with acoustic clicks showed a broad area of activation in the inferior colliculus with little spatial selectivity.

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930 Localized Activation of the Cochlea Using Visible Light

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Alternative stimulation strategies and technologies are needed to improve frequency specific sensorineural activation of cochlear implants. One promising approach is the use of optical stimulation of the peripheral auditory system. In a previous report (Wenzel et al 2007), we demonstrated that green laser light (532 nm) can effectively activate the cochlea. We herein present our follow-up study assessing if visible light stimulation of the cochlea achieves frequency-specific activation of higher auditory pathways.

Frequency-specific ABRs were recorded preoperatively in ketamine-anesthetized guinea pigs to confirm normal hearing. An electrode array (16 linear sites) was then positioned along the tonotopic gradient of the inferior colliculus central nucleus (ICC) based on acoustic-driven responses and fixed to the skull to ensure its proper placement prior to further ear surgery. After opening the bulla, a 50 µm diameter optic fiber was positioned into the round window niche directed towards the basilar membrane and used to apply 532 nm laser pulses (10 ns duration, 50 repetitions, 1 pulse/s; Nd:YAG laser, Quantel Brilliant B, France). ICC activity was recorded in response to each laser pulse.

Local field potentials and spike activity were elicited within the ICC for pulse energy levels between 1 and 23 µJ. At low levels, neural activity was localized to a high frequency region of the ICC consistent with the stimulated high frequency region of the cochlea near the round window. As the energy level was increased, greater activity appeared across the tonotopic gradient of the ICC.

These findings suggest that green laser light stimulation has the potential for a new type of auditory prosthesis that can achieve localized activation of the cochlea. Further studies are needed to investigate the effects of optical stimulation of lower frequency cochlear regions and how to safely elicit frequency-specific auditory activation with multiple fibers positioned within the cochlea.

931 Topographic Guidedance for Neuronal Cells on Auditory Implants

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Effective transmission of the stimulation signal from the cochlear implant electrode to the cochlear nerve could be improved by positioning it as near as possible to the modiolus as well as a decreasing impedance. Therefore, the focus of our research project is the physical modification of the border between the implant and tissue, the electrode surface. Our aim is to create a guiding structure for neuronal cells to decrease the distance between electrode contact and neuronal cells.

We used a femtosecond laser to produce microstructures (gradually increasing widths of 1 to 10 µm and a depth of approx. 1 µm in linear configuration) in the electrode material (carrier material: silicone, contact material: platinum). The silicone material was structured directly using laser ablation and indirectly using microstructured glass molds. Neuronal cells (PC-12, primary spiral ganglion cells) were cultured on the samples for 6 days resp. 2 days. The length and direction of outgrowing neurites comparable to the micro structure were determinated.

The microstructuring of the implant material led to an oriented neuronal outgrowth parallel to the microstructures. They showed a significant guidance of neurite outgrowth on platinum ($p<0.01$) and on molded silicone ($p<0.01$) compared to control. Whereas on the direct laser structured silicone material no oriented neurite outgrowth could be observed. This may be effected by the

better quality of the molded microstructure comparable to the laser ablated microstructure.
In conclusion, the topographical functionalization of electrode materials offer new possibilities in the optimization of auditory implants.We show that the geometrical distance between electrode contact and neural cells can be decreased with laser induced microstructure on implant carrier materials. Furthermore, by using a microstructured molds, well defined and reproducible structured silicone carriers could be manufactured commercially.
The project was supported by the DFG, SFB 599.

932 Surface Patterns on Cochlear Implant Electrode Arrays to Permanently Reduce Fibrous Tissue Growth

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During the first three weeks after implantation of a cochlear implant electrode array, the electrical impedance at the electrode contacts increases. This increase is typically explained by the formation of fibrous tissue around the electrode array. Fibrous tissue formation is needed to safely close the cochlea after electrode insertion, but it also increases the resistance for the electrical current and influences the stimulation of the nerve cells. To improve the electrode nerve interface, it is aimed to reduce the tissue formation after implantation around the electrode carrier but not at the cochleostomy. This can be achieved by a physical surface patterning of the electrode array. In cell culture experiments a reduction in fibroblast growth was shown for linear structures with a width of 4-7 µm on Platinum surfaces. The aim of the current investigations is to transfer these patterns to real (animal) electrode arrays.
To achieve reproducibly patterned electrode surfaces, femtosecond laser technology is used to create electrode molds that carry a negative image of the final pattern of the silicone surface. In a first step, these molds were used to produce electrodes with patterned silicone surfaces. Additionally, ring contacts of straight animal electrode arrays are directly patterned by laser radiation and later used to produce active electrode arrays. A third prototype combines structured silicone and contact surfaces. After implantation in guinea pigs, the influence on fibrous tissue growth around the electrode array was investigated by impedance monitoring.
Molding of patterned silicone surfaces results in highly reproducible surface structures. Transfer of these patterns to three-dimensional molds was challenging as the production process had to be revised. Nevertheless, we were able to produce functional electrode arrays with patterned surfaces on silicone and Pt contacts. First in vivo impedance measurements with patterned electrode arrays are currently done.
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933 Biofunctionalisation of the CI-Electrode Surface for Drug Delivery to the Inner Ear as Future Perspective for Enhancing the Benefits of Electrical Stimulation?

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The benefit of the cochlea implant depends also on the efficiacy of the electrode-nerve-interactions. Studies demonstrated survival effects of neurotrophic factors (NTF) in combination with electrical stimulation on spiral ganglion neurons (SGN) following ototrauma. Functionalisation of electrodes with cells providing NTF to the SGN to induce survival mechanisms may be an approach to realize drug delivery. In the present study we first established an in vitro model for the cellular delivery of GDNF enabling the induction of neuronal-like outgrowth in PC12 cells. NIH3T3 cells were lentivirally modified to synthesize the green fluorescent protein (GFP), BDNF (NIH3T3/BDNF) and GDNF (NIH3T3/GDNF), respectively. Free GDNF from the supernatant of the NIH3T3/GDNF cells were added to the PC12 cells. The neurite outgrowth of PC12 cells was determined for 10 d and the GDNF release was quantified by ELISA. Statistical data revealed significant axonogenesis induction on day 3, which increased up to 10 days following GDNF application (3.7fold extension) indicating the feasibility of recombinant fibroblasts as NTF delivery system and the bioactivity of the released GDNF on outgrowth rate. As follows, silicone dummies, used as a CI-electrode model, were coated with NIH3T3/BDNF to characterise their ability to proliferate on the surface and to produce BDNF during 14 d of cultivation. As early as 3 d >50% of the surface of the dummies were covered with NIH3T3/BDNF cells. The proliferation rate increased following 7 d. The BDNF measurements by using ELISA revealed an increased BDNF expression level between days 4 (0,25 ng/ml ±0,017) and 7 (1,34 ng/ml ±0,259). The bioactivity of BDNF was tested by co-cultivation of the dummies coated with NIH3T3/BDNF cells with SGN explants resulting in strong SGN neurite outgrowth. Our data indicate a rise of the viability of NIH3T3/BDNF cells and an effective release of BDNF on round-shaped surfaces which may be sufficient for SGN survival in vivo.

934 Dependence of Otoconial Biomineralization Upon Otoconin 90 in Vitro and in Vivo

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We have recently initiated in vitro studies on the growth of calcite crystals, modified by the otoconial matrix protein Otoconin 90(OC90). In vitro experimentation has the

advantage that effects of individual proteins can be assessed and experimentally manipulated in isolation. Zhao et al (2007) ablated the OC90 gene and studied the effects upon morphogenesis of otoconia. Apart from its many advantages, gene ablation has the disadvantage that it takes place in a background of complex interacting processes. Consequently interpretation of pertinent comparative studies requires a great deal of judgment. A) Modification of growth parameters of calcite crystals by OC90 in vitro exhibits a conspicuous degree of negative correlation with the effects of ablation of the corresponding gene. Absence of OC90 in vivo results in nucleation of only a small number of mineral particles with a far larger than normal size (giant otoconia) and a spindle-like morphology. The giant otoconia are transparent and highly soluble, evidently due to reduced matrix incorporation. For the most part, ablation data are exactly opposite to the effects of addition of OC90 upon calcite growth in vitro: OC90 facilitates nucleation, inhibits crystal growth and induces a highly distinctive morphologic change. Moreover addition of OC90 results in substantial incorporation of the protein and reduction of solubility. Comment: Seeding of otoconia in the absence of OC90 can be explained by the observed presence of FetuinA - a powerful nucleator, whereas generation of giant otoconia is consistent with absence of inhibition of calcite growth by OC90. B) Deep Etch studies by Lins et al (2000) indicated that the organic otoconial core consists of a network of 15 nanometer fibrils. Subsequent TEM studies (Lins et al, 2003) indicated that OC90 labeled gold particles are aligned with a fine hexagonal network of fibrils, most likely consisting of Otolin, considering its similarities to collagen X. Corresponding in vitro studies demonstrated a clear interaction of Otolin with OC90. C) Mann et al (1983) found that mammalian otoconia consist of minute crystallites arranged in a highly ordered mosaic. Our recent in vitro experiments indicate that OC90 induced a massive reduction of calcite crystal size, comparable to that of the crystallites of native otoconia.

935 Matrix Recruitment and Calcium Sequestration Lead to Spatial-Specific Otoconia Formation

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Despite the importance of otoconia in motion sensing and bodily balance and the detrimental effects of ectopic calcification, the mechanism underlying the spatial-restriction of extracellular matrix (ECM) mineralization to otoconia in the vestibule has not been studied. Only recently the spatial-specific mineralization to bone has been resolved despite much endeavor of the bone field. Although otoconia calcite crystals share some components with bone apatite crystals, the vestibular milieu is drastically different from that of bone in that the endolymph has very low Ca(2+). In this study, we have expressed otoconial proteins Oc90 and otolin in HEK293 cells, and have used co-immunoprecipitation to demonstrate that Oc90 recruits otolin through its interaction with the C-terminal domain of otolin. These matrix components are able to cause ECM calcification in cell culture and lead to an enrichment of Ca(2+) in the luminal ECM of the utricle and saccule in wildtype mice. Absence of this ECM complex in Oc90 null mice results in profound reduction of ECM-Ca(2+) above the macular epithelia. Therefore, Oc90 recruits otolin to form the otoconial matrix complex, which sequesters Ca(2+) for spatial-specific calcification of otoconia.

936 Mechanistic Difference Between Otoconial Matrix Protein Otoconin 90 and Functionally Related Invertebrate Biomodulators

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Mammalian biomineralization is dominated by the skeletal and dental systems which depend on phosphate based calcification and an enormous amount of pertinent information was assembled over the years. By contrast extremely little is known about the biomineralization of otoconia, which constitute the only mammalian carbonate based calcification system. Information derived from the skeletal and dental systems can't readily be applied to otoconia in view of major mechanistic differences. Current concepts about otoconial biomineralization are largely shaped by the well characterized invertebrate CaCO3 system. To overcome the prohibitive constraints of insufficient availability of native otoconial material, we generated recombinant OC90 and characterized essential calcite crystal growth parameters and solution state properties. OC90 has an unusually high MW (100 KD) with only moderate acidity of the protein backbone (pI 4.4) compared to functionally related invertebrate molecules (pI 2.5). The structure of OC90 is dominated by two large domains coopted from the lipolytic enzyme sPLA2. For the role as calcite growth modulator, OC90 is modified by substitution by several anionic and hydrogen bonding residues, but the characteristic disulfide bonds of sPLA2 are retained, thereby evidently providing the necessary rigidity to the surface interacting with the mineral phase. Alpha helices dominate the secondary structure and undergo significant conformational changes upon binding of Ca2+ and CO32- ions, a feature of considerable functional significance. Homology based molecular modeling of the two sPLA domains demonstrates a spherical arrangement of negative elctrostatic potential, whereas the surface of the parent sPLA2 template appears electrostatically neutral. The effectiveness of the paradigm exemplified by OC90 appears to be based primarily on the alpha helical structure in combination with strategic placement and clustering of anionic and hydrogen bonding residues. This arrangement should result in a restructuring of water thereby greatly enhancing the interaction of calcium ions with the calcite lattice. Thus the relatively low overall acidity would be compensated by effective placement of charged and uncharged polar residues.

937 Atlas of Otolith Formation in the Zebrafish

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The otoliths of ray-finned fishes and the orthologous otoconia of tetrapods are essential structures of the inner ear that convey linear accelerations to sensory hair cells. Their displacement or absence results in disequilibrium, such as in benign paroxysmal positional vertigo. Otoliths and otoconia are completely acellular biominerals that contain at least 95% CaCO3 by weight and up to two dozen proteins, most of which have not been identified. Both nucleate early during embryonic ear development; otoconia grow until hatching or birth, while otoliths continue to grow throughout life. In fishes, the three otoliths—the lapillus in the utricle, the sagitta in the saccule, and the asteriscus in the lagena—have elaborate and species-specific shapes, whereas otoconia are innumerable, small, and generally barrel-shaped. Most importantly, the formation of both otoliths and otoconia is likely to be governed by their constituent proteins. The zebrafish is an excellent animal model for studies of otolith formation because its ear is highly similar to that of other vertebrates, including humans, and because it easily lends itself to embryological and molecular-genetic laboratory studies. As a reference for future studies, we have established an atlas of otolith formation in the zebrafish. Otolith size and shape were recorded by light microscopy, scanning electron microscopy, and X-ray computed tomography, and calcium content was measured by inductively-coupled-plasma mass spectrometry. Our results show that the size of zebrafish otoliths correlates more closely with fish size than with age; that otoliths are the first structures to calcify during development and initially contain up to two-thirds of the body's calcium; and that the transition from the smooth spheroids in the embryo to the extravagant organ-specific shapes in the adult coincides with the elaboration of the labyrinth at around two weeks of age.

938 Expression of Recombinant Otolin 1 and Assessment of Aggregation Behavior by Rotary Shadowing Electron Microscopy

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Morphogenesis of otoconia depends critically upon Otolin 1, the principal scaffold protein, which is essential for control of oriented growth of calcite crystals. Otolin is a homolog of Collagen X which is abundant in mature chondroblasts where it forms a hexagonal network of fine fibrils. Lins et al (2003) observed an analogous fibrillar network in the organic otoconial core by TEM. As prerequisite for in vitro assembly of a corresponding network, we generated recombinant Otolin by means of a mammalian expression system. The Otolin cDNA was amplified by RT-PCR using RNA from E16.5 mouse otocysts. Stable transfects expressing Otolin-Flag were selected by means of G418 for preparation of recombinant Otolin from ammonium sulfate precipitates of conditioned medium. Precipitates were desalted and incubated with anti-Flag M2 Agarose antibody and eluted. Fractions containing rOtolin were concentrated and desalted and subsequently identified by Commassie staining and Western Blotting with anti-Flag antibody. Recombinant mouse Otolin-Flag has an apparent MW of 85 KD and a calculated amino acid backbone of 48 KD. Electron Microscopy following Rotary Shadowing reveals small molecules with fine tails and globular heads at one end. Based on the homology to Collagen X, we conclude that the tail represents the somewhat shorter helical domain of Otolin including the N-terminal, whereas the head group corresponds to the highly interactive globular C-terminal C1Q domain. Apart from monomers, rOtolin tends to form dimers, trimers to multimers based exclusively on assembly mediated by the C-terminal C1Q domain. We are currently extending our study of the in vitro assembly behavior of the molecule to test the hypothesis that rOtolin is indeed able to form a hexagonal network like that observed in the three dimensional network of the otoconial core.

939 Characterizing Utricular Stimulation During Natural Behaviors of the Turtle, *Trachemys Scripta*

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Recent work in a number of sensory systems suggests that sensory neurons encode and process naturally occurring stimuli more efficiently than artificial stimuli. In order to determine if this is also the case for vestibular neurons, we have developed a method to quantify the stimulus waveforms experienced by vestibular end organs during natural movements in turtles in order to utilize these waveforms in neurophysiological experiments.

This approach requires precise measurements of linear and angular motion of the animal's head during natural behaviors as well as precise knowledge of the orientation of each end organ within the skull. We have used a combination of high speed digital cinematography of freely behaving turtles, high resolution imaging of the orientation of the utricle in the skull, and computational methods to quantify patterns of utricular stimulation that occur during a variety of natural behaviors.

Freely behaving turtles were filmed in two orthogonal views at 1000 frames per second. The positions of three landmarks on the head were digitized from the video sequences, and time varying 3-dimensional acceleration vectors were calculated by double differentiation of the measured position vectors. These were combined with a gravity vector to produce a gravito-inertial (GI) vector that

describes the net acceleration experienced by the head in earth centered coordinates.
High resolution micro-CT scans were used to establish the relationship between the plane defined by the 3 head landmarks and a plane fitted to the otoconial layer of the utricle. The component of the GI vector in this plane was calculated using linear algebra and subjected to wavelet analysis to analyze its frequency content. These waveforms can be used to calculate the frequencies and accelerations experienced by utricular afferents at any location on the epithelium during natural behavior and implemented in neurophysiological experiments to study mechanisms of stimulus encoding.

940 Supporting Cell Deficits Underlie the Rapid Balance Deterioration of the Twister Mice

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In the inner ear, sustaining the proper function and number of sensory cells is essential to maintaining normal balance and hearing. While supporting cells (SCs) are well known for their potential to transdifferentiate into hair cells, their role in sensory relay has barely been studied. Here we have discovered age-related deterioration of balance function due to rapid reduction in the macular sensory input in the twister mouse, Otog(twt)/Otog(twt), and have identified compromised SCs as the likely primary cause. Twister SCs have normal morphology at a young age, as are other vestibular structures including hair cells and ganglia, but have profoundly reduced cell adhesion and significantly reduced BrdU incorporation in primary epithelial culture. Although older mice also have easily detachable otoconial membrane (OM) and a mild loss of otoconia crystals, such loss cannot account for the degree or rate of sensory reduction when their linear vestibular evoked potentials (VsEPs) are compared with those of other vestibular mutants that had much worse otoconia loss. Work is underway to further assess the sensory deficits arising from the suboptimal SCs, and to identify ultrastructural and molecular changes in older twister mice that coincide with the functional degeneration.

941 Vestibular Development of Dominant-Negative Connexin26 Transgenic Mouse

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Heredity deafness affects about 1 in 2,000 children and mutations in the connexin 26 gene (GJB2) are the most common genetic cause of congenital bilateral non-syndromic sensorineural hearing loss. On the other hand, it is known that a considerable number of children with congenital deafness have balance function disorder. We previously reported transgenic mice carrying human connexin 26 (Cx26) with R75W mutation that was identified in a deaf family with autosomal dominant inheritance. In this study, we analyzed morphological development of the vestibular organ in the transgenic mice (R75W+) between 0 and 14 days after birth, which was compared with that of littermate control mice (non-Tg). Balance disorders such as head tilt and swimming abnormality were not observed in R75W+ mice as well as non-Tg mice. No morphological differences between R75w+ and non-Tg mice were observed in utricule and saccule by HE staining at 0-14 days after birth.

942 Supporting Cells Regulate Synapse Formation in the Vestibular Epithelium

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Supporting cells of the vestibular epithelium are closely associated with hair cells and afferent nerve terminals, making them potential contributors to vestibular development and function, but this remains to be shown. Vestibular supporting cells express erbB receptors and sensory neurons express the erbB ligand neuregulin 1. We tested the potential roles of this pathway in the vestibular system using a transgenic mouse line in which erbB receptor signaling in vestibular supporting cells is blocked by expression of a dominant-negative erbB receptor (DN-erbB4).
Adult DN-erbB4 mice display behaviors consistent with vestibular dysfunction (ataxia, spinning behavior, inability to swim). Evoked potential recordings showed that vestibular function is severely affected by P21, even though macular epithelia are normal in size and general structure. FM1-43 dye uptake and neurofilament staining are normal in mutant mice, indicating that hair cell mechano-transduction and afferent innervation are unaffected. In contrast, synaptic site numbers (defined as the colocalization of RIBEYE and GluR2/3 staining) are dramatically reduced, suggesting a synaptic defect. Analysis of synapse numbers at different postnatal ages showed that the number of synaptic puncta increases by 5 fold between birth and P21 in wild types, but this does not occur in the mutants. Molecular analysis showed that the synaptic alterations are accompanied by a dramatic reduction in BDNF and we found that over-expression of BDNF in vestibular supporting cells after birth using tamoxifen-induced transgene activation rescues the functional and anatomical phenotypes of DN-erbB4 mice. Together these results indicate that vestibular supporting cells contribute to the formation/maturation of synapses in the postnatal vestibular maculae and that this is mediated by NRG1-erbB and BDNF-TrkB signaling.

943 A Re-Examination of the Striated Organelle in Vestibular Endorgans

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The striated organelle (STO) is a structure located in the subcuticular region of hair cells and consists of alternating thick and thin bands (Friedman et al., 1965; Ross and

Bourne, 1983). Although present in all cochlear and vestibular hair cells, the STO is particularly well-developed in type I hair cells, where it is shaped like an inverted open ended cone that contacts the cell membrane along its entire circumference and is separated from the cuticular plate by a layer of mitochondria. In other hair cells, it is a much smaller structure and appears to be free-floating. We studied its structure in EM tomography of the type I hair cell. In three-dimensional reconstructions, we found that it is connected to at least some actin rootlets. It may also be associated with microtubules, mitochondria and smooth ER. Confocal immunohistochemistry places Yotiao (an AKAP protein) in the same area as the STO and the actin-binding protein, alpha fodrin (non-erythroid spectrin), where the STO contacts the cell membrane. The contact with the rootlets suggests that the STO might regulate hair-bundle stiffness. Its association with the cell membrane suggests that the STO may help in the formation of the constricted neck characteristic of type I hair cells.

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944 Calretinin Stains Bouton Terminals in Turtle Utricular Striola

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The calcium-binding protein calretinin (CR) is generally thought to be specific for calyceal afferents in mammals. Because these afferents are also the most irregularly discharging afferents, it is unclear whether CR is a marker for a specific terminal morphology, a specific pattern of discharge regularity, or both. In turtle utricle, antibodies against CR label a subset of calyceal terminals in the utricular striola (zone 3) as they do in mammals. But CR also labels a population of bouton terminals in an adjacent subdivision of the striola (zone 2).

Zone 2 is a band of type II hair cells, which differ from extrastriolar type II hair cells in having somata that are calretinin-negative (CR^-) but are positive for a second calcium-binding protein, calbindin (CB^+). Some bouton terminals in zone 2 are CR^- and have a varicose structure. But other bouton terminals in zone 2 are CR-positive (CR^+). These CR^+ terminals arise from parent axons that ascend in zone 2 and end in claw-like structures similar to those described on bouton afferents of some fish. Often the “claws” arise at irregular intervals from a process that runs long distances within the epithelium, parallel to zone 2. The apices of these CR^+ terminals reach half way toward the neck of the apposed hair cells. Some terminals approach adjacent CR^+ calyces, but preliminary data suggest that CR^+ bouton terminals and CR^+ calyces typically do not arise from the same parent axon: 1) CR^+ bouton terminals are largely or completely CB^-, unlike adjacent CR^+ calyces, and 2) automatic 3D direct volume-rendering frequently reveals a distinct separation between CR^+ bouton terminals and CR^+ calyces. Our data indicate that CR is not specific for calyx afferents in turtle utricle. Physiological differences between striolar afferents giving rise to CR^+ and CR^- bouton terminals may provide insight into the distinctive role of CR in vestibular afferent signaling.

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945 Signaling Mechanisms at the Type I Hair Cell/Calyx Synapse

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The close association between type I vestibular hair cells and their afferent calyx terminals has prompted speculation that there may be unusual modes of intracellular communication between the two cells. Furthermore both quantal and non-quantal forms of transmission are reported at this synapse (Holt et al. *J. Neurophysiol.* 98:1083-1101, 2007). We made whole cell patch clamp recordings from both cell types with Lucifer Yellow dye in the electrode solution in order to visualize cells and investigate possible electrical coupling. Type I hair cells and calyces were isolated from gerbil semicircular canals and identified as described previously (Rennie & Streeter *J. Neurophysiol.* 95: 26-32, 2006). Gigaseals were made on the outer face of calyces surrounding type I cells and following membrane rupture fluorescent calyx terminals were seen as horseshoe-shaped structures surrounding the basolateral membrane of type I cells. Transient inward Na^+ currents and inactivating outward K^+ currents were recorded from dye-filled calyces and were similar to those seen with normal electrode solution. The same intracellular solution was used to record from type I hair cells. Whole cell access was apparent from fluorescence throughout the hair cell and hair bundle and a low input resistance (40-155 MΩ). As described previously a low voltage-activated K^+ current was present in type I cells and outward currents exceeded 4 nA following steps to potentials above 0 mV. Calyx terminals did not express low voltage-activated current and the mean input resistance was 0.62 ± 0.16 GΩ (n = 8), which was significantly different from type I cell values. We found no evidence for electrical continuity between cells. In current clamp spontaneous excitatory postsynaptic potentials in calyces were 3-5 mV in size, but spontaneous action potentials did not occur. Mechanisms underlying non-quantal transmission at this synapse remain to be determined.

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946 Simultaneous Pre- And Post-Synaptic Recording from the Peripheral Vestibular Calyx and Its Included Type I Hair Cell

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The form of synaptic transmission between type I vestibular hair cells and their surrounding calyces has been under investigation. Recordings obtained from “calyx

bearing" afferent nerves have thus far demonstrated only action potentials and miniature postsynaptic potentials (mEPSPs), but have not provided data to address hypotheses concerning alternate forms of synaptic transmission. To better understand type I hair cell-calyx terminal synaptology, we have performed simultaneous pre- and post-synaptic recordings from the calyx and its included type I hair cell. The utricular macula was removed from the labyrinth of the turtle (Pseudemys (Trachemys) scripta elegans) and placed in a recording chamber on the stage of a compound microscope. Transmitted light and a video microscopic system were used to visualize the epithelium, individual type I hair cells, and their surrounding calyx terminals. Microelectrodes, ca. 200 megOhms, impaled hair cells apically, while calyces were patched basolaterally using borosilicate patch pipettes. Whole cell records from calyx terminals demonstrated inward, presumed sodium currents and outward, presumed potassium currents. Spontaneous inward, presumed miniature synaptic currents (mIPSCs) were also observed. When penetrated hair cells were depolarized, the frequency of these spontaneous events increased. Histograms of mIPSC amplitudes indicate the presence of at least two classes of events, those of large and those of small amplitude. To date, no alternate forms of transmission between the type I hair cell and its surrounding calyx have been observed.

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947 Alteration of the Vestibular System Function in the Prosaposin KO Mice

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Prosaposin, a precursor of four glycoprotein activators (Saposin A, B, C and D) for lysosomal hydrolases, has been shown to have both lipid transfer properties and neuritogenic activity. Our previous studies have demonstrated that prosaposin KO mice develop a progressive deafness that starts at P19 and show abnormal afferent and efferent innervation patterns, suggesting that prosaposin may be required for normal adult cochlear innervations and consequently the maintenance of normal hearing (Akil 2006).

During these initial studies, we also noted that the prosaposin KO mice behave similarly to mice that have vestibular dysfunction (circling, unsteady gait, and difficulties in maintaining balance). In the present study now formally assesses the vestibular system in the prosaposin KO mice. The goals of this study were to: 1) Determine the prosaposin expression in the vestibular end-organs (ampullae, saccuole, utricle and Scarpa's ganglia) in normal mice using RT-PCR and Western blot; 2) Study the histology of the vestibular end-organs of the prosaposin KO mice compared to the wild type littermates, 3) Evaluate associated neuronal changes in the KO mice through immunofuorescence using neurofilament (NF200) and synaptophysin; and 4) Study the vestibular response using several simple behavioral tasks – the air righting reflex, contact righting reflex, the rearing and circling test (ossenkopp 1990) and the swimming test (Sawada 1994).

The RT-PCR and the western blot demonstrate the presence of Prosaposin mRNA and protein in all vestibular end-organs and Scarpa's ganglia. At the light microscopy level KO mice demonstrated an exuberant cellular proliferation and vacuolization below the vestibular hair cells causing disruption of the supporting cells. Immunofluorescence suggests that the cellular proliferation corresponds to afferent/efferent (NF200) and efferent (synaptophysin) neurite overgrowth. However no abnormality was seen in Sarpa's ganglia. Lastly, the battery of behavioral tasks tested strongly suggests serious vestibular dysfunction in the prosaposin KO mice as compared to the wild littermates.

These data suggest that prosaposin plays a role not only in the maintenance of normal hearing but also plays an important role in the neuronal maturation processes of the vestibular sensory epithelium and the maintenance of normal vestibular system function.

948 Estrogen Receptors in the Rat Vestibular Periphery

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Estradiol elicits its effects through binding to two distinct receptors, estrogen receptor a and ß (ERa and ERß). RT-PCR of vestibular ganglion and crista ampullaris RNA extract revealed that both estrogen receptors are expressed in these tissues. In situ hybridization of complementary RNA ERa and ERß probes to sections of vestibular ganglia and crista ampullaris showed that the ERa and ERß mRNAs are expressed in primary afferent vestibular neurons and in hair cells and nerve terminals of the crista ampullaris. Western blot analysis of vestibular ganglia and crista ampullaris protein extract with anti- ERa antibodies revealed bands at 66, 46 and 36 kDa. The 46 kDa and 36kDa isoforms of ERa may function as plasma membrane-based estrogen receptors that mediate rapid, non-genomic estrogen signaling. Immunohistochemistry showed that ERa-like immunoreactivity was in nuclei of neurons and hair cells and in possibly nerve terminals. ERß-like immunoreactivity was mostly cytoplasmic in the neurons and in what appeared to be nerve fibers in the epithelia. One possible mechanism for the rapid estradiol action is its binding to membrane-inserted ERa or ERß and subsequent activation of metabotropic glutamate receptors. Since metabotropic glutamate receptors are present on vestibular afferent terminals, estradiol may influence their response to stimuli. These data indicate estradiol regulate gene expression in the vestibular periphery and suggest it may influence excitability in the epithelium. Supported by NIDCD grants DC02971.

949 Intrinsic Electrical Properties of Vestibular Afferents Can Shape Spike Timing Regularity

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In mammalian vestibular afferents, remarkable variation in the regularity of inter-spike intervals correlates with zone of innervation, afferent morphology, and response dynamics. To investigate the proposal (Smith & Goldberg, 1986) that intrinsic ion channels shape spike timing, we recorded with the perforated-patch whole-cell method from neuronal somata isolated from the superior vestibular ganglion of rats and mice (postnatal days, P, 0 - 16). Current steps evoked three response types: transient (1-2 spikes), sustained (a train of spikes), and resonant (a spike followed by voltage oscillations). Resonant responses may be a sub-class of sustained responses. Response classes were robust, occurring in rat and mouse, at different temperatures (22-37°C), and from P0 to P16.

The isolated neuronal somata lacked spontaneous activity. To investigate the interaction of synaptic input and intrinsic channels on spike timing, we drove spiking with simulated excitatory postsynaptic currents (pseudo-EPSCs) at pseudo-random intervals. For similar pseudo-EPSCs trains, evoked firing was more regular in the sustained neurons than in the transient neurons. We conclude that intrinsic electrical properties of vestibular afferents do help set spike timing and propose that transient and sustained patterns of step-evoked activity correspond to irregular and regular firing patterns in vivo.

In voltage-clamp recordings, we find that transient neurons have a non-inactivating K+ current that activates over 10 mV more negatively than the major K+ currents of sustained neurons. This current may explain the more negative resting potentials, lower input resistances and shorter membrane time constants of transient neurons relative to sustained neurons. Such a current is well suited to influence spike timing. A candidate channel is a KCNQ channel localized to the terminals of very irregular (calyx) afferents.

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950 Repetitive Discharge in Vestibular Nerve Afferents

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Discharge regularity has served to classify vestibular-nerve afferents (VNAs) (Goldberg, 2000). Past work has shown that discharge regularity and the sensitivity of the postsynaptic spike encoder to depolarizing inputs are related (Goldberg et al., 1984). To explain the sensitivity-regularity relation, a cumulative afterhyperpolarization (AHP) model was developed (Smith and Goldberg, 1986). Support for the model was based on circumstantial evidence, particularly the response of VNAs to externally applied galvanic currents. The assumptions of the model have now been confirmed in intracellular recordings from VNAs near their peripheral terminations in the turtle posterior crista. Specifically, discharge regularity is related to the depth and time course of AHPs and to the size of synaptic quanta. Based on these observations, the model has been refined. Nevertheless, the model remains unrealistic in that the AHPs are assumed to be based entirely on an sK conductance. Recordings from vestibular ganglion cells (Chabbert et al., 2001; Limón et al., 2005) indicate that there is a rich repetoire of K+ currents that could contribute to the AHP. A role for persistent Na+ conductances must also be considered (Holt et al., 2007).

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951 The Effect of CNQX on Single-Unit Responses of Horizontal Canal Afferents in the Chinchilla

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In order to address the question of whether hair cell synaptic input is necessary for the resting rate and response dynamics of vestibular nerve afferents, we recorded from 5 horizontal canal afferents in anesthetized chinchillas before and after the administration of the AMPA antagonist CNQX. The drug or artificial perilymph (AP) was introduced through a fenestra in the superior canal. Other than a small transient change in background activity (probably due to a pressure change) the AP had no effect on the background activity.

Resting activity of every afferent declined after the administration of CNQX. The time course of decline was roughly related to the concentration of CNQX. The time over which resting rate declined to 0 varied from 4 minutes in one afferent to > 20 minutes in a separate afferent. CV* increased over time after the administration of CNQX. The average CV* for regular afferents (n=3), pre-CNQX was 0.027 ± 0.005 and post-CNQX was 0.20 ± 0.11 when recorded at the last point at which resting rate was > 10 sp/s. The average CV* for irregular afferents (n=2) was 0.22 ± 0.08, which increased to 0.42 ± 0.23 post-CNQX. Afferents also began to resemble irregularly firing afferents in their dynamic responses (i.e. an increase in adaptation to currents, larger phase leads with respect to head velocity). The silencing of afferents due to CNQX suggests that glutamate release from hair cells is important for spontaneous firing of vestibular afferents.

When resting activity had declined to -80 ± 5 %, rotational sensitivity (n=4) had changed very little (-10 ± 28%). Galvanic sensitivity increased by 70 ± 50 % when measured at a comparable decline in resting rate in 3 afferents (2 regular, 1 dimorphic irregular). Galvanic sensitivity of 1 irregular (calyx-only) afferent decreased -44% when its resting rate had been reduced by -39%. These findings indicate that membrane properties of the afferent can be affected by synaptic activity. (Supported by NIH RO1 DC02390)

952 Quantitative Study of the Peripheral Vestibular System in Down Syndrome

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[Introduction] Down syndrome (21 trisomy) is the most common genetic disorder. Previous studies show that vestibular and semicircular canal anomalies are common in Down syndrome. However there is no quantitative study that evaluates the peripheral vestibular system.

[Material and Method] Sixteen temporal bones from 8 patients with Down syndrome and 15 control temporal bones from 8 individuals were selected. The number of Scarpa's ganglion cells in the superior and inferior division was counted individually under a magnification of ×200. The number of vestibular hair cells was expressed as density (/ 0.01 mm^2). The densities in the lateral semicircular canal crista and utricular and saccular maculae were examined. Types I and II vestibular hair cells were counted separately under differential interference contrast (Nomarski) microscopy at a magnification of ×1,250. Statistical analysis was performed between Down syndrome and control groups and between those with and without hypoplasia of the lateral semicircular canal.

[Result] The number of Scarpa's ganglion cells in the Down syndrome group was significantly smaller than in controls in both divisions. The density of vestibular hair cells in the Down syndrome group was significantly lower than in controls in all portions. A decrease in the number of vestibular hair cells was mainly found in the otolithic organ. In the Down syndrome group, 8 temporal bones (4 cases) had hypoplasia of the lateral semicircular canal. There was no significant difference between these 2 groups.

[Discussion and Conclusion] There was no laterality in the peripheral vestibular system in the Down syndrome group. Furthermore they had a smaller number of ganglion cells and smaller density of hair cells than controls. We should pay attention to vestibular dysfunction as well as hearing dysfunction in patients with Down syndrome.

953 Effects of Commissural Pathway Transections on Vestibular Nuclear Amino Acid Concentrations

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Studies have shown the existence of three major sources of input to the vestibular nuclei that involve amino acid neurotransmitters. Our previous studies on removal of pathways from the cerebellum and labyrinth found significant changes in concentrations of amino acids, especially GABA, glutamate, and aspartate, in vestibular nuclei. Here, we examined the effects of transecting the third major pathway, the commissural connection between the two vestibular nuclear complexes. A 3.5-mm-long, 1.5-mm-deep midline cut was made at the level of the cochlear nuclei in nine adult rats. Three rats were euthanized at each of 2, 7, and 30 days after surgery. Two sham-lesioned animals were exposed for surgery but no cut made. Freeze-dried coronal sections at 3 rostral-caudal levels through the vestibular nuclei were microdissected to obtain samples of superior (SuVN), dorsal and ventral lateral (LVNd and LVNv), dorsal and ventral medial (MVNd and MVNv), and spinal vestibular nuclei (SpVN). Amino acid concentrations were measured by HPLC. The largest changes of amino acid concentrations were for GABA, which decreased by 60% in LVNd and 40% in MVNd by 2 days. Reductions of GABA concentrations continued through 30 days except in MVNv. Glutamate concentrations decreased by almost 40% in LVN and 25% in MVN, but not in SuVN or SpVN, then showed recovery toward control values by 30 days. Changes of aspartate concentrations resembled those of glutamate but with less recovery by 30 days. Glutamine and taurine concentrations increased in almost all regions after the transections. Glycine concentration decreased by 10-15% in MVN. Our results suggest involvement of GABA, glutamate, aspartate, and to a minor extent glycine, in commissural connections of the vestibular nuclei. However, results of our prior studies suggest that some of the effects on amino acid concentrations, especially for LVNd, result from damage to cerebello-vestibular pathways.

954 Co-Localization of 5-HT1F Receptor and Glutamate in Rat Vestibular Nuclei (VN)

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Co-morbidity of migraine with balance disorders has been an object of interest for many years. Activation or blockade of serotonin (5-hydroxytrypatmine, 5-HT) receptors is closely related with migraine and its associated vestibular symptoms. Of a variety of 5-HT receptors, 5-HT1F receptor has recently attracted attention in the treatment of migraine. Potent agonist for 5-HT1F receptor, LY334370 is very effective in the treatment of migraine and acts centrally since it has lack of vasoconstrictive effect. Meanwhile, a release of the excitatory neurotransmitter glutamate from trigeminal neurons has been implicated in migraine. Glutamate is co-localized with 5-HT1B, 1D, and/or 1F receptors in trigeminal neurons. Given that there is a co-localization of the 5-HT1F receptor and glutamate in the vestibular nuclei (VN) of rats, serotonergic mechanism in the vestibular pathways are presumably parallel to trigeminal pathways involved in migraine. Adult male Long-Evans rats were deeply anesthetized with sodium pentobarbital and perfused transcardially with phosphate-buffered saline followed by PLP fixative. Alternate sets of 35 micrometers thick frozen sections were first stained immunocytochemically for rabbit polyclonal anti-5-HT1F antibody (1:500; Imgenex, San Diego, CA, USA) and rabbit polyclonal anti-glutamate

(1:500; Chemicon International, Temecula, CA, USA), a biotinylated secondary antibody and standard ABC-peroxidase methods. Double immunofluorescence (DIF) was then employed to investigate the co-localization of 5-HT1F receptor and glutamate in rat VN. Each the 5-HT1F receptor and glutamate was expressed differentially in 4 major VN in immunohistochemical staining study. In addition, the majority of 5-HT1F receptor positive neurons were also glutmate positive in DIF staining. These results suggest that the 5-HT1F receptor might modulate the release of glutamate from VN centrally. It seems likely that the 5-HT1F receptor agonists alone could contribute to the treatment of migraine and balance disorders.

955 Adaptive Changes in Spatial Properties of Central Otolith-Only Neurons

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Spatial temporal convergence (STC) properties of central vestibular neurons are attributed to convergence of peripheral inputs with static and dynamic inputs. Whether the dynamics come from the otolith organs (Angelaki et al. 1992) or from the semicircular canals (Yakushin et al. 2006), all models agree that STC behavior arises from inputs, which are out of phase by close to 90„a. We recently demonstrated that the direction of the response vector orientation (RVO), which is a projection of the otolith polarization vector onto the head horizontal plane, can be altered by prolonged head reorientation re gravity (Eron et al., 2008). Preliminary data indicate that changes in RVO can enhance or abolish STC characteristics of canal-otolith convergent neurons (Eron et al., 2008). Whether similar adaptation alters the spatial properties of otolith-only neurons is unknown. Changes of RVO in otolith-only neurons were determined in cynomolgus monkeys after 2 hrs in side-down positions. Combinations of static and dynamic otolith inputs were tested by oscillating animals about a spatial horizontal axis in different head orientations in the XY-plane. STC properties were verified using oscillation frequencies from 0.05 to 0.25 Hz. Data were compared to an extension of a model of STC behavior (Yakushin et al. 2006), which utilizes both static and dynamic otolith input before and after RVO adaptation. Otolith-only neurons had similar but smaller shifts in RVO than canal-convergent neurons. The maximal sensitivity of most of these neurons was significantly changed after adaptation. Thus, otolith-only neurons have more narrowly tuned projections from afferents and are more sensitive to small head deviations from gravity than canal-otolith convergent neurons. Prolonged head reorientation may also change the STC properties of otolith-only neurons.

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956 Role of Thoracic Spinal Interneurons in Generating Vestibulo-Autonomic Responses in Decerebrate Cats

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Stimulation of vestibular receptors produces changes in respiratory muscle activity as well as alterations in sympathetic outflow. Unlike vestibular reflexes acting on the limbs, vestibulo-respiratory and vestibulo-sympathetic responses are elicited by otolith organ inputs, particularly those generated by body rotations in the pitch (sagittal) plane, with little contribution of signals from semicircular canals. Recent studies have shown that thoracic interneurons make connections with respiratory motoneurons and sympathetic preganglionic neurons, but the responses of these cells to vestibular stimulation have not been explored. In the present study, recordings were made from thoracic interneurons in decerebrate, paralyzed cats. Approximately 60% of thoracic interneurons were activated by electrical stimulation of vestibular afferents at short latency (average of 9±1 msec). The majority of these cells exhibited response dynamics similar to those of otolith organs. Over 75% of the units were best activated by tilts in the roll plane or the planes of the vertical semicircular canals; few cells responded best to pitch rotations. These data show that vestibular signals to thoracic interneurons are distinct from those transmitted to the cervical and lumbar spinal cord; however the spatial properties of the responses of the cells to body rotation differ from that of respiratory and sympathetic outflow. Presumably, the latter responses are synthesized through the integration of signals partially relayed via interneuronal pathways.

957 The Changes of Calbindin Expression in the Flocculus After Unilateral Labyrinthectomy in Rat

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The role of the flocculus in vestibular compensation is still a longstanding controversial issue. Calbindin regulates intracellular signaling and consequently can alter the sensitiviey of Purkinje neurons to synaptic signals, recently is reported to be a more reliable marker of human Purkinje cell. Therefore, we examined the changes of calbindin expression in the ipsilateral and contralateral flocculus after loss of vestibular sensory organ. We used Spargue-Dawley rats that underwent Unilateral labyrinthectomy(UL), we examined the change of calbindin expression by immunohistochemistry at 2 hr, 6 hr, 24 hr, 48hr and 72 hr following UL.

Both the staining intensity and number of calbindin-positive Purkinje cells of calbindin in the ipsilateral flocculus decreased 6hr after UL compared with the control and contralateral side and returned to control level 48hr after UL and asymmetric expression in both flocculus also

subsided. Thus, our results suggested that transient reduction of calbindin expression in the ipsilateral flocculus might reflect a decrease in GABAergic inhibition of the ipsilateral VNC via floccular Purkinje cell projections to the vestibular nuclear complex during vestibular compensation. Thus, It was thought that we could identify the role of flocculus during vestibular compensation.

958 Changes in Glycinergic Synaptic Transmission and Neuronal Excitability in Mouse Medial Vestibular Nucleus Neurons During Early Vestibular Compensation

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Behavioural recovery after removal of a peripheral vestibular apparatus by unilateral labyrinthectomy (UL) is referred to as "vestibular compensation". Immediately after UL, *in vivo* studies have shown an asymmetry in discharge rate between ipsi and contralesional neurons in the medial vestibular nucleus (MVN). As behavioural symptoms subside, similar discharge rates are re-established. Fast inhibitory inputs ($GABA_A$- and glycinergic) are thought to play a role in mediating this compensatory process. Therefore, we investigated whether $GABA_A$ergic and glycinergic quantal synaptic transmission are altered during recovery from UL. Methods: Mice underwent surgical UL. Horizontal and anterior ampullae were removed, and the other organs disrupted. Immediately after surgery, animals displayed behaviour consistent with UL. At three time points after UL (4 hours, 2, and >7 days), whole cell recordings were obtained from 300 µm thick coronal brainstem slices. Discharge rates, and $GABA_A$ergic and glycinergic quantal currents were recorded using KCH_3SO_4 and CsCl-based internal solutions. Results: The discharge rate of contralesional type B MVN neurons was significantly reduced at all time points. There were no changes in $GABA_A$ receptor function following UL. In contrast, there were significant changes in glycine receptor function, 4 hours post-UL. mIPSC amplitude increased on the contralesional side versus control (70.2 ± 16.1 vs. 37.3 ± 6.6 pA, $p < 0.05$), whereas mIPSC frequency increased on both sides (ipsi- 0.6 ± 0.2, contralesional, 0.7 ± 0.2, vs. control, 0.2 ± 0.03 Hz). Conclusions: Our results suggest that glycine receptor mediated inhibition plays a significant role in altering neuronal excitability during the early phase of vestibular compensation.

959 Neural Network Model for the Horizontal Vestibulo-Ocular Reflex

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A neural network model for eye movements caused by stimulation of the horizontal semicircular canal, the vestibulo-ocular reflex (VOR), has been developed. The VOR is one example of eye-head coordination, whereby movement of the eyes serves to compensate for head movement, thereby enabling one to fixate on a region of space or an object of interest while he/she is moving. The model is being used as a theoretical framework to evaluate abnormalities of the VOR that are caused by inner ear disease or dysfunction of neural pathways and populations of neurons in the brainstem and cerebellum caused by the genetic family of neurodegenerative diseases, the spinocerebellar ataxias. The first aims are (a) to identify those neurons which might be involved in a disease process causing a specific pattern of abnormal behavior of the VOR and (b) to predict the extent to which the network could compensate for or adapt to the abnormal behavior and which neurons/pathways would be involved. The initial development and testing of the model uses artificial neural network techniques, while the connectivity among neurons and input-output characteristics of single neurons is based on physiology of the horizontal VOR. Matlab, simulink, and the neural network and parallel computing toolboxes serve as a software platform for custom network programs. Future development will test models for otolith-canal interactions and motor learning involving sensory-motor transformations from a three-dimensional perspective. Supported by U MN Supercomputing Institute and Lions Foundation.

960 Mechanisms Underlying Compensation of the Vestibulo-Ocular Reflex: Evidence for Sensory Substitution in the Vestibular Nuclei

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Compensation in the vestibulo-ocular reflex (VOR) pathway results in normal gains for rotations at low frequencies and velocities, following unilateral labyrinthectomy. Behavioral studies have provided evidence that extravestibular inputs could play an important role in this process. For example, VOR gains are enhanced during active head rotations (i.e., in the presence of neck afferent and efference motor command signals) compared to passive whole body rotations. In the present study, we recorded from position-vestibular-pause (PVP) neurons (type I and II) in the vestibular nuclei of alert rhesus monkeys, following contralateral labyrinthectomy. We used whole body rotation and body-under-head rotation (BUH) to test vestibular and neck sensitivities, respectively. To study the interaction of vestibular and non-vestibular signals we also used passive (PHB) and active head-on-body rotations. Acute stage (<day 5): We measured a 50-80% decrease in vestibular sensitivity of both type I and II PVPs. Surprisingly, ~70% of neurons also showed neck sensitivity during BUH rotation, which was antagonistic to the vestibular sensitivity for most of the cells. Notably, before labyrinthectomy PVPs did not show any neck sensitivity. The vestibular and neck signals interacted during PHB rotations, such that responses could be predicted by summation of the vectors of the two sensitivities. Finally, during active head rotations, when the gaze was stabilized, responses were similar to those observed during PHB rotations. Chronic stage (up to day 60): Over the next 60 days, vestibular sensitivities improved to about 60-80% of normal values. During the

same period, the percentage of cells with neck sensitivity decreased to ~40%. Although responses during PHB could still be predicted by addition of vestibular and neck sensitivities, those measured during active head movements were higher than predicted values (~30%). Conclusion: Our findings provide evidence for contribution of extravestibular signals in VOR compensation. The sensory substitution that occurs at the level of vestibular nuclei in both acute and chronic stages is likely to underlie the improved VOR response observed during active head movements.

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961 Visual-Vestibular Stimulation Interferes with Auditory Information Processing Task Performance in Older Persons

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The purpose of this study was to extend a prior study of interference between visual-vestibular stimulation and cognitive task performance. This study evaluated an older subject population, compared a spatial reaction time (RT) task to a non-spatial RT task, and focused on otolithic stimulation. Subjects were healthy young (n=19; 24+2.7yrs), old (n=15; 68+-2.9yrs), and old-old (n=12; 79+-2.9yrs) adults. Testing consisted of a dual-task paradigm. Subjects performed one of two different auditory choice RT tasks while during visual-vestibular stimulation. The RT task consisted of either 1) a frequency discrimination task or 2) a lateralization task. Performance was based on RT. Visual-vestibular conditions consisted of a non-movement baseline, sinusoidal earth-vertical axis rotation (EVAR) in darkness, off-vertical axis rotation (OVAR) at a constant velocity, OVAR with a sinusoidal profile, EVAR with a lighted visual surround, constant velocity optokinetic stimulation, and sinusoidal optokinetic stimulation. Testing required two experimental visits per subject, one for each type of RT task. Results indicated that RT while exposed to visual-vestibular stimulation was slower than that recorded while stationary in darkness. This effect was more pronounced in the oldest subject group. The prolongation was similar for each of the two RT tasks. Otolithic stimulation was associated with greater slowing of RT compared to visual stimulation, which was associated with greater slowing of RT as compared to semicircular canal stimulation. Combining semicircular canal with otolithic or visual stimulation had no additional effect beyond otolithic or visual stimulation alone. This study suggests that interference between vestibular stimulation and cognitive tasks is especially prominent in older individuals and with otolithic simulation. The spatial characteristics of the task do not appear to influence the interference.

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962 Low Level Blast Overpressure Exposures Initiate Gene Expression for Brain Microvascular Remodeling and Repair

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In the war on terror, blast-induced, traumatic brain injury (BI-TBI) has reached unheralded proportions. Sequelae of mild BI-TBI include tinnitus, hearing loss, emergent and delayed post-traumatic balance disorders, and migrainous disorders in the absence of overt histological or radiological evidence of damage. The pathophysiology of BI-TBI is still poorly understood.

This study provides the first data on the effects of low intensity (10-11 psi or 15-17 psi) blast overpressure (BOP) in female Sprague-Dawley rats. Brain tissues were analyzed with quantitative PCR arrays and histological markers of injury at 2, 24 and 72 h after BOP exposure. Behavioral measurements were performed on other animals out to 21 days post-BOP exposure. A battery of rat vascular remodeling mRNAs and interleukins showed BOP related up- or down-regulation (≥1.8-fold) of greater magnitude and/or duration with increasing BOP intensity (ANOVA, $p<0.01$). A shorter duration and/or smaller response appeared with increasing BOP for a battery of C-C and C-X-C motif chemokine mRNAs. Stress-related mRNAs revealed modest responses after low BOP, but considerably augmented responses by 72 hrs after higher intensity BOP. These changes were consistent with reactions to diffuse microvascular wounds that increased with greater BOP exposure. BOP exposure-related up-regulatory responses include mRNAs for Id1, ECM constituents, fibroblasts, endothelial growth/differentiation, Timp2&3, brain specific angiogenesis inhibitors, angiopoietin-1,VEGFa,b and d, lymphocyte docking, platelet derived growth factors, several interleukins and plasminogen activator-urokinase. Chemokine mechanisms for angiostasis (e.g., Cxcr3 + ligands), angiogenesis (e.g.., IL8b/Cxcr2 + ligands), hypoxia-induced factor 1 alpha were unchanged with blast intensity. Several C-C motif chemokine mRNAs and Cx3cr1 mRNA were reduced indicating vascular or perivascular cell impairment or death at the higher BOP levels. A late up-regulation (72 h post-BOP) of many stress genes (e.g., Bax, Nos1, Sod1, Sod2, Hsp family genes, Hmox1 and Hmox2) at the higher BOP level suggests more severe direct and secondary injury. Post-exposure behavioral responses showed decreased Rotorod performance (-30%) and open-field activity (-50%) for both BOP exposures.

963 Modification of the Torsional Cervico-Ocular Reflex (COR) by Canal-Plugging

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The yaw and pitch cervico-ocular reflex (COR) have low gains in normal animals. These gains increase to compensate for the angular VOR (aVOR) at low frequencies of head oscillation after canal plugging (Boehmer and Henn). Since the gain of the aVOR is smaller for roll than for pitch and yaw, it is not clear that the roll COR adaption would be similar to that of yaw and pitch. Here, we characterized the gain and phase of the roll COR and compared it to that of the roll aVOR in normal monkeys and those with all six semicircular canals or with only the lateral canal plugged. During experiments animals sat in a chair that could be rotated about the head in three-dimensions. The body was oscillated about body-yaw and body-pitch axes over a frequency range of 0.05-6 Hz, with amplitudes <10°. For normal animals, roll eye velocities were compensatory to the relative velocity of the head with respect to the body. The roll COR gains were 0.1-0.2 at frequencies below 1 Hz and decreased to zero as stimulus frequency increased above 1 Hz. In all six-canal plugged animals, roll COR gains were close to 0.7 at low frequencies, decreasing to 0.2 for stimulus frequency above 3 Hz. Roll aVOR gains in the canal-plugged animals tested at the same frequency range were negligible at low frequencies but were about 0.4 at higher frequencies. Taken together with our previous finding on yaw and pitch COR, this study demonstrates that the adaptation of COR gain is tuned to a frequency range at which the aVOR is compromised by the canal plugging in three-dimensions as for pitch and yaw.

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964 Canal Plugging Affects Lateral Control of Stepping During Normal Locomotion

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Canal plugging preserves the resting discharge of canal afferents, but changes the frequency response of the cupula/endolymph system, so that low frequencies (<2Hz) are blocked while high frequencies (>2Hz) are maintained. We studied how loss of low frequency canal afferents affects limb, body and head movements during quadrupedal locomotion of cynomolgus monkeys. Head, body and limb movements were recorded in 3-D with a motion detection system (Optotrak), while the animals walked on a treadmill. All six canals were plugged in two animals and both lateral canals in a third, reducing the canal time constants from ≈4.0 s to ≈0.07 s. Cycle-averaged yaw, pitch and roll head rotations, and translations were significantly larger and more erratic after than before surgery. There were major changes in the control of the limbs after surgery. Fore and hindlimbs were held farther from the body, producing a broad-based gait, and swing phase trajectories were inaccurate and splayed. Control of medial-lateral limb movement was erratic and animals' bodies lurched from side to side in successive steps. These changes in gait were present immediately after surgery, and also15 mos, 2.5 and 5.5 yrs later. Thus, control of the limbs in the lateral direction along the Y-axis was permanently defective after loss of the low frequency semicircular canal input and never recovered. We postulate that loss of the ipsilateral extension and contralateral flexion of the limbs in response to low frequency canal activation sensed by the canals during normal locomotion was responsible for the splaying of the swing phases, and model this by an extension of a previous model of forward (X-axis) stepping (Osaki et al. 2007, 2008) to include vestibular control of lateral motion of the swing phases. We conclude that the semicircular canals provide critical low frequency information that maximizes the accuracy of stepping and stabilizes the head during normal quadrupedal locomotion.

965 I^{KLT} and I^{h} May Explain Rapid and Short-Term Spike-Rate Adaptation in Auditory Nerve Fiber Responses to Cochlear Implant Stimulation

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Several physiological studies have shown that auditory nerve fibers in deafened ears exhibit adaptation in their spike rate in response to electrical pulse train stimuli from cochlear implants. Standard models of the auditory nerve membrane, such as the Hodgkin–Huxley, Frankenhaeuser–Huxley, Chiu, and Schwarz–Eikhof models, do not produce spike rate adaptation. In this study we investigate inclusion of low-threshold potassium (I_{KLT}) channels and hyperpolarization-activated cation (I_h) channels in a stochastic Hodgkin–Huxley model. Pulse-train responses were examined for pulse rates of 200, 800, and 2000 pulse/s at three different current levels. Simulation results show that the I_{KLT} channels generate a rapid spike-rate adaptation that is most apparent in model responses to the higher pulse rates. In contrast, I_h channels produce slower "short-term" adaptation in the spike rate that is noticeable also at the lower pulse rates. Interestingly, this adaptation occurs even in trials where the spike rate is quite low in response to the early part of the pulse train, consistent with experimental observations. This indicates that the observed phenomenon is a form of stimulus-dependent adaptation or accommodation rather than a spike-dependent adaptation of spike rate. Analysis of the model's ionic channels and the membrane potential behavior throughout the pulse train during different stimulus presentations indicates that subthreshold membrane responses, as well as spikes, lead to deactivation of the I_h channels, reducing excitability progressively throughout the pulse train.

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966 Sub-Threshold Electric Stimuli Can Enhance or Reduce Auditory Nerve Responsiveness

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At the peripheral level of auditory nerve fibers (ANFs), supra-threshold stimuli are known to cause post-stimulatory changes in excitability associated with refractoriness or adaptation, while the influence of sub-threshold stimuli has been thought to be limited to short-term effects, associated with stimulus integration, that persist for a few milliseconds. We have observed another category of sub-threshold effects. Pulse trains as brief as 100 ms and too low to elicit ANF responses can reduce responsiveness to subsequent supra-threshold stimuli. Recovery from the "sub-threshold masking" caused by such stimuli can require tens of milliseconds. This phenomenon contrasts with the assumption that rate adaptation is contingent upon prior spike activity. We examined this effect with low-rate (250 pulse/s) and high-rate (5000 pulse/s) trains of charge-balanced pulses delivered to chemically deafened cochleae. Trains with durations of 50-400 ms were used as forward maskers, followed by low-rate (10 pulse/s) probe pulses. The following effects were observed. First, "sub-threshold masking" was induced by high-rate, but not low-rate, pulse trains. Second, for supra-threshold trains that evoked similar numbers of responses (spikes), the high-rate trains caused greater post-stimulus effects. Third, the extent of sub-threshold masking could be increased with increases in pulse-train duration. These observations suggest that reduced responsiveness is related to a characteristic of the stimulus (e.g., the pulse train's total energy) rather than a characteristic of the neural response to that stimulus. Finally, we observed that sub-threshold high-rate trains could also facilitate greater responsiveness for a brief (<10 ms) period immediately after the train's offset, suggesting that two separate mechanisms are involved in these sub-threshold phenomena.

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967 Enhanced Transmission of Temporal Fine Structure Using Penetrating Auditory Nerve Electrodes

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Resolution of temporal fine structure through a conventional cochlear implant is surprisingly limited. For instance, most implant users can distinguish electrical pulse rates only up to ~300 pps. We tested the hypothesis that electrodes implanted in the auditory nerve, intraneural electrodes (IN), might transmit temporal fine structure more effectively than do conventional intrascalar (IS) electrodes. In anesthetized cats, we stimulated with electrical pulse trains at various rates using IN and IS electrodes. We recorded phase-locked activity in the central nucleus of the inferior colliculus (ICC). The limiting pulse rate was defined as the highest rate at which IC units showed significant phase locking. Consistent with our hypothesis, IN stimulation produced limiting rates of 300 pps or higher in about twice as many ICC neurons as did IS stimulation. At 600 pps, about 13% of IC units showed significant phase locking to IN stimulation compared to only ~4% for IS stimulation. One possible explanation for the higher limiting rates observed with IN stimulation is that high limiting rates might be associated with low characteristic frequencies (CFs) and that IN electrodes provide better access to low-CF fibers from the cochlear apex than do IS electrodes. Indeed, higher limiting rates correlated strongly with lower CFs, and the statistical difference in limiting rates of phase locking to IN vs IS stimulation was eliminated after accounting for the difference in the sample of apical-vs-basal cochlear fibers. Another characteristic associated with both higher limiting rate and lower CF was a tendency for short first-spike latencies in the condition of electrical stimulation, which eliminated the contribution of the cochlear traveling wave that is present with acoustic stimulation. In summary, the results indicate that temporal fine structure is transmitted most effectively by short-latency pathways originating in the apical cochlea and that such pathways can be activated by selective low-threshold stimulation of low-CF auditory nerve fibers using IN electrodes. The relatively poor transmission of temporal fine structure observed with conventional IS stimulation might be due to its failure to provide frequency-specific low-threshold stimulation of low-CF pathways originating in the cochlear apex.

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968 Improved Temporal Pitch Discrimination in Cochlear Implants with a Single Sideband Encoding Strategy

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To better encode temporal fine structure in cochlear implants, we proposed a sound processing strategy that can spectrally shift a band-limited signal towards a low-frequency band and subsequently generate pulse trains based on its time waveform. The frequency transformation is analogous to the coherent demodulation in single sideband radio receivers and it is called the single sideband encoder (SSE). Two variants of the 8-channel SSE strategy were implemented on the Nucleus NIC2 platform using NMT toolbox from Cochlear Corporation. One strategy (SSE_VR) used a variable rate to capture the waveform peaks. Another strategy (SSE_FR) used a fixed rate (1,900 pps) to modulate the half-wave rectified base-band signal. For comparison, an 8-channel CIS strategy was also implemented at the rate of 1,900 pps. Customized fitting software was designed to measure the threshold and most comfortable levels for each electrode.

We initially conducted acute experiments with 4 Nucleus Freedom implant users by presenting pre-generated pulse trains through the Laura-34 processor. The preliminary results suggested that SSE produced comparable

performance on melody and musical instrument identifications, and spondee word recognition in noise. However, all patients rated the SSE higher in musical quality, 5.8 (SSE) vs. 3.8 (CIS) on average in a 0-10 scale. Subjects' comments on SSE include "pitched", "distinguishable", "pleasant to listen to", etc.

Temporal channel interaction and the lack of sufficient training could contribute to the above observations. To further study whether a patient can perceive the temporal cues presented in SSE, we designed a single-channel pitch pattern discrimination experiment. Each pattern consists of three acoustic tones that are randomly roved by +/-4 dB SPL and then passed through the SSE or CIS processing. Each tone can be either a high-pitch or low-pitch signal falling within a target band. Three electrodes (E22, E13 and E7) were tested. All three patients were able to discriminate the 8 pitch patterns at a level of 50-100% correct for SSE, while the CIS strategy produced only performance at the chance level (12.5%). This remarkable improvement in the single-channel stimulation paradigm suggests that temporal fine structure can be encoded through the SSE strategy for a single channel and that efforts to minimize channel interaction in SSE could substantially improve multi-channel sound perception.

969 Temporal Pitch Perception of Dual-Channel Stimulation in Cochlear Implants

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One of the most commonly-cited reasons for the limitations experienced by cochlear implant (CI) subjects in a range of tasks is the spread of current between neighboring electrode channels. One source of evidence for this comes from the finding by McKay and McDermott (1996) that, when two different amplitude modulated pulse trains are presented to two electrodes separated by < 1.5 mm, patients perceive the aggregate temporal pattern. We attempted to replicate this general finding and to test whether dual-electrode stimulation would extend the upper limit of temporal pitch perception in CIs. We asked six CI subjects to rank a series of dual-channel stimuli differing in their rate (ranging from 92 to 516 pps on each individual channel) and in their inter-channel delay (pulses on the two channels being either nearly synchronous or delayed by half the period). We showed that, for an electrode separation of 0.75 or 1 mm, a) the perceived pitch was on average slightly higher for the long- than for the short-delay stimuli but never matched the pitch corresponding to the aggregate temporal pattern; b) the upper limit of temporal pitch did not increase using long-delay stimuli c) the pitch differences between short- and long-delay stimuli were relatively insensitive to channel order (basal or apical first) and to electrode configuration (narrow bipolar or monopolar). These results are consistent with the idea that each channel excites a largely discrete set of neurons, and that, even at quite narrow channel separations, the perceived temporal pattern is not strongly influenced by neurons that respond to the aggregate temporal pattern applied to neighboring channels.

McKay and McDermott (1996), JASA 100, pp. 1081-1092.

970 Investigating a Multi-Rate Speech Processing Strategy and the Benefits of Tuning in Cochlear Implants

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Speech recognition in noisy conditions and music appreciation remain a challenge for cochlear implant users. Using acoustic models, researchers have demonstrated that utilizing multiple carrier frequencies on each channel to encode more spectral information may lead to improvements in speech recognition performance (Nie et al. 2005; Throckmorton et al. 2006). This improvement has not yet been observed in actual cochlear implant listeners, and it has been hypothesized that subject-specific tuning may be necessary for users to obtain the maximum benefit from multi-rate strategies (Throckmorton et al. 2006; Stohl et al. 2008a). In this study, the Multiple Carrier Frequency Algorithm (MCFA) is compared to the clinically available Advanced Combination Encoder strategy (Kiefer et al. 2001) using speech materials (vowels, consonants, and open-set monosyllabic words) presented in quiet and varying levels of speech-shaped noise. Speech materials were processed with ACE, a generic, untuned version of MCFA, and implementations of MCFA in which an electrode-rate pitch map was applied and/or the rate update duration was modified. Tuned versions of MCFA were based on psychophysical data that were collected for previous psychophysical studies (Stohl et al. 2008a,b). Using pitch ranking data, band-pass filter outputs were assigned to electrode-rate combinations that would ideally result in a monotonic pitch structure. The minimum duration assigned to any single pulse rate was systematically varied according to previously obtained data. Preliminary results suggest that a multi-rate algorithm that has been tuned to a specific user may provide an increase in the amount of information transmitted to the user when compared to the clinically available ACE strategy under certain conditions. Results will be presented as both a function of percent correct as well as information received by the listener for comparison between the fixed-rate ACE strategy, untuned MCFA, and tuned MCFA.

971 Enhanced Fundamental Frequency Coding in Cochlear Implants

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Cochlear implant (CI) recipients show very poor pitch perception performance which impedes music perception and appraisal. A new signal processing algorithm F0mod is proposed that implements fundamental frequency (F0) coding in the electrical stimulus for improved pitch perception. An F0-extraction algorithm together with a voiced-unvoiced decision component was embedded into the signal processing chain of F0mod. For voiced signals the filter bank outputs were low pass filtered and then amplitude modulated at the frequency of the extracted F0. The F0mod scheme is compared with the Advanced Combination Encoder (ACE) strategy (clinical standard

provided in Cochlear devices) in psychophysical music perception related tasks. We used the same filter bank configuration in both schemes under study thereby eliminating possible spectral cues and ensuring identical processing of unvoiced sound segments.
In the psychophysical evaluation procedure we focused on music perception. First of all, our test battery contained Pitch Ranking (PR) and Familiar Melody Identification (FMI) of isochronous nursery songs. These two tests can be considered as standard procedures for the assessment of music perception in CI users. Furthermore, we included the Melodic Contour Identification (MCI) test. This test addresses the perception of complex and music related stimuli but does not assume any familiarity with the stimuli allowing the assessment of purely musical abilities. Consequently, with these tests we focus on different aspects of music perception at different levels of complexity. To ensure a systematic comparison between both processing schemes differences in loudness were examined and minimized in adaptive loudness balancing procedures.
Significant improvements in the PR, MCI and FMI tests were demonstrated with the new F0mod scheme. The results indicate a potential benefit from consistently mediated envelope pitch cues, i.e. via amplitude modulations provided in-phase across channels and at full modulation depth. However the loudness balancing results demonstrated that these cues significantly influence the loudness percept. This has to be taken into account when considering a real-time implementation.

972 The Effects of Stimulus Duration on Amplitude Modulation Frequency Discrimination in Cochlear Implants

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Contemporary cochlear implant (CI) devices primarily provide pitch information via temporal amplitude modulation (AM) of electrical pulse trains. CI users' recognition of segmental and supra-segmental speech information has been significantly correlated with their AM processing capability. The present study investigated temporal integration processes that underlie CI users' AM frequency discrimination. AM frequency discrimination thresholds (FDTs) and upward AM frequency sweep discrimination thresholds (FSDTs) were measured on single electrode in 4 post-lingually deafened adult CI users, as a function of modulation frequency (50, 100, and 200 Hz) and stimulus duration (50, 100, 200, and 400 ms). Reference stimulation levels were at 50% of the dynamic range; the carrier stimulation rate was 2000 pulses per second. Sinusoidal AM with 30% modulation depth and amplitude roving of 1 dB were applied to the reference levels. The results showed that FSDTs were highly correlated with FDTs, whereas mean FSDTs were 1.8 times larger than FDTs. Significant interactions were found between standard frequency and stimulus duration for both FDTs and FSDTs. Discrimination thresholds for 50 Hz were significantly higher with the 50 ms duration, possibly due to the small number of modulation periods within the stimuli. Thresholds for 100 Hz were not significantly different across durations. Interestingly, thresholds for 200 Hz were significantly higher with longer durations, possibly because the amplitude roving may have had a more pronounced effect given longer durations. Follow-up testing showed that, indeed, 200 Hz FDTs without amplitude roving were not significantly affected by stimulus duration. These preliminary results suggest that similar temporal integration processes are used to discriminate both static (FDTs) and dynamic (FSDTs) changes in AM frequency. As such, temporal integration greatly limits CI users' sensitivity to rapid pitch changes over a short period of time.

973 Effects of Electrode Configuration on Across-Site Variation in Cochlear Implant Function: Stimulus Detection Versus Modulation Detection

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Psychophysical detection thresholds (T levels) for electrical stimulation of single channels of cochlear implants are highly variable from channel to channel when narrow bipolar (BP+0) stimulation is used but much less variable across channels with monopolar (MP) stimulation. The across-site variation (ASV) has been attributed to local variation in the condition of the auditory neurons and /or variation in the pathways from the electrodes to the neurons. The effect of the electrode configuration on ASV might be related to differences in the longitudinal extent of activation of neurons by the two configurations. One theory is that MP stimulation activates more neurons, resulting in a greater diversity in the activated neural population and greater overlap between populations activated by adjacent electrodes and that these conditions result in more similar average responses across stimulation sites. In contrast, BP+0 stimulation is hypothesized to activate spatially restricted neural populations and thus be more sensitive to localized variation in the conditions of the neurons and the electrical pathways.
In the current study, we tested this hypothesis as applied to modulation detection thresholds (MDTs) for cochlear implant stimulation. In seven subjects with Nucleus Contour implants, T levels and MDTs at 30% of the dynamic range (DR) were measured for all available stimulation sites using BP+0 and MP electrode configurations. All subjects showed marked reductions in ASV of T levels but no consistent reduction in ASV of MDTs with MP stimulation compared to BP+0 stimulation. Thus, in searching for cochlear regions were MDTs are poor, there is no advantage to using BP+0 stimulation rather than MP stimulation. These results suggest that either (1) MDTs are not affected by spatial extent of excitation or (2) spatial extent of excitation for BP+0 and MP stimulation are similar on average at 30% DR.
Supported by NIH Grant DC004312 and a CTSA from the U. of M.

974 Can Species Difference in Intensity Coding Strategies Be Explained by Differences in Hair Cell-Afferent Fiber Synaptic Organization?

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The synaptic organization of auditory hair cells exhibit structural differences between species. Chick basilar papilla hair cells have 12 to 15 synaptic ribbons that identify sites of neurotransmitter release. Only two afferent nerve fibers contact each hair cell, and thus the avian post synaptic afferent fiber receives input from multiple putative release sites The PSTH of cochlear nerves attached to these synapses, and derived from a dual segmented tonal stimuli (100 ms at +20 dB re CF threshold immediately followed by 100 ms at +40 db re threshold), showed classical adaptation from the first segment. The sudden increase in second segment intensity caused a second well defined adaptation function. These observations contrast that seen in mammalian inner hair cells, which have a similar number of synaptic ribbons per cell, but each ribbon is associated with only one post synaptic auditory nerve afferent fiber. Stimulation with a nearly identical dual segment stimulus produced an adaptation function to the first segment. However, in mammals like the gerbil, neuron discharge rate during the second segment was unchanged even though stimulus intensity was increased +23 dB. The adapted level of discharge activity reached during the first segment persisted throughout the duration of the second segment (Westerman and Smith, 1987). If adaptation results from synaptic vesicle depletion, then chick hair cells compared to mammalian inner hair cells, appeared to recruit the release of additional vesicles in signaling a sudden change in stimulus intensity. Birds may utilize the innervation of a single afferent fiber by multiple synaptic ribbons to improve their ability to detect sudden changes in the intensity of ongoing sounds.

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975 Forward-Masking of CAP Responses Suggests a Unique Organization of the Auditory Papilla in Pygopod Lizards

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CAP recordings from legless lizards of the Australian gecko family Pygopodidae show frequency responses over 13kHz in species of the genus *Delma* (ARO 2008 MW meeting, #598). Further data collected in the field and using forward masking of CAP responses suggest a remarkable division of the pygopod papilla into two frequency ranges.

Freshly-caught Pygopods, *Delma pax*, *D. haroldi* and *D. fraseri* were gas anesthetized and a silver-wire electrode inserted deep into the mouth close to the “round window” to record CAP responses to 10ms tones (rise-fall 1ms) from the auditory nerve. Stimulation and recording were under the control of a laptop computer using Labview software and a PCMCIA card. I/O functions were measured using 3dB steps and CAP thresholds (noise+2SD) were close to 0.6µV and about 3dB more sensitive than visual detection thresholds. In forward masking, tones were preceded (3ms) by a narrow-band noise pulse (1000Hz-bandwidth, rise-fall 3ms) for 30ms.

Delma CAP thresholds increased to a high value at 8kHz, generally becoming somewhat more sensitive again above that. Remarkably, the CAP suppression tuning for tones above 3kHz often showed two sensitivity peaks. CAP responses to 4 and 7kHz were not only suppressed by tones in this same range but also by frequencies above 8kHz and vice versa. The I/O functions were always steepest at 8kHz. This strong recruitment and the double-peaked suppression curves suggest that each higher-frequency nerve fiber responds to two frequency ranges, below and above 8kHz.

The high-frequency region of the papilla of pygopods and other geckos is divided by a hiatus into two parallel hair-cell areas. Our data suggest that the two frequency-response ranges are represented in these two areas. Remarkably, the responses from the non-innervated post-axial hair cells must be indirect, through a micromechanical affect on pre-axial cells.

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976 A Chimera Analysis of the Prestin Mutation in Mice

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A chimera is a genetic composite containing a unique mix of cells derived from more than one zygote, allowing one to learn how cells of contrasting genotype functionally interact *in vivo*. For example, how does changing the relative proportion of prestin-containing outer hair cells (OHC) affect cochlear amplification? In order to address this question, we developed a prestin chimeric mouse in which both wildtype ROSA26 (WT) and prestin knockout (KO) genotypes are expressed in a single cochlea with the proportion of each genome equally likely. Because WT ROSA26 mice express a reporter gene, beta galactosidase, a blue reaction product develops when the substrate for this enzyme (LacZ) is added, allowing one to identify cells originating from the WT genome. However, the number and size of beta-galactosidase positive inclusions is variable, which complicates their precise quantification. Prestin antibody staining was, therefore, used to document the number and distribution of OHCs expressing prestin and derived from the WT genome. Anatomical examination indicates no propensity for OHCs of similar genotype to group together, consistent with reports that early aggregation mouse chimeras produce a mosaic in all tissues examined so far, including the cochlea. Cytocochleograms were compared with physiological data including thresholds and tuning curves for the compound action potential recorded in anesthetized mice using a round-window electrode. Analysis of these

measures did not reveal mixed phenotypes in which the proportion of prestin-containing OHCs impacted sensitivity and frequency selectivity to different degrees. By reducing the number of prestin-containing OHCs, it is possible to demonstrate phenotypes intermediate between WT and KO response patterns. In other words, there appears to be a quasi-proportional reduction in sensitivity and tuning as the number of OHCs derived from the KO genome increases. (Supported by NIDCD #DC00089 and NIMH #5R44MH066670).

977 Outer Hair Cell Loss in V499G/Y501H Prestin Knockin Mice

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A prestin knockin (KI) mouse model was developed in order to determine if threshold shift and loss of tuning are observed in mice with normal outer hair cell (OHC) length and stiffness but without motor function. Measurements for the compound action potential (CAP) measured at the round window indicate that V499G/Y501H KI mice show a large threshold shift and loss of tuning at ~P30 (Dallos et al., 2008). These animals also exhibit basal OHC loss. Consequently, we endeavored to examine the degree of OHC loss in order to determine if cell death might contribute to the change in phenotype. Cytocochleograms were constructed for mice at P42 to quantify the location and extent of OHC loss. When compared to the original prestin KO mouse (Liberman et al., 2002; Wu et al., 2004), the V499G/Y501H KI suffers a significantly greater loss of OHCs. In fact, at P42, there are no OHCs in the basal half of the cochlea. Cytocochleograms were also obtained in heterozygotes (hets). These animals show a wide variability in OHC loss. For example, at P42 some hets exhibit OHC loss patterns that are similar to the original KO, some to V499G/Y501H KIs. CAP thresholds and CAP tuning curves in hets at P42 indicate that mice with OHC loss patterns similar to KIs have a KO-like phenotype except at very low frequencies where presumably more functional OHCs are present at the apex of the cochlea. However, at ~P30 hets demonstrate CAP sensitivity and frequency selectivity similar to WT controls. This result implies that a combination of 50% WT and 50% V499G/Y501H prestin is adequate for normal cochlear function in the absence of OHC loss and assuming no genetic modifiers, strain variations, etc. (Work supported by NIDCD Grant #DC00089).

978 Gentamicin Decreases NADH Fluorescence in Outer Hair Cells of the Organ of Corti

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Gentamicin is an aminoglycoside antibiotic that is extremely effective in the treatment of gram negative bacteria infections, but has been known to result in both temporary and permanent hearing loss. For the first time we are able to investigate the effect of gentamicin on the metabolism of the organ of Corti. Two-photon fluorescence microscopy of NADH was used to monitor changes in NADH concentration after the administration of gentamicin. It was found that outer hair cells (OHCs) exhibited a significant decrease in NADH fluorescence over the course of a half hour of 300 μg/ml gentamicin treatment, when compared to controls. Inner hair cells and pillar hair cells did not exhibit this decrease, even though all cell types took up gentamicin as determined by confocal microscopy using gentamicin conjugated with the fluorescent dye Texas Red (1 μg/ml). The OHCs of the basal turn exhibited the most rapid drop in NADH fluorescence while the apical turn OHCs exhibited the least. The drop in NADH fluorescence may be due to changes in metabolism or to opening of the mitochondrial permeability transition pore (MPTP). Using cyclosporine A to block the MPTP should allow us to differentiate between these possibilities.
Supported by NIH DC008995, DC04555, and the American Hearing Research Foundation.

979 Characterization of Inner Ear Dysfunction in Caspase-3 Deficient Mice

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Caspase 3 is one of the most downstream enzymes activated in different apoptotic pathways. In Capase-3 deficient mice, loss of cochlear hair cells and spiral ganglion cells have been observed, coinciding closely with progression of hearing loss. In contrast to the auditory phenotype, the vestibular phenotype has not been characterized. We report the characterization of auditory and vestibular phenotype in correlation with inner ear histology in a novel construct of Caspase-3 deficient mice.
Auditory function, by auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAE), and vestibular function, by vestibulo-ocular reflex (VOR), as well as histology of the inner ear were compared among Caspase-3 homozygotes (Casp3-/-) (n=8), heterozygotes (Casp3+/-) (n=5), and wild type (Casp3+/+) (n=5) mice.
For ABR, averages of 500 responses to click stimuli were recorded in descending 5-dB stimulus steps to determine the threshold. For DPOAE, distortion product (DP) of 2F1-F2 were obtained (F2; 0.5- to 16-kHz). For VOR, two dimensional eye movements were recorded with various stimuli. Velocity gain and phase shift were compared.
Average ABR thresholds of Casp3-/-, Casp3+/-, and Casp3+/+ mice were 29.0 dB, 42.9 dB, and 88.3 dB respectively at 3 months of age. In DPOAE, DP was significantly decreased in Casp3-/- mice, whereas Casp3+/- and Casp3+/+ mice showed normal and comparable thresholds to each other. Most Casp3-/- mice were hyperactive and exhibited counter-clockwise circling behavior. In VOR testing, Casp3-/- mice had no response to any of the stimuli tested, whereas Casp3+/- and Casp3+/+ mice had normal responses.

Preliminary anatomical analysis revealed gross malformation of the lateral semicircular canal, mostly in the left ear.
These results indicate that Caspase 3 is essential for correct functioning of the cochlea as well as the vestibule. Further studies will be done to evaluate the histology of the inner ear.

980 Mondini-Like Malformation of the Cochlea in a Pendred Syndrome Mouse Model Is Due to an Enlargement of Scala Media and an Incomplete Ossification

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Pendred syndrome, due to mutations of *SLC26A4*, is characterized by an enlargement of the vestibular aqueduct, Mondini-like malformation of the cochlea, and congenital or progressive hearing loss during childhood. Knockout mice, *Slc26a4*$^{-/-}$, resemble the human disease including a prenatally developed enlargement of scala media and a failure to hear. Here we determine whether Mondini-like malformations occur in *Slc26a4*$^{-/-}$ mice. Cochlear morphology was evaluated in cryosections and in microdissected specimens. Ossification was evaluated by Alizarin Red staining. Gene expression of collagens *Col2a1* and *Col10a1*, which are markers of proliferative and hypertrophic chondrocytes and of *Bglap*, which is a bone matrix protein, were determined by quantitative RT-PCR using SYBR green for detection and 18S rRNA for calibration. Sex-matched *Slc26a4*$^{-/-}$ and *Slc26a4*$^{+/-}$ littermates were investigated at postnatal (P) age P3-8, which is before the onset of hearing, P22-35, which is after weaning, and at ~P80, which is a young adult age. At P3-P7, scala media of the cochlea of *Slc26a4*$^{-/-}$ mice was 10-fold enlarged. At P8, mineralization was lacking in the modiolus and in interscalar septa but no difference in mineralization of phalangeal and metatarsal toe bones was observed. At ~P22 and at ~P80, the differences in cochlear mineralization became less evident. At P8, cochlear expression compared to toe expression was 100-fold lower for *Col10a1*, 10-fold higher for *Bglap* and nearly equal for *Col2a1*. No differences between *Slc26a4*$^{-/-}$ and *Slc26a4*$^{+/-}$ mice were found for *Col10a1*, *Col2a1* and *Bglap* at ages P8 or P35 in the cochlea or in toes. In conclusion these data demonstrate a Mondini-like appearance of the cochlea in *Slc26a4*$^{-/-}$ mice that is due to an enlargement of scala media and an incomplete ossification. Whether the reduced ossification is due to reduced osteoblast or increased osteoclast activity remains to be determined.
Supported by NIH R01-DC01098 and NIH-P60-RR017686.

981 Expression of the Ascorbic Acid Synthesis Enzyme *Gulo* and the Ascorbic Acid Transporter *Slc23a2* During Early Postnatal Development of the Murine Cochlea

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The antioxidant ascorbic acid has been shown to protect the cochlea against noise and drug-induced damage. Ascorbic acid is synthesized in mice by the enzyme gulonolactone oxidase (*Gulo*) and transported across cell membranes by the Sodium-dependent Vitamin C transporters, Svct1 (*Slc23a1*) and Svct2 (*Slc23a2*). The first aim of the present study was to determine whether the cochlea expresses *Gulo* and/or *Slc23a2.* The second aim was to determine whether the expression of *Gulo* or *Slc23a2* is altered in a Pendred syndrome mouse model (*Slc26a4*$^{-/-}$) in which oxidative stress has been shown to be present in stria vascularis. Expression of *Gulo* and *Slc23a2* mRNA was determined by quantitative RT-PCR using SYBR green for detection and 18S rRNA for calibration. Total RNA was isolated from whole cochleae of *Slc26a4*$^{+/-}$ and *Slc26a4*$^{-/-}$ mice at postnatal (P) ages P2, which is before the onset of K^+ secretion, P6, which is after establishing mature K^+ gradients but before the generation of the endocochlear potential, and P15, which is after establishing a mature endocochlear potential and nearly adult levels of hearing sensitivity. Expression of Svct2 protein was determined by immunohistochemistry. Low amounts of *Gulo* mRNA were found in the cochlea at P2 and expression declined with further development. In contrast, high amounts of *Slc23a2* were found in the cochlea and no changes in expression occurred with development. No differences in mRNA expression were found between *Slc26a4*$^{+/-}$ and *Slc26a4*$^{-/-}$ mice. Svct2 protein was detected in the cochlea most prominently in outer sulcus and root cells, in interdental cells, in nerve terminals underneath inner hair cells and in spiral ganglion cells. In conclusion, these data suggest that cells in the murine cochlea rely on uptake of ascorbic acid via Svct2 (*Slc23a2*) and that oxidative stress, that is limited to stria vascularis, does not lead to an upregulation of *Slc23a2* mRNA expression.
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982 Commonality and Diversity in Cochlear Protein Profiles of Wistar, Sprague-Dawley and Fischer 344 Rats with Normal Hearing

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Differences in expression of cochlear proteins are among the important factors that determine the suitability of animal models for different types of auditory pathology. Identification of abundantly expressed proteins in inner ear may facilitate the search for markers and interventional targets. This study analyzed the cochlear protein profile of Wistar, Sprague-Dawley and Fischer 344 rats using a

broad spectrum antibody microarray. Normal hearing function of the animals was ascertained using distortion product otoacoustic emissions (DPOAE). Of 725 antibodies screened in whole cochlea, more than 80% are present in all three strains. However, there is striking difference in the levels at which they occur. Among the proteins that are expressed at levels ≥ 2 fold that of actin, only 7.5% are present in all three strains. Of these, myosin light chain kinase was immunolocalized in the cuticular plate of outer hair cells (OHC) while mitogen activated protein (MAP) kinase – ERK1 was detected in OHC, pillar cells, marginal cells of stria and in foci of spiral ligament. A review of the literature indicated that 8 of the 16 most abundantly expressed cochlear proteins have not yet been studied in the inner ear. One of these abundant, but unstudied proteins, MAP kinase activated protein kinase2, shows strong immunolabeling in inner hair cells (IHC) and is also expressed in OHC, supporting cells, neuronal cells of the spiral ganglion and marginal, intermediate and basal cells of stria vascularis. Protein profiling combined with immunolabeling may aid in the identification of key proteins involved in cochlear pathology and functional pathways that could be used to develop novel drug therapies. The diversity in the expression of abundant proteins in these 3 strains may contribute to differences in susceptibility of these strains to aging, noise or ototoxic drugs.

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983 Determining the Makeup of K+ Channel Currents in Spiral Ganglia Neurons

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To confer diversity of K^+ channel currents, neurons employ multiple sub-classes of pore-forming subunits, as well as differential splicing of genes. The diversity of functional phenotypes emanating from the alternative splicing could be further deepened by the ensuing diverse regulation and/or modulation of each subunit individually. Additionally, functional K^+ channels are tetramers and provide a clear example of the fine control of channel activity, which leads to diverse physiological consequences. Random heteromerization of the splice variants of K^+ channels and their indiscriminate functional interaction with other classes of K^+ channels may proliferate further functional diversity. However, solitary mutations in channel isoform/s may produce global impairment of K^+-mediated currents, leading to significant pathology. Such is the case for mutations in Kv7 channel in the auditory setting, which causes progressive hearing loss.

To understand the underlying molecular and functional mechanisms of membrane excitability in spiral ganglia neurons (SGNs), we investigated Kv channels in SGNs for their profiles, compositions, and properties during development and alteration with age. K^+ channel subunit profile was studied using immunostaining on primary culture of SGNs at different ages. To determine the physiological makeup of K^+ channel currents in SGNs, we used siRNA gene silencing of single subunits and dominant-negative mutant subunit expression, which were delivered into primary cultured SGNs using the lentivirus expression system. Potassium currents were recorded using an extracellular solution (in mM, NaCl 125, KCl 6, $CaCl_2$ 0-8, D-glucose 10, $MgCl_2$ 1, HEPES 10), and intracellular solution (in mM, KCl 120, Na_2ATP 5, $MgCl_2$ 2, HEPES 10, EGTA 1-10, or BAPTA 1-10 D-glucose 10). We will present data which demonstrates that multiple K^+ channel subclass are expressed by SGNs. Also important, the diversity of K^+ current properties in SGNs is established by the promiscuous interaction between different classes of K^+ channels.

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984 Change of Cochlear Mechanics in Acute Otitis Media and Otitis Media with Effusion

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One of major challenges to otologists is to discriminate acute otitis media (AOM) and otitis media with effusion (OME). Misdiagnosis of these two middle ear diseases results in inappropriate treatments and causes serious sequelae. The purpose of this study is to identify different changes of cochlear mechanics in these two diseases by experiments and statistical analysis. In this study, AOM was created by inoculating the left ear of guinea pig with Streptococcus pneumoniae (ATCC 6303) and OME was created by injection of lipopolysaccharide into the middle ear. The vibration of basilar membrane (BM) at the basal turn and auditory brainstem response (ABR) was measured in control and treated ears. The change of cochlear mechanics was found by performing Student t-tests ($P<0.05$) between the control and experimental data of BM movement and ABR measurements. The difference of cochlear mechanics change between AOM and OME was confirmed by ANOVA test ($P<0.05$) in the data obtained from measurements. This study provides new and useful data of cochlear mechanics change in AOM and OME and the analysis brings us new insight into discrimination of AOM and OME. (Work supported by OCAST HR-036 and NIH/NIDCD R01DC006632)

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