ABSTRACTS OF THE TWENTY-SIXTH ANNUAL MIDWINTER RESEARCH MEETING OF THE

Association for Research in Otolaryngology

February 22-27, 2003

Daytona Beach, Florida, USA

Peter A. Santi, Ph.D.
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. To understand current concepts of function of normal and diseased ears and other head and neck structures.
2. To understand current controversies in research methods and findings that bear on this understanding.
3. To 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. Robert Shannon, the President, Dr. Donata Oertel, and President-Elect, Dr. Edwin M. Monsell 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:
Christove, Gomez and Anderson, Genotype-phenotype Correlations for the Horizontal VOR in Spinocerebellar Ataxia 1-8, Assoc. Res. Otolaryngol., Abs.: 1077.

President's Message

Welcome to the 26th Midwinter meeting of the Association for Research in Otolaryngology! For the first time the meeting is held in Daytona Beach. It is a testament to the success of the organization and its MidWinter Meeting that a move had to be made from small quarters on the west coast of Florida to larger quarters on the east coast. Even with more abstracts than ever before, the poster sessions will now all be warm and dry, independent of the weather.

A perusal of the abstracts shows that the meeting promises to be diverse and interesting. Before the meeting officially begins, there will be a short course entitled "Vestibular System 101: Introduction to Vestibular System Structure and Function for Non-Experts." The meeting will begin with the Presidential Symposium on the "Calyceal Synapses of the Brain Stem." This symposium will highlight synapses whose specializations are not only remarkable for the role they play in hearing but that have also made possible biophysical studies of neurotransmission in the mammalian central nervous system. Speakers include E. Neher, Nobel Laureate, from Germany, J.G.G. Borst from the Netherlands, P. X. Joris from Belgium as well as L.G. Wu, E. von Gersdorff, L. O. Trussell, and R. Shannon from the United States. Other symposia occur over the following days. On Monday these include "Development of the Inner Ear" organized by D. Wu, "Tinnitus: Mechanisms, Models and Therapy" organized by M. C. Liberman, and "Afferent Synaptic Transmission in the Cochlea" organized by T. Parsons. On Tuesday there will be two symposia, "Functional Organization of the Auditory Cortex in Humans and Primates" organized by D. Hall and A. Palmer, and "Auditory Learning: Principles, Applications and Mechanisms" organized by D. Moore and B. Wright. On Wednesday a symposium entitled "Stem Cells and Progenitors: Identification, Isolation and Use" will be presented under the guidance of N. Segil and A. Groves.

The winner of the Award of Merit is David Kemp, whose work on otoacoustic emissions has not only contributed to an understanding of the cochlea but has also led to the development of sensitive, objective hearing tests. On Sunday afternoon he will present the Presidential Lecture, "Otoacoustic Emissions – A 25 year Overview." His contributions will be celebrated on Tuesday evening at the Award of Merit Ceremony.

The Program Book provides details about other highlights. These include workshops and special events.

The ARO owes its vitality to the people it attracts and to the support it receives. It is fortunate to be served by a group of exceptionally talented, generous and committed people. Much of the work of organizing this meeting and running the ARO is done by members of the Council and by those who serve on committees. The MidWinter Meeting of the ARO is supported in part by a grant from the National Institute for Deafness and Other Communication Disorders. Support for students and residents comes from the National Institute for Deafness and Other Communication Disorders as well as from the American Academy for Otolaryngology-Head and Neck Surgery Foundation and from the Deafness Research Foundation. The ARO also gratefully acknowledges the contributions of Springer Verlag, the publisher of the Journal of the Association for Research in Otolaryngology (JARO) and host of a reception on Sunday evening. The ARO is ably managed by the Talley Management Group, Inc..

Donata Oertel, PhD

David T. Kemp, Ph.D.

2003 Award of Merit Recipient

David T. Kemp Ph.D.
2003 Award of Merit Recipient

David T. Kemp was trained initially in physics and then in geophysics at Kings College, University of London. His graduate studies on naturally occurring low frequency electromagnetic radiation led to a number of novel observations. These included developing a method for global thunderstorm mapping and for localizing individual lightning bolt strikes, along with the characterization of rare, massive electrical discharges. These latter resonant events have since been identified as being giant electrical discharges from clouds that extend up to the ionosphere. Soon after completing his doctoral studies, to follow up on his interests in audio-frequency signals, Dr Kemp joined the research group at London's Royal National TNE Hospital that was attempting to quantify auditory perception in children. He soon extended his research to include the study of low-level hearing in adults, and it was his observations of the fine 'microstructure' of auditory sensitivity that led him to postulate sound 'reflection' inside the cochlea. Dr Kemp further hypothesized the reality of a biologically 'active' signal-processing mechanism, and predicted the consequential existence of evoked otoacoustic emissions (OAEs) from the ear. As the result of a series of experiments conducted in 1977, he demonstrated that the human ear really did generate sound as a part of the normal hearing process. After describing the existence of stimulus-frequency OAEs that mimicked the psychoacoustical microstructure and supported his internal-reflection hypothesis, he went on to observe both spontaneous and distortion product OAEs. To illustrate this new class of physiological response to the hearing field, Dr Kemp devised the transient evoked OAE method and described it, in 1978, in the initial peer-reviewed report on emitted responses. This original publication led to a series of grant awards from the United Kingdom's Medical Research Council, which permitted him to establish the auditory biophysics laboratory at the University of London that focused on further exploration of the biophysics of cochlear function using OAEs. There is no question that Dr Kemp's discovery of OAEs led to new avenues of research into the fundamental mechanisms of hearing including the subsequent finding by others of the outer hair cell's electromotility. In addition, the discovery of OAEs gave new life to the concept of the 'cochlear amplifier' and stimulated further research on the biomechanical nonlinearities of the cochlea. Moreover, the breakthrough finding of OAEs also led to the development by Dr Kemp of a new objective hearing test based on the detection of OAEs. By introducing the first commercial OAE instrument, he set the 'gold standard' for developing such devices for clinical testing. Another major contribution that Dr Kemp has made to clinical service has been his commitment to universal newborn hearing screening. There is no doubt that his interest in, and support of, this important endeavor have contributed to its visibility around the world. Most recently, Dr Kemp along with his auditory research colleagues at University College London (UCL), has spearheaded the development of a new facility called the UCL Centre for Auditory Research. This center, due to open in early 2004, will be a major national and European focus for integrated cross-disciplinary hearing research aimed at understanding the causes of deafness. In all, Dr Kemp has received a number of awards and prizes for his considerable scientific work on OAEs from professional societies, learned institutions, charitable foundations, and industry. It is fitting that the Association for Research in Otolaryngology honor David T. Kemp as the recipient of the 2003 Award of Merit for his significant contributions to both the basic and clinical hearing sciences.

Brenda Lonsbury-Martin
Susan Norton

Association for Research in Otolaryngology

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1999	Murray B. Sachs, PhD
2000	David M. Green, PhD
2001	William S. Rhode, PhD
2002	A. James Hudspeth, MD, PhD
2003	David T. Kemp, PhD

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*** Indicates presenting author**

1 Short-term Synaptic Plasticity at the Calyx of Held

*Erwin Neher1, Ralf Schneggenburger[1], Takeshi Sakaba[1], Felix Felmy[1], Volker Scheuss[2] [1]Membrane Biophysics, Max-Planck-Institut fuer biophys. Chemie, Goettingen, Germany, [2]Neurobiology, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724

The Calyx of Held is a large glutamatergic synapse in the auditory pathway, which offers unique possibilities for studying synaptic transmission. It allows simultaneous whole-cell patch clamping of the pre- and postsynaptic compartments. We established that under 100 µM CTZ (to protect from desensitization) and 1mM kynurenic acid (to avoid saturation) the post-synaptic current is a reliable sensor of transmitter release and we developped a specialized deconvolution method to calculate transmitter release rates from postsynaptic currents (Sakaba and Neher, J. Neurosci., Vol. 21, 462-476, 2001). We used Ca++-uncaging to elevate presynaptic free [Ca++] in a controlled manner and established the relationship between transmitter release rates and [Ca++] (Schneggenburger and Neher, Nature 406, 889, 2001). Using these methods we could show that during facilitation the sensitivity of the release apparatus is not changed. Rather, it seems that the effectiveness of Ca influx in increasing [Ca++] is enhanced. This effect might be mediated by the saturation of endogenous Ca++ buffering molecules in the presynaptic nerve terminal.

We found that short-term depression is partially mediated by postsynaptic desensitization (in the absence of (CTZ) and partially by depletion of a pool of release-ready vesicles (Scheuss et al., J. Neurosci. 22. 728-739, 2001). Remarkably, the pool of release-ready vesicles is heterogeneous, with about half of the vesicles releasing at a given ICa almost 10 times faster than the rest. Following depletion of the pool, the two components recover with distinct time courses, the more sensitive component being regulated by cAMP (Sakaba and Neher, PNAS 98, 331-336, 2001) and Ca++/Calmodulin. Depletion of the 'readily releasable pool' of vesicles and this heterogeneity of its components allow to explain the relatively complex relationship between short-term depression and stimulation frequency.

2 Vesicle Recycling in a Calyx-type Synapse of the Rat Auditory Brainstem

*J. G. G Borst, A.D.G. de Roos, R.P.J. de Lange Dpt of Neuroscience, Erasmus University, Rotterdam, Netherlands

The number of vesicles that are released following an action potential depends on the number of available vesicles and their average release probability. We studied vesicle pool dynamics in a giant terminal, the calyx of Held, in slices of the rat auditory brainstem. The calyx of Held is part of a fast auditory relay in the medial nucleus of the trapezoid body (MNTB), which is involved in sound localization. To gain insight in the sizes of different vesicle pools and the kinetics between pools, we labeled synaptic vesicles with the fluorescent styryl dye RH414. Electron microscopy after photo-conversion of RH414 showed that a maximum of ~50% of the vesicles could be labeled by a long (15-30 min) incubation in the presence of a high extracellular K+ concentration. In contrast, following afferent stimulation for 20 min at 5 Hz or for 5 min at 20 Hz, only ~5% of vesicles were labelled, despite the fact that the number of vesicles released was much larger than 5% of the total number of vesicles. Our data therefore suggest that in the calyx of Held, recently endocytosed vesicles are preferentially released.

3 Synaptic Vesicle Endocytosis At A Calyx-Type Synapse

*Ling-Gang Wu Department of Anesthesiology, Washington University, St. Louis, MO

Vesicle endocytosis provides a continuous supply of vesicles to maintain synaptic function. The kinetics of endocytosis may be critical to active synapses, such as the calyx-type synapse in the medial nucleus of trapezoid body (MNTB), which often relay high frequency trains of action potentials from the presynaptic to the postsynaptic neuron. Here we studied the kinetics of endocytosis at the MNTB synapse. We monitored endocytosis by measuring the membrane capacitance at the calyx of Held. We found that the time constant of 'quantal endocytosis', i.e., endocytosis after single vesicle fusion, was 56 ms. The endocytic time constant was about 115 ms following a single action potential or trains at £2 Hz, but increased gradually to tens of seconds as the frequency and the number of action potentials increased. These findings may reconcile a long-lasting debate on the rate of endocytosis between two schools championed by Heuser and Ceccarelli, respectively. Existing models that may account for slowed endocytosis include Ca2+ inhibition and saturation of a limited endocytic capacity. In contrast to these models, we found that a higher rate of vesicle fusion at active zones induced a slower rate of endocytosis, most likely via an accumulation of unretrieved vesicles at the plasma membrane. These novel mechanisms may slow vesicle recycling and thus may contribute to short-term synaptic depression.

4 Optimizing Synaptic Architecture and Efficiency for High-Frequency Transmission

*Henrique von Gersdorff[1], Holger Taschenberger[1], Ricardo M. Leão[1], Kevin Rowland[2], George Spirou[2] [1]The Vollum Institute, OHSU, Portland, OR, [2]Sensory Neuroscience Research Center Departments of Otolaryngology and Physiology, West Virginia Univ. Sch. of Med, Morgantown, WV

High frequency bursts of neuronal activity are transmitted more effectively as synapses mature. However, the mechanisms that control synaptic efficiency and short-term plasticity during development are poorly understood. In our presentation we will summarize studies of the postnatal changes in synaptic ultrastructure and exocytosis in the calyx of Held synapse in the medial nucleus of the trapezoid body. In mature mammals this excitatory synapse is capable of prolonged, high-fidelity transmission at near-kHz frequencies. How is this possible? How does it become optimized for the prodigious amounts of exocytosis necessary to sustain such high rates? How does it avoid synaptic depression and AMPA receptor desensitization and saturation?

We combined electron microscopy, presynaptic membrane capacitance (Cm) measurements, and excitatory postsynaptic current (EPSC) recordings in rat brainstem slices to study developmental alterations from postnatal day 5 (P5, shortly after calyx formation) to P14, just after the onset of hearing (about P12 in rats). Vesicle pool size, exocytotic efficiency (amount of exocytosis per Ca influx), quantal size, and the number of active zones (AZs) increased with age, whereas AZ area, number of docked vesicles per AZ, and release probability decreased with age. These changes, together with a remodeling of AZ cleft geometry, lead to AZs that are less prone to multivesicular release, resulting in reduced postsynaptic AMPA receptor saturation and desensitization. A greater multiplicity of small AZs with few docked vesicles, a larger pool of releasable vesicles, and a higher efficiency of release thus promote prolonged high-frequency firing in mature calyx-type synapses.

5 Regulation Of Synaptic Strength By Presynaptic Receptors In MNTB

*Larry Trussell[1], Rostislav Turecek[2], Gautam Awatramani[2] [1]Oregon Hearing Research Center, L-335A, Oregon Health & Science University, Portland, OR, [2]Oregon Hearing Research Center, Oregon Health & Science University, Portland, OR

Most auditory brainstem nuclei receive GABA- and glycinergic input. These are thought to play diverse roles in information processing, through their activation of postsynaptic GABA and glycine receptors. Recently we described the presence of presynaptic glycine and GABA-A receptors in the medial nucleus of the trapezoid body (MNTB) of the rat. This work has addressed 1) what are the consequences of activation of these receptors for release of the excitatory transmitter glutamate, 2) what is the mechanism of action of these receptors, and 3) how are these receptors regulated developmentally.

Neurons in brain slice preparations were voltage clamped. Bath application of 100 uM glycine or GABA isoguvacine (a GABA-A

agonist) reversibly increased the amplitude of evoked excitatory postsynaptic currents (EPSCs) by up to 60%. This effect was developmentally regulated, so that isoguvacine was most effective in < P10 and glycine is most effect > P11. Recordings from calyces of Held revealed the presence of presynaptic glycine and GABA-A receptors, which also had a reciprocal developmental pattern of expression.

Studies of inhibitory postsynaptic currents (IPSCs) revealed additional developmental regulation. In P5-7 rats, IPSCs were mediated primarily by GABA. By P14, the amplitude of the IPSCs increased 10-fold, their decay time dropped sharply, and they become largely glycinergic. These changes resulted largely from increase in the number of glycinergic synapses, which activate fast-gating glycine receptors. We also observed a developmental switch in the remaining GABAergic IPSCs: these increased in decay time significantly.

Thus, in parallel with the rise in presynaptic glycinergic effects, there is the acquisition of a fast postsynaptic inhibitory mechanism in MNTB. Current studies are exploring the significance of these mechanisms on the relay function of the MNTB.

Supported by grants from the National Institutes of Health (NS28901 and DC4055)

6 Calyceal Synapses in Two Binaural Circuits

**Philip X. Joris* Laboratory for Auditory Neurophysiology, K.U.Leuven, Leuven, Belgium

Two well-studied binaural circuits in the mammal contain calyceal synapses.

Spherical bushy cells in the anteroventral cochlear nucleus (AVCN) receive calyceal endings from the auditory nerve, and project bilaterally to the medial superior olive (MSO). MSO shows a bias towards low frequencies, and contains cells that compare the timing in their afferent inputs from left and right side. Comparison of responses of bushy cells to auditory nerve fibers shows that bushy cells form a monaural preprocessing stage which improves the temporal information delivered to the MSO.

Cells in the medial nucleus of the trapezoid body (MNTB) receive calyceal endings from contralateral globular bushy cells in the AVCN, and inhibit the homolateral lateral superior olive (LSO), which is excited by spherical bushy cells in the ipsilateral AVCN. The LSO shows a bias towards high frequencies, and its cells are sensitive to interaural level differences. Besides calyceal endings, this circuit show several other specializations which suggest that the temporal dimension is critical to its function. There is no definitive answer what the role of timing in this circuit is, but several possibilities will be discussed.

Supported by the Fund for Scientific Research - Flanders (G.0083.02) and Research Fund K.U.Leuven (OT/10/42).

7 The Auditory Smorgasbord: Perceptual Use of Temporal Neural Information

**Robert V. Shannon* Auditory Implants and Perception, House Ear Institute, Los Angeles, CA

The auditory nervous system can encode temporal information in the phase-locked discharges up to nearly 5 kHz and can represent interaural timing differences of 100s of microseconds. Neurons that respond to stimulus onsets may be able to encode timing information to resolutions of 100s of microseconds. However, it is not completely clear how this neural information is used in auditory perception. Recent work suggests that temporal information faster than 20 Hz contributes little to speech recognition. However temporal information in the 500-1500 Hz range is important for residue pitch and for localization. Clearly, different perceptual tasks have different requirements for temporal information. This talk will review the different types of temporal information coded in the auditory nervous system. Perceptual studies will be reviewed in terms of the use of temporal information. Rather than a serial, cumulative pathway, we suggest that it is more useful to think of auditory processing as a smorgasbord, in which auditory perceptual tasks can pick and choose the neural information needed for the task. Not all of the wealth of neural information is necessary or useful for every perceptual task.

8 Otoacoustic Emissions - A 25 Year Overview

**David T. Kemp* UCL Centre for Auditory Research, Institute of Laryngology and Otology, London, United Kingdom

The idea of sound actually emanating from an ear still fascinates the layman but in today's auditory physiology labs and audiology clinic it has become an everyday tool.

Twenty five years ago nothing could have been further from the truth. At that time a few researchers in the UK, Netherlands and the USA (including Wilson, Wit and Zurek) were wrestling with a group of weird acoustic and psychoacoustic phenomena which just didn't make sense – at least not as the auditory system was understood at that time. Preceding these observations throughout the 1970's were indications of mechanical non-linearity in the cochlea – not least those reported by Rhode, Pfiffer, and Kim. In parallel, both Lim and Flock were postulating a motile capacity to outer hair cells. All these observations were hotly disputed and in any case seemed to contribute little to a major goal of auditory research at that time which was to discover the basis of fine cochlear tuning. The scientific climate in 1978 paralleled that in1948 when Gold first proposed a tuning-enhancing cochlear amplifier powered by the endocochlear potential via a supposedly piezoelectric tectorial membrane. But this time the idea caught on once the existence of oto-acoustic emission was confirmed.

With the demonstration of hair cell electro-motility by Brownel and the confirmation of basilar membrane nonlinearity by Johnstone and Patuzzi in the early 1980s a new view of the cochlea - as a "nonlinear and active signal imaging device"- began to come very slowly into focus That heralded an explosion of research by psychoacousticians, physiologists, mathematicians, and audiologists- which continued right through the 1990s to today.

Thanks to all their work sound emission by the cochlea is now well (if not yet perfectly) understood leading to its exploitation in applications ranging from newborn hearing screening to auditory cell biology and genetics. Twenty five years is perhaps a good time to ask if there are lessons still to be learned and to speculate on what surprises the biophysics of the cochlea might still hold in store!

9 The Animal Rights (AR) Movement Is Rapidly Becoming a Mainstream Movement: Do Scientists Have a Plan to Counter It?

**Patti Strand* President, National Animal Interest Alliance, Portland, OR

The AR philosophy is growing, not because of the strength of its arguments, but because the people who could challenge it are not doing so. Organizations that should lead the fight seem more concerned over turf than over winning; more concerned over who should speak than in making sure that the message gets out; and more interested in getting credit than in assuring victory for all the parties they represent. Worse, many national organizations work to silence grassroots efforts by warning that anyone not appointed by them or reading from their script could undermine the effort. The result is that few voices are heard refuting animal rights nonsense.

Scientists, the most credible group among the targeted parties, too often remove themselves from the debate. Some are uncomfortable engaging in emotional debates or cannot reconcile working with other animal organizations whose missions they feel to be less noble than their own. Others believe that maintaining the proper image of a scientist is tantamount to the advancement of science itself and find it demeaning to respond to the lies of the animal rights fringe. Still others regard the topic as unworthy of serious consideration. They determine that animal rightists have little credibility, offer dishonest and outrageous claims to support their arguments and present themselves in ways that antagonize the public. They discount the possibility that animal rights philosophy

could ever become part of mainstream thought. They cannot see the forest for the trees.

Meanwhile, the AR movement is winning, not because its arguments are sound, but because it is organized, has a plan and because its leaders understand that repetitive messages no matter how false, when coupled with emotional (shocking) images alter perceptions of issues and eventually change public opinion. Public opinion is what eventually gets drafted into law.

The scientific community will continue losing ground in this debate until they make the animal rights issue a top priority. Scientists must come out of their laboratories and take on the intellectual dishonesty and overall corruption of the animal rights movement. If they do not, they face a world in which their values will take second place to the values of people who claim that an ant and a child deserve the same moral consideration.

10 NIDCD Research and Training Workshop for NEW INVESTIGATORS

*Amy M. Donahue NIH, NIDCD, Rockville, MD

This year's workshop will focus EXCLUSIVELY ON NEW INVESTIGATORS.

Following a brief overview of NIH funding mechanisms for new investigators, attendees will choose one of three breakout sessions:

NIH 101: How does the NIH system work?

Training and Career Development (Fs and Ks): What do I need to know?

Transitioning to Independence: Should I apply for an R03 or an R01?

The ultimate aim of the workshop is to provide information for a successful transition from the trainee/new investigator status to the independent investigator (R01).

NIH 101 will provide practical information on how the NIH/NIDCD works (e.g., institute and study section assignments, application timelines, reviewer assignments, funding paylines, council activities, and the role of program and review staff).

Training and Career Development will include a profile of research training and career development mechanisms appropriate for new investigators, including mechanisms for clinically trained investigators (K08 and K23) as well as the individual fellowship awards (F31/F32).

Transitioning to Independence will provide guidance on the appropriate grant mechanisms for early career stages, and will focus on the NIDCD Small Grant Award (R03). This session will also include a discussion of how to avoid mistakes commonly observed in the review process.

The breakout sessions are intended to allow for ample time for questions and answers. Handouts from all three breakout sessions will be available.

11 The Federal Appropriation Process for NIH Funding: How the Payline Gets Set

*Maureen Hannley[1], James Battey[2], Dominic Ruscio[3], Mary Wooley[4]
[1]American Academy of Otolaryngology, Head and Neck Surgery Foundation, Alexandria, VA, [2]National Institute on Deafness, and Other Communication Disorders, Alexandria, VA, [3]Cavarocchi, Ruscio, and Dennis Associates, Alexandria, VA, [4]Research!, America, Alexandria, VA

Many researchers depend on funding from federal agencies to support their work, but have little understanding of the annual appropriations process that determines the amount of funds available to those funding agencies. More importantly, they are either unaware that they are able to influence that process and hence the size of the appropriation, or are uncertain as to the best or most appropriate process to exert that influence. Everyone understands that The Payline largely determines which grants will be supported. The payline, of course, depends on the amount of money available to an institute, as well as a number of other factors. Determination of the next fiscal year's payline begins well in advance of that year with the appropriations process in the Senate and House of Representatives committees, continues with testimony and lobbying to attach report language, and culminates with processes and decisions in the Institute. This team presentation will include information on the Congressional committee structure and function; lobbying, appropriations hearings, and report language; the influence of advocacy groups; and the Institute planning process and payline determination. The expert panel will then discuss questions from the audience.

12 Expression Profile of the Human Inner Ear Genes and Identification of Candidate Genes for Genetic Deafness

*Zheng-yi Chen[1], Cynthia C. Morton[2], David P Corey[3] [1]Department of Neurology, Harvard Medical School, Boston, MA, [2]Department of Pathology, Harvard Medical School, Boston, MA, [3]Department of Neurobiology, Harvard Medical School, Boston, MA

Despite the progress in the human genome project and the efforts to identify the genes expressed in the mammalian inner ear, our knowledge of the genes expressed in the inner ear is very limited. Microarray technology has provided a powerful tool that can be used to study the inner ear gene expression.

Oligonucleotide array was used to survey genes expressed in the fetal human inner ear. Sub-tissues (the organ of Corti, the ligament) from the inner ear were collected to evaluate the regional expression patterns. In total ~27,000 genes and ESTs were classified as being expressed in at least one of the samples, representing 43% of genes and ESTs surveyed. A majority of genes were found to be expressed in all the tissues, with a subset of genes showed distinct patterns associated with each sub-tissue.

Other human tissues were also studied using the same chip set in order to identify the inner ear enriched genes. The tissues included the human brain, heart, kidney, testis and retina. ~2600 (4% of total number of genes surveyed) genes were identified as the inner ear enriched, being either expressed in the inner ear only, or predominantly expressed in the inner ear. Gene ontology representation showed many enriched genes encode for signal transducers, enzymes and transporters. Of 39 deafness genes on the chip 41% came from the inner ear enriched genes, indicating that other genes within the group are more likely to be involved in many forms of deafness. We have mapped all the inner ear enriched genes and their mouse orthologs to the human and mouse chromosomal locations, respectively. This approach will result in the enrichment of candidate genes for deafness by as much as 10 fold. In addition our work also identified genes enriched in other human tissues, thereby providing candidate genes for genetic disorders involving those tissues.

13 Identification of Candidate Genes Underlying a Critical Period of Afferent Dependence in Mouse Anteroventral Cochlear Nucleus Using a cDNA Microarray

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Cochlear ablation in young animals results in dramatic neuronal cell death in the anterovental cochlear nucleus while the same manipulation performed in older animals does not result in significant cell loss. The molecular basis underlying this critical period of differential susceptibility to loss of afferent input remains largely unknown. We used cDNA arrays spotted with the National Institute of Aging 15K mouse clone set to identify genes that are differentially expressed in the cochlear nucleus (CN) of mice within the critical period at postnatal day 7 (p7) compared to after the critical period at p21. Total RNA was isolated from 40 cochlear nuclei at each age to prepare cDNA probes

for hybridization. The experiments were done in triplicate. Approximately half of the 15,000 clones on this array are known genes. We identified 856 (5.6%) genes and expressed sequence tags that are differentially expressed in the CN (greater than 1.8 fold) between these developmental ages. 525 of these are known genes. 260 are more highly expressed in p7 CN and 265 have a higher expression level in p21 CN. These differentially expressed genes have a variety of functions including roles in apoptosis, cell cycle, energy and metabolism, signal transduction, and heat shock. Further analysis revealed 29 genes with a higher expression level in p7 CN and 44 genes with a higher expression level in p21 CN that have a previously described role in cell death, survival, or plasticity. This group includes caspase 3 in the p7 CN and several genes with antioxidant or redox roles more robustly expressed in the p21 CN. Expression levels of various genes will be validated using in situ hybridization and/or RT-PCR. These genes are included in a list of candidate genes that could potentially have a role in defining the critical period and the response to afferent deprivation in the CN.

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14 Identification of Genes that Influence Susceptibility of Zebrafish Lateral Line Hair Cells to Neomycin-Induced Death

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The susceptibility of inner ear hair cells to noise, aging and ototoxic drug exposure is strongly influenced by genetic variation. However, very little is known about specific genes that influence hair cell susceptibility and resistance. We have begun a genetic screen in zebrafish designed to identify genes that influence susceptibility of lateral line hair cells to death induced by exposure to neomycin. We made use of a method to efficiently examine lateral line hair cells in the live zebrafish using the vital dye DASPEI. This method was used to examine the dose-response relationship between neomycin concentration and lateral line hair cell death and was validated against both phalloidin labeling and acetylated tubulin labeling (Harris et al., in press, JARO). This dose-response relationship is now used in 5-day-old zebrafish larvae to conduct a screen for dominant mutations that alter the susceptibility of hair cells to neomycin-induced death. Adult male zebrafish were mutagenized with ethylnitrosourea, and F2 offspring were examined for their susceptibility to neomycin-induced hair cell death. At 5 days of age, each F2 clutch was divided into two groups of larvae. To find genes that increase sensitivity, one group of larvae was treated with 75 μM neomycin, a dose that causes very little hair cell death in wildtype larvae. To find genes that increase resistance, the second group of larvae was treated with 200 μM neomycin, a dose that causes near-maximal hair cell death in wildtype larvae. Of 479 F1 crosses screened to date (~10,000 fish), we have identified three mutations that increase resistance to neomycin. We are in the process of further defining the nature of these mutations and determining the parameters of resistance. One of these mutations, res1, significantly ($p<0.0001$) alters the entire neomycin toxicity dose-response curve relative to wildtype larvae.

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15 Eleven Mouse Mutants with Vestibular Defects all Map to Centromeric Chromosome 4

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Our laboratory has taken advantage of two large-scale ENU mutagenesis programmes which use a genome wide phenotype-driven strategy to identify mice with balance and hearing impairments, with the aim of discovering novel morphologies and genes involved in mammalian ear development.

Previous work in our laboratory characterised 7 independent mouse mutants derived from the Harwell mutagenesis programme that all exhibited a similar circling, head-bobbing and hyperactive behaviour. Morphological analysis revealed that all 7 mutant mice possessed a truncated or absent lateral semi-circular canal. Genetic mapping of these mutants linked the mutations to the centromeric region of chromosome 4 (Kiernan et al 2002, Mamm. Genome, 13: 142-148), where the mutant mouse Wheels had previously been mapped (Alavizadeh et al 2001, Dev. Biol. 234: 244-260).

Subsequently, 3 additional mouse mutants Carousel, Whirligig and Flouncer derived from the Neuherberg mutagenesis programme also showed similar abnormal behaviour. The aims of this project were to establish whether the three new mutants belonged to the group of chromosome 4 mutants and to find the gene responsible. Morphological analyses of Carousel, Whirligig and Flouncer have revealed they all possess abnormal lateral semi-circular canals. Genetic mapping has linked all three mutations to the centromeric region of chromosome 4. The region, with a physical distance of approximately 1.8Mb, is now being scrutinised for candidate genes in order to identify the mutation(s) responsible.

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16 Characterisation of the New Hearing-Impaired Mouse Mutants *Cloth-ears*, *Whisper* and *Hush*

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A major ENU mutagenesis programme has been underway over a number of years at Harwell, UK for the identification of new mouse models of human genetic disease. A major focus of the programme was to employ screens to identify new mouse deafness and vestibular mutants. A large number of new deaf mouse mutants have been recovered and characterised, of which the underlying genes for several have been identified by positional cloning. Hearing loss was identified by a reduced or absent Preyer reflex (ear flick) and startle response upon exposure to a 90dB, 20kHz noise, generated by a clickbox. Three new mouse mutants showing hearing loss have recently been identified. The autosomal dominant mutant *Cloth-ears* has reduced hearing with an age of onset from 30 days. A craniofacial feature and reduced body size appear to be variably present. The autosomal dominant mutant *Whisper* has reduced hearing, a blunt face, and reduced body size. Finally, the recessive mutant *Hush* shows deafness from around 40 days. These mutants represent opportunities for the discovery of new deafness genes. We are taking these mutants into positional cloning projects and will present phenotypic and genetic data for each of them.

17 Phenotypic Analysis of a New Mouse Model for Genetic Deafness: "Hush Puppy"

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Phenotypic and genotypic analysis of mouse mutants with hearing or balance problems has helped with the identification of the genes involved in deafness, and has contributed to the understanding of mechanisms of normal hearing. We describe a new mouse mutant, Hush Puppy, which shows abnormal pinnae and deafness. This mutation shows semi-dominant inheritance, and was generated by mutagenesis using ethylnitrosourea.

A total of 8 mutants and 8 littermate controls aged from 30 to 36 days and 16 newborns were investigated. Hearing was assessed by Preyer reflex. The morphology of their middle and inner ears were investigated by microdissection, clearing using glycerol, paintfilling of the labyrinth and scanning electron microscopy (SEM). Skeletal staining of skulls was performed to assess the craniofacial dimensions.

Hush Puppy mutants showed deafness from an early age (14 days)with no vestibular deficit. They have unilateral or bilateral small and bat- like pinnae, mild anomalies of the malleus, various degrees of abnormalities of the incus including small short process, malformed lenticular process, and absent posterior crus of the stapes, demonstrating patterns of incomplete penetrance and variable expression. Paintfilling of newborns' inner ears revealed no morphological abnormality, although half of the mice studied were expected to carry the Hush Puppy mutation. Under SEM, hair cells of the organ of Corti were normal in both mutants and controls at 29 days old. Skeletal staining showed that the Hush Puppy mutants have shorter snouts and mandibles.

These results indicate developmental problems of the first and second branchial arches due to a single gene mutation. Similar defects are found in humans, such as Goldenhar syndrome1, and Hush Puppy provides a mouse model for genetic analysis of such defects.

Reference

1. Beighton P, Sellars S (1982) Cranio-facio-cervical malformation syndromes (In: Emery A (ed) Genetics and Otology, p 44, Churchill Livingston, NY.)

18 Physical Mapping of Circling Mouse, and Complimentation Test With Spinner Mouse

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Circling mice were recorded to display profound deafness and a head-tossing and bidirectional circling behavior, showing an autosomal recessive mode of inheritance. In addition, the histological examination of inner ears revealed that the region around organ of Corti, spiral ganglion neurons and outer hair cells showed definite abnormality. On the other hand, a genetic linkage map was constructed in an intraspecific backcross between *cir* and C57BL/6J mice. The *cir* gene was mapped to a region between *D9Mit116/D9Mit15* and *D9Mit38* on the mouse chromosome 9. Estimated distances between *cir* and *D9Mit116,* and between *cir* and *D9Mit38* are 0.70 ± 0.40 and 0.23 ± 0.23 cM, respectively. The markers in order was defined as follows: centromere-*D9Mit182- D9Mit51/ D9Mit79/ D9Mit310- D9Mit212/ D9Mit184- D9Mit116/ D9Mit15- cir- D9Mit38- D9Mit20- D9Mit243- D9Mit16- D9Mit55/ D9Mit125- D9Mit281*. Based on genetic mapping, we constructed for a YAC contig across *cir* region. They covered the entire region of *cir* and *cir* gene was located on between the lactotransferrin (*ltf*) and the macrotubule-associated protein (*map4*). It is known that sr gene is localized in 64cM of mouse chromosome 9. The two mouse were found to be allelic by complementation test. Recently the spinner mouse has been mapped to our cir region, and tmie gene were elucidated. And further study will be needed in circling mouse to prove tmie gene mutaiton.

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19 Genetic Modifiers of the Inner Ear Phenotype in Jagged1 Heterozygote Mice

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Previous studies have shown that ENU-induced point mutations in the 2nd EGF-like repeat of the Jagged1 (>*Jag1*) gene lead to a dominant head-shaking behavior, indicating the presence of a vestibular defect (Kiernan et al Proc Natl Acad Sci 98:3873-8., 2001; Tsai et al Hum Mol Genet 10:507-12, 2001). Further analysis confirmed the presence of vestibular and cochlear defects in the *Jag1* heterozygous mice. Interestingly, heterozygote mice with a targeted deletion of the *Jag1* gene do not demonstrate the head-shaking behavior that was observed in the ENU-induced alleles.

Since the *Jag1* targeted allele was maintained on a C57BL/6J (B6) background whereas both ENU-induced *Jag1* alleles were maintained on a C3H background, we tested whether the phenotypic differences were due to genetic background. B6- *Jag1* +/- mice were crossed onto the C3H background to determine whether the head-shaking behavior could be recovered. F1 mice from this cross did not show any head-shaking behavior. However, a proportion of the N2 (backcross) mice did, suggesting there is either recessive enhancer modifier loci in the C3H background or dominant repressor loci in the B6 background. Approximately 32% (55/171) of the N2 mice showed robust head-shaking, suggesting the presence of only a small number of modifying genes. Phenotypic analysis of the B6- *Jag1* and F1- *Jag1* mice showed that, surprisingly, these mice demonstrate truncations in the posterior canal and missing posterior ampullae. However, N2 mice that exhibited the head-shaking behavior displayed truncations in both the anterior and posterior canals, and both respective ampullae were absent. In order to localize these modifier(s), a genome scan and QTL analysis will be performed. Identifying these modifier(s) will be important for uncovering new genes involved in sensory specification in the ear and possibly novel players in the Notch signaling pathway.

20 Characterization of GS3786 (FAM3C) in the Mouse Inner Ear

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A novel gene, GS3786, has recently been mapped near the DFNB17 interval at chromosome 7q31. Recently GS3786 was reported to be a member of a novel cytokine-like gene family and re-named FAM3C. The precise function of this gene remains unknown. FAM3C and its mouse orthologue, D6Wsu176e (mouse chromosome 6), share 88.3% homology at the RNA nucleotide sequence level and 94% of identity at the protein level. FAM3C was expressed in a human embryonic (16-22 weeks) cochlea library and mouse embryonic (day 15) inner ear library as full-length transcripts. We examined the expression of D6Wsu176e as an initial attempt to define a potential role for this gene in the mammalian inner ear. RT-PCR and Northern blot analysis of D6Wsu176e showed ubiquitous expression in several tissues that can suggests the gene involvement in a fundamental role essential for physiological functions in a variety of cell types. No alternative splicing was noted in the transcripts. Expression in the inner ear was demonstrated from embryonic day 10 (E10) through postnatal periods. Mutation analysis by direct sequencing of DNA from DFNB17 patients excluded the coding region of the FAM3C from being involved in the pathogenesis of DFNB17. Discussion will also include preliminary in situ hybridization data on D6Wsu176e suggesting a role for FAM3C during mouse inner ear morphogenesis.

21 A New Spontaneous Mutation in the Mouse Ames Waltzer Gene, *Pcdh15*

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The Ames waltzer (*av*) mouse is a model system to study the sensorineural defects associated with Usher syndrome type 1F. We report a new recessive allele of Ames waltzer caused by mutation of *protocadherin 15* (*Pcdh15*) that arose spontaneously on a C57BL/6J background strain. Auditory-evoked brain stem response (ABR) evaluation of mutants revealed a complete absence of auditory response and these mutants display a circling, head-tossing behavior. The mutant phenotype was genetically mapped near marker *D10Mit186* and shown by complementation testing to be allelic with Ames waltzer ($Pcdh15^{av}$). The mutation has been identified as a single nucleotide insertion at position 2099 of *Pcdh15*. The altered reading frame introduces a stop codon 17 amino acids downstream of the insertion site predicting a protein product truncated after the sixth extracellular cadherin ectodomain. Scanning electron microscopy revealed abnormal stereocilia on inner and outer hair cells of the organ of Corti as early as postnatal day 0. Adult mutants show severe degeneration of the cochlear neuroepithelium. The new allele of Ames waltzer is designated $Pcdh15^{av\text{-}Jfb}$.

22 *Jeff*, a Single Gene Model of Otitis Media.

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Otitis media is the most common cause of hearing impairment in children and is primarily characterised by inflammation of the middle ear mucosa. Yet nothing is known of the underlying genetic pathways predisposing to otitis media in the human population. As part of the UK mutagenesis programme we have identified a novel deaf mouse mutant, Jeff (Jf). Jeff maps to the distal region of mouse chromosome 17 and presents with fluid and pus in the middle ear cavity. Jeff mutants are 21% smaller than wild-type littermates, have a mild craniofacial abnormality and elevated hearing thresholds. Middle ear epithelia of Jeff mice show evidence of a chronic proliferative otitis media. 3D reconstruction of the middle ear cavity and Eustachian tube showed the cavity to be reduced in size and the Eustachian tube bent. In order to determine if a pathogen-free environment would affect the development of otitis media in Jeff mutants, we rederived mice into both conventional and pathogen-free facilities. Surprisingly, we found that rederived mice in both environments developed deafness and affected mice had glue in their middle ears as observed in sections. We compared the immune status of Jf/+ and wild-type mice raised in isolators. Age matched Jf/+ and +/+ mice were compared at 43-44 days and 121-127 days for lymphocytes, neutrophils, monocytes, lymphocyte cell surface markers CD3, CD4, CD19, and MHC class II cell surface expression. At 43-44 days the Jf/+ mice born in isolators have a significantly higher number of neutrophils compared to control mice. Both Jf/+ and +/+ mice show similar low levels of MHC class II cell surface expression that may indicate an inflammatory response occuring in Jf/+ mice in the absence of an antigenic stimulus (Hardisty et al., JARO in press).

The Jeff mutant should prove valuable in elucidating the underlying genetic pathways predisposing to otitis media.

23 The *Tommy* Mouse Mutant is a New Allele of *dfw* That Displays Semi-Dominant Age Related Hearing Loss.

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A genome wide phenotype driven ENU mutagenesis approach was adopted by our facility to describe, amongst others, novel dominantly inherited single locus deafness disorders in the mouse. One such mutant *Tommy, Tmy,* presents with progressive hearing loss with onset between 100 and 220 days of age. Homozygote animals are deaf and display a hesitant wobbly gait. Initial genetic analysis mapped the *Tmy* locus to distal mouse chromosome 6 overlapping the deafwaddler, *dfw,* critical region. Mutation screening of Atp2b2 discovered a G1750A change in the coding frame resulting in a Glu584Lys substitution in the predicted ATP binding domain. The presence of an age related hearing loss phenotype in heterozygotes of this mutant on a C3H homozygous 'protective' background at the modifier-of-deafwaddler, *mdfw,* locus establishes *Tmy* as an important tool with which to dissect the interaction of *dfw* and *mdfw* and the underlying molecular pathology of age related hearing loss in the mammalian ear.

24 A Mutation in the *Evi1* Locus Predisposes Late Onset Otitis Media With Effusion (OME) in the Mouse Deafness Mutant *Junbo*

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A genome wide phenotype driven ENU mutagenesis approach was adopted by our facility to describe, amongst others, novel dominantly inherited single locus deafness disorders in the mouse. One such mutant *Junbo* presents with progressive hearing loss with onset between 40 and 140 days of age. Histological examination of affected animals showed that the deafness appears to result from effusive matter filling the middle ear cavity providing evidence that *Junbo* represents a single locus model of otitis media with effusion (OME). Among other phenotypic characterstics *Junbo* also displays partial polydactyly, reduced body weight and craniofacial abnormalities. Here we report the positional cloning of this mutant and the elucidation of the causal mutation, A2318T (N763I) in the transcription regulator *Evi1* and present a model of how such a change in an oncogene commonly associated with leukaemia may also predispose to OME, the most common cause of hearing loss in children in the developed world.

25 Characterisation of the Progressive Deafness Mouse Mutant Oblivious

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Progressive hearing loss has been estimated to affect more than a third of individuals over the age of 60 and it is likely to have a significant genetic component. Mouse mutagenesis programs have provided a valuable resource to investigate the genetics of many hereditary diseases. Mutations are induced using the chemical N-ethyl-N-nitrosourea (ENU) and offspring are subsequently screened for specific phenotypes, including hearing and balance defects. Initial characterisation of the dominantly-inherited progressive deafness mutant Oblivious, identified from such an ENU mutagenesis program, is presented.

Oblivious (Obl) mutants exhibit a normal ear flick response (Preyer reflex) to a calibrated 90dB SPL 20kHz tone-burst at two weeks of age.

A progressive reduction in this reflex is seen in Obl mutants from 1-2 months, with most Obl mutants exhibiting no Preyer reflex by two months. Obl mutants do not exhibit circling behaviour characteristic of a balance defect and cleared samples of Obl mutant inner ears suggest that the morphology of the inner ear is normal. Scanning electron microscopy (SEM) analysis of the organ of Corti in Obl mutants at three months revealed missing outer hair cells (OHC) throughout the length of the cochlea, with most extensive degeneration and OHC loss present in the base. There also appears to be some inner hair cell loss in the base. The remaining hair cells look normal. This phenotype does not look like any other known mouse mutant, suggesting that the Oblivious locus encodes a novel progressive deafness gene.

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26 Aging Amplifies the Effects of Early Noise Exposure in CBA/CaJ Mice

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Interactions between age-related and noise-induced hearing loss remain poorly understood. The short life span of the mouse, as well as the availability of strain-specific variation in cochlear aging, make this an attractive model in which to address these questions. We evaluated noise-induced permanent threshold shifts (PTSs) by ABR and DPOAE in 3 groups of CBA/CaJ mice: 1) 'Expose Young-Test Young' animals were exposed at 4 wk and tested at 6 wk; 2) 'Expose Old-Test Old' animals were exposed at 12-18 mo and also tested 2 wk post-exposure; 3) 'Expose Young-Test Old' animals were exposed at 4 wk and tested at 18-20 mo. Age-matched, unexposed cagemates served as controls. Middle ear function was assessed by laser vibrometry.

In the Expose Young-Test Young group, a 2 hr exposure (8-16 kHz OBN, 100 dB SPL) produced ~25-30 dB maximum PTS. The same exposure was minimally traumatic to older animals: PTSs for the Expose Old-Test Old group remained below 10 dB. Unexposed controls demonstrated age-related threshold elevations: At 12 mo, shifts were ~15 dB or less; by 18 mo, thresholds were elevated 15-25 dB from levels at 6 wk. Age-related changes in middle ear function were observed, but could not account fully for age-related threshold shifts.

Animals in the Expose Young-Test Old group showed much larger PTSs than expected from simple addition of the noise induced- and age-related shifts seen in other groups: PTSs were 50+ dB across the entire frequency range. These data suggest that early noise damage renders the ear significantly more vulnerable to aging, and that interactions between age-related and noise-induced hearing loss can be studied systematically in the mouse model.

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27 Redefining the DFNB17 Interval in Consanguineous Indian Families

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We previously mapped the DFNB17 locus to a 3-4 cM interval on human chromosome 7q31 in a large consanguineous Indian family with congenital profound hearing loss. To further refine this interval, 30 new highly polymorphic markers and 8 SNPs were analyzed against the pedigree. Analysis in the original DFNB17 family determined the interval to be more centromeric and larger than previously reported (9 cM or about 11 MB on the physical map). A second consanguineous family with congenital deafness from the same geographic region was found to map to the redefined interval, limiting the area of shared homozygosity-by-descent (HBD) to approximately 4MB between markers D7S2453 and D7S525. Analysis of a genome-wide screening revealed no other significant areas of shared HGD. Included in this interval is the *SLC26A4* gene; previous sequencing of the coding and splice site regions of this gene did not reveal mutations in either family. DNA sequence analysis of the 5' and 3' non-coding regions and a putative transcription promotor site likewise revealed no mutations. Nineteen known genes and over 20 other cDNAs have been identified in the redefined DFNB17 interval. We have determined the cDNA sequence and genomic structure for 4 other cochlear-expressed genes that map to the DFNB17 interval. These include 2 isoforms of the basement membrane proteins in the laminin family, another anion transport gene in the SLCA family and a novel synaptophysin-like protein presumed to be involved in vesicle transport in the neural system. Mutation analysis for these genes is currently underway. In conclusion, DFNB17 has been relocated to a more centromeric location and, with the identification of another family mapping to this region, the interval has been narrowed to approximately 4MB by physical mapping. Mutation analysis in *SLC26A4* has not yet determined the cause of DFNB17, while analysis of several other candidate genes is in progress.

28 Refining the Genetic Interval for an Autosomal Dominant Hearing Impairment Locus, DFNA27.

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DFNA27 is a locus for autosomal dominant nonsyndromic hearing loss on chromosome 4q12-13. This locus is defined by genetic mapping analysis of a large North American family segregating progressive nonsyndromic sensorineural hearing loss. The reported age of onset for hearing loss was variable, ranging from pre-teens to late twenties. Audiogram data showed that affected individuals under the age of 40 years exhibit moderate to profound hearing loss (30-90 dB). In older affected individuals hearing loss was profound (> 90 dB). To map the gene causing hearing loss in this family, we first excluded linkage to known deafness loci and then performed a genome wide scan. Significant evidence for linkage was found to genetic markers in a 15 cM interval between D4S428 and D4S393 (maximum LOD = 4.76 at q = 0 for D4S3248) on chromosome 4q12-13. Additional genotyping analyses of polymorphic markers within this region refined the critical interval to 5.7-cM between D4S1592 and D4S1541. The DFNA27 physical map spans 8.8 Mb of DNA and includes at least 19 predicted and known genes. In order to identify the DFNA27 causative gene, candidate genes will be selected for mutation detection using DNA sequencing analysis. Future efforts include ascertaining additional DFNA27 families in order to reduce the 5.7-cM genetic interval and the identification of a mouse model.

29 Gene Identification for the DFNA37 Locus

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We have previously reported linkage of dominant progressive hearing loss in a 4-generation American family to chromosome 1p21. Linkage analysis with markers from ABI Prism panels produced a LOD score of 8.29 for marker D1S495 at è=0.0. This new locus is designated as DFNA37. Affected individual experiences an early-onset high

frequency hearing loss, which progresses with age to include middle and lower frequencies. The craniofacial features of affected family members are entirely normal, and there is no history of ocular abnormalities nor cleft palate in the family.

Using the cochlear ESTs, we identified 4 candidate genes: KIAA0893 (an unknown protein), GPR88 (G-protein coupled receptor), ABCD3 (ATP-binding cassette), and CNN3 (Calponin 3, acidic) in the DFNA37 region. We identified several SNPs in the genomic sequences of these genes by searching the GenBank sequences. These sequence variations will be used to verify segregation of the candidate genes with the DFNA37 locus. Mutation screening will be performed on the gene(s) which are linked to this deafness locus.

30 Genetic Mapping and Identification of an Autosomal Nonsyndromic Recessive Deafness locus *DFNB37*

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Autosomal recessive profound nonsyndromic deafness in a large consanguineous family from Pakistan (PKDF10) was found during a genome-wide scan to co-segregate with markers on chromosome 6, defining a novel locus *DFNB37*. A fully informative linked marker supported a LOD score of 6.53 at θ=0. Subsequently, linkage to *DFNB37* was found in two additional Pakistani families (PKDF71, PKSR14) segregating recessive nonsyndromic deafness. The three families are unrelated and carry different haplotypes for the markers tested in the linkage region. In addition to pure-tone audiometry, participating family members underwent medical history interviews, physical balance tests (Romberg and tandem gait tests), and, in a subset of hearing impaired subjects, funduscopy, electronystagmography and electroretinogram examaninations were performed. Upon sequencing a candidate gene from the *DFNB37* linkage region, a single base pair insertion in the open reading frame, predicted to cause a frameshift leading to premature termination, was homozygous in affected individuals of family PKDF10. Affected individuals of family PKDF71 have a homozygous nonsense mutation in the second to the last exon of the same gene. The deaf individual of family PKSR14 has a homozygous missense mutation in a non-conserved amino acid. This mutation was not found among 351 normal representative DNA samples from the same ethnic group or from the Human Diversity panel (Corriell Cell Repositories). The two other mutations were not found by sequence analysis among DNA from 100 normal control samples from Pakistan. Screening of DNA samples indicate a prevalence of 1.5% of *DFNB37* among Pakistani families segregating severe to profound deafness.

31 Genetic Linkage Analysis in a Pedigree with Progressive High-Frequency Sensorineural Hearing Loss

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We are characterizing a large four generation family with moderate-to-profound bilateral sensorineural hearing loss. Auditory dysfunction in this pedigree is noticed typically in the high frequency ranges by the second decade of life and progresses to the mid and low-frequencies by the fourth decade. Most affected older family members are profoundly deaf across all frequencies. One 78-year-old family member has undergone cochlear implantation. The mode of inheritance in this large pedigree appears to be autosomal dominant. A candidate loci and genome-wide linkage analysis have been initiated on 29 DNA samples from this pedigree.

32 Candidate Gene Screening for the DFNA41 Locus

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We have recently mapped the 41rst gene locus for autosomal dominant hearing loss to a 15cM region on chromosome 12q24-qter. Affected subjects show gender difference in the pattern of hearing loss, suggesting the presence of a modifier. Linkage data and recombination events between D12S1609 and D12S343 in two affected individuals place this novel locus distal to DFNA25 in an interval defined by D12S1609 and the telomere of the q arm of the chromosome 12. The DFNA41 candidate region is syntenic to the mouse chromosome5 containing Bronx walzer (bv), a mouse recessive deafness mutation. Public databases search identified numerous known genes, Unigene clusters as well as program predicted genes in the candidate genomic region. We are screening three potential functional candidate genes within this interval by direct sequencing: a) the frizzled 10 gene, which encodes a seven-transmembrane-receptor with the C-terminal Ser/Thr-Xxx-Val motif that believed to be a binding site for scaffold proteins with multiple PDZ domains, b) the Zing Finger Protein 10 gene (ZNF 10) that may function as a transcription factor, c) the epimorphin gene encoding a protein with T-Snare coiled-coil homology domaine. We are also using 3 single nucleotide polymorphisms (2 in 3'untranslated and 1 in coil-coil domain) of the epimorphin gene for association study. Direct sequencing of the ZNF 10 coding sequence in DFNA41-affected individuals revealed no pathogenic sequence changes. These results provide evidence for the exclusion of this candidate as theDFNA41-causative gene. In a separate study we have identified over 22,000 genes and ESTs expressed in the human inner ear. Cluster analysis has identified ~2,000 of them (~10%) to be highly enriched in the cochlea. Interestingly 4 cochlea-enriched genes are located within the interval of DFNA41. Analysis of involvement of the candidate genes as well as SNPs association study is currently underway.

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33 Refined Mapping of the Familial Laryngeal Abductor Paralysis Locus on Chromosome 6q16-21 and Candidate Gene Mutation Screening

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Familial laryngeal abductor paralysis(FLAP) is an autosomal dominant inherited disorder with variable penetrance. Our previous linkage analysis studies of a family with this order identified the first genetic locus for this disease in the chromosome 6q16-22 region.

Further genetic linkage and haplotype analysis with both known and novel tandem repeat markers of our own design has defined a 3.6 Mb critical region for FLAP between D6S1692 and AL591516.5. Additional novel repeat markers within this region also display significant linkage to FLAP with lod scores > 3.0, consistent with our previous data.

The FLAP region contains at least 18 known and putative genes according to current human genome mapping data. Candidate genes have been prioritized for mutation screening by function and tissue expression. Single strand conformation polymorphism gel analysis and direct sequencing of BVES, POP3, PREP and FOX03A exons and splice sites have identified some single nucleotide polymorphisms in this family, but none have been identified that solely segregate with the affected individuals.

34 Genetic Mapping Study of Acoustic Startle and Prepulse Inhibition

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The present study investigates the genetics of central auditory processing in mice using two auditory behaviors, the acoustic startle response (ASR) and prepulse inhibition (PPI), widely accepted as a measure of central "sensory gating." Five inbred strains were chosen on the basis of data obtained in a survey of 40 mouse strains being used in the Mouse Phenome Project. AKR/J and SM/J strains exhibit extremely large acoustic startle responses (ASRs) and very strong prepulse inhibition (PPI), whereas strains SJL/J and MOLF/Ei exhibit small ASRs and poor PPI. Because all four strains have normal threshold sensitivity, the differences are assumed to reflect central brain mechanisms. A fifth strain, C57BL/6J (B6) is used as well because it has "average" ASRs and PPI and is the focus of mutagenesis and other genetic studies. F1 hybrids and N2 backcross or F2 intercross mice are being produced using the five strains. Genome-wide scans for linkage will be undertaken to map the underlying genes as quantitative trait loci (QTLs). The individual contribution of each new QTL and its interactive effects with other loci will be examined by producing defined genetic combinations on uniform strain backgrounds.

Most of the possible F1 combinations have now been tested. Among the more interesting results, all hybrids with MOLF/Ei, a strain with weak ASRs/PPI likewise exhibit weak ASRs/PPI. However, the results with the other weakly responding strain, SJL/J are more complicated: when crossed with C57BL/6J, F1 hybrids have weak ASRs/PPI but when crossed with AKR/J, responses are strong. Other crosses exhibit behaviors whose strength is intermediate with respect to the parental strains. Backcrosses between selected F1 hybrids and inbred strains are being employed for mapping.

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35 A Human Mitochondrial GTP Binding Protein Related to tRNA Modification May Modulate the Phenotypic Expression of the Deafness-Associated Mitochondrial 12S rRNA Mutation

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Human mitochondrial 12S rRNA A1555G mutation has been found to be associated with aminoglycoside induced and non-syndromic deafness. However, putative nuclear modifier gene(s) has been proposed to regulate the phenotypic expression of this mutation. In yeast, mutant alleles of MSS1, encoding a mitochondrial GTP binding protein, manifest a respiratory-deficient phenotype only when coupled with mitochondrial 15S rRNA PR454 mutation corresponding to human A1555G mutation. This suggests that MSS1-like modifier gene may influence the phenotypic expression of the A1555G mutation. Here we report the identification and characterization of human MSS1 homolog, GTPBP3, the first identified vertebrate gene related to mitochondrial tRNA modification. The Gtpbp3 is the mitochondrial GTPase evolutionarily conserved from bacteria to mammal. Functional conservation of this protein is supported by the observation that isolated human GTPBP3 cDNA can complement the respiratory deficient phenotype of yeast mss1 cells carrying PR454 mutation. GTPBP3 is ubiquitously expressed in various tissues as multiple transcripts, but with a markedly elevated expression in tissues of high metabolic rates. We showed that Gtpbp3 localizes in mitochondrion. These observations suggest that the human GTPBP3 is a structural and functional homolog of yeast MSS1. Thus, allelic variants in GTPBP3 could, if they exist, modulate the phenotypic manifestation of human mitochondrial A1555G mutation.

36 Skewed X-Inactivation in Carrier Females and Refined Mapping of a Novel Deafness-Mental Retardation Syndrome

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We previously reported three related males with congenital deafness, mental retardation, short stature, and multiple other anomalies who share a common haplotype on the X chromosome, between markers DXS1003 and DXS1220 (Martin et al, 2000). The precise genetic lesion in this family has not yet been identified. For this study, we performed PCR analysis with multiple polymorphic markers within the nonrecombinant interval, and excluded chromosomal deletions 5 Mb or greater. Mutations in exons 8, 9 and 10 of the XNP gene, which cause ATR-X syndrome, were excluded by direct sequencing. Obligate carrier females in this family are clinically normal, and the small pedigree size has precluded extensive genetic mapping. Using a previously defined assay for X-inactivation at the HUMARA locus, we identified skewed X-inactivation in peripheral leukocytes of obligate carrier females. We ascertained carrier status in several females in the pedigree by skewed X-inactivation, and obtained linkage to the pericentromeric region of the X chromosome (LOD = 3.01 at DXS988). Interestingly, there is a statistically significant preference for transmission of the X chromosome carrying the mutation ($p = 0.004$), as well as suppression of recombination within the nonrecombinant interval ($p = 0.02$). We hypothesize the presence of a chromosomal abnormality within the nonrecombinant region that suppresses recombination and is preferentially transmitted to the developing ovum during female meiosis. Identification of the causative mutation in this family will offer clues to the genetic bases of hearing, cognition, hematopoiesis, and normal growth and development.

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37 Chromosomal Localization and Characterization of the Nicotinic Acetylcholine Receptor alpha9 (CHRNA9) Gene

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The human nicotinic acetylcholine receptor (nAChR) subunit alpha9 gene product (CHRNA9) codes for a component of the AChR in hair cells of the inner ear. While no direct evidence presently links this gene to known hearing disorders, it may underlie individual susceptibility to acoustic inner ear injury, and is associated with the autoimmune skin disorder Pemphigus vulgaris. Future studies will depend upon a thorough characterization of the nAChR alpha9 gene.

The nAChR alpha9 gene was localized to chromosome 4p4p14-15.1 by FISH analysis. Radiation hybrid mapping further localized the gene between markers D4S405 and D4S496 (Stanford G3 panel), and between markers WI-3875 and D4S1231 (Genebridge 4 panel), representing a distance of approximately 3.1 cR. The D4S405 marker has been linked to a non-syndromic form of hereditary hearing loss, DFNB-25.

The gene contains 5 exons, separated by 4 introns. Exons1-5 are 78, 145, 154, 532 and 877 bases, respectively. Introns 1-4 are 294, 1239, 11517, and 4571 bases, respectively. The intron-exon splice junction sites correlate identically with those of the rat alpha9 gene and are nearly identical to those of the human alpha10 gene.

Sequence promoter analysis reveals a number of potential regulatory elements, including several in common with the nAChR alpha10 gene, whose expressed protein is assumed to combine with alpha9 in the inner ear.

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38 Positional Cloning and Characterization of the Head Bobber Locus

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Little is known of the developmental regulation of the hearing and balance systems, and of the genes involved in these processes. Head Bobber is a transgenic mouse generated by a random transgene integration. Mice homozygous for the hb locus (hb/hb) are characterized by deafness, circling behavior and repetitive head tilting. These mice have malformed inner ears with absence of semicircular canals and a small cochlea. The behavioral phenotypes found in hb mice are likely contributed by malformation of the semicircular canals. Since it is clear that hb mice have behavior and phenotypes of ear defects, we expect the gene(s) involved to be crucial in regulating development and functional maturation of the inner ear. We have cloned candidate genes from the putative transgene integration site on chromosome 7, where the transgene has been localized by FISH analysis. We are currently identifying the specific gene or genes affected in hb mice with respect to rearrangement of the genomic structure and the expression pattern of the hb gene(s), particularly in structures of the inner ear.

39 Does Hair Cell Espin Determine the Steady-state Length of the Stereocilium?

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The mechanosensory functions of hair cells require the existence of precise variations in stereocilium length according to position in the hair bundle and according to hair cell position in the cochlea or vestibular system. Since the parallel actin bundle scaffold at the core of the stereocilium has recently been shown to undergo continuous renewal through treadmilling, we reasoned the steady-state length of the stereocilium would increase according to the number of hair cell espin cross-links it contained in its parallel actin bundle. Hair cell espin is an actin-bundling protein we have localized to stereocilia and shown to be the target of the jerker deafness mutation. Consistent with this hypothesis, we observed a gradient of hair cell espin expression in outer hair cells from base to apex that correlated with the known increase in stereocilium length. To test the hypothesis, we examined the effects of hair cell espin on the length of a related parallel actin bundle-containing process, the microvillus, in transfected epithelial cells of the LLC-PK1 clone 4 (CL4) line. The hair cell espin became localized to the microvilli of CL4 cells and caused a dramatic (up to 10-fold) increase in their length. The effect required the known actin-binding sites of hair cell espin and was not observed upon over-expression of a different actin-bundling protein, fimbrin. The use of a series of expression constructs of graded promoter strength revealed a positive correlation between the level of hair cell espin expression and microvillus length. These data suggest that hair cell espin cross-links can increase the steady-state length of the parallel actin bundle at the core of a microvillus or stereocilium and afford an explanation for the degeneration of stereocilia observed in espin-deficient jerker mice.

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40 Continuous Renewal of Stereocilia Based on an Actin Molecular Treadmill.

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Auditory and vestibular mechano-reception is initiated at the tips of stereocilia bundles, which are the sensory organelles of inner ear hair cells. It is known that stereocilia develop before or immediately after birth according to a hair cell developmental program, which generates a stereocilia staircase bundle precisely specified with respect to number and height. Previously, mature stereocilia were thought to be extremely stable because they are supported by a core structure consisting of a rigid array of cross-linked parallel actin filaments of uniform polarity. Structural stability is deemed important because the mechano-reception apparatus is anchored to the actin filaments and is sensitive to displacements at the nanometer scale. We now describe experiments that show for the first time the rapid turnover of the structural core of the stereocilia. To determine the locus of actin polymerization during stereocilia development and to assess the degree of actin turnover we transfected cultured hair cells with beta-actin-GFP. We show that the actin filament arrays are continuously being remodeled. We calculated that actin filaments in developing stereocilia are polymerizing at a rate of at least 50 times the reported rate of stereocilia growth. Mature bundles continued to incorporate actin-GFP progressively from the tips to the base such that labeling along the entire stereocilia was reached after 48 hours. The progressive and uniform incorporation in these fully developed stereocilia indicated that the entire actin filament bundles and associated proteins were treadmilling towards the base as new actin monomers were incorporated into the filaments at the tip. Recognition of this dynamic aspect of stereocilia is essential to understanding their development and maintenance. This renewal mechanism could help understand the molecular basis of several genetic, environmental and age related inner ear disorders that involve malformation or disruption of stereocilia.

41 The Hair-Cell Antigen/PTPRQ is Required For the Normal Development of Hair Bundles and the Long-Term Survival of Hair Cells in the Mouse Cochlea

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The 275 kDa hair-cell antigen (HCA), a protein associated with hair-bundle shaft connectors, was identified as a receptor-like protein tyrosine phosphatase called PTPRQ. Transgenic mice with targeted deletions in the transmembrane or catalytic domains of PTPRQ were used to study the role of the HCA. In wild type mice, antibodies to the intracellular domain of PTPRQ stain vestibular hair bundles from E13.5, inner hair cell bundles from E17.5, and outer hair cell bundles from E18.5. In mice that are homozygous for either deletion, hair bundles are present in both the vestibule and cochlea at birth, but fail to stain with PTPRQ intracellular domain antibodies. Transducer currents can be recorded from the OHCs of homozygous PTPRQ transmembrane domain mutants at P5-P7 and, although reduced in size relative to those in wild type cells, show clear signs of adaptation. Severe anomalies in hair bundle structure, including fusion and loss of stereocilia, become readily apparent in the inner and outer hair cells of both PTPRQ mutants by P8. By P80, hair cells disappear from the basal end of the cochlea. In the mature vestibule of homozygous PTPRQ mutants, hair bundle defects and an obvious loss of hair cells are not observed. Mature, homozygous PTPRQ mutant mice do not exhibit any overt signs of vestibular malfunction but fail to respond to a 20 kHz click at 100 dB SPL. Although vestibular hair cells appear to be grossly unaffected by the mutations, transmission electron microscopy of

samples fixed in the presence of ruthenium red reveals a complete loss of shaft connectors.

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42 Pharmacological Clues to the Nature of the Mechano-Electric Transducer Channel.

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Hair cell mechano-electric transducer (MET) channels are one of few channels not to be identified at the molecular level. Many properties including activation kinetics, voltage sensitivity, mechanical sensitivity, calcium sensitivity and adaptation may not be intrinsic to the MET channel but rather a function of accessory proteins and the machinery that couples the channel to its stimulus. One property that is intrinsic to the channel is the pore. Identifying chemicals that block or interact with the pore will be useful in determining if expressed channels are the MET channels. MET channels are nonspecific cation conductances that have an appreciable permeability to calcium, show no voltage dependence and have a large single channel conductance (Ohmori,1985; Crawford et al.,1991). Several classes of channels have similar properties, nicotinic acetylcholine channels, cyclic nucleotide gated (CNG) channels, and those channels in the vanniloid family. Potassium channel antagonists such as TEA and 4AP were ineffective blockers of the MET current while calcium channel antagonists diltiazem (IC50 236±50μM) and D600 (IC50 111±10μM) were effective. Nimodipine another calcium channel blocker was ineffective. Diltiezam and D600 are known antagonists of CNG channels as is tetracaine (a local anesthetic). Tetracaine was effective at antagonizing MET currents (IC50 608±50μM). Curare, hexamethonium and decamethonium were also effective antagonists of the MET channels. A hypothesis was developed based on the common structure of these drugs, a tertiary amine, suggesting that the charged amine will permeate the channel and the R-group attached to the amine will serve to block the channel. All of the compounds tested with tertiary amines either permeated or blocked the MET current.

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43 Salicylate Affects on Mechano-electric Transduction

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In higher vertebrates salicylate has been used as a probe for identifying the site of the cochlear amplifier. In vivo, salicylate alters otoacoustic emissions (OAEs), a byproduct of the cochlear amplifier. In vitro, salicylate shifts the voltage-dependence of the outer hair cell (OHC) motor, thus implicating outer hair cell motility as the site for the generation of the cochlear amplifier. Lower vertebrates and birds also produce OAEs, despite the lack of OHCs. In addition, auditory thresholds are comparable or better than mammalian cochlea, supporting the hypothesis that an amplification process exists in these hearing organs. Recent evidence has demonstrated that salicylate also alters OAEs from lizard in a manner comparable to that of mammalian systems. An active process residing in the sensory cell hair bundle associated with mechano-electric transducer channels (MET) and fast adaptation has been postulated. This hypothesis was tested by studying the effects of salicylate on MET and fast adaptation. Hair cells from the intact auditory papilla of the turtle were voltage-clamped and responses to mechanical deflection of the hair bundle measured in the absence and presence of 3 or 10 mM salicylate. Salicylate increased the magnitude of the MET current by 18±4%. Fast adaptation was enhanced with adaptation getting more complete and faster. Effects on the activation curve were varied but tended to shift the curve to the right. Data is consistent with a charge screening effect of salicylate increasing calcium flow through the MET channels. Consistent with this interpretation is a shift in the activation curve of the voltage-dependent calcium current. These results implicate the hair bundle as a site of amplification and generation of OAEs.

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44 Complete Sequence and Immunohistochemical Localization of an N-type Calcium Channel in the Hair Cell Epithelium of the Trout Sacculus

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The predominant voltage-gated calcium channel (VGCC) of the hair cell is believed to be the neuroendocrine, L-type (α1D or Ca_v1.3) isoform. However, non-L-type calcium channels have been electrophysiologically identified in hair cells of the chick cochlea (Kimitsuki et al., Acta Otolaryngol. 114: 144-148, 1994) and bullfrog sacculus (Su et al., Hearing Res. 87: 62-68, 1995), showing N-type (α1B or Ca_v2.2) sensitivity to ω-conotoxin (Rodriguez-Contreras and Yamoah, J. Physiol. 534: 669-689, 2001). Such non-L-type channels appear to contribute to the calcium current of certain hair cells. In the present investigation, we have obtained the full-length cDNA sequence for Ca_v2.2, including 5' and 3'untranslated regions, allowing identification of functional domains, and further have immunolocalized Ca_v2.2 within the trout saccular sensory epithelium. PCR, RACE, and cloning of PCR products were compared for the isolated hair cell preparation, trout brain, and a trout brain λZAP II cDNA library. The entire saccular Ca_v2.2 sequence was found to be 2,378 amino acids in length with 62% identity to rat and human brain Ca_v2.2. This sequence manifests major molecular features characteristic of the N-type channel: ω-conotoxin binding region, synaptic protein interaction site, and a relatively elongated II-III intracellular loop. There are three $G\beta\gamma$ binding domains: two in the I-II intracellular loop and one near the carboxy-terminal end. We also observed the expression of an 11-amino-acid exon in the $G\beta\gamma$ binding region in an identical location to that reported for chick dorsal root ganglion Ca_v2.2 (Lu and Dunlap, J. Biol. Chem. 274: 34566-34575, 1999). Selected peptide sequence from the carboxy-terminal region was employed to yield antigen for the development of a polyclonal antibody, which was protein-A purified and characterized by Western blot. Immunohistochemical studies demonstrated immunoreactivity in the saccular hair cells, concentrated on the cell membranes, apical and lateral. The molecular characteristics for hair-cell Ca_v2.2 inferred from cDNA sequence, including the 11-amino-acid exon of the I-II cytoplasmic loop within the $\beta\gamma$ binding region, should be predictive of function for an N-type calcium channel contributing to VGCC conductance of saccular hair cells.

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45 Serotonin and Its Receptors May Modulate Hair Cell Afferent Transmission in the Trout Saccule

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Evidence was previously obtained with HPLC and electrochemical detection of biogenic amines that serotonin (5-HT) and its metabolites were present in a model hair cell preparation from the trout saccule (Drescher et al., *ARO Abstr*. 21: 113, 1998). Further, cDNA expression of a 5-HT receptor was suggested by RT-PCR (Oh et al., *ARO Abstr*. 21: 111, 1998). We have now cloned the PCR products for the teleost

hair cell layer and brain from use of degenerate primers targeting amino acid sequence for 5-HT1A conserved across vertebrates. Sequencing of clones indicated that the primers promoted amplification of both alpha and beta subunits of serotonin receptors. The relative frequency for alpha to beta for the hair cell layer was 3:1 while that for brain was 2:7. The beta subunit expressed for both the hair cell layer and brain was the 5-HT1A receptor beta subunit. However, the message expressed for the alpha subunit was different for the hair cell layer and brain. The alpha subunit for brain bore highest similarity to the alpha subunit for 5-HT1A across vertebrates. The alpha subunit for the hair cell layer most closely resembled the alpha subunit for 5-HT1F expressed across vertebrates, and for fish, the alpha subunit for 5-HT1A. Hence, a form of 5-HT receptor appears to be expressed uniquely in the hair cell preparation, not detected in brain. Further, immunohistochemical studies have been carried out to localize the transmitter molecule 5-HT in the saccular sensory epithelium. A complex pattern of immunoreactivity has emerged. Immunostaining was detected in a neural tract, tentatively termed efferent, with fibers making contact with the base of hair cells. In addition, a subpopulation of hair cells, corresponding to peripheral saccular hair cells, both caudal and rostral, in longitudinal sections of the trout saccule, display immunoreactivity for a serotonin-like molecule. Overall, the results support the hypothesis that serotonin may modulate/mediate hair cell afferent transmission in this saccular end organ.

46 Serotonin (5-HT) and Norepinephrine (NE) in Rat Organ of Corti Analyzed by HPLC and Immunohistochemistry: Co-localization with Synaptophysin in Association with Outer Hair Cells of the Apical Turn

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Previously we demonstrated, with RT-PCR performed on microdissected subfractions of the rat cochlea, expression of cDNA for 5-HT receptors 1A, 1B, 2B and 6 in the organ of Corti (Oh et al., *Mol. Brain Res*. 70: 135-140, 1999). Biogenic amine analysis of the isolated organ of Corti by reversed-phase HPLC and electrochemical detection has provided evidence that the small-molecule neurotransmitters, 5-HT and NE, are present in the organ of Corti, suggesting that these molecules may play a role in cochlear sensory transmission. Moreover, we have observed a specific pattern of immunoreactivity in the rat organ of Corti with primary polyclonal antibodies targeting 5-HT and NE, in comparison with immunoreactivity for synaptophysin, a synaptic complex protein and marker for efferents. Immunoreactivity for 5-HT and NE is associated with the base of outer hair cells, appearing to be concentrated in nerve fibers of small caliber. A spiral pathway was observed for the immunostaining, following the Deiters' cells to the base of the sensory epithelium. The immunoreactivity appeared to demark peripheral pathways, above and parallel to the basilar membrane progressing towards the outer sulcus and towards the Hensen's cells. This pattern of immunoreactivity was also observed with application of a monoclonal primary antibody targeting synaptophysin. Immunoreactivity for NE and 5-HT in the outer hair cell region decreased from apical to basal turn. Staining was observed for both neurotransmitters beneath the inner hair cell in the apical turn, possibly coincident with the lateral efferent innervation of type I afferents, but in considerably less abundance than labeling by synaptophysin. Participation of 5-HT in modulation of hair cell signaling was further suggested by the presence of serotonin receptor 5-HT1A immunoreactivity in afferent cell bodies of the spiral ganglion in the apical turn. Overall, the evidence indicates a role for 5-HT and NE in modulation of the afferent signal in the cochlea.

47 Extrasynaptic Colocalization of and Interaction Between Cav1.3 and BK Channels in Mouse Inner Hair Cells

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The consecutive activation of colocalized voltage-activated Ca2+ channels and Ca2+-activated K+ (BK) channels in hair cells (HC) of lower vertebrates leads (besides synaptic transmission) to electrical resonance and enables frequency coding in those animals. Little is known about the interaction of the two channel types in mammalian HCs in which frequency coding occurs mechanically. We performed immunocytochemistry using two antibodies against the predominant Ca2+ channel Cav1.3 (or a1D) of IHCs (anti-Cav1.3-1 and Cav1.3-2, the latter recognizing only a long C-terminal splice variant) and one against the BK channel (Alomone Labs). Dot-like anti-Cav1.3-1 immunoreactivity along the latero-basal surface was observed in neonatal inner hair cells (IHCs) at P6 and vanished upon further maturation. Anti-Cav1.3-2 staining started around P12 in the IHC "neck" region between cuticular plate and nucleus. Anti-BK staining appeared at P12 and became more intense until P18. Unexpectedly, the staining was found in the same "neck" region as the Cav1.3 staining. Neither of the antibodies gave a staining at the basal pole of the IHCs where the synapses reside, a fact that might have been caused by the tight packing of channels and presynaptic proteins in these areas.

Immunostaining was also performed in Cav1.3-/- mice. No Cav1.3 staining could be dected, but BK staining was lacking, too. Whole cell current measurements revealed that no fast BK current component was present in IHCs of Cav1.3-/- mice though BK mRNA was present as shown by in-situ hybridisation.

Taken together, (i) Cav1.3 channels undergo a change in the splice variant(s) during development, (ii) Cav1.3 channels and BK channels show extrasynaptic colocalization and (iii) a posttranslational step in BK channel expression (biosynthesis or membrane targeting) requires functional Cav1.3 channels.

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48 A KCNQ-type Potassium Current in Cochlear Inner Hair Cells

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Cochlear inner hair cells (IHCs) transduce sound-induced vibrations into a receptor potential (RP) that controls afferent synaptic activity and, consequently, frequency and timing of action potentials in the postsynaptic auditory neurons. The RP is thought to be shaped by the two voltage-dependent K^+ conductances $I_{K,f}$ and $I_{K,s}$ that are carried by BK-type and K_V-type K^+ channels, respectively. In contrast, outer hair cells (OHCs) function as mechanical amplifiers of cochlear vibration by virtue of their unique electromotility. Membrane conductance of OHCs is dominated by a K^+ current with an unusually negative activation range ($I_{K,n}$) that is mediated by KCNQ4 channels.

Using whole-cell voltage-clamp recordings in the acutely isolated mouse cochlea we show that a similar K^+ current is also present in IHCs. This current is active at the resting membrane potential (–72 mV) and deactivates upon hyperpolarization. It is potently blocked by the KCNQ-channel blockers linopirdine and XE991, while it is insensitive to tetraethylammonium (TEA) and 4-aminopyridine (4-AP), that inhibit $I_{K,f}$ and $I_{K,s}$, respectively. Immunocytochemistry showed expression of

the KCNQ4 subunit in IHCs, indicating that the novel K^+ current is mediated by KCNQ4 channels. In current-clamp experiments, block of the KCNQ4 current shifted the resting membrane potential by about 7 mV to –65 mV and led to a significant activation of BK-channels. Using BK channels as an indicator for intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), it is shown that the shift in IHC resting potential observed upon block of KCNQ4 leads to an increase in $[Ca^{2+}]_i$ to micromolar concentrations. In conclusion, our results show that KCNQ4 channels set the resting membrane potential in cochlear IHCs and, thereby, contribute to the maintenance of low $[Ca^{2+}]_i$. Destabilization of the resting potential and increase in $[Ca^{2+}]_i$ as may result from impaired KCNQ4 function in IHCs provide a novel and straightforward explanation for the progressive hearing loss (DFNA2) observed in patients with defective KCNQ4 genes.

49 Ion Channel Clustering and Synapse Formation in the Normal and Sound-damaged Bullfrog Amphibian Papilla

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L-type calcium channels (L-VGCCs) control, via their association with BK potassium channels, frequency tuning in electrically tuned hair cells (HCs) and synaptic transmission between these cells and auditory neurons. We used fluorescent derivatives of dihydropyridine (Fl-DHP) and charybdotoxin (Fl-CbTx) to examine the relationship between channel clustering and synapse formation in the amphibian papilla (AP), verifying with patch-clamp recordings that these derivatives retained their specificities for L-VGCC and BK channels. We then labeled APs for 15 mins with Fl-CbTx and/or Fl-DHP, immunolabeled with antisera against myosin VI and neurofilament proteins, and used confocal and electron microscopy to study the acquisition, distribution and composition of channel clusters.

Mature HCs had varying numbers of puncta on their basolateral surfaces, indicating that they possessed both L-VGGC and BK channels. These puncta, which ranged from <0.25-1.0 um in diameter, were restricted to the subnuclear region and strongly co-localized with each other. Co-localized clusters also were associated with synaptic active zones, providing a mechanism by which HCs could increase the number of these channels without affecting their stoichometric ratio. L-VGCC channels in immature HCs were confined to the supranuclear region, not necessarily co-localized with BK channels, and not associated with synaptic endings.

Caudal HCs were sublethally and lethally damaged by exposure to high-intensity sound, with the former cells undergoing repair and the latter being replaced by regeneration. Sublethally damaged HCs, although deprived of synaptic endings, retained co-localized L-VGCC and BK channels. Regenerating HCs, like developing HCs, acquired co-localized L-VGCC and BK channels prior to synaptic innervation.

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50 Two Photon Imaging of Synaptic Release in Inner Hair Cells Maintained in the in situ Cochlea

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Inner hair cells (IHCs) precisely communicate sound features to the postsynaptic auditory nerve by release from about 20 synaptic sites.

Using trans-epithelial stimulation in the intact cochlea, we assessed synaptic release by two-photon imaging of synaptic vesicle populations labelled with FM 1-43 (Griesinger et al., J Neurosci 22:3939, 2002). Up to 7 release sites per IHC were monitored simultaneously. Stimulation was achieved by depolarising current pulses (20 ms) delivered at 20 to 100 Hz with amplitudes between 100 to 250 μA.

When stimulated, signal decayed by 25% within 5 s and recovered within 30 s. Remarkably only 60% of release sites responded during a stimulus train irrespective of the stimulation frequency. When delivering three consecutive trains, some sites responded to every stimulus, while others were unaffected during one or two of the trains. Release sites within one cell also differed with regard to the extent of release triggered: some destained completely, others only partially. Inhibiting vesicle suppy to release sites by blocking kinesin dependent trafficking with monastrol (50 μM) increased the decay amplitude to about 40% and blocked signal recovery but did not affect destaining kinetics. Yet, stimulus frequency affected kinetics. Higher frequencies lead to faster destaining while the number of activated sites was unchanged. Release was dependent on L-type calcium channels. Blocking the channels with 10 μM Nimodipine strongly reduced stimulus-dependent destaining.

The data indicate that IHCs use L-type calcium channels to trigger synaptic release. However, only a fraction of an IHC's release sites is activated at a given time. This is compatible with the previously observed heterogeneity of release site thresholds. Alternatively, IHCs might use information divergence to ensure that not all release sites are depleted of vesicles at the same time.

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51 Ca^{2+} Channels in Hair Cells that Mediate Transmitter Release: Permeation, Gating and The Relevance of Membrane Oscillations on Channel Properties

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We have demonstrated that Ca channels in hair cells consist of at least two subtypes (Rodriguez-Contreras & Yamoah, 2001). Here, we report the permeation properties and kinetics of gating of Ca channels in hair cells. The channels behave as multi-ion pores, exhibiting anomalous mole fraction effects at low concentrations of divalent cations, and the single channel conductance of Ca channels at relevant physiological conditions is ~2 pS. The channels showed distinct gating modes with high- and low-open probability (Po) interspersed with periods of quiescence: mean maximum Po for 70 and 5 mM Ba at 0.71 + 0.11 and 0.60 + 0.10, and for 70 and 5 mM Ca at 0.20 + 0.10 and 0.24 + 0.12, respectively. The activity of the channel was ion- and concentration-dependent. The half-activation voltage shifted in the hyperpolarizing direction from high to low permeant ion concentrations consistent with charge screening effects. However, the differences in the slope of the voltage shifts between Ca and Ba (slope values 0.23 and 0.13 mV mM-1, respectively), suggest that channel-ion interaction may also contribute towards the gating of the channel. There were subtantial differences between the profile of ensemble-averaged currents in Ba and Ca, raising the possibility that the channels undergo slow Ca-dependent inactivation. Using membrane oscillation stimuli to activate Ca and Ba currents in hair cells, subtle features of the channels became apparent. A kinetic scheme with parallel multiple closed and open states is proposed to describe the gating mechanisms of the channel. Finally, we will address the relationship between the Ca channel properties and Ca handling in hair cells.

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52 Endolymph Calcium Concentrations are Reduced in *dfw^{2J}* Mice

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In vertebrates, transduction of sound into an electrochemical signal is carried out by hair cells that rely on calcium to perform specialized functions. The apical surfaces of hair cells are surrounded by endolymphatic fluid containing calcium at concentrations that must be maintained by active transport. The mechanism of calcium transport into the endolymph is unknown, but an ATP-dependant pump is believed to participate. Mutation of the *Atp2b2* gene that encodes plasma membrane calcium ATPase type 2 (PMCA2) produces the deaf, ataxic mouse: deafwaddler^{2J} (*dfw^{2J}*). We hypothesized that PMCA2 might transport calcium into the endolymph, and that *dfw^{2J}* mice would have low endolymph calcium concentrations. First, using immunocytochemistry, we found that PMCA2 is present in inner and outer hair cell stereocilia where it could pump calcium into the endolymph. Second, using an aspirating microelectrode and calcium sensitive fluorescent dye, we found that the endolymph calcium concentrations of *dfw^{2J}* mice are significantly lower than those of control mice. These findings suggest that hair cell PMCA2 contributes to endolymph calcium maintenance.

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53 Disrupted Endolymphatic pH Homeostasis in Pendrin Knockout Mice

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Pendred's syndrome, one of the most prevalent causes of hereditary deafness, is caused by a dysfunction of pendrin. Pendrin in the cochlea is localized in the apical membrane of spiral prominence, outer sulcus and spindle-shaped stria cells (White et al., ARO 2003). Pendrin knockout mice (*Pds* -/-) are deaf, lack a normal endocochlear potential but have a normal endolymphatic K^+ concentration (Wu et al., ARO 2002). The observations that pendrin mediates DIDS-sensitive Cl^-/OH^- or Cl^-/formate exchange (Soleimani et al., 2001) and HCO_3^- secretion (Royaux et al. 2001) raised the hypothesis that pendrin mediates pH regulation in the cochlea. The perilymphatic and endolymphatic pH and potential (EP) were measured with double-barreled ion selective electrodes. The cytosolic pH (pHi) of pendrin-expressing cells was monitored by ratiometric confocal microfluorometry. The endolymphatic pH in *Pds* -/- mice (pH 7.3) was markedly acidic compared to *Pds* +/+ mice (pH 7.7) although the perilymphatic pH values were similar in both mice (pH 7.7). The EP in *Pds* -/- and *Pds* +/+ mice were -6 and 99 mV, respectively. Measurements of pHi were performed in the gerbil. In HCO_3^- free media, pHi was 6.7. Cl^- steps (150 to 15 mM) caused an alkalinization (ΔpH 0.13) that was reduced by 1 mM DIDS and enhanced by 1 and 10 mM formate. These observations are consistent with the hypothesis that pendrin is an anion exchanger that mediates HCO_3^- secretion necessary for pH homeostasis although it is conceivable that the acidic pH of endolymph in *Pds* -/- mice, which lack the EP, is simply the result of the eliminated driving force. In either case, the data suggest that Pendred's Syndrome is associated with a disturbance of endolymphatic pH homeostasis.

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54 Pendrin, KCNQ1, KCNE1 and ZO-1 in the Gerbil and Mouse Inner Ear: Confocal Immunolocalizations

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Pendred's syndrome, one of the most prevalent causes of hereditary deafness, is caused by a dysfunction of pendrin. Pendrin knockout mice (*Pds* -/-) are deaf, lack a normal endocochlear potential, have an acidic endolymphatic pH and a normal endolymphatic K^+ concentration (Wu et al., ARO 2002; White et al., ARO 2003). Interestingly, pendrin has been shown to mediate HCO_3^- secretion in the kidney (Royaux et al. 2001). In the cochlea, pendrin is expressed in the spiral prominence and outer sulcus and in the vestibular labyrinth in transitional cells (Everett et al., 1999). The cellular localization of the pendrin protein is undetermined and the relationship between pendrin and the endocochlear potential is currently unclear. The goal of the present study was to localize the pendrin protein. Pendrin, KCNQ1 and KCNE1 (two subunits of the K^+ channel in apical membrane of strial marginal cells) and ZO-1 (a tight junction protein) were localized by confocal immunocytochemistry in inner ear cryosections from gerbils, normal (*Pds* +/+) and pendrin knockout mice (*Pds* -/-). Pendrin was localized exclusively in the apical membrane of spiral prominence, outer sulcus and spindle-shaped stria cells of the cochlea and transitional cells of the vestibular labyrinth. Further, pendrin was found in the root cells of the cochlea. Spindle-shaped stria cells were identified by their proximity to ZO-1 labeled basal cells and clearly distinguished from marginal cells by the absence of KCNQ1. Pendrin was absent in cochleae of *Pds* -/- mice. Colocalization of KCNQ1 and KCNE1, which has previously been shown in normal mice and gerbils (Albrecht et al., ARO 2002), was found also in *Pds* -/- mice. This observation is consistent with the normal K^+ concentration in endolymph of these mice. The localization of pendrin raises the hypothesis that pendrin mediates pH regulation of endolymph through HCO_3^- secretion.

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55 Simulation of Corticosteriod Pharmacokinetics in the Inner Ear Fluids

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The delivery of drugs to the inner ear by direct application to the round window membrane is a promising way for the treatment of human inner ear disorders. To design well-controlled clinical trials using the method, preclinical studies are necessary that document pharmacokinetics, toxicity and dose-effect relationships. Since pharmacokinetic measurements in the human cochlea are not possible, computer simulations provide a valuable tool for the interpretation and planning of animal studies, for evaluating the effect of changes in application protocols and drug delivery systems, and for extrapolating the results from animal studies to the human. The present study has analyzed quantitatively the prior published data of measurements of concentration time courses of corticosteroids in the cochlear fluids (Parnes et al, Laryngoscope, 109,1,1999; Bachmann et al., HNO 49,538,2001). This was performed by calculations with an established finite element computer model of the cochlear fluids, which is available in the public domain at http://oto.wustl.edu/cochlea/. The time course of corticosteroid pharmacokinetics could be approximated for each study by simulation of movements of substances in inner ear fluids. Although the experimental studies reported considerably different drug levels in the fluid samples taken from the cochlea, these differences could largely be explained by considering the very details of the experimental design of the respective studies. The likely perilymph concentrations derived for each of the studies were within a factor of two of each other. The

simulations demonstrate that the duration that drug remains in the middle ear after an intratympanic injection is a dominant factor in the drug level achieved. It can be concluded that small differences in clinical application protocols may cause large changes in the drug levels achieved in the inner ear fluids.

56 Quantification of Cerebrospinal Fluid Contamination of Perilymph Samples Taken from the Basal Cochlear Turn.

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Our knowledge of the perilymph kinetics depends largely on results obtained by the analysis of perilymph samples taken from the cochlea. In the present study we have quantified the degree of carebrospinal fluid (CSF) contamination of perilymph samples taken from the basal turn of the guinea pig cochlea using the ionic marker trimethylphenylammonium (TMPA). In each experiment, TMPA solution was irrigated across the round window membrane while a TMPA-selective electrode sealed into scala tympani continuously monitored perilymph TMPA concentration. After a period of TMPA loading, a single perilymph sample was aspirated and its TMPA content was determined. The sample concentration and the measured TMPA time course during loading and perilymph sampling were interpreted using a finite-element computer model for simulation of solute movements in the inner ear fluids, available in the public domain at http://oto.wustl.edu/cochlea/. The experimental results were consistent with the aspirated fluid sample from the cochlea being replaced by CSF drawn into the perilymphatic space through the cochlear aqueduct. Samples taken through the round window membrane showed contamination varying from 20% for a 1 uL sample to 65% for a 5 uL sample. The relationships between sample purity, the cochlear location of sampling, and the sample volume withdrawn were defined. Quantification of the relationships will aid in the design and interpretation of experiments that utilize perilymph sampling.

57 Cochlear Responses to Acute Endolymph Volume Changes

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Endolymph volume disturbances are believed to be a primary factor contributing to the cochleovestibular disturbances associated with Meniere's disease. The present study was designed to document the effects on inner ear function of endolymph volume disturbances, induced by microinjection of artificial endolymph into scala media. Volumes up to 1.2 µL were injected over a 15 min period into the second cochlear turn of normal guinea pigs, with up to 3 injections performed in each preparation. Function was monitored at 30 sec intervals using Tucker-Davis hardware controlled by custom software. Measurements included the endocochlear potential (EP) from the second turn, cochlear microphonics (CM), summating potential (SP) and action potential (AP) amplitude in response to 4 kHz, 90 dB tone bursts, AP thresholds at 8 kHz and 2.8 kHz and the CM to a single phase, 500 Hz, 90 dB stimulus. Single phase CM was analyzed for harmonic distortion and operating point (Kirk and Patuzzi, Hear. Res. 112: 69, 1997). During the first injection, the EP, CM amplitude and thresholds at 8 kHz were almost unaffected, while thresholds at 2.8 kHz showed a reversible elevation of about 15 dB. SP, CM distortion and CM operating point showed substantial changes, although the direction and magnitude of the change varied from animal to animal and with repeated injections. The results showed that even though endolymph volume disturbances cause only minor elevation of AP thresholds, other aspects of cochlear physiology, most specifically measures of CM distortion, were markedly influenced. The development of diagnostic methods for abnormal endolymph volume states requires a greater understanding of volume-induced changes.

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58 L-type Calcium Channel a1C Subunit is Expressed in Spiral ligament Fibrocytes

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It is widely recognized that spiral ligament fibrocytes (SLFs) are actively involved in maintaining K+ homeostasis in the inner ear. Recent studies from our group have demonstrated that a big conductance voltage- and Ca2+-dependent K channel (BK) is the dominant membrane conductance in type I SLFs and that the function of BK channels is influenced by extracellular Ca2+ levels. Although the mechanisms regulating Ca2+ homeostasis in type I SLFs have not been established, it is known that these cells express an intracellular Ca-ATPase. The aim of this study was to investigate the possible expression of L-type Ca2+ channels in type I SLFs, which may contribute to Ca2+ homeostasis in these cells by moving extracellular Ca2+ into an intracellular compartment.

The expression of L-type Ca2+ channels was screened by Western blot analysis using polyclonal antibodies against the a1C, a1D and a1E subunits. Only the a1C subunit was identified with a single band near 100 Kd. Expression of the a1C subunit was further confirmed by RT-PCR analysis. Total RNA was extracted from cultured type I SLFs, freshly isolated SLFs and gerbil heart as a positive control tissue. All three samples showed a single PCR product of the expected size, which was conclusively identified as the L-type Ca2+ channel a1C subunit by DNA sequencing.

These results demonstrate the expression of the functional a1C subunit of the L-type Ca2+ channel both in cultured type I SLFs and SLFs in vivo. The physiological properties and relevance of the L-type Ca2+ channel to SLF function remain to be determined.

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59 Aquaporin 2 in the Epithelium of Human Endolymphatic Sac

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The endolymphatic sac may participate to endolymph homeostasis by a double mechanism of fluid reabsorption and luminal secretion of osmotically active molecules. In most epithelia, transepithelial water fluxes occur through membranous water channels named aquaporins. Aquaporin 2 (AQP-2) has been localized in the renal collecting duct and its membranous insertion is regulated by antidiuretic hormone. The aim of the present study was to detect the presence of AQP-2 in a human endolymphatic sac.

The sac was sampled during a translabyrinthine approach for removal of a vestibular schwannoma and immediately fixed in 10% formaline. Rabbit polyclonal anti-AQP-2 antibody was used (Cluzeaud et al., Am J Physiol 1998; 275:C1602-9). Positive immunostaining was observed in a subset of epithelial cells, mostly in the cytosol and more rarely in the basolateral membrane. In situ hybridization is currently underway to localize AQP-2 mRNA.

The presence of AQP-2 within the endolymphatic sac suggests that this channel is involved in water transport through the sac epithelium. Considering the predominant cytosolic localization of AQP-2, water

flux through this channel is probably low or null in the endolymphatic sac of this patient. In other cases, membranous insertion may be upregulated by antidiuretic hormone, as receptors to this hormone have been localized in the rat endolymphatic sac. In conclusion, in human endolymphatic sac, the presence of AQP-2 suggests that this water channel is involved in the regulation of the volume and/or composition of endolymph.

60 Effect of Calmodulin Inhibitors on Gap Junctional Coupling in Isolated Hensen-Cells of the Guinea Pig Cochlea

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In the mammalian organ of Corti supporting cells are responsible for the recycling of endolymphatic potassium ions released by the sensory cells during mechanosensory transduction. Thus supporting cells, which show strong gap junctional coupling, play an essential role for the maintenance of endolymphatic potential and of cochlear ionic homeostasis. In order to evaluate the physiological mechanisms and the corresponding signal transduction pathways which influence gap junctional coupling in supporting cells the double whole-cell patch-clamp technique was applied to Hensen-cells of guinea pig organ of Corti.

Calmodulin (CaM) is an ubiquitous Ca2+ -binding protein that controls many cellular events, including the activation of several proteins, enzymes and ion channels. The work was focused on the effect of calmodulin inhibitors on gap junctional coupling in isolated Hensen-cells. Addition of either a conventional calmodulin antagonist (W7 and trifluoperazine) or of a calmodulin inhibitory peptide (MLCK peptide) caused a decrease of gap junctional conductance. Monitoring the cytoplasmic free calcium concentration ([Ca2+]i) by Fura-2 showed no significant change of [Ca2+]i by W7. Neither chelation of [Ca2+]i by 10 mM BAPTA nor use of nominally Ca2+- free external bath suppressed the W7-induced gap junctional uncoupling. The results show that calmodulin inhibitors induce gap junctional uncoupling at unchanged global [Ca2+]i. Therefore it is suggested that a CaM-dependent gating mechanism is involved in gap junctional coupling in isolated Hensen cells.

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61 Bumetanide-induced Enlargement of the Intercellular Space in the Stria Vascularis is Dependent on Perilymphatic Na^+

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It is known that loop diuretics such as furosemide and bumetanide inhibit Na^+-K^+-$2Cl^-$ cotransporter. These inhibitors cause not only a decline in the endocochlear potential (EP) but also acute morphological changes in the stria vascularis (Pike and Bosher, 1980, Hear Res 3: 79-89). One of the morphological changes is a marked enlargement of the intercellular space in the stria vascularis (intrastrial space). The enlargement of the intrastrial space is likely to be caused by accumulation of solutes, most likely ions, which drag water osmotically. We have reported that the bumetanide-induced enlargement of the intrastrial space is dependent on the activity of Na^+-K^+-ATPase (Azuma et al., 2002, Acta Otolaryngol, in press). In this study, we examined the role of perilymphatic Na^+ for this morphologic change. Guinea pigs were anesthetized and perilymphatic perfusion was performed from the scala tympani of the basal turn to the scala vestibuli of the basal turn at a flow rate of 10 μl/min. The EP was measured from the second turn. Morphological changes in the stria vascularis of the second turn were examined by transmission electron microscopy. Perilymphatic perfusion with a control perfusate did not cause apparent changes in both the EP and the strial morphology. Perilymphatic perfusion with 100 μM bumetanide dissolved in the control perfusate caused a decline in the EP and a marked enlargement of the intrastrial space. Perilymphatic perfusion with a Na^+-free perfusate for 30 min caused a decline in the EP by 15-30 mV. Perilymphatic perfusion with the Na^+-free perfusate followed by additional 40-min perfusion with 100 μM bumetanide (dissolved in the Na^+-free perfusate) did not cause the enlargement of the intrastrial space. These results indicate that perilymphatic Na^+ is responsible for the bumetanide-induced enlargement of the intrastrial space.

62 Regulation of Endocytosis in the Marginal Cell by ROCK and MLCK

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The marginal cell of the stria vascularis (SV) is thought to have pivotal role in the regulation of the endolymph metabolism. If the tracers were injected into the cochlear duct, the marginal cell endocytoses CF, the marker of receptor-mediated endocytosis, and microperoxidase (MPO), the marker of fluid phase endocytosis. The actin filament is required for the endocytosis in the marginal cell. Myosin motor activity is controlled by the regulatory light chain of myosin (MLC). Phospharylation of MLC plays a critical role in controlling actin-myosin interaction. MLC phospharylation is regulated by myosin light chain kinase (MLCK) and myosin phosphatase. MLCK is phospharylated by several serine/threonine protein kinase including ERK, ROCK, PKC, PKA, PAK and DAPK. ROCK dose not only phospharylate MLC directly, but also inhibit myosin phosphatase.

Rho-family small GTPase are key modulators of the cytoskeletal dynamics. Cdc42 is required for cell polarity, whereas Rac promotes lamellipodial extensions via to align filamentous actin. ROCK is the downstream effector of Rho A activity and also the upstream signal of Cdc42 and Rac 1. Thus the cellar effects of ROCK on the actin filament could be mediated through Rho-family.

In this study, we investigated the signal cascade to regulate the receptor-mediated and the fluid phase endocytosis in the SV. To show that MLC has a pivotal role in the endocytosis, MLCK specific (ML-7) and ROCK specific (Y-27632) inhibitor were used. The tracer, CF or MPO, in the artificial endolymph containing ML-7 or Y-27632 were infused into the cochlea duct of guinea pigs. After 30 min infusion, the SV was harvested and treated as routine procedures of EM.

The results show that each of ML-7 and Y-27632 moderately inhibit the endocytosis of CF and MPO. These results suggest that MLC has an important role in the receptor-mediated and fluid phase endocytosis, and that MLC exists downstream of both ROCK and MLCK and controlled by the two signal systems.

63 Crosstalk Between Na/K-ATPase and Na-K-2Cl Cotransporter Revealed by Genetic Engineering

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The basolateral isoform of the Na-K-2Cl cotransporter NKCC1 and the Na/K-ATPase isoforms α1 and α2 are present in the mammalian cochlea and are active in K+ cycling and homeostasis.

We use ABR, DPOAE, LM, SEM, and EP measurements to evaluate the auditory phenotypes of heterozygous $NKCC1^{+/-}$, $\alpha1^{+/-}$, and $\alpha2^{+/-}$ mutant mice. We find that single deletion of either NKCC1, α1, or α2 independently shows progressive hearing loss with minimal deterioration of cochlear morphology with age. Double heterozygote

deletion of NKCC1 with α1 shows progressive deterioration in ABR threshold with age. However, double heterozygote deletion of NKCC1 with α2 shows apparent preservation of hearing threshold with age, with DPOAE levels blunted at high frequencies but preserved at low frequencies. Cochlear morphology under LM and SEM is grossly preserved over age in both groups of double heterozygotes.

We induce a pharmacologic model of the double heterozygote mutation by administration of furosemide and digoxin to wild type mice. We find that mice treated with NKCC1 antagonist furosemide show deterioration in ABR threshold, while mice treated with Na/K-ATPase antagonist digoxin prior to furosemide challenge show preservation of ABR threshold.

We invoke a model of cochlear K+ cycling whereby NKCC1 acts in parallel with α1 and functions as the rate limiting step in K+ secretion from the intrastrial compartment to the endolymph. α2 may serve as the rate limiting step in K+ uptake from the endolymph. Functional suppression of either protein causes deterioration of auditory function. However, suppression at both sites simultaneously can hinder deterioration of auditory function with age.

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64 Corticosteroids Stimulate Sodium Absorption via Epithelial Sodium Channels (ENaC) in Semicircular Canal Duct (SCCD) Epithelium

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SCCD is known to secrete Cl into vestibular endolymph. We sought to determine whether SCCD was also capable of cation absorption in response to stimulation by corticosteroid receptors. SCCD cells of neonatal rats were cultured to confluence on permeable supports and transepithelial voltage (VT) and resistance (RT) were measured in an Ussing chamber at 37°C. Dexamethasone (DEX) and aldosterone (ALDO) increased short circuit current (Isc = VT/RT) over a 10 – 15 h interval with an EC50 of 17 nM and 236 nM, respectively. The time course is consistent with genomic regulation of Isc by DEX. Isc was blocked and RT increased by apical amiloride (IC50 ~1 μM) and benzamil (IC50 ~0.1 μM) but not EIPA, consistent with Isc carried by ENaC. The amiloride sensitivity was nearly abolished when apical Na was replaced with K, demonstrating a high Na-selectivity of Isc. DEX (100 nM) stimulated Isc was inhibited by the glucocorticoid receptor (GR) antagonist Mifepristone (MIF; 1 μM) but not by the mineralocorticoid receptor (MR) antagonist spironolactone (SPI; 10 μM), suggesting stimulation of Isc by activation of GR. Hydrocortisone (HC)-stimulated amiloride (AMIL)-sensitive Isc with an EC50 of ~0.1 μM, which is in the physiological range of plasma concentration (0.1-1 μM). Similar to DEX, the effect of HC was not inhibited by SPI. Aldosterone (ALDO) stimulated AMIL-sensitive Isc and the effect of ALDO (1μM) was partially blocked by 1 & 10 μM SPI and completely blocked by either 100 μM SPI or 100 nM MIF (n=1), consistent with stimulation of Isc by activation of both GR and MR. It was found that amiloride in corticosteroid-treated epithelia did not prevent stimulation of Isc by forskolin, a stimulator of Cl secretion. We conclude that SCCD contribute to the homeostasis of endolymph via bi-directional transport of Na via ENaC under control of GR and MR and transport of Cl under control of β2-adrenergic receptors coupled to intracellular cAMP.

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65 Characteristics of Blood-Labyrinth Barrier

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The blood-labyrinth barrier (BLB) is an important homeostatic mechanism that protects most of the intricate biological structures of the inner ear. It is well established that the maintenance of a constant composition of the inner ear fluids is essential for the functional integrity of the inner ear. In earlier studies of the BLB, we have reported that small molecular weight molecules entered the perilymph in a dose and time dependent manner. Several ototoxic drugs have been shown to cross the BLB and enter the perilymph with a rate of elimination from perilymph that is much slower than from serum. In experimental animals, numerous biological substances such as osmotic agents, aminoglycoside antibiotics, diuretics, radiolabeled ions and salicylates have been injected into the blood and recovered from perilymph at a rate that was inversely related to the molecular weight of the substance. When BLB is disrupted, leakage of the blood components results in damage to the ion transport system in the inner ear tissues. Bacterial inoculation (S. pneumoniae) into the middle ear or systemic injection of osmotic agents are known to cause the disruption of BLB in the lateral wall of the cochlea. This disruption can damage the ion transport system of the lateral wall of the cochlea and lead to the disturbance of inner ear homeostasis resulting in functional anomaly of the auditory system. The results of on-going projects related to the characteristics of the BLB will be presented and discussed.

66 Functional β-Adrenergic Receptors in the Gerbil Inner Ear

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Sympathetic stimulation during a fight or flight reaction may put the sensory systems for hearing and balance into a state of heightened alert. Sympathetic activation was found to stimulate K^+ secretion in stria vascularis via β_1-adrenergic receptors (β_1-AR) and to enhance cochlear blood flow. The aim of this study was to localize β-AR in the gerbil inner ear. β_1-AR were localized by confocal immunocytochemistry and characterized by Western immunoblots. Functional β-AR were localized pharmacologically by measuring cAMP production in microdissected inner ear tissue fractions. Staining for β_1-AR was found in strial marginal cells, inner and outer hair cells, outer sulcus cells, and spiral ganglia cells of the cochlea, as well as in dark, transitional and supporting cells of the vestibular labyrinth. Receptors were characterized in five microdissected inner ear tissue fractions (stria vascularis, non-strial lateral wall, organ of Corti, modiolus, and vestibular labyrinth) as 55 kDa and as 160 kDa species. Functional pharmacological studies using the β-AR agonist isoproterenol and the subtype-specific antagonists CGP-20712A and ICI-118551 revealed that the predominant β-AR subtype in stria vascularis and organ of Corti is the β_1-AR and that the predominant β-AR subtype in modiolus is the β_2-AR. These observations demonstrate that β-AR are present in tissues that mediate K^+ cycling, sensory transduction and auditory processing. Our studies support the hypothesis that β-AR enhance the performance of the inner ear during fight or flight situations.

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67 Filling of Capillaries in the Stria Vascularis with Red Blood Cells After a Short-Time Stasis of Blood Flow: Contribution by Active Ion Transport

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It has been reported that strial capillaries are markedly filled with red blood cells (RBCs) when the tissue is chemically fixed a few minutes after decapitation by perilymphatic perfusion of a fixative. In contrast, the filling of capillaries with RBCs is not observed when the cochlea of a living animal is chemically fixed by the same method (Watanabe, Ann Otol Rhinol Laryngol 95: 427-431, 1986). We speculated that ion transport conducted by strial cells during stasis of blood flow might be related to the filling with RBCs, and examined the role of Na^+-K^+-ATPase for the filling with RBCs. Albino guinea pigs were deeply anesthetized, and artificially ventilated. The cochlea was exposed, and the stapes was dislocated to secure the drainage of fixative and/or

artificial perilymph. Animals were divided into three groups. In the experiment#1 (Ex#1), cochleae were fixed in vivo. In the experiment#2 (Ex#2), cochleae were fixed three minutes after decapitation. In the experiment#3 (Ex#3), perilymphatic perfusion with the artificial perilymph containing 1mM ouabain was performed prior to decapitation. Then cochleae were fixed three minutes after decapitation. Thin sections for transmission electron microscopy were made. The ratio of RBC volume to the volume of capillary lumen (V_{RBC}/V_{CL}) was estimated by the point counting method (Weibel, Stereological Method. Academic Press, New York, 1979). Data were presented as mean ± SEM (n, number of capillary sections). The V_{RBC}/V_{CL} values were 0.64±0.04 (Ex#1, n=17), 0.96±0.02 (Ex#2, n=23), and 0.81±0.01 (Ex#3, n=10). These results indicate that Na^+-K^+-ATPase by itself and/or mechanisms dependent on the electrochemical gradient of ions created by Na^+-K^+-ATPase contribute to the filling of RBCs in strial capillaries.

68 Measurement of Cochlear Blood Flow by Digital Power M-Mode Doppler Ultrasound Flowmetry

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A Doppler ultrasound device has been developed to measure cochlear blood flow in the spiral modiolar vein. This pulse Doppler device employs a 12 MHz sub-millimeter catheter-mounted transducer, and delivers a 16 cycle transmit burst at 8 kHz pulse repetition frequency to interrogate blood flow at up to 11mm from the tip of the transducer. Signal averaging is employed to improve SNR and simultaneously improve velocity resolution over the range from 0 to 4mm/s. The probe is advanced to the round window membrane and observes flow in the spiral modiolar vein through a 20 micron layer of bone and the scala tympani. The digital signal processor in the instrument receiver section incorporates 33 sample gates to be analyzed and displayed simultaneously in an M-mode format. This unique display, referred to as power M-mode, allows the user to locate quickly the blood flow signals up to 11 mm from the face of the transducer. The device was tested in a guinea pig model as a prelude to human clinical trials. Cochlear blood flow was imaged in a total of twenty guinea pigs, and hemodynamic responses to vasoactive and ultrasound contrast agents were demonstrated. Results of these tests are presented.

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69 Multi-mechanisms Mediate Acetylcholine-induced Hyperpolarization and Relaxation in Smooth Muscle Cells of the Spiral Modiolar Artery

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Acetylcholine (ACh) dilates cochlear blood vessels and muscarinic antagonists reduce cochlear blood flow, suggesting a role of cholinergic control of the inner-ear circulation. We recently showed that cells in isolated guinea pig cochlear spiral modiolar artery (SMA) exhibit bi-stable resting potential (RP) near –40 or –75 mV, called low and high RP, respectively, and ACh induces hyperpolarization only in the low RP cells but a depolarization in high RP cells. Only the hyperpolarization in muscle cells was suppressed by a gap junction blocker suggesting an endothelium-origin of the response. Using intracellular recording, propidium iodide dye labeling and vasodiameter-tracking techniques on the in vitro SMA, we found that: 1) myoendothelial dye-coupling was often detected after an intracellular recording longer than 30 min; 2) ACh hyperpolarized both endothelial and smooth muscle cells when they had a low RP level. Ba+ (100 μM) or combined with 10 μM ouabain attenuated the ACh-hyperpolarization in muscle cells but not in endothelial cells; 3) the ACh-induced hyperpolarization and dilation both were blocked by 50 nM 4-DAMP or charybdotoxin. The ACh-hyperpolarization was also blocked by 10 μM nifedipine but not by Nω-nitro-L-arginine methyl ester, glipizide, indomethacin and ions of Ni or Cd. We conclude that, in the SMA, ACh-induced hyperpolarization originates from endothelial cells via activation of M3 receptors and calcium-activated potassium channels, and is independent of releasing NO or cyclo-oxygenase products. The calcium influx is via dihydropyridine-sensitive Ca-channels. ACh-induced hyperpolarization and relaxation in the muscle cells involve two mechanisms: 1) electrical spread of the hyperpolarization from the endothelium and 2) activation of inward rectifier K-channels and Na-K-ATPase pump current by elevated interstitial K-ion released from the endothelial cells.

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70 Tonically Released Nitric Oxide Activates ATP-Sensitive K-Channels of Smooth Muscle Cells and Dilates Guinea Pig Spiral Modiolar Artery

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Nitric oxide (NO) is a potent vasodilating agent implicated in cochlear blood flow regulation. We recently demonstrated that exogenously applied NO-donor DPTA-NONOate hyperpolarizes both endothelial and smooth muscle cells of the spiral modiolar artery (SMA) via activation of ATP-sensitive K-channels (K_{ATP}). Bath application of DPTA-NONOate (0.1 - 30 μM) caused a concentration dependent hyperpolarization in all the low resting potential (RP,~-40 mV) cells, with an EC_{50} of 1 μM. The hyperpolarization was completely blocked by glipizide, a blocker of K_{ATP}, but not by blockers for other K-channels. Using intracellular recording techniques, vaso-diameter video tracking method and NO-sensor measurement, we investigated a possible tonic release of NO in this in vitro preparation and its role in maintaining the vascular tone. With a calibrated NO-sensor (WPI), we found that the freshly prepared 0.1, 1, 10 and 30 μM DPTA-NONOate solution produced about 1, 10 100 and 300 nM of NO in the recording bath. But the 3 h old solutions produced NO concentrations that were only a third of those by the fresh ones. Secondly, 300 μM L-NAME, a NO-synthase inhibitor, and 3 μM glipizide caused a depolarization of 4.5 and 3.2 mV, respectively in low RP cells. Finally, L-NAME but not glipizide produced 2-4 μm reduction in the SMA diameter (~40 μm) in the majority of freshly isolated SMAs. In the presence of glipizide, L-NAME still produced a vaso-constriction. We conclude that 1) the EC_{50} of DPTA-NONOate produces ~10 nM NO, indicating a high NO-sensitivity of the SMA, and 2) there is a tonic release of NO in the in vitro SMA that activates K_{ATP}, thus contributes several millivolts to the resting membrane potential and several micrometer diameter relaxation in the vasotone, but the K_{ATP} activation plays little role in the relaxation.

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71 Pharmacology of Acetylcholine-Induced Hyperpolarization and Depolarization in Guinea Pig in vitro Spiral Modiolar Artery

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Acetylcholine (ACh) induces hyperpolarization and depolarization in the spiral modiolar artery (SMA) depending on the high or low level resting potential of the recorded cell (Jiang et al. J. Physiol. 2001). The ACh—hyperpolarization is an endothelium-dependent event while the ACh-depolarization originates at least partially in muscle cells. ACh also produces vasodilation and constriction dual effects in various vessels. Using in vitro SMA and intracellular recording methods, we examined pharmacological profiles of these opposite membrane effects. We found: 1) ACh induced hyperpolarization in nearly all low RP cells

tested (RP=~-37 mV) with an EC_{50} of 0.93 μM while elicited depolarization in all high RP cells (RP=~-77 mV) with an EC_{50} of 0.26 μM. But both responses had maximums at similar concentrations (30-100 μM). 2) The ACh-induced hyperpolarization was near completely (98.6%) blocked by 25 nM 4-DAMP, partially (50.6%) blocked by 50 nM pirenzepine and not significantly changed by 100 nM methoctramine. 3) The ACh-depolarization was near completely (97.6%) blocked by 50 nM 4-DAMP, 17% reduced by 50 nM pirenzepine and 13% attenuated by 100 nM methoctramine. 4) The estimated K_D were less than 100 pM and 10 nM of 4-DAMP and pirenzepine, respectively, for ACh-hyperpolarization, and less than 1 nM and 10 nM of 4-DAMP and pirenzepine, respectively, for ACh-depolarization. We conclude that M3 receptor is the main mediator for both ACh-induced hyperpolarization and depolarization in the SMA, whereas a small role of M2 receptor may involve in ACh-depolarization. The different affinity for the M3 antagonist between the receptors responsible for the hyperpolarization and depolarization may reflect distinct molecular structures of the M3 receptors expressed in the SMA endothelial vs. muscle cells.

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72 "Alport Syndrome" Mice Exhibit Lateral Wall Dysfunction Following Acoustic Overstimulation

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Several animal models of human diseases associated with high frequency hearing loss such as Alport syndrome, diabetes mellitus and systemic lupus erythematosus exhibit an abnormally thick basement membrane in capillaries of the stria vascularis. Strial capillary basement membranes of the mouse model of Alport syndrome (129Sv-Col4a3) contain increased amounts of collagen IV, laminin, and entactin. We suspect that matrix protein accumulation in the strial capillary basement membrane confers a susceptibility to environmental stress that culminates in the high frequency hearing loss observed in many Alport patients. Our previous work (Gratton et al, 2002, AR0 25:251) revealed a heightened susceptibility to noise in the "Alport" mouse as contrasted with normal littermates. The acoustic sensitivity displayed by the 129SV-Col4a3 mice is thought to reflect dysfunction of the lateral wall since hair cells showed no damage from the noise. The present study was undertaken to investigate whether differences in strial function exist between the "Alport" mice and their normal controls.

The magnitude of the endocochlear potential was monitored in the basal turn of nine-week old 129Sv-Col4a3 "Alport" mice and normal littermates. A subset of each group was exposed to the same noise used to document noise susceptibility with the ABR technique (8-16 kHz, 106 dB SPL, 10 Hr). The EP data was analyzed with ANOVA followed by a posthoc Student-Newman-Keuls. The average EP value in the Alport mice was substantially lower than that of normal counterparts. Noise exposure significantly lowered the EP in Alport mice, but had no effect was noted in the EP magnitude in the normal mice. The decreased EP in the Alport mice suggests that accumulation of matrix proteins in the strial capillary basement membrane may compromise lateral wall function.

73 Pro-Inflammatory Cytokine Receptors in the Lateral Wall of the MRL-*Fas*^{lpr} mouse

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The MRL-*Fas*^{lpr} (Lupus) mouse, a model of the multisystemic autoimmune disease, exhibits elevated evoked potential thresholds, decreased endocochlear potentials, antibody deposition in strial capillaries, and strial degeneration. However, these changes occur in the absence of an inflammatory response in the stria vascularis. Cytokines, particularly those in the Tumor Necrosis Factor (TNF) family, can mediate cell death in the absence of a classical inflammatory response. Cytokine dysregulation with progressively increasing levels of circulating proinflammatory cytokines is noted in this murine model. We hypothesize that the observed cell death of strial intermediate cells is mediated by cytolytic cytokines. In order for cytokines to mediate cytolytic processes, the target cells must express appropriate high affinity cytokine receptors. Thus, if elevated circulating cytokines play a role in the observed strial pathology, at least one of their receptors must localize to capillaries of the stria vascularis.

Temporal bones of young (8-9 week) and adult (20 week) MRL-*Fas*^{lpr}, MRL/+, and B129Sv mice were perfused, decalcified and paraffin-embedded. Sections cut in the mid-modiolar plane were immunostained with polyclonal antibodies directed against five different cytokines receptors: TGFß-R1 and R2, TNF-R1 and R2, and IL-1R1. Capillaries of the stria vascularis reacted with antibodies for TGFß-R2, TNF-R1 and R2, and IL-1R1 in the three mouse strains. While the level of reactivity varied among the receptors, staining appeared equally strong among all strains with the exception of TGFß-R2, which was most intense in the MRL-*Fas*^{lpr} strain. The presence of these receptors on strial capillaries provides initial support for the hypothesis that circulating TGFß, TNFa and/or IL-1 are involved in mediating strial cytolysis in Lupus mice.

74 Age-Dependent Uptake of Fluorescently-Conjugated Gentamicin by Murine Cochlear Hair Cells.

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Previous studies have shown that remote injections of fluorescently-conjugated gentamicin are accumulated by hair cells in the inner ear of bullfrogs, chicks, guinea pigs and neonatal mice. Other researchers have shown that the fluorescent molecule FM1-43 enters hair cells and a variety of other tissues known to contain mechano-sensitive channels (Meyers et al, 2002 ARO abstracts, #619). In this study we used Texas Red-conjugated gentamicin (GTTR) (a 1:300 molar dilution in 500 mg/ml gentamicin) to compare the in vivo distribution of this conjugate in 6-day and 21-28 day old mice.

In both 6 and 21-28 day old mice, GTTR preferentially accumulated in kidney proximal tubule cells, neuronal somas in the dorsal root ganglion, and sensory cells in the tongue, whisker bulb, and Merkel cells.

In 6-day old mice, considered to have immature auditory function, GTTR fluorescence could be observed in cochlear hair cells within 30 minutes of a single 300 mg/kg gentamicin (as previously described). In contrast, in 21-28 day old mice, considered to have functionally mature auditory function, little GTTR fluorescence could be observed in cochlear hair cells when dosed with a daily injection of 300 mg/kg gentamicin for 1-3 days.

These studies show that GTTR enters a wide variety of tissues known to contain mechano-sensitive channels, and that entry into cochlear hair cells appears to be dependent on the maturation of the blood-labyrinth barrier.

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75 Unilateral Transtympanic Gentamicin Uptake in the Inner Ear: Distribution and Apoptotic Cascade Activation

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There is increasing evidence of inner ear ganglion cell damage following aminoglycoside administration. Using a chinchilla model we

explored the effects of minipump (Alza 2002, 1 ul/hr) unilateral transtympanic injections of gentamicin (10mg/ml) on vestibular and spiral ganglion cells. Histological sections of decalcified temporal bones were stained immunohistochemically to identify the distribution of gentamicin (anti-gentamicin, Sigma) and markers of activation of the apoptosis cascade (anti- cleaved caspases 3, 7, 9 and intact PARP, Cell Signaling Technologies). Intense anti-gentamicin immunoreactivity was observed within 4 hours of injection in spiral and Scarpa's ganglia cells, stria vascularis, and intracellular connective tissues of the injected ear. Peak anti-gentamicin staining occurred 8 hours post injection, with persistence after 14 days in ganglion cells only. All ipsilateral ear structures (including bone cells) were positive at 1:500 antibody dilution; only soft tissues were positive at 1:5,000-50,000 dilutions. Contralateral ganglion cells showed less intense gentamicin staining. Contralateral inner ear gentamicin uptake was confirmed by autoradiographic localization of tritiated gentamicin. No significant evidence of a gradient of anti-gentamicin staining was found in either the ipsilateral or contralateral spiral ganglion ($p > 0.1$). Cleaved caspases 3, 7, 9, and intact PARP were expressed by both spiral and Scarpa's ganglion cells with peak expression at 48-72 hours after injection. Transtympanically injected gentamicin is retained in ganglion cells of the inner ear for an extended period and is associated with activation of pro-apoptotic signaling pathways. Such a process may have profound implications on both the success of cochlear implantation in patients following systemic aminoglycoside therapy and on the advisability of clinical practices of transtympanic gentamicin therapy and of ototopic aminoglycoside treatment.

76 Oxidative Reactions Promoted by Aminoglycoside Antibiotics

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Aminoglycoside antibiotics are widely used for the treatment of serious infections caused mainly by Gram-negative and some Gram-positive bacteria A major problem in therapy with aminoglycosides is their potentially high oto- and nephrotoxicity.

Our previous studies have shown that adverse affects of aminoglycosides could be related to interaction with transition metals ions like Fe(II)/Fe(III), Cu(II) and oxidative reactions promoted by forming metal complexes [1,2,3,4].

To gain deeper knowledge of the mechanism of oxidative reactions facilitated by aminoglycoside antibiotics we performed systematic in vitro studies using variety of the reactive oxygen species indicators. We examined in detail reactions of gentamicin and its iron and copper complexes (in the presence and absence of H2O2 and O2·-) with arachidonic acid, cytochrom C, epinephrine, N,N-di-methyl-p-nitrosoaniline, nitro blue tetrazolium and lucigenin. The sum of the results indicate that both gentamicin alone and its metal complexes facilitate reactions with reporter molecules in the presence of O2·-. On the contrary only metal-gentamicin species are reactive towards H2O2.

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4) WOJCIECH LESNIAK, WESLEY R. HARRIS, JOSLYN YUDENFREUND-KRAVITZ, JOCHEN SCHACHT, VINCENT L. PECORARO Coordination pattern and reactivity of copper(II)-gentamicin complexes submitted

77 Necrotic and Apoptotic Hair Cell Death Induced by Kanamycin in vivo

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The initial biochemical toxic events triggered by aminoglycosides (e.g., formation of reactive oxygen species) are followed by downstream pathways of cell death or survival. A considerable body of literature implicates apoptosis in hair cell loss in cochlear and vestibular organ culture. Both apoptosis and necrosis may occur in vivo. Lysosomal pathways of cell death should be of special interest in attempts to explain aminoglycoside toxicity, because lysosomes are a site of drug accumulation both in the kidney and the inner ear.

We investigated transcription factors and components of cell death pathways in a mouse model. CBA mice received 700 mg kanamycin bid for 3, 7 or 14 days, and markers of cell death pathways were assessed at these times as well as 1, 2 and 3 weeks after the end of treatment. Hair cell loss in the basal turn was barely evident at 7 days, reached about 30% at 14 days of treatment and was almost complete 3 weeks thereafter. ABR was essentially normal at 7 days but showed significant functional deficits at 14 days; the final threshold shift was around 50 dB (at 24 kHz).

Results can be summarized as follows: Markers for apoptotic pathways (TUNEL, JNK, caspase and PARP1-fragments 23 and 89 kDa) were absent during treatment but were seen in a small number of cells 1 week after the end of treatment. In contrast, noise-exposed cochlea showed intense staining for apoptosis markers. Apoptosis-inducing factor, a marker for caspase-independent apoptosis or necrosis-like apoptosis was not translocated into the nucleus at any time. Expression of cathepsin D, a lysosomal protease implicated in necrosis, and its precursors was increased during treatment with kanamycin. Necrosis may be a major form of early outer hair cell death in kanamycin treatment, possibly linked to lysosomal mechanisms.

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78 Calpain Plays More Important Roles than Caspase in Aminoglycoside Induced Hair Cell Death

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To find methods to prevent aminoglycoside ototoxicity, it is necessary to understand the mechanisms. Until some years ago, it had still not been determined what type of cell death, apoptosis or necrosis, occurred in cochlear hair cells following aminoglycoside treatment. But recently it has been shown that aminoglycoside induces apoptosis in hair cells. The most famous protease associated with apoptosis is caspase. More recently, however, evidence has accumulated that noncaspases, including calpain, also have important roles in mediating and promoting cell death. Calpain, a family of calcium dependent cysteine proteases, is known to promote cell death in several organs. To investigate the apoptotic mechanisms of ototoxic and/or acoustic injury, protease inhibitors such as leupeptin which is a calpain inhibitor, and BAF (Boc-Asp(Ome)-fluoromethyl ketone), which is a caspase inhibitor, are generally used. In the present study, we cultured cochlea explants, which were prepared from P6 mice, with or without protease inhibitor. After culture periods, these specimens were stained by Phalloidin-FITC and observed with a laser microscope. We then counted the surviving hair cells and compared the protective effects of the inhibitors. Additionally we stained other specimens by the TUNEL method to detect DNA fragmentation. The result was that leupeptin significantly protected against aminoglycoside induced hair cell loss, however, the protective effect of BAF was negligible. Also we showed that leupeptin, but not BAF, prevents apoptotic DNA fragmentation after aminoglycoside treatment, using the TUNEL method. These results suggest that the hair cell loss induced by aminoglycoside is apoptosis,

and that compared with caspase, calpain plays a more important role in this pathway.

79 JNK Signaling in Hair Cell Death and Regeneration

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The c-Jun-N-terminal kinases (JNK) have been implicated in hair cell death following aminoglycoside-treatment (Pirvola et al., J Neurosci, 20:43) and in tissue repair (Ramet et al., Dev Biol 241:145). We examined the effects of CEP-11004, an indirect inhibitor of JNK activation, on cell death and regenerative proliferation in the avian utricle.

Chick utricles were cultured for 24 hours with 1mM neomycin and varying concentrations of CEP-11004. Hair cells were identified by immunoreactivity to calretinin and hair cell densities were quantified. CEP-11004 promoted hair cell survival in a dose-dependent manner with maximal protection at 1.6 μM. Utricles treated at that dose contained 95±5% of hair cells in the extrastriolar region vs. 48±3% in cultures treated with only neomycin. Other studies examined the presence of phosphorylated c-Jun after neomycin treatment. Increased numbers of p-c-Jun-labeled hair cells were observed at 3-12 hours after neomycin treatment, whereas increased pyknotic nuclei were observed at 12-24 hours. Ototoxic hair cell death can be prevented by treatment with the caspase inhibitor BAF (Matsui et al., J Neurosci, 22:1218). Treatment with BAF did not reduce the number of p-c-Jun-labeled cells, but treatment with CEP-11004 inhibited both p-c-Jun and caspsae-3 activation, suggesting that c-Jun phosphorylation occurs upstream of caspase activation.

The role of JNK in supporting cell proliferation was also examined. The density distribution of p-c-Jun-labeled supporting cells closely paralleled the density distribution of cell proliferation in epithelial cultures. Treatment with 1.6 μM CEP-11004 reduced proliferation in epithelial cultures to 30±6% of control values. JNK activation may serve a dual role in the avian ear, in both hair cell death and in regenerative proliferation.

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80 Gentamicin Treatment Increases Expression of mRNA Encoding the Proapoptotic Factor BAD in the Organ of Corti

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Recent evidence suggests that apoptosis is involved in the death of hair cells exposed to ototoxins such as the aminoglycoside antibiotics. The bcl-2 family of proteins play a critical role in the intrinsic pathway of apoptosis, by influencing the integrity of the mitochondrial membrane. To explore factors that may contribute to apoptosis, we evaluated the expression of mRNA encoding the pro-apoptotic bcl-2 family member BAD in rat organ of Corti exposed to gentamicin in vitro. Organ of Corti was harvested from postnatal day 4 rats and divided into basal, middle and apical turns. The samples were placed in tissue culture with or without 50 [micro]M gentamicin, for 24 hours. Cell death at this dosage is not observed until approximately 48 hours. Six samples of each turn were pooled for mRNA extraction and cDNA generation. The levels of BAD and beta actin cDNAs were assessed using real-time PCR, by comparison with standard curves. Levels of BAD cDNA were normalized by beta actin expression in each sample set. The experiment was replicated four times. In all four experiments, the level of relative BAD cDNA was higher in the gentamicin-treated than in the control samples from basal turn organ of Corti. Relative BAD cDNA was higher in three out of four experiments in the gentamicin-treated middle and apical turn cDNA samples. The results suggest that increases in BAD expression may be involved in gentamicin-induced damage to the organ of Corti.

81 Streptomycin and Gentamicin Have No Effects ON Outer Hair Cell Motility

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The cochlear outer hair cell (OHC), which plays a crucial role in mammalian hearing through its unique voltage-dependent motile responses, has been well established as primary targets of the ototoxic actions of aminoglycoside antibiotics. Although the ototoxicity eventually leads to hair cell loss, these polycationic drugs are also known to block a wide variety of ion channels such as mechanotransducer channels, purinergic ionotropic channels and nicotinic ACh receptors in acute preparations. In this study, we attempted to study the effects of streptomycin and gentamicin on OHC motility by measuring motility and nonlinear capacitance simultaneously from isolated guinea-pig OHCs. Stair-step voltage stimulus from –140 to 50 mV in 10 mV steps was used to evoke motility. Motility was measured by a photodiode-based measurement system. Nonlinear capacitance was determined by transient analysis of currents induced by the staircase voltage stimulus, and the capacitance function was fit to the first derivative of a two-state Boltzmann function relating nonlinear charge to membrane voltage. Streptomycin and gentamicin were applied extracellularly through a puff pipette or intracellularly through patch pipettes. Motility and nonlinear capacitance were measured before and after streptomycin or gentamicin was applied to the extracellular medium for 5 minutes. No significant changes in motility and nonlinear capacitance were observed with 1 or 5 μM concentrations. We also measured motility and nonlinear capacitance after gentamicin or streptomycin was applied intracellularly. Motility and nonlinear capacitance remained essentially the same after 5-minute perfusion. Our study suggests that gentamicin and streptomycin have no effects on OHC motility.

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82 Antioxidant Gene Therapy Against Aminoglycoside Ototoxicity

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Aminoglycoside induces oxidative stress and causes hair cell (HC) loss in the inner ear, leading to hearing loss that is permanent in mammals. Several molecules such as iron chelators, neurotrophic factors and antioxidant drugs have been applied systemically and showed protective effects in the inner ear against several kinds of oxidative stresses. Because of the short half-life of the antioxidant enzymes in the plasma and the lack of uptake of the proteins into cells, gene transfer is a promising approach to deliver these drugs into cells of the inner ear. We set out to determine the extent of protection provided by antioxidant gene therapy against ototoxicity. We inoculated adenoviral vectors that contain the genes encoding for human catalase or superoxide dismutase (SOD2) (designated as Ad.cat and Ad.SOD2, respectively) into the guinea pig cochlea. Before the inoculation, we established base line ABR thresholds. For control, we inoculated artificial perilymph (AP) or adenovirus containing no gene cassette (Ad.empty). Five days after adenovirus inoculation, all the animals were treated bilaterally with a combination of kanamycin and ethacrinic acid. Seven days later, ABRs were measured and the animals euthanized. In Ad.cat and Ad.SOD2 inoculated groups, hearing and the HCs of the inoculated (left) ears were significantly protected compared to the contralateral ears. Ad.cat and Ad.SOD2 provided better preservation of hearing and HCs than the control inoculations. Ad.SOD2 provided better protection than Ad.cat. In conclusion, we demonstrated the feasibility for antioxidant gene therapy in the inner ear and that SOD2 protects HCs effectively against ototoxic insult.

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83 IB1/JIP-1 Peptides Protect Hearing And Auditory Hair Cells From Noise And Aminoglycoside-Induced Apoptotic Death.

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Hearing loss can be caused by a variety of factors including acoustic trauma or ototoxic insults that principally affect the sensory hair cells which die via an apoptotic pathway associated with the c-Jun amino terminal kinase (JNK), a member of the stress-activated family of MAP kinase. We have studied the effects of IB1/JIP-1, a scaffold protein that prevents the interaction between JNK and its numerous targets such as c-Jun , on the traumatized cochlea. As model systems, we used guinea pigs cochleas exposed to an acoustic trauma and organotypic cultures of neonatal mouse cochleae. In organotypic cochlear cultures, IB1/JIP1 prevented totally neomycin-induced hair cell loss. This finding and the observed increase of phosphorylation of one JNKs target, the transcription factor c-Jun, in stressed hair cells are demonstrations of the ability of IB1/JIP-1 peptides to prevent hair cell loss. In vivo, IB1/JIP1 was delivered to one ear via a mini-osmotic pump in guinea pigs exposed to a sound trauma (6 kHz, 120 dB SPL, 30 min). Protection was assessed physiologically by the change in 8th nerve compound action potential threshold and histologically by hair cells survival counts. Animal ears that were perfused with IB1/JIP1 peptides showed less threshold shift and less hair cell loss than non-treated contralateral ears. These results indicate that JNK pathway is involved in both neomycin and sound induced hair cell loss and that its blocking by a small permeable peptide acting on the intracellular signaling cascade, such as IBI/JIP-1 might be of therapeutic value to confer morphological and functional protection.

84 The Effects of Glucocorticoid on Protection Against Aminoglycoside Ototoxicity in the Guinea Pig

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Steroid hormones are the mainstream of treatment, however, because of the existence of the blood-labyrinthine barrier, the local dose of cochlea and therapeutic mechanism are still unclear. Fundamental study designed direct intra-cochlea infusion of pharmacologic agents with osmotic pumps was evaluated. We investigated the effect of Dexamethasone (Dex) following trauma by coadministration of kanamycin (400mg/kg) and ethacrynic acid (40mg/kg) on guinea pigs, using brainstem response (ABR) and survival rate of hair cells. For the experimental pre treatment groups (Group 1), Dex was administered using implanted osmotic pumps filled with 0.1, 1 and 10ng/ml Dex delivered via a catheter to the left scala tympani. ABR recordings were performed on day 0 and 28. Infusion of Dex was before and after KM/EA treatment for a total of 28days. On the 1ng/ml Dex infused ear, the mean ABR threshold after ototoxicity was reduced about 20dBSPL and showed significant higher OHC survival rate compared to contralateral ear. Dex (1ng/ml) showed the most effective result on both ABR threshold and OHC survival rate. For the experimental concurrent and post treatment groups, we examined two patterns of Dex infusion. Group 2 received AP for 14 days and then 1ng/ml Dex for 14 days; animals were deafened with KM/EA on day 14. Group 3 received AP and Dex with the same protocol as group 2;animals were deafened on day 12. ABR threshold shifts in the pump-implanted ears were significantly smaller than in the contralateral ears in group 2, but these were not significantly in group 3. The OHC survival rates of the implanted ears of group 2 and 3 were significantly greater than that of the contralateral ears. This result suggests that early infusion of Dex is more effect against aminoglycoside ototoxicity. Our studies have demonstrated that local administration of Dex directly to the inner ear, preceding aminoglycoside administration, is effective in attenuating both auditory physiology and morphology.

85 The Effect of the Pan-caspase Inhibitor in the Guinea Pig Inner Ear During Intracochlear Administration of Gentamicin

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On a disorder of inner ear hair cell of various drug including aminoglycoside drug, the probability that apoptosis is involvement of the cell death is reported. We administered gentamicin and pan-caspase inhibitor to the perilymph of the guinea pig cochlea to investigate its protective effect on the apoptosis of inner ear hair cell.

We assessed auditory brain stem response (ABR) thresholds to evaluate cochlear function and observed the sensory epithelium using fluorescent microscopy. Fourteen days after treatment, gentamicin only caused ABR threshold shift, but threshold remained moderately. The ABR threshold showed an more slowly increase in the guinea pigs treated gentamicin and pan-caspase inhibitor than that of gentamicin only. Histopathological examination revealed severe hair cell damages in cochlear endorgans in guinea pigs given 12 mg gentamicin after 14 days. In guinea pigs given gentamicin and pan-caspase inhibitor, outer hair cells were survival in the third turn. These results suggest that pan-caspase inhibitor may play an important role in cochlear protection of inner ear disorder by topical therapy.

86 Outer Hair Cells are Protected by IGF-I Against Aminoglycoside Ototoxicity

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Once cochlear hair cells (HCs) are lost they are not replaced. It is therefore important to protect HCs against degeneration due to insults such as overstimulation, ototoxic drugs and infections. Insulin-like growth factor-I (IGF-I) is a potent survival factor in a variety of cells and tissues. In the mammalian inner ear, IGF-I and its binding proteins have been shown to change their expression levels following an ototoxic insult. The aim of the present study was to determine the extent of protection of IGF-I against cochlear HC degeneration due to aminoglycoside-induced ototoxicity. Mature female pigmented guinea pigs were used. Normal hearing was confirmed and baseline auditory thresholds established with ABR audiometry. On day-1, an osmotic mini-pump was filled with human recombinant IGF-I (or vehicle solution) and implanted in the interscapular subcutaneous tissue. The cannula tip was placed in the basal turn of the left cochlea via cochleostomy at the scala tympani, so that IGF-I was infused continuously (0.5 µl/hr, from day-1 through day-11) into the perilymphatic space. Hearing was not significantly changed by the surgical process. On day-4, the animals received a combination of kanamycin (250 mg/kg sc) and ethacrynic acid (40 mg/kg iv). On day-11, ABRs were measured, animals sacrificed and cochleae processed for HC counts. Comparing ABR thresholds between right (contralateral) and left (IGF-I infused) ears revealed better hearing in the left ears, with significant differences at 4 kHz ($p<0.05$) and 20 kHz ($p<0.005$). The average loss of OHC in left ears was less than 2%; whereas in the control ears it was between 27% and 40%. The difference was statistically significant ($p<0.05$). We conclude that exogenous IGF-I can protect mammalian HCs from aminoglycoside ototoxicity.

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87 The MAPK Pathway Initiates Oxidative Stress-Induced Apoptosis of Auditory Sensory Cells

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Oxidative stress is generated by a wide variety of insults which include: loss of trophic support; aminoglycosides; cisplatin; sound trauma; presbycusis; and exposure to toxic substances in the enviroment. Toxic compound damage to auditory sensory cells can trigger the initiation of an apoptotic cascade and loss of sensory cells. An important step towards formulating effective therapies to counteract damage-initiated loss of sensory cells is to more fully understand the sequence of events that results in their death. To accomplish this we have utilized in vitro and in vivo models of oxidative stress using 3 insult paradigms: neurotrophin withdrawl; 4-hydroxy-2-nonenal; and cisplatin. To define one of the signal pathways that participates in the apoptosis of auditory sensory cells we used inhibitors of the MAPK signal pathway and antisense oligonucleotides directed against molecules of this pathway. The results of these studies have identified the MAPK pathway as a major initiator of auditory sensory cell apoptosis for the insult paradigms tested. These results support other studies that have utilized inhibitors of the MAPK pathway (e.g. CEP1347 and D-JNKI-1) to stop damage-induced cell death of auditory sensory cell both in vitro and in vivo. The results of this study support a perilymph-based therapuetic approach that targets the MAPK pathway to prevent the loss of oxidative stress-damaged auditory sensory cells.

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88 Activation of BK Channels Mediates Cisplatin-induced Apoptosis in Spiral Ligament Fibrocytes

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The ototoxic effects of cisplatin have been attributed mainly to cell death mediated by apoptosis. Moreover, it is well established that activation of K conductance by apoptotic agents is an early hallmark of apoptosis in CNS neurons. The current study was designed to test: 1) if cisplatin can induce apoptosis in spiral ligament fibrocytes (SLFs) in vitro and 2) if cisplatin-induced apoptosis is mediated by activation of K conductance. Secondary cultures of SLFs derived from gerbil were incubated with 0 (control), 5, 10, 20 and 40 mM cisplatin for 72 hrs. A dose-dependent pattern of apoptosis was observed both by annexin V-FITC/ PI (propidium iodide) fluorescence microscopy and flow cytometry. The corresponding apoptotic ratios were 8¡À1 (control), 19¡À2, 21¡À2, 26¡À3 and 76¡À9 % (N=10), respectively. Co-incubation of 10 mM tetraethylammonium with 5, 10, 20 or 40 mM cisplatin for 72 hrs significantly reduced apoptotic ratios to 11¡À2, 13¡À1, 12¡À3 and 28¡À5 % (N=10), respectively. Incubation with 80 mM cisplatin for 72 hrs significantly increased DNA fragmentation from 30¡À8 (control) to 82¡À10 % (N=6). Gel analysis of DNA fragmentation showed a smear pattern. Whole-cell patch clamp showed that incubation with 10 mM cisplatin for 20 hrs significantly shifted the voltage activation curve towards a more active value in about 30% of SLFs. The average change of the half maximal voltage activation was 50 ¡À8 mV (N=3). The activated whole cell current was inhibited (97%) by 0.8 mM iberiotoxin, indicating the involvement of endogenous BK channels. These results demonstrate that cisplatin can induce dose-dependent apoptosis in cultured SLFs, which appears to be dependent on the activation of endogenous BK channels. This information may provide potential new therapeutic strategies for preventing apoptotic cell death.

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89 Protective Effect of EUK-134, a Synthetic Superoxide Dismutase and Catalase Mimetic, Against Cisplatin-induced Damage in the Organotypic Culture of Organ of Corti

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There is considerable interest in identifying compounds that can block the ototoxic effects of cisplatin without interfering with its antineoplastic efficacy. Since cisplatin ototoxicity may arise from the generation of reactive oxygen species (ROS), compounds that scavenge these toxic molecules have the potential to protect against cisplatin ototoxicity. EUK-134, a salen-manganese complex, exhibits both superoxide dismutase (SOD) and catalase activities thereby suppressing the toxic effects of superoxide and hydrogen peroxide. Since salen-manganese complexes protect against several animal models of ROS-associated disorders such as stroke, Alzheimer's and Parkinson's disease, we assessed the ability of EUK-134 to protect against cisplatin-induced hair cell damage in P3 rat cochlear organ cultures. Cochleae were cultured for 24 hrs and then treated with EUK-134 (10, 20, 40, 100 uM), cisplatin (10ug/ml) or cisplatin plus EUK-134 for an additional 48 hrs. Afterwards, the cultures were fixed and the stereocilia bundles stained with FITC-conjugated phalloidin. Hair cells were then counted with an epifluorescence microscope. The 10ug/ml dose of cisplatin caused significant damage to the stereocilia bundles and destroyed IHCs and OHCs. EUK-134 provided partial protection against cisplatin-induced hair cell loss in the cochlear cultures. Additional studies are currently underway to determine if protects against gentamicin-induced hair cell damage.

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90 L-Methionine Protects the Ototoxicity of Cisplatin by Changing HMG1 Expression

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Cisplatin (CDDP) is an effective chemotherapeutic agent for cancers. Studies demonstrate that either systemic or local delivery of L-Methionine (L-Met) to the round window membrane niche of the rat inner ear can protect against CDDP ototoxicity. Although much attention has been directed at developing methods of protection against CDDP ototoxicity, the mechanisms by which CDDP produces its ototoxcity and by which L-Met provides otoprotection are not well understood. HMG1, a sub-family of the HMG protein family, plays an important role in regulation of DNA transcription, repair of damaged DNA, and the mediation of CDDP antitumor activity. In this study, we investigated the relationship between HMG1 and CDDP ototoxicity, and the effects of L-Met on the expression of HMG1 in CDDP treated rats. Thirty rats were divided into three groups: Group I, untreated control (n=10), Group II, CDDP treated (12mg/kg) (n=10) and Group III, CDDP (12mg/kg) +L-Met (300mg/kg). The right cochleae were collected for Western blot analysis. Left cochleae were divided into two sub-groups; one sub-group for immunohistochemical analysis and one sub-group for scanning electron microscopy. L-Met protected hair cells against CDDP otoxicity, as evidenced by scanning electron microscopy. Immunohistochemical and Western blot analysis demonstrated that HMG1 expression in the cochlea was up-regulated in Group II (CDDP

treated) rats in comparison to the cochlea in Group I (untreated rats)rats. Although levels of expression of HMG1 were elevated in the cochleae of Group III (CDDP+L-Met) rats when compared to HMG1 levels in Group I (untreated control) rats, the up-regulation in group III was statistically less than in Group II (CDDP treated). Our findings suggest that HMG1 plays a role in mediating the otoxicity of CDDP, and provide promise for the ability to ameliorate CDDP ototoxicity by manipulating the expression of HMG1.

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91 Local Therapeutic Strategy Against Cisplatin-Induced Ototoxicity in Guinea Pig.

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Cisplatin is an effective cytotoxic drug, used in the treatment of a range of neoplasms. Unfortunately, sensorineural hearing loss is a serious side effect of cisplatin chemotherapy which greatly impairs patients' quality of life. One way to protect the cochlea without affecting the antitumoural activity of cisplatin is to apply protective agents directly into the cochlea. The aim of this study was to design therapeutic strategy using local application of putative protective agents.

In the guinea pig, cisplatin treatment (2 mg/kg, I.P., for 5 days) results in high-frequency hearing loss (up to 60 dB), and the loss of outer hair cells from the basal turn. Morphological analyses and specific DNA labelling reveal fragmented hair cell nuclei and cytochrome c redistribution into the cytoplasm of the hair cells of the basal turn, suggesting an apoptosis mechanism of hair cell death. Two strategies were used to rescue the cochlea. The first was aimed at preventing the toxicity of cisplatin by using sodium thiosulfate, a drug known to bind to the platinum molecule of cisplatin to form an inactive platinum-thiosulfate complex which is not taken up into cells. The second strategy aimed at comparing the efficiency of anti-apoptotic agents. Here, we show that an intracochlear perfusion of sodium thiosulfate as well an intracochlear perfusion of z-DEVD-fmk, a specific caspase-3 inhibitor, rescued hair cells from apoptotic death. In contrast, D-JNK, a cell-permeable peptide inhibitor of the c-Jun-N-terminal kinase, acting upstream in the apoptotic cascade does not. This is probably because the activation of the NH2-terminal Jun kinase is required for DNA repair.

In human, local application of drugs is achieved through the tympanic membrane using a catheter placed into the round window niche. The present results, especially those obtained with sodium thiosulfate, constitute a great hope for prevention of deafness in those patients having to undergo cisplatin chemotherapy.

92 Round Window Application of the P53 Inhibitor, Pifithrin-Alpha, Protects against Cisplatin Ototoxicity

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Cisplatin is a commonly used chemotherapeutic agent with several dose-limiting side effects such as ototoxicity, nephrotoxicity, and myelosuppression. Salvi, et al. demonstrated that Pifithrin-alpha (PTF), a P53 inhibitor, protects against cisplatin-induced hair cell loss in organotypic cochlear and vestibular cultures. No in-vivo studies have been done to investigate the role of p53 in cisplatin ototoxicity. The goal of our study was to determine the role of P53 in cisplatin ototoxicity, in-vivo, using PTF.

Auditory brainstem responses (ABRs) were measured in adult chinchillas, in response to 2,4,8 and 16 kHz tones, before and 3 days after round window (RW) application. The RW was exposed and 10 µl of PTF (0.5, 1 or 2mM) or 10 µl of the vehicle solution was applied. After 30 minutes, the remaining solution was removed from the round window and 2 µl of cisplatin (0.66 mg/ml) was applied to each RW. Hearing loss was determined by measuring ABR thresholds changes.

The ears pretreated with 2mM PTF were substantially protected from hearing loss when compared to the vehicle plus cisplatin group. Threshold changes in the 2mM PTF-treated ears were 13.3 ± 3.3, 11.7 ± 1.6, 6.7 ± 1.6, 11.7 ± 2.0 and 18.3 ± 6.3, for 1, 2, 4, 8 and 16kHz, respectively. These results were significantly different from the vehicle plus cisplatin group ($p<0.001$) which had threshold changes of 56.7 ± 3.2, 61.7 ± 6.0, 66.7 ± 2.0, 65 ± 3.1 and 60 ± 2.6, for 1, 2, 4, 8 and 16kHz, respectively. The otoprotection afforded by PTF was dose-dependent with moderate to complete protection as the dose of PTF was increased from 0.5 mM to 2 mM. Vehicle or PTF alone had no effect on ABR thresholds.

These results support the role of P53 and the apoptotic pathway in the mechanism of cisplatin ototoxicity. Further studies will be needed to determine the clinical applications of these results.

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93 Influence of Cochlear pH Modulation on the Ototoxicity of Systemically Applied Cisplatin.

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Cisplatin is a commonly used antineoplastic agent that produces a number of dose-limiting side effects, including ototoxicity. Prior studies in vitro have shown that cisplatin toxicity may be modulated by pH. At the 2002 ARO mid-winter meeting, we reported that the ototoxicity of locally applied cisplatin is inhibited by the local application of basic phosphate buffered saline (PBS) and potentiated by acidic PBS. In the present study, we investigated the effect of locally applied PBS of various pH on the ototoxicity of systemically applied cisplatin.

Rats were anesthetized and auditory brainstem responses (ABR) were recorded. The auditory bullae were surgically opened, and neutral (pH=7.4) or basic (pH=9.0) PBS was applied to the round window niche (RWN) and 60 minutes later any remaining solution was removed. At 30 minutes after PBS application, cisplatin (13 mg/kg) was injected intraperitoneally with a 30 minute perfusion. After 3 days, follow up ABRs were performed. Basic PBS significantly reduced cisplatin-induced threshold changes ($p<0.00001$ by ANOVA). The ABR threshold changes for the neutral PBS + cisplatin group were 18.3 ± 3.1, 20.0 ± 5.8, 16.7 ± 4.9, 18.3 ± 6.5, 31.7 ± 6.0 and 36.7 ± 8.4 dB (mean ± SEM) for click, 2, 4, 8, 16 and 32 kHz, respectively. Those for the basic PBS + cisplatin group were 5.0 ± 5.0, 6.7 ± 4.9, 3.3 ± 2.1, 5.0 ± 3.4, 13.3 ± 5.6 and 15.0 ± 6.7 dB for click, 2, 4, 8, 16 and 32 kHz respectively.

These results support our previous findings that cochlear pH can affect the ototoxicity of cisplatin, and that this effect is not due to changes in window permeability. Our data suggest that care should be taken regarding the pH of any solution that is applied to the round window membrane.

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94 Systemic Treatment with Alpha-Melanocyte-Stimulating-Hormone Delays Ototoxicity Caused by Local Administration of Cisplatin

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Previous work has demonstrated that ototoxicity induced by systemic cisplatin in guinea pigs is reduced by concomitant administration of melanocortins, e.g., alpha-MSH. However, these experiments were hampered by large interanimal variability. Therefore, we have developed a model that involves intracochlear administration of cisplatin into the scala tympani via an osmotic pump system in

combination with chronic, daily recordings of the compound action potential (CAP) from a permanent round-window electrode. In this model we have re-investigated the protective effects of systemic alpha-MSH treatment. Guinea pigs were implanted with a round window electrode and a microcannulation-osmotic pump system operating at 0.5 µl/h. The pumps were filled with saline or cisplatin in saline (15 µg/ml). Co-treatment consisted of either SC injections of alpha-MSH (75 µg/kg/day) or saline (similar volume) for one week or until the electrocochleogram showed a criterion decrease in CAP output (40 dB threshold shift at 8 kHz). The animals then were sacrificed and the cochleas processed for histological examination and OHC counts. After 2-3 days of cochlear application, cisplatin alone caused a threshold shift at all frequencies (2-16 kHz). alpha-MSH co-administration consistently delayed the threshold shift by 1-2 days. Since pathological assessment followed a 40 dB criterion threshold shift for both alpha-MSH and saline co-treatment groups, similar hair cell loss was observed. These data show that alpha-MSH delays cisplatin ototoxicity, but does not prevent OHC loss. Nevertheless, a delay of 1 to 2 days is of potential clinical significance. Moreover, because of the local administration of a fixed amount of cisplatin in this model, it is likely that the alpha-MSH effect involves a cochlear target and is not caused by changes in cisplatin pharmacodynamics.

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95 Some Geometrical Aspects of Cochlear Interferometry

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We compare the results of bench-top measurements made with three vibrometers: a commercial laser-Doppler vibrometer made by Polytec, a laser-feedback vibrometer, and an optical-coherence-microscope (OCM) vibrometer.

The Polytec and the laser-feedback interferometer (LFI) use 638.6 nm laser light. The OCM uses a red diode with a nominal coherence length of 10 nm. For each system, we have measured the spatial resolution for scans along the optic axis using a target made of two cover slips mounted on adjacent piezoelectric stacks, such that each target can be vibrated independently. The target separation is 75 microns. This allows us to determine the extent of the interference between the two surfaces. This same experiment determines the resolution and interference of phase measurements for adjacent surfaces.

By placing a 400-micron diameter pinhole in front of the target, we are able to determine the effects of a restricted numerical aperture, such as exists when imaging the basilar membrane through a small hole in the cochlear wall. This demonstrates the benefits of an OCM vibrometer, whose resolution is primarily based on the coherence length of the source, and not on the optical resolution of the system.

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96 Prediction of Two Types of Pressure Waves in the Cochlea

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When fluid pressure fluctuation is induced by vibration of the stapes, two types of pressure waves occur in the cochlea. One is a fast wave which propagates at the velocity of sound, and the other is a slow wave which follows a traveling wave on the basilar membrane (BM). As the organ of Corti (OC) is driven by these pressure waves, it is important to understand their characteristics. However, there have been no reports of observation of these pressure waves, because it is difficult to measure them separately. In this study, a finite-element model of the OC at the basal turn of the gerbil cochlea was constructed. Geometry of the model was determined based on measurement of the hemicochlea (Edge et al., 1998, Hear. Res. 124: 1-16). By comparing numerical results with experimental data of intracochlear pressure (Olson, 2001, J. Acoust. Soc. Am. 110: 349-367), the magnitude and phase of the fast wave and those of the slow wave were estimated when sound pressure in front of the tympanic membrane was 80 dB SPL. The magnitude of the fast wave showed a moderate increase as a function of frequency, and that in the scala tympani was smaller than that in the scala vestibuli due to the stiffness and damping of the BM. The slow wave grew gradually to the point of resonance (CF: f = 16 kHz) with increasing frequency, and then fell sharply. When these pressure waves were applied to the model, the numerical results showed good agreement with the experimental data of the velocity of the BM vibration (Wilson and Johnstone, 1975, J. Acoust. Soc. Am. 57: 705-723). Therefore, predicted characteristics of the two pressure waves appear to be reasonable.

97 Reconstruction of Noisy Speech Signals Based on a Cochlear Model.

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One dimensional cochlear model with embedded outer hair cell (OHC) has been recently presented (Cohen and Furst, ARO 2002). The cochlear fluid dynamic equations were solved in the time domain, and the OHC contribution to the basilar membrane motion was integrated into the time domain equations. The model output was presented by the basilar membrane's velocity as a function of time. One of the most interesting properties of the model is its response to noisy sounds. The background noise dramatically decreases when the signal exists. Those properties are highly dependent on the OHC gain. The purpose of the present research is to use the dynamic properties of the model in order to reconstruct noisy speech signals with improved SNR. A special algorithm for signal reconstruction was developed. The algorithm includes an estimation of both the traveling wave delay and weight of each part along the cochlear partition. The reconstructed signal is obtained in the time domain as a weighted sum of all the cochlear partition outputs. The algorithm performances were evaluated for different types of inputs such as sine waves, chirps, and speech sounds with different SNRs. The reconstruction quality was highly dependent on the OHC gain.

98 Filtering in the Cochlear Amplifier?

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We have measured the frequency response of the basilar membrane (BM) velocity in the cochlea of anesthetized guinea pigs. The stimulus signal was wide-band noise, and the response is derived from cross-correlation functions between the stimulus signal and the response. The frequency response vfBM(f) (where f is frequency) was converted into the response vxBM(x) of a three-dimensional model of the cochlea, where x is the longitudinal coordinate. By way of the "inverse solution" the BM impedance ZBM(x) was derived; when the model is provided with this impedance, its response is compatible with the response vxBM(x). The BM impedance consists of two components, one is the impedance corresponding to the "dead" cochlea, the other one is the contribution assumed to be due to the outer hair cells (OHCs). We call the latter one the "active" part Zact(x) of the impedance.

Associated with Zact(x) is the pressure pact(x), close to the BM. We considered the hypothesis that pact(x) is a weighted sum of contributions of BM velocity around the location x. Contributions from more basal locations – governed by weighting factors h(j) with negative j – constitute "feed-forward", those from more apical locations – weighting factors h(j) with positive j – "feed-backward". The coefficients h(j) were assumed to be REAL, which means that no form of frequency- or space-dependent filtering was involved. The weighting factors h(j) were determined with a specialized minimum-square-absolute-error method. We found that in 28 out of 36 experiments model responses computed on the basis of the reconstructed pact(x) are compatible with experimental data. In all cases both feed-forward and feed-backward components are included. It should be noted that an

extended model, in which the OHCs are assumed to be nonlinear transducers, displays all the frequency- and amplitude-dependent properties of the real cochlea. In particular, we show that it displays the near-invariance of zero-crossings of the impulse response when stimulus intensity is varied.

99 Theoretical and Experimental Considerations for the Study of Anisotropic Elastic Moduli of the Mammalian Tectorial Membrane

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The issue of anisotropy of the tectorial membrane (TM) has been raised by Zwislocki and Cefaratti (Hear Res 42;1989) and, more recently, by Abnet and Freeman (Hear Res 144;2000). Classical models of contact mechanics, which have been applied in the analysis of indentation data obtained using the atomic force microscope (AFM), are invalidated by considerations of anisotropy. Moreover, the anisotropy that is disregarded by classical methods may be fundamental to the functioning of the TM. Nonetheless, AFM is rapidly emerging as an important tool in auditory mechanics. In our current work, we have developed a mathematical model that predicts surface displacement patterns in response to normal and shear point forces in a transversely isotropic elastic half-space (the simplest model of anisotropy). By incorporating 3 elastic parameters, this model provides a framework for studying anisotropic materials and suggests new AFM experiments on the TM. Our 3D finite element model validates and generalizes the utility of this mathematical paradigm and allows the analysis of an anisotropic contact problem by providing descriptive and predictive parameters that can be readily tested in vitro. To facilitate and objectify the analysis of AFM data, we have automated the analysis of force-distance curves. Our program reconstructs cantilever height/deflection data and automatically detects the initial contact point. Comparing the resultant curve to theoretical data, the program converts cantilever deflections to sample deformations, which are fitted to an appropriate theoretical model. This enables the computation of directional elastic moduli of a material under a variety of different assumptions, and facilitates a comprehensive parametric consideration of how calculated tissue properties are affected by the use of different analysis techniques.

100 Hysteresis in Cochlear Transduction Observed from Low-frequency Modulation of DPOAEs.

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Recently, we (Bian et al., 2002, JASA, 112:198) developed a noninvasive method of estimating a cochlear transducer function (f_{tr}) using low-frequency modulated distortion product otoacoustic emissions. This method is based on the approximation that the magnitude of the cubic difference tone (CDT, 2f1-f2) is proportional to the absolute value of the third derivative of the f_{tr} at low primary levels. An experiment in gerbils verified such a relation between the CDT and a cochlear f_{tr}. However, this procedure is time-consuming, since it requires measuring the CDT at twenty-one bias levels. To significantly reduce the data collection time, the CDT can be measured using one high-level bias tone which provides all the bias levels necessary for deriving the f_{tr}. Specifically, the CDT magnitude and corresponding bias level can be extracted within a short-time window that moves along the time series. In an experiment in gerbils (n = 9), a two-tone signal (f1=3968, f2=5120 Hz) with levels from 50 –70 dB SPL was biased by a 25 Hz tone at 20 Pa. The CDT magnitudes were obtained with a 512-point FFT window moving along three cycles of the bias tone. The CDT magnitude as a function of bias level showed two distinct functions both demonstrating the shape of the absolute third derivative of the f_{tr}. Depending upon the directions of the change in bias tone amplitude (increasing or decreasing), the CDT-bias function shifted to positive or negative sound pressure directions. The distance between the two modulation patterns was about 10 Pa for all primary levels. The separation of the CDT-bias functions according to the phase of the bias tone indicates hysteresis in cochlear transduction processes. A model incorporating reactive force generation within the cochlear transducer fit the CDT data well (r2 = 0.6 – 0.9) for different primary levels.

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101 The Basilar Membrane Velocity for Electrical Stimulation of the Organ of Corti at the Measured Place

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Electrical stimulation at the round window membrane of the guinea pig cochlea causes electrically evoked otoacoustic emissions (EEOAE) having a wide frequency bandwidth. The upper frequency limit of the EEOAE (defined by the noise floor level of output for a 35 microampere RMS stimulus) is about 40 kHz. When the organ of Corti is stimulated locally by electrodes near the basilar membrane (BM) or by electrode pairs across the cochlear duct, the EEOAE is much more tuned. Moreover, acoustic energy does not appear to reverse propagate for the frequencies above the best frequency of the electrode place. With an electrode pair across the cochlear duct at the first cochlear turn, the upper frequency limit of the EEOAE was 15-20 kHz. We measure the local BM velocity for stimulation at the round window or across the cochlear duct of the first turn. Reflective beads of 20 or 3 micrometer diameter were placed on the BM at about the 18 kHz location for BM velocity measurements. The magnitude spectrum of the local velocity response can be divided into three frequency regions. The low frequency region below BF has a sharply tuned appearance similar to a conventional mechanical tuning curve. The second region, from BF to about 25 kHz, is low-pass with a fairly steep roll-off and nearly zero phase slope. This region may represent a rapidly extinguished evanescent wave. Above 25 kHz, the local displacement response extends in frequency to 100 kHz (for 100 microampere, RMS). When the bead is at the radial location of the outer hair cells (OHC), this 'ultrasonic' part of the spectrum has a magnitude minimum at 40-60 kHz with a sharp phase change. When the bead is at the radial location of the tunnel of Corti, there is a peak in the spectrum at 40-60 kHz. Local ultrasonic response suggests that in vivo OHCs create sufficient force to move the BM at frequencies to 100 kHz and that local mechanical properties result in resonance around 50 kHz.

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102 Measurement of Volume Velocity Of Basilar Membrane Vibration in the Sensitive Cochlea

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Our current knowledge of cochlear mechanics in sensitive cochlea is based mainly on the measurement of basilar membrane (BM) vibration at a single point on the cochlear partition. A cochlear amplifier has been proposed to interpret the nonlinearity and sharp frequency tuning of BM responses. However, the single-point measurement of velocity or displacement of BM vibration provides limited information on power flow in the cochlea. Towards a direct quantitative measure of the power flow in the sensitive cochlea, a technique for the volume-velocity measurement of BM vibration was developed in this study. We implemented a scanning laser interferometer microscope by combining a sensitive heterodyne interferometer, a computer-controlled 3D positioning system, and a microscope. Magnitude and phase of BM vibration in response to a single tone were measured as functions of longitudinal and radial locations on the BM. The volume velocity was calculated by integration of transverse velocity over an area half a wavelength long and by the width of the BM centered at the characteristic frequency location. The volume velocity of stapes vibration was measured by multiplying the point velocity of the stapes footplate by its area. The volume velocity gain of BM vibration was

obtained as the ratio of the BM volume velocity to that of the stapes. The data demonstrate that this scanning laser interferometer is sensitive enough to measure cochlear partition vibration at low sound pressure level, in the sensitive cochlea without placing a reflecting object on the BM. Our data also show the traveling wave as a function of time or location in three dimensions in the living cochlea.

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103 Comparison of the Traveling Wave Velocity Between CAP Experiment and Numerical Method.

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In our previous report the two dimensional cochlea model was solved numerically using MAC method and have shown that the phase velocity Vp of the traveling wave along the cochlea is solely a unique function of the distance from the base and independent of the frequency, i.e., $V_p=V_{po}exp(-x/0.71)$ where x is the location.

Although a body of studies on the propagation velocity exist, however, it is still open problem because the multipoint measurement is difficult owing to the geometry of the cochlea. It seems that CAP (compound action potential) measurement is a candidate which will provide a position sensitive information without scanning the observation points. The basic ideas are as follows.

- The CAP is considered as an integrated signal originating from a group of simultaneously firing afferent nerves, main contributing portion of which is those within the cochlea region.

- When a tone pip with a carrier frequency ω is applied, IHCs located in the CF region of ω bear a strong displacement from BM after an intrinsic latency, which, in turn, give rise to firing of nerves from IHC.

Consequently, it is expected that the CAP signal position in the time domain will contain the latency.

In experiment, we used a guinea pig, whose bulla was opened with a drill. Under the microscope, a silver ball electrode was placed on the round window, and the electrical signal was recorded in vivo.

It was confirmed in our experiment that the latencies indeed depend on input frequencies, and latency of the signals decreased with frequency.

This finding qualitatively agrees with above speculation and early work by Prof. J. J. Eggermont.

104 Investigating Energy Propagation In The Cochlea: Time Domain Measurements Of Otoacoustic Emissions

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We have previously reported that the delay of the 2f1-f2 DPOAE from its CF place is consistent with a round trip delay from this place, and presented preliminary data that does not show a systematic increase in delay for the 2f1-f2 DPOAE arising from the generator region as f2/f1 (in a fixed-f2 paradigm) decreases below 1.15 (Withnell, et al., 2002). In the present study, we use a pulsed-tone paradigm (Talmadge et al., 1999) to examine signal-front delays, and extend previous work, to investigate the propagation time of backward-traveling waves in the long-wave region and the effects of basilar membrane filtering on propagation times as f2/f1 approaches 1, as suggested by theoretical considerations (e.g., Tubis et al., 2000).

105 The Mechanical Basis of the Resonance in the Mustached Bat's Cochlear: Mode-Hopping and Timing of Cochlear-Feedback.

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A sharply tuned mechanical resonance (~61 kHz) in the mustached bat cochlea resolves fine Doppler-shifts in returning echoes. Cochlear microphonic (CM) responses, which are sharply tuned to the primary cochlear resonance, were analysed to reveal how the basilar membrane (BM) and tectorial membrane (TM) interact and when in each cycle their vibrations are amplified. Within 5 kHz of the primary resonance frequency CM responses at the tone onset are broadly tuned with linear magnitude level functions. CM measured during the tone and in the ringing at the tone offset is 50 dB more sensitive, sharply tuned, with compressive level functions. The resonance appears to be due to interaction between two resonators. A broadly tuned resonator responds first to a tone, followed by a narrowly tuned resonator whose responses take 1 – 3 ms to build-up and ring at the resonance frequency at the tone offset. CM responses are amplified during maximum BM velocity for frequencies within 2 kHz of the primary resonance. The broad resonator has several frequency-modes. This is evident from hopping of the response frequency between discrete steps that are 2-3 kHz apart. There are phase-transitions and magnitude-minima at the transition frequencies. Within a frequency band slightly above the primary resonance both resonators, presumably the tectorial and basilar membranes, move together in phase which results in minimal hair bundle displacement and CM magnitude during stimulation and only the on and the off responses remain.

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106 Medial Olivocochlear Efferent Fast Effects on Basilar Membrane Motion in Guinea Pigs

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To aid in understanding the mechanisms by which medial olivocochlear efferents produce their effects, we measured basilar membrane (BM) motion in response to tones in the basal turn of anesthetized guinea pigs, with and without electrical stimulation of efferents by brainstem shocks. We selectively measured efferent fast effects by averaging BM responses over 8-16 bursts of 100 ms shock trains and compared average BM motion in time windows before and at the end of the shocks-on period.

The fast efferent effect produced (1) a reduction in BM motion for low-level tones near the characteristic frequency (CF), (2) an enhancement of BM motion for high-level tones above-CF, and (3) at most small effects for tones an octave or more below CF. Similar effects were previously reported for stimulation that evoked both fast and slow efferent effects. In addition, we found consistent changes in BM phase: (1) a phase lead at CF increasing to about 45 degrees above CF, and (2) below CF, small phase lags at low levels, sometimes becoming phase leads at high levels.

For the basal cochlea (CF>10 kHz), the findings of only small efferent-induced changes in guinea-pig BM motion at low frequencies, but up to 10 dB changes at 2-3 kHz in cat auditory-nerve fiber responses (Stankovic & Guinan, 1999 J. Acoust.Soc. Am. 106:857), suggests that

efferents change the micromechanics of the cochlear partition. To explain efferent enhancement of BM motion, we hypothesize that at high levels and above CF, BM motion is due to an efferent-inhibited component with compressive growth and a long group delay that interferes with a less-compressive (perhaps linear) component with a short group delay. When these two components are out of phase and similar in magnitude, inhibition of one component increases the sum and thereby increases BM motion.

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107 The Time Course of the Medial Olivocochlear Efferent Reflex in Humans

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The time course over which the medial olivocochlear efferent (MOC) acoustic reflex affects the cochlea must be tied to the function of the reflex in hearing. Nonetheless, relatively little work has been done to ascertain the time course of MOC reflexes in humans. We quantified MOC effects by measuring changes in stimulus frequency emissions, ΔSFOAEs. SFOAEs are advantageous because they are produced at a single cochlear place and the probe sound elicits little or no efferent activity.

SFOAEs were evoked by continuous 40 dB SPL tones near 1 kHz. Efferent activity was elicited by 2.5 s, 60 dB SPL noise bursts with 5 ms rise/fall times presented every 5 seconds. Group delay tests eliminated subjects in whom 60 dB SPL noise bursts elicited ΔSFOAEs dominated by middle-ear-muscle activity. Since SFOAEs are suppressed by sound in the measurement ear at frequencies near the probe frequency, we concentrated on the time course of the contralateral reflex.

ΔSFOAEs produced by broad-band contralateral noise bursts were well characterized (on a linear magnitude scale) by an onset delay followed by a saturating single-exponential rise, and an offset delay followed by a single-exponential fall. For 11 ears from 7 subjects, the rising time constant was 380 ms (SD 140 ms), the falling time constant was 170 ms (SD 50 ms) and both onset and offset delays were on the order of 20 ms.

The initial delay is near the shortest expected on the basis of the small number of synapses thought to be in the reflex, the conduction time in the reflex pathways, and the delay for ΔSFOAEs to arrive in the ear canal. The slow build up of efferent effects (hundreds of ms) limits the ability of the reflex to shape cochlear properties on the time scale of the fastest features in speech.

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108 Deriving a Cochlear Transducer Function using the Summating Potential

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The summating potential (SP) is a dc component in the electrical response of the cochlea to a sinusoid. The response originates from the nonlinearity of mechano-electric transduction (MET). The relationship between SP and the cochlear transducer function (f_{tr}) characterizing MET was first described by Cheatham and Dallos (1997, Hear. Res. 108,191-212), who suggested that the SP is related to the shape of the second derivative of the f_{tr}. The long-term goal of our research is to quantify cochlear MET in normal and pathologic ears to provide a more accurate description of sensorineural hearing loss than presently available. In this study we introduce a method to construct a cochlear f_{tr} from the SP.

The dc component is generated from the even-order terms of a Taylor series that approximates a nonlinear function. Mathematical exploration suggested that the magnitude of the dc component is proportional to the second derivative of the nonlinear function when the signal level was small. By using a bias tone to position a probe tone at different places along the cochlear f_{tr}, the SP amplitude can be used to derive the second derivative of the f_{tr}.

A probe tone (5888 Hz) ranging from 50 to 75 dB SPL and a 25 Hz bias tone of various magnitudes was presented to gerbils. The SP was recorded from the round window. Results showed that the SP amplitude as a function of the bias levels, demonstrated a shape similar to the second derivative of a sigmoid-shaped function representing nonlinear cochlear transduction. The correlation coefficient between the second derivative of a second-order Boltzmann function and the SP amplitude was highest (.96) at the probe tone level of 75 dB SPL. This suggests that the low frequency modulated SP amplitude can be used to estimate a cochlear transducer function.

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109 Modeling Outer Hair Cells

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Outer hair cells (OHC) play a critical role in sensitivity of the ear. It is believed that somatic force generation of the OHC is the main source of cochlear amplification, providing for the sharpness of frequency filtering and large gain seen for low amplitude sound. *In vitro* and *in vivo* experiments have shown that OHC are capable of high frequency (up to 100 kHz) voltage induced length and stiffness changes and exhibit strong nonlinear piezoelectric behavior.

In recent years, various constitutive laws have been proposed for outer hair cell transduction (e.g., Iwasa 1994 and Raphael 2000). In this study, the various laws are reconciled and placed into a common mechanics based framework (Spector 2000). General nonlinear constitutive theories for OHC tissue are presented. These constitutive theories include piezoelectric-like electromotility and voltage dependent stiffness. Expressions for the thermodynamic potential functions are derived for different constitutive models and analyzed. The two state motor probability model with linear and nonlinear elasticity is investigated in detail. Expressions for whole cell stiffness and nonlinear capacitance of the basolateral membrane are obtained. Model predictions of individual OHC stiffness and dynamic frequency response are compared to *in vitro* experimental results.

110 Mechanical Properties of Sensory and Supporting Cells in the Organ of Corti of the Guinea Pig Cochlea: An Atomic Force Microscopy Study

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Mammalian ear has high sensitivity, broad dynamic range and fine tuning. These functions are based on the vibration of the organ of Corti, which contains outer hair cells (OHCs), inner hair cells (IHCs), Deiters' cells, Hensen's cells, pillar cells and so on. As the vibration of the organ of Corti is considered to be related to mechanical properties of the cells in the organ of Corti, it is important to understand these properties. Recently, it was reported that Young's modulus of pillar cells, which mainly consist of microtubules, was 2×10^9 Pa using a three-point bending test (Tolomeo and Holley, Biophys. J. 73: 2241-2247, 1997). Sugawara et al. (Hear. Res., in press) reported that Young's modulus of OHCs in the apical turn was 2.0 ± 0.81 kPa, whereas that of the OHCs in the basal and second turns was 3.7 ± 0.96 kPa, using the contact mode of an atomic force microscope (AFM). However, mechanical properties of the other cells in the organ of Corti have not been clarified yet. Therefore, in this study, an attempt was made to measure mechanical properties of cells in the organ of Corti with an AFM. Results revealed that Hensen's cells and Deiters' cells were more than twice as soft as OHCs in the apical turn of the cochlea.

111 Mena Does Not Compensate VASP in Pillar Cells of VASP-Knock-Out-Mice

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Vasodilator stimulated phosphoprotein (VASP) and Mena are members of the Ena/VASP-family associated with regulation of the actin cytoskeleton and signalling pathways. Both proteins have been found to be co-localized in most mouse tissues suggesting similar function and ability to compensate for each other. VASP and Mena expression in the adult mouse cochlea analysed by PCR-examination, Western blot, mRNA-in-situ-hybridization and immunohistochemistry is reported. While Mena is co-localized in pillar cells with VASP in wild type mice, both proteins are not detectable in pillar cells of VASP -/- knock-out mice. Loss of Mena in VASP -/- knock-out mice was most surprisingly limited to the pillar cells. Mena was still found in VASP -/- knock-out mice to be expressed in various other cochlear cells. Click-ABR, frequency-dependent ABR and DPOAE in VASP -/- knock-out mice proved normal hearing function. Hearing measurements in VASP -/- knock-out mice in comparison to wild type mice included noise exposure experiments. Specific noise exposure (11.1 kHz, 86 dB SPL, 24 hours) resulted in statistically significant temporary threshold shift at 7.9 kHz (p=0.006, d=2.3) in VASP knock-out mice. Loss of VASP and Mena in the pillar cells of VASP -/- knock-out mice may explain this specific functional difference as VASP -/- knock-out mice have shown otherwise no major functional deficit. Pillar cells of VASP knock-out mice are therefore highly interesting cells as they show loss of both proteins without the ability of Mena to compensate for VASP. Reduced noise protection in VASP -/- knock-out mice gives a hint for pillar cell function in cochlear mechanics. Changes in stiffness of the pillar cells due to loss of VASP and Mena affecting the actin cytoskeleton are suggested as a possible mechanism for reduced hearing protection in VASP -/- knock-out mice.

112 Stimulus-Induced Volume Change in the Subtectorial Space of the Organ of Corti

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The electrically-induced vibration pattern of the organ of Corti (CO) was measured throughout its depth, using a laser-interferometer. The laser-interferometer works like a confocal interferometer, with a depth resolution of ± 1.8 µm from the focus plane (at -10 dB). Data were obtained at several points within the OC in the radial direction of: i) the basilar membrane (BM), ii) the reticular lamina (RL) and iii) the upper and lower surfaces of the tectorial membrane (TM).

The results derive from an in-vitro preparation of the third turn of the guinea-pig cochlea. The other turns of the cochlea were removed. Reissner´s membrane was intact over this turn, allowing better preservation of the TM. For electrical stimulation, two platinum electrodes and one gold electrode were used; the latter was also used as a mirror. The frequency range of the stimulus was 480 Hz to 74 kHz.

The RL exhibited a significant phase shift between the inner and outer hair cells and also between the outer hair cells and Hensen´s cells. A phase shift was not found on the TM, its motion was in phase with that of the RL at the site of the outer hair cells. The TM vibration amplitude above the outer hair cells was, on average, four times larger than that above the inner hair cells. The different phases on the RL and TM over the inner and outer hair cells result in a volume change in the subtectorial space over the inner hair cells. This volume change must induce a radial fluid flow which superimposes on the usually assumed fluid shear.

The amplitude of the BM vibration was much less (more than five times) than that of the RL, implying that the BM is stiffer than the RL. The largest BM amplitudes were encountered at the site of the tunnel of Corti.

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113 Tectorial Membrane Vibrations at the Tip of the Hair Cell Stereocilia

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Contributions of the tectorial membrane (TM) in conversion of sound-induced vibrations of inner ear structures into receptor potentials of hair cells are not fully understood. While some believe the TM acts as a rigid beam, others suggest that the TM provide a second frequency place map to the basilar membrane. The present study will present experimental data on TM vibrations at the tip of the hair cell stereocilia.

Gerbil hemicochleae were placed on the stage of an upright microscope. While mechanical vibrations were induced via a paddle immersed in the fluids below the basilar membrane, images of the magnified view of the TM, stereocilia bundles, and reticular lamina were captured during one stimulus cycle. Using a video flow technique, displacements of selected structures were measured. Vibration amplitudes and orientation were determined from the major axes of the Lissajous figures, magnitudes and phases of the movements from the transversal and radial components of the vibration.

Across frequencies, movement amplitudes revealed a tuned response with a single maximum, which occurred at the same stimulus frequencies for any location selected. The orientation of the Lissajous figure was different for measuring sites at the TM and the reticular lamina. Movements of the TM were mostly transversal above the outer hair cells and at the Hensen's stripe, whereas reticular lamina vibrations were predominantly radial at the pillar heads becoming increasingly transversal towards the third row of outer hair cells. At the Hensen's stripe the movements of the TM and the tip of the inner hair cell stereocilia bundle were almost perpendicular to each other. Moreover, movements at the tip of the stereocilia bundle were significantly larger in comparison to those obtained from the inner hair cell cuticular plate for frequencies below and close to best frequency of the measuring site and were similar in size above best frequency.

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114 Comparing Material Properties of Tectorial Membranes From Normal and COL11A2 -/- Deficient Mice

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Collagens are the primary structural proteins of the tectorial membrane (TM) (Thalmann et al., 1986). Mutations in COL11A2, which encodes one of the proteins that make up type XI collagen, cause non-syndromic hearing loss (DFNA13) in humans and a 40-50 dB hearing loss in homozygous COL11A2-deficient mice (McGuirt et al.,1999). In the COL11A2 -/- mouse mutant, the TM has a disorganized fibril structure in electron microscopic images. These results led McGuirt et al. to hypothesize that hearing loss in the COL11A2 -/- mouse mutants results from changes in the material properties of the TM. To determine how large these changes are, we measured the stress/strain relation of isolated TMs from normal and mutant mice. TMs were isolated and placed on a microscope stage, and volume changes were measured by tracking the positions of carboxylated beads attached to the TM surface. In contrast to their appearance in fixed tissue, isolated TMs from COL11A2 -/- mouse mutants were typically half the thickness of normal TMs. To a first approximation, the radial fibrillar structure and

the longitudinal fibers of the marginal band in TMs of the COL11A2 -/- mouse mutants appeared normal in light microscopic images. Osmotic stress was applied by bathing the TM in artificial endolymph solutions containing various concentrations of polyethylene glycol (PEG) with a molecular weight of 20 kDa. For a given PEG concentration, the TMs of COL11A2 -/- mouse mutants shrank less than the TMs of control mice. With 10 mmol/L PEG in the bath, the thickness of the TM for both mutants and controls was comparable. These results show that changes in TM structure in COL11A2 -/- mouse mutants that are only visible at the electron microscopic level can radically alter the material properties of the TM.

115 Tectorial Membrane Bulk Stiffness Measurements in the Gerbil Hemicochlea

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It is widely agreed that the tectorial membrane plays an important role in delivering sound-induced vibrations of the inner ear structures to the hair cell stereocilia bundles. However, basic properties and micromechanics of the tectorial membrane (TM) remain unclear. Experimental data on TM micromechanics and material properties are sparse. This study presents measurements on tissue properties of the gerbil TM. Bulk stiffness (κ) was determined at two locations in the gerbil hemicochlea and is compared to previous data obtained from isolated mouse TMs by Masaki et al. (Abstr. Assoc. Res. Otolaryngol. 25, 916, 2002).

Gerbil hemicochleae were placed on the stage of an upright microscope. Pictures of the TM were taken in 1-4 minute intervals with a CCD camera attached to one port of the microscope, while artificial endolymph and artificial endolymph containing Polyethylene Glycol (PEG) 1, 2, 3, 4, 5 mM were perfused into the experimental chamber. PEG (MW: 20,000) is a large molecule unlikely to diffuse into the TM, promoting a concentration gradient between TM and surrounding fluid space. Using the PEG-concentration gradient, the osmotic pressure (P) on the TM could be calculated. Resulting changes in TM cross-sectional area, width and height were measured 2.5 mm (basal turn) and 6.8 mm (middle turn) from the basal end of the basilar membrane. Volume changes dV were estimated using the changes in cross sectional area.

Values for bulk stiffness (κ=-P/(dV/V), of the gerbil TM differed at the two measuring sites, 34 kPa at the basal cut edge and 27.6 kPa at the middle cut edge. Moreover, changes in width and height were different at the two measuring sites. At both locations the changes in TM height were larger than the changes in TM width. The ratio between changes in TM width and changes in TM height increased towards the apex of the cochlea, but remained independent of PEG concentration.

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116 A Microfabricated Orthotropic Cochlear Partition for a Physical Cochlear Model

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Basilar membrane motion in animals and mathematical models of the cochlea provide an understanding of the physics of the cochlea. However, due to the complexity of the Organ of Corti, this is often not sufficient to explain the system. As a result, we turn to simplified physical models to either verify critical assumptions or to provide an explanation of a function in a controlled environment. In this work, we are developing a fabrication method to build a passive uncoiled cochlea model.

Iurato (JASA Vol. 34, No. 8, Part 2 Sept. 1962) describes the basilar membrane as consisting of a supporting layer made up of filaments arranged in a transverse direction. The transverse fibers create highly orthotropic (direction dependent) material properties. Our efforts to create a life-sized uncoiled cochlea model have resulted in the development of a composite material to more closely represent the highly orthotropic basilar membrane properties. Thin layers of polyimide (several microns) serve as the matrix and aluminum (fractions of a micron) is used for the fibers. The fiber dimensions and distribution were selected to account for the stiffness change from basal to apical ends. Immersing the membrane in a fluid environment and exciting with a piezoelectric sound source shows some spatial tuning and a traveling wave.

This technology can be extended for additional study of various basilar membrane boundary conditions. The fabrication method is compatible with additional processing needed to achieve our long term goal of an active cochlear model.

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117 Calculation of Inner Head Acoustic Field for Bone-Conducted Sound

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Bone-conducted ultrasound is known to be perceivable not only to the normal hearing but also to the profoundly deaf person. Activation of auditory cortex has been reported for the stimulation of bone-conducted ultrasound and some of the central mechanisms of its hearing has been revealed over the last few years; meanwhile, that of peripheral's has remained unclear.

In order to elucidate the peripheral perceptual mechanism of the bone-conducted ultrasonic hearing, it is essential to reveal the physically occurring phenomenon within the body for the bone-conducted stimulation. Here, we attempt to show the calculation of acoustic field formed within the head using the initial numerical head model and the finite-difference time-domain (FD-TD) technique. Bone-conducted stimulation of audible and ultrasonic frequency was simulated; transducers were placed at three slightly different locations near the left mastoid. Calculated sound fields at the plane including the cochleae showed considerably different characteristics between those frequencies. For audible frequency stimulation, their distribution negligibly differed for each stimulation location. On the contrary, for ultrasonic frequency stimulation, their distribution shifted considerably for each different stimulation location. Perceptible directional difference of the sound for bone-conducted ultrasound and audible sound can be accounted for by these differences of the sound field formed.

118 Title: Protective Effect of Edaravone Against Streptomycin-Induced Vestibulotoxicity in the Guinea Pig

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Aminoglycoside antibiotics enjoy widespread clinical use as highly effective antimicrobial agents. However, a serious limitation to the use of these drugs is their side effect of ototoxicity. Streptomycin causes preferential damage to the vestibular system. Free-radical scavengers have been shown to protect against aminoglycoside-induced ototoxicity. Edaravone, a free radical scavenger, attenuates reactive oxygen species and has also been used in clinical practice to treat cerebral infarction. This study investigated alleviation of streptomycin-induced vestibulotoxicity by edaravone in guinea pigs. Streptomycin was administered to the inner ear by osmotic pump for 24 hours, and edaravone (n = 8) or saline (n = 6) was intraperitoneally injected once a day for 7 days. We observed horizontal vestibulo-ocular reflex (HVOR)

gains as a marker of postoperative vestibular function. We calculated HVOR gains using our method in the preoperative state and postoperative days. Our method used an infrared charge-coupled device (CCD) camera and our macro for analysis of guinea pig eye movement. Animals injected with saline showed statistically smaller gains than those injected with edaravone. These results suggest that edaravone suppresses streptomycin-induced vestibulotoxicity. It is expected that edaravone will be used to treat streptomycin-induced balance loss.

119 AMPA-Induced Vestibular Disorder in the Guinea Pig

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Ischemic injury is one of the major causes of inner ear diseases. The ischemic injury induced elevation of glutamate concentration in the cochlear perilymph. Glutamate is the most likely neurotransmitter between hair cells and primary afferents in the inner ear. But excessive glutamate also has toxic effects on the inner ear. The aim of this study was to make an animal model of peripheral vestubular disorder like ischemic injury.

Twenty-five Hartley guinea pigs with normal PreyerÕs reflexes and normal tympanic membranes were used in this study. Intracochlear administration of AMPA (10 mM) was performed at 0.6 ml/hr for 5 minutes by a syringe pump (n = 15). As a control group, a same amount of artificial perilymph was administered intracochlearly (n = 10). In the AMPA group, 13 animals out of 15 showed spontaneous nystagmus, and it disappeared within 18 hr after operation. In 5 animals, caloric test was performed, and it revealed significantly decreased caloric response in the lesioned side compared with intact side. In the control group, all animals showed no static symptom. One week after operation, in 5 animals of each group, caloric test was performed. Animals in both groups showed no statistical difference in caloric responses between the lesioned side and the intact side. But in the AMPA group, a mean value of caloric response time in the lesioned side/ caloric response time in the intact side was statistically smaller than that in the control group.

Our results indicate the possibility that proper intracochlear administration of AMPA can produce an animal model with partial and reversible peripheral vestibular disorder.

120 Characterization of Gαi2 Splice Variants in the Rat Vestibular Periphery

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The response of vestibular afferent neurons to efferent modulation is regionally variable within the end organs, and this complex efferent/afferent interaction is subserved by a variety of neurotransmitter/neuromodulators receptors as well as second messengers. Previously we identified a novel partial cDNA encoding a G-protein alpha subunit, termed Gαi2(vest), as well as two forms of Gαs (Wackym et al., *Neurosci Lett* 2000;280:159-162). Using a normalized *Rattus norvegicus* vestibular cDNA library (Wackym-Soares) we identified expression of Gαi2, Gαs, Gαolf, and Gαo. This normalized rat vestibular cDNA library was subsequently enriched for Gαi2 using the RecActive protocol (Active Motif, Carlsbad, CA). After colony filter hybridization, nucleic acid sequencing was performed on positive clones. Sequencing revealed two additional alternatively spliced transcripts of Gαi2 (pA8 and pV2). Analysis of the sequence data suggests that pA8 and pV2 could encode for truncated versions of the Gαi2 protein that may serve altered cellular functions. Additional clones are currently being characterized and these data will be presented.

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121 Opioid Regulation of cAMP Levels in the Mammalian Vestibular Epithelia

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We investigated the effects of μ opioid receptor (MOR) activation in the rat vestibular epithelia by measuring changes in cAMP levels in vitro. MOR was stimulated by bath application of DAMGO (10, 100 and 1000 nM final concentration), and cAMP levels where measured with a sensitive solid phase assay (Assay Design, Inc.). DAMGO increased dose cAMP levels in buffer treated vestibular epithelia (unstimulated cAMP levels) in a dose dependent fashion. Treatment with DAMGO had no effect on cAMP levels in forskolin treated (receptor independent stimulation of cAMP levels). To study the effects of MOR activation on cAMP levels stimulated through a Gαs coupled pathway, vestibular epithelia were treated with CGRP (calcitonin gene-related peptide) prior to treatment with DAMGO. DAMGO did not inhibit CGRP stimulated cAMP levels. MOR is coupled to Gαi/o, which inhibits adenylyl cyclase. Therefore, stimulatory effects of MOR activation on unstimulated cAMP levels suggests that it is through the βγ subunit complexes of G proteins. Since isoforms AC2 and/or AC4 of adenylyl cyclase are stimulated by the βγ subunit complexes, these data suggest MOR is colocalized with AC2 and AC4.

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122 Kappa Opioid Receptor Activation Inhibit Calcium Current in Type II Hair Cells.

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It has been found that kappa opioid receptors decrease the afferent activity in the isolated vestibular system. This study was designed to determine the actions of kappa receptors on the ionic currents from hair cells of the axolotl (Ambystoma tigrinum). Hair cells were isolated from the semicircular canals and ionic currents studied using the whole cell patch clamp technique. The kappa receptor agonist U-50488 (10 pM to 10 μM, n = 20) produced a significant concentration-dependent decrement of the outward component of the total ionic current. The inhibitory action of 0.1 μM U-50488 was blocked in a reversible form by the prior application of 1 μM naloxone (n = 4) or 1 μM nor-binaltorphimine (n = 4) indicating an U-50488 specific interaction with kappa receptors. The perfusion of 300 μM CdCl2 (n = 6) blocked the effect of U-50488 10 μM indicating that its action was due to a blockade of the calcium current and subsequent lack of activation of the calcium activated potasium current. To prove that indeed U-50488 was acting on the voltage dependent calcium currents, we registered this current. The application of U-50488 (10 pM to 1 μm, n = 28) produced a concentration-dependent, and reversible decrement of the calcium current with an IC50 of 2.4 nM, with non-significantchanges in the current activation kinetics. Possible interactions of U-50488 with ACh receptors were studied in experiments in which 10 μM atropine, 10 μM d-tubocurarine (n = 3) or 1 μM strychnine (alfa9/alfa10 ACh antagonist, n = 4)) were applied by bath perfusion during 3 min, and 0.1 μM U-50488 was then co-applied by microperfusion to isolated hair cells (n = 3). The inhibitory action of U-50488 showed no significant modification in the presence of the cholinergic antagonists.

These results shows that kappa opioid receptors modulate the calcium current and the subsequent neurotansmitter release from vestibular hair cells.

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123 A Survey of Vestibular Function in Mutant Mouse Strains

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The purpose of this research is to identify vestibular deficits in mice using linear vestibular evoked potentials (VsEPs). VsEP thresholds, peak latencies and peak amplitudes from 25 strains with known genetic mutations have been analyzed and descriptive statistics generated for each strain. Response parameters from homozygotes were compared with heterozygote controls and all animals were compared to normative ranges. Previous work established average values for normal VsEP response parameters at +6 dBre:1g/ms: P1 = 1.3ms, P2 = 2.2ms, P3 = 2.8ms; P1/N1 = 2μV; P2/N2 = 1.6μV. Normal thresholds averaged –8 dB re:1g/ms. Homozygotes of the following mutations had absent VsEPs at the ages tested: *je, tlt, dfw^{2J}, qv^{Ind2J}, qv^{3J}, sh1, sr, jc, mh, av^{J}, av^{2J}, av^{3J}, av^{Tg}, v^{2J}, kcne1, pou3f4*. These results suggest profound gravity receptor deficits for these homozygotes; consistent with the structural deficits documented for many of these strains. Homozygotes of *pa, cdf, mu, sw, stg, qk* , and *shi* strains had measurable VsEPs but one or more response parameters differed from their respective controls or were outside normal ranges. For example, *qk* and *shi* homozygotes showed significantly prolonged latencies consistent with the demyelination known for these strains. Prolonged latencies may suggest deficits in neural conduction, elevated thresholds suggest reduced sensitivity and reduced amplitudes may suggest reduced neural synchrony. One mutation, *jv*, had all VsEP response parameters within normal limits; an expected finding since the abnormality in *jv* is restricted to the lateral semicircular canal. Interestingly, some heterozygote strains also showed abnormalities in one or more VsEP response parameters suggesting that vestibular dysfunction, although less severe, may be present in heterozygotes.

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124 A Comparison of Vestibular and Auditory Function in Inbred Mouse Strains

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The purposes of this research are: 1) to survey vestibular function in inbred mouse strains; and 2) compare vestibular and auditory function. Vestibular function was measured using vestibular evoked potentials (VsEPs). VsEP thresholds, peak latencies and amplitudes were quantified. VsEP thresholds were compared to ABR thresholds from age and strain matched mice (Zheng et al. 1999, Hear. Res. 130:94-107). Vestibular data have been collected for 15 inbred strains ($n \leq 5$). B6 (35d (days), 190d) and C3H/HeSnJ (120d) mice had VsEPs comparable to normal (Jones & Jones, 1999, Hear. Res. 136:75-85). Abnormalities in one or more parameters were found for NOD.NONH2nb1 (28d, 185d), BUB/BnJ (84d), BALB/cByJ (71d), A/J (211d), CBA/J (62d), DBA/2J (73d), NOD/LtJ (66d, 190d), A/WySnJ (60d), MRL/MpJ (68d), A/HeJ (120d), CAST/Ei (72d), SJL/J (58d), and CE/J (91d). These results suggest that vestibular function varies among inbred strains and identify several strains that show vestibular deficits by age 70 days. Potential genes responsible for early onset vestibular impairment remain to be identified. ABR thresholds were compared to VsEP thresholds for 12 age-matched strains. B6 mice showed normal ABRs and VsEPs up to 190 days old. Three strains had significant hearing loss (BUB/BnJ, NOD/LtJ, A/J) and elevated VsEP thresholds. Four strains (DBA/2J, A/WySnJ, NOD.NONH2nb1, A/HeJ) had elevated VsEP thresholds (including absent VsEPs) but relatively little change in ABRs. Three strains (MRL/MpJ, Ce/J, SJL/J) had significant vestibular loss with no concomitant hearing loss. These results suggest that functional change in one sensory system does not obligate change in the other. Furthermore, we hypothesize that genes responsible for early onset hearing loss may affect vestibular function, yet the time course of change in the sensory systems may vary. In addition, some genetic mutations may produce solely vestibular deficits.

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125 Hair Cell Mechanics of Three-Dimensional Utricular Bundles

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Most models of hair cell bundles have adopted the stereocilia-in-a-row configuration, ignoring the three-dimensional aspects of a bundle. We used finite element models of real three-dimensional vestibular bundles to analyze their mechanics. Using six selected bundles from different regions in the utricle of the red eared turtle, we subjected each bundle model to a point-force applied to the kinocilium tip, ranging from 0 to 500 pN, and analyzed the resultant deformations and tip link tensions.

The cells divided into two groups based on an order-of-magnitude difference in bundle stiffness: a compliant group and a stiff group. Although both groups exhibited stiffness values that increased with applied load, the values of the compliant group increased less than those of the stiff group. Two obvious structural differences existed between the groups that caused the differences in stiffness. The stiff group had more cilia than the compliant group, and a more significant factor was the smaller difference in height between the tallest stereocilia and the kinocilium. We also found that decreasing the cilia diameters in a bundle by 10% resulted in a 20% decrease in stiffness.

A similar distribution of tip link tensions was discovered in all six cells: parallel tension behavior was common near the edges of the bundle, and serial tension behavior was found near the center of the bundle toward the kinocilium. Increasing the elastic modulus of the tip links tended to push the overall distribution in the parallel direction, but no matter how much the tip link modulus was increased, completely parallel behavior could not be achieved.

126 Spatial Patterns in the Structure of Otolithic Membranes

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In vertebrate utricles, the otolithic membranes (OMs) deliver mechanical stimuli to hair cells. One important question is whether the OMs move as a rigid plate, thereby delivering a uniform stimulus to all receptors. To answer this question, it would be useful to know whether the OMs are structurally uniform across the entire sensory surface.

We characterized these spatial patterns in the OMs using fixed, but not dehydrated utricles of a turtle, *T. scripta*. Utricles were processed using artificial endolymph (65μM Ca^{2+}), stained with wheat germ agglutinin to visualize OMs and with phalloidin to visualize hair bundles, and viewed as wholemounts or slices using confocal microscopy.

There are marked regional differences in all OMs. The **column filament layer** (between epithelial surface and the underside of the gel layer) in the medial extrastriola (MES) is 10.8 $\pm$ 1.4 μm thick and continuously stained, suggesting that it is occupied by numerous,

uniformly distributed filaments. In the striola it is thinner (7.1 ± 1.0 μm), appearing as isolated, widely-spaced strands. The **gel layer** is thin in the MES (2.6 ± 0.6 μm). In the striola it is much thicker (11.1 ± 1.9 μm). It is densely stained and honey-combed with irregularly shaped channels. Many of these channels are continuous with interstices in the compact filament layer and penetrate to the otoconial layer. Bundles are eccentrically placed in these channels; the kinocilial end of each bundle is nearest its channel wall. The **otoconial layer** is thick (83 ± 3.3 μm) in the MES with variably sized (17.9 ± 9.4 μm^2) otoconia; it is thinner over the striola with smaller (12.6 ± 7.8 μm^2) crystals.

Our data indicate the OMs are not spatially uniform. If these regional differences in structure have mechanical *sequelae*, then stimuli delivered to utricular bundles may differ with location on the macular surface.

127 Hair Bundles of the Utricular Striola in a Turtle, *Trachemys scripta*.

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The striola is a specialized region of all vertebrate otolith organs, but its definition and functional significance are unclear. To address these issues we use scanning microscopy and autocorrelation analyses to characterize mechanically significant features of striolar arrays (number, spacing, and geometry of stereocilia), and we combine confocal microscopy with hair cell and afferent labelling to assign bundles to different hair cell types.

This approach distinguishes lateral (zone 1) and medial (zone 4) extrastriola from the striola (zones 2, 3). Extrastriolar hair cells are exclusively type II. They outnumber striolar hair cells (85-15%) and differ significantly from them in stereocilia number (15-70 vs. 65-130), spacing (0.31 vs. 0.27 μm), and array regularity. Striolar hair cells form two distinct zones. Zone 2 is a 35-40 μm band of type II hair cells that spans the line of polarity reversal. Bundles in zone 2 have 20-85, widely spaced (0.32 ± 0.01 μm) stereocilia. Kinocilia and the tallest stereocilia are the same height. Zone 3 is a 35-45 μm band medial to zone 2. Bundles in zone 3 have significantly more numerous (65-130), closely-spaced (0.29 ± 0.02 μm) stereocilia than zone 2 bundles, and relative kino- and stereocilia heights are heterogenous. Type I hair cells are restricted to zone 3. They account for over 50% of receptors in this striolar subdivision and 3% of total utricular hair cells. They supply single (24%) or complex calyces with 2-7 hair cells (76%).

Our data suggest that striolar and extrastriolar bundles are structurally distinct and that the striola is highly differentiated, with location- and perhaps type-specific bundle morphologies. These structural differences likely have mechanical consequences (Silber et al., 2003, ARO), suggesting that subsets of utricular hair cells may respond distinctively to head movements.

128 Responses of Primary Afferents to Mechanical Indentation of the Posterior Canal in the Turtle, *Trachemys scripta*.

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Posterior canal afferents in the turtle exhibit a range of response dynamics to rotational stimuli (Brichta and Goldberg, 2000). We are using an *in vitro* turtle preparation to assess whether this diversity can be replicated with mechanical indentation of the posterior canal. A probe was placed directly on the posterior duct, about 2 mm from the ampulla, via a small hole in the overlying skull and bony canal. The probe was attached to a Burleigh piezzo-electric device that delivered sinusoidal indentations at frequencies ranging from .01 to 100 Hz and with amplitudes ranging from .5 to 12.5 μm. Afferent responses in the form of sinusoidal modulations of firing rate were recorded in phase histograms, and the amplitude and phase of the modulations calculated by discreet Fourier transform. Posterior canal units were excited by decreasing indentation of the canal and inhibited by increasing indentation. Bode plots for frequencies from .01 to 2 Hz generally resemble those observed for rotational stimuli, with phase leading minimum indentation by 50-100 deg at .01 Hz, and the magnitude of the phase lead declining with increasing frequency. In many units, at frequencies above 2 Hz, continuous modulation of firing rate within a cycle gave way to burst-like firing patterns, with burst duration progressively decreasing with increasing frequency. At the highest frequencies, the response was typically reduced to a single, phase-locked spike per cycle. In Bode plots, this behavior appeared as a progressive phase advance at frequencies from about 2-5 Hz up to 100 Hz. In a minority of units, response phase continued to decline with frequency even at 100 Hz.

129 Synaptic Mechanisms Underlying Afferent Responses to Efferent Activation in the Turtle Posterior Canal.

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In the turtle posterior crista, there is heterogeneity in afferent responses to electrical stimulation of efferent fibers. Bouton afferents near the torus (BT) are inhibited while those at intermediate positions (BM) show mixed inhibitory-excitatory responses. In calyx-bearing units (CD), efferent stimulation results in a large excitation. Afferent action potentials were abolished after iontophoresing the charged lidocaine deriviative, QX-314, from the recording microelectrode into the afferent. This allowed us to record postsynaptic potentials in isolation while stimulating efferent fibers. Efferent inputs to hair cells were distinguished from inputs to afferents using response latencies, modulation of quantal rate, and sensitivity to the glutamate antagonist CNQX. We have deduced that efferent responses in BT and BM units are comprised of a hair-cell excitation coupled with a hair-cell inhibition. The inhibition most likely results from the activation of alpha9/alpha10-containing nicotinic receptors (a9/10nAChRs) functionally coupled to the activation of small-conductance, calcium-dependent potassium channels (SK). Consistent with this mechanism, the voltage response to a single efferent shock in BT and BM afferents consist of a brief increase in quantal activity followed by a prolonged decrease in quantal activity. Both components were simultaneously blocked by a9/a10nAChR antagonists and CNQX, and the initial increase in quantal activity was enhanced and isolated using SK blockers. BM units show an additional hair-cell post-inhibitory excitation, and both BT/BM show a postsynaptic component following CNQX. These components are all equally sensitive to drugs known to block a9/10nAChRs. CD units also respond to single shocks of efferent fibers. The kinetics of these single shock responses suggest at least two excitatory components. The efferent responses in CD fibers are also sensitive to cholinergic antagonists.

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130 Modes of Afferent Non-quantal Transmission in the Turtle Posterior Crista

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Transmission from hair cells to afferents may involve both quantal and non-quantal components. In our previous work, we studied the responses to 0.3-Hz sinusoidal indentation of the canal duct. Quantal transmission was indicated by brief, randomly timed mEPSPs, which were abolished in low Ca2+ and by the glutamate-receptor antagonist, CNQX; non-quantal transmission, by a periodic modulation in intracellular potential that persisted after quantal transmission was blocked. Afferents were classified as calyx-bearing (CD) or bouton (B) based on their responses to electrical stimulation of efferent fibers. Quantal and non-quantal transmission were more conspicuous in B and CD units, respectively. We now report that stimulation at higher

frequencies reveals two forms of non-quantal transmission. During stimulation at 30 Hz, there is a slow, depolarizing shift in potential upon which are superimposed faster potentials synchronized to the sinusoidal stimulus. Slow shifts are conspicuous only in CD units and may reflect the accumulation of K+ ions in the cleft between the type I hair cell and the calyx ending. The faster, synchronized potentials are present in both CD and B units and, judged by the actions of CNQX, consist of both quantal and non-quantal components. Once again, the quantal components are more conspicuous in B units. It has been suggested that the depolarization of type I hair cells due to K+ accumulation is necessary for quantal transmission involving calyx endings. Two observations do not support the suggestion: 1) A slow shift is not seen at 0.3 Hz, even when quantal transmission is present in CD fibers. 2) Increasing extracellular K+ to 10 mM, rather than enhancing quantal transmission in CD fibers, severely curtails it. Evidence is required to confirm that the slow shift is due to K+ accumulations. The mechanisms responsible for the synchronized potentials need to be elucidated.

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131 Comparative Analysis of Afferent Dendritic Architectures within the Otolithic Maculae.

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We have previously reported that dendritic arbors of primary afferent neurons projecting to the lateral extrastriolar region of chinchilla utricular maculae exhibit architectures that are aligned with the morphologic polarization vectors (mpvs) of hair cells to which they project (Hoffman, *Abs. Soc. Neurosci.* 27, '01). In the present study we explored whether this characteristic is a general feature of afferent dendritic morphology in other species, particularly nonmammals. To accomplish this, dendritic architectures of utricular afferents were studied in bullfrogs. We also tested whether the mpv-specific alignment of afferent architectures is found among saccular afferents.

Primary afferent dendrites were labeled through extracellular injections of tetramethylrhodamine-biocytin into the anterior vestibular nerves of anesthetized bullfrogs. Following a 24-hour incubation period, the utricular and saccular maculae were harvested following transcardial perfusion and postfixation with 4% paraformaldehyde. Neuroepithelia were then labeled with fluorophore-conjugated phalloidin (i.e. Alexa-488 phalloidin), which illuminated the stereocilia and tight junctions of hair cells for determination of their mpvs and hair cell densities. Afferent dendrites and the overlying neuroepithelial surface were then imaged via confocal microscopy. Dendrites were reconstructed and hair cell mpvs determined from these optical stacks using *Neurolucida* (Microbrightfield, Inc.) and *NIH Image*.

Among the arbors examined thus far, the variance of hair cell mpvs within bullfrog utricular afferent terminal field areas tended to be associated with projection locus within the neuroepithelium. This variance tended to be greater than that found among chinchilla utricular afferents. However, terminal field areas, and hair cell mpv variance within these areas, of bullfrog utricular afferents were smaller than areas corresponding to saccular afferents. This suggests that the factors contributing to dendritic architecture may be different in these two sensory receptors.

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132 Response Properties of Regenerated Vestibular Afferents following Regeneration from Ototoxic Damage

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Common to all animals, pigeon vestibular afferents innervating the semicircular canals and otolith receptors are spatially tuned to directional motion and have been well characterized in their dynamic responses. What happens to these response properties when peripheral receptors of the vestibular system are damaged, replaced with the new cells, and subsequently re-innervated by the afferents after treatment with aminoglycoside antibiotics? The primary objective of the present study was to determine the functional capacity of the regenerated vestibular afferents in pigeons. Neural recordings from vestibular afferents were obtained by linear acceleration and rotational motion, in both normal birds and animals undergoing regeneration from 4 days to 1 year post-treatment. Each afferent was characterized as semicircular canal or otolith fibers using rotations in different planes and linear motion & OVAR. Once identified, the responses to sinusoidal stimuli at frequencies ranging 0.02 – 4.0 Hz (for canals) and 0.03 to 10.0 Hz (for utricle) were obtained. The direction of maximum sensitivity for each afferent was determined. To date, responses from canal afferents show that following 9 months to 1 year of post-treatment regeneration, the mean gains of regenerated horizontal and vertical canal afferents are not significantly different from normal values. Response phases of regenerated afferents had also returned to normal values. In contrast, the dynamic response properties of regenerated otolith afferents were different than normal, with lower gain and more advanced phases. Anatomical reconstructions of fully regenerated afferents show that the three main types of afferents return, including calyx, dimorph, and bouton fibers. However, the innervation patterns of regenerated fibers are different than normal afferents, with smaller terminal fields, smaller calyces, and fewer boutons.

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133 Characterization of 3D Eye and Head Movements under Headfixed and Headfree Conditions in the Normal and Regenerated Pigeon.

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To fully appreciate the effects of hair cell regeneration in avians, one must characterize the return of function after aminoglycoside insult. In pigeons, it is thought that head movement plays a major role in the stabilization of gaze and vision, although it has been studied far less than the VOR. From previous studies in our laboratory, it was determined that the VOR responses to both rotational and linear motion were under compensatory in headfixed animals, especially for translational motion. Under headfree conditions, others have shown that gaze stabilization is completely compensatory. To provide head stability, several mechanisms appear to be working in concert, including the VCR and CCR reflexes, as well as head inertia. It was reported that the VCR response supplemented the VOR in the dark and more so in the light. However, these studies were limited to a single plane of stimulation at low frequency.

In the present study, both normal and long-term regenerating adult pigeons were implanted with dual eye and head search coils and placed beak-forward in a three-field AC magnetic coil system atop a motion delivery device under dark conditions. Frequency dynamic responses to rotational stimuli ranging from 0.01 - 2 Hz yaw (20 deg/s), 0.02 - 4 Hz pitch and roll (20 deg/s), as well as OVAR (10 - 60 deg/s) were obtained under both headfree and headfixed conditions. For yaw and pitch stimuli, the regenerated eye, head, and gaze gains were lower than those of normal birds, especially at lower frequencies, while the regenerated phases were slightly advanced. At higher frequencies, regenerated and normal gains were similar. Under OVAR conditions, normal gains were flat compared to regenerated gains, especially in the torsional and vertical directions.

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134 Development Of Vestibular Otolith Receptors In Microgravity

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Development of the utricle and saccule vestibular otolith organs was examined in embryonic quails raised from fertilization in either normal 1g or microgravity (0g). Fertilized eggs were arrested from development by cooling, placed into an incubator (ADF - SHOT, Inc), and flown on STS108 for 12 days in space. Upon orbital insertion (2 hours), the ADF temperature was raised to 99ºF, with a constant 66% humidity. Group 1 eggs were exposed to 0g and group 2 eggs underwent constant velocity centrifugation at 1g in the same ADF in space. Four 0g quail embryos and six 1g embryos were recovered alive at E12 upon shuttle landing. Ten additional control embryos were raised in a normal ground ADF. The otolith organs were harvested from all E12 embryos for study. The 0g embryos had smaller mean body weights (2.4 gms) as compared to the 1g controls (3.5 gms). The ratios of otoconial stone mass weight/body weight increased by 40% in 0g embryos as compared to both flight and ground 1g controls. In addition, the mean saccular epithelium area was smaller in 0g embryos as compared to 1g embryos. Examination of the hair cell stereocilia polarizations showed that normal organizational arrangements were present for both 0g and 1g sacular maculae. Number of hair cells and type are currently being assessed. Neural tracing (HRP) experiments were performed on all E12 embryos, with vestibular nerve afferent innervation of otolith organs being examined. Group 1 0g fibers were highly branched with dense arborizations and numerous terminals as compared to 1g controls. Initial electron microscopic observations reveal that the synapse type and number for type I and type II hair cells may differ for the 0g and 1g embryos. Quantitative comparisons of EM and innervations will be presented, as currently double blind evaluations are being performed.

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135 Hair Cell Counts in Rodent Cristae Ampullares

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Though numerous studies have been conducted in mammalian cristae organs (Fernández et al., *JNP* 60:167, 1988; Lysakowski and Goldberg, *JCN* 389:419, 1997), precise estimates of hair cell and supporting cell numbers using a single method are lacking. Using the disector method, we counted the numbers of type I, type II, and supporting cells in the central, intermediate, and peripheral zones of the mouse, rat, gerbil, guinea pig, chinchilla, and tree squirrel cristae. We have previously presented our findings in the macular organs of these species (Desai et al., *ARO Abst* 2002).

Horizontal (N=3 for each species) and vertical cristae (N=3 for each species) were dissected from mouse, rat, gerbil, guinea pig, chinchilla, and tree squirrel and stained using calretinin immunochemistry. Sensory organs were osmicated, dehydrated, embedded in Araldite resin, serially sectioned at 2 μm thickness, and counterstained with Richardson's stain. Sections were photographed and digitized for computer-assisted disector counts. The total number of type I, type II, and supporting cells were determined.

Overall numbers (mean ± SE) of type I (I) and type II (II) HCs, and supporting cells (SC) follow: mouse (I, 760 ± 45; II, 653 ± 47; SC, 1968 ± 152), rat (I, 1323 ± 50; II, 1171 ± 42; SC, 2919 ± 144), gerbil (I, 1635 ± 88; II, 1540 ± 70; SC, 3740 ± 206), guinea pig (I, 2563 ± 41; II, 2875 ± 59; SC, 6417 ± 107), chinchilla (I, 3196 ± 57; II, 2988 ± 49; SC, 7234 ± 134), and tree squirrel (I, 3308 ± 48; II, 3025 ± 23; SC, 7536 ± 120).

The ratios of type I to type II HCs in the central (C) and peripheral (P) zones, and overall (O) for each species are: mouse (C, 60:40; P, 47:53; O, 54:46), rat (C, 60:40; P, 47:53; O, 53:47), gerbil (C, 58:42; P, 45:55; O, 52:48), guinea pig (C, 64:36; P, 38:62; O, 47:53), chinchilla (C, 54:46; P, 47:53; O, 52:48), and tree squirrel (C, 54:46; P, 49:51; O, 52:48). The number of cells in all of the cristae of a species were pooled after it was determined that there is no significant difference between horizontal and vertical cristae (F test > 0.05).

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136 Transient Expression of Voltage-Gated Na+ Channels and Activity-Dependent BDNF Release During Late Synaptogenesis in Rat Utricle.

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Electrophysiological maturation of vestibular hair cells occurs in parallel with the expression of brain-derived neurotrophic factor (BDNF), the main neurotrophin implicated during vestibular epithelium development. In the present study, we investigated whether a correlation exists between these two developmental processes.

Using the whole-cell configuration of the patch-clamp technique and single-cell RT-PCR, we characterized in the rat utricle, the electrophysiological properties of the voltage-gated sodium currents and the molecular characteristics of their related channels. We then performed intracellular recordings to test whether INa may underlie the generation of action potentials in neonatal hair cells. Using ELISA in situ we studied the capacity of utricle to induce activity-dependent secretion of BDNF at the same period.

We report the presence of a voltage-gated tetrodotoxin (TTX)-sensitive Na+ current that displays most of the characteristics of neuronal sodium current. Single-cell RT-PCR reveals that this current is supported by different mRNA alpha subunits isoforms, with a major expression of Nav1.2 and Nav1.6. This current present at birth decreases dramatically from P1 to P8 and becomes undetectable at P21. This transient expression is confirmed at the protein level by immunocytochemical studies. In addition, we demonstrate that depolarizing current injections provide to the neonatal hair cells the capability to fire TTX-sensitive action potentials. Finally, we provide evidence of a TTX-sensitive activity-dependent BDNF release on utricle explants at P0, whereas only a constitutive secretion remains at P21.

Taken together, our results suggest that during postnatal period, the rat utricle hair cells transiently express sodium channels that underlie electrical activity and an activity-dependent BDNF release, required for the synaptogenesis processes.

137 Uncoupling Protein Expression in the Rat and Primate Inner Ear

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Uncoupling proteins (UCPs) are a proton transporter family located in the mitochondrial inner membrane. UCPs play important roles in expenditure of energy and heat production. UCP1 is the classical thermogenic form in brown adipose tissue. UCP2 is distributed more widely, including the central nervous system; UCP3 is expressed most heavily in muscle.

The mechanisms of caloric-induced nystagmus during the caloric test have been believed due to the endolymphatic convection. However, caloric-induced nystagmus under microgravity appeared toward the same direction as on the earth, implying a direct conductive effect via sensitive inner ear thermoreceptors. Since the inner ear is thermally isolated from the external environment, it is hypothesized that regulated changes in local heat generation could alter inner ear function. This

study examined UCP1, UCP2 and UCP3-like immunoreactivity (-LIR) (Santa Cruz; diluted 1:1000) in rat and primate temporal bones.

Neither UCP1 nor UCP2-LIR was detected in neural or non-neural tissues of the inner ear. However, UCP3-LIR was clearly observed as puncta within Scarpa's ganglion cells and vestibular hair cells in the maculae and cristae. UCP3-LIR was also observed both in spiral ganglion and cochlear hair cells. The neural UCP3-LIR was co-localized with a mitochondrial marker, MitoFluorGreen (MFG; Molecular Probes; 10nM). In the monkey, cochlear tectorial membraneous limbus was heavily stained with UCP3-LIR. These results provided the first evidence for a role of mitochondrial UCP3 in inner ear function. According to previous reports, UCP3 uncouples oxidative phosphorylation, contributes to free fatty acid degradation and may be thermogenic or thermoregulatory. Hence, it is suggested that local thermogenesis by neuronal mitochondrial UCP3 may modulate the eighth nerve excitability via thermoreceptive mechanisms that also produce caloric responses.

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138 Frequency Responses of the Cetacean Vestibular Labyrinth

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The vestibular labyrinth of whales and dolphins is significantly reduced compared to land mammals of similar size. The radius of the semicircular canal in the massive blue whale (*Balaenoptera musculus*), for example, is equal in length to that of man despite the whale outweighing humans one thousand fold. Cetaceans therefore broke with the long-observed allometric relationship between canal dimensions and body mass when they re-entered the sea during the Eocene period. This adaptation has been suggested to have reduced the gain of their vestibular systems and given them a selective advantage in their aquatic habitat.

Using ultra-high-resolution computed tomography scans of extant cetacean tympano-periotic bones, the exact dimensions of the semicircular canals of cetaceans were measured. The frequency responses of a variety of cetacean labyrinths were estimated from these measurements using a torsion-pendulum model of the semicircular canal. In a small odontocete such as the Atlantic whitesided dolphin (*Lagenorhynchus acutus*), the short time constant corresponded to a frequency of 88 Hz, while the long time constant corresponded to a frequency of 0.1 Hz. In the much larger sperm whale (*Physeter catodon*), the upper frequency was 52 Hz and the lower frequency 0.04 Hz. In comparison, the range in man has been calculated as approximately 0.02-50 Hz.

These findings imply that cetaceans probably preserved the frequency response profile of their vestibular system despite returning to the ocean. Anatomic features, such as a horizontal tail and fused cervical vertebrae, that distinguish these creatures from other animals may not have changed the requirements of their vestibular system as significantly as previously believed.

139 pH Sensitivity of the Vestibular Endorgans

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Extracellular pH exerts important regulatory influences in excitable and non-excitable tissues. In this work, we studied the pH sensitivity of vestibular endorgans. For this we used the axolotl (Ambystoma tigrinum) inner ear in three different experimental conditions: 1) in-vitro vestibular endorgan preparation, 2) isolated semicircular canal hair cells and 3) isolated afferent neurons from the Scarpa´s ganglion. In the in-vitro inner ear we studied the electrical activity of the afferent neurons using a multiunit extracellular recording technique. pH variations between 5 to 7.4 significantly decreased the resting and mechanically evoked spike discharge (maximum decrease of 75%). pH between 7.4 and 10 significantly increased the spike discharge (maximum increase of 50%). Data were adjusted by a sigmoid curve with pH50 = 7.17. To define whether variations of pH are capable of modifying the afferent neurons response to the hair cell afferent transmitter, the response to 1 mM NMDA (n = 5) and 100 μM AMPA (n = 7) were studied at pH 7.4 (control), and at pH of 7.0 and 7.8. Response to both excitatory amino acid agonists increased at pH 7.8 (41% and 22% respectively) and decreased by perfusion of the preparation with a saline solution with pH 7.0 (28% in both cases). Voltage clamp of the isolated hair cells showed that hair cells are unresponsive to extracellular pH. In contrast, voltage clamp of isolated afferent neurons showed that extracellular pH acidification evoked a non-selective (Erev = 0 mV) inward current. These results demonstrate that the vestibular peripheral system displays a significant sensitivity to pH changes, which modified the response to the afferent transmitter and activates a pH sensitive current.

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140 Fluid Dynamics of Ground-based and Microgravity Caloric Tests

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This paper is concerned with the fluid and structural dynamics of the lateral semicircular canal system during both 1g and microgravity caloric stimulations. Robert Barany received the 1914 Nobel prize in medicine for describing the cupular deflections caused by the ampullopetal and ampullofugal flows of the hot and cold irrigations in terms of a buoyancy-driven natural convective mechanism. Microgravity caloric tests aboard the 1983 SpaceLab1 mission produced nystagmus with an intensity comparable to those elicited during post- and pre- flight tests, thus contradicting the basic premise of Barany's convection hypothesis. In this work, we present a finite element numerical model for the caloric stimulation of the lateral semicircular canal based on two simultaneous driving forces for the endolymphatic flow: natural convection driven by the temperature-dependent density variation in the bulk fluid and expansive convection caused by direct volumetric displacement of the endolymph during the thermal irrigation. The two-dimensional fluid-structural model includes a rigorous two way coupling between the endolymph and the cupula. Direct numerical simulations indicate that on earth the natural convection mechanism is dominant. But in the microgravity environment of orbiting spacecraft where buoyancy effects are mitigated, the expansive convection becomes the sole mechanism for producing cupular displacement. A series of transient numerical simulations are presented to delineate the intricate differences between the dynamics of 1g natural convection and those of microgravity expansive convection. The resulting fluid-structural interactions are analyzed parametrically based on time evolution of cupular displacements and cupular velocities during microgravity and 1g cold and hot caloric stimulations.

141 Origin of Intense Sound Exposure Induced Hyperactivity in the Dorsal Cochlear Nucleus of Hamsters

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Intense sound exposure, the most common tinnitus inducer, is known to cause the elevation of spontaneous activity in the dorsal cochlear nucleus (DCN). This hyperactivity persists after removal of cochlear input to the DCN (Zacharek et al., 2002). The current study aimed to determine whether the persistence of hyperactivity relies on inputs from higher levels of brainstem nuclei. Ten adult hamsters were exposed to a 10 kHz tone to their left ears at 127 dB SPL for 4 hrs. Another 11 age-

matched unexposed animals served as controls. Thirty to forty days later, a circumferential transection was performed around the left DCN, which was to remove the central inputs to the DCN. Spontaneous activity was recorded on the DCN surface 20-30 min after the transection. Mean spontaneous rates (SRs) for both groups were plotted as a function of distance from the lateral margin of the DCN. At the end of recording, the brain of each animal was processed histologically and Nissl stained to examine the degree of efferent sectioning. The results showed that the DCN was separated less than 50% from the brainstem tissue that underlies the DCN along the dorsoventral axis in 4 control and 4 exposed animals, but more completely (50% up to 100%) in 7 control and 6 exposed animals. After both partial and more complete efferent sectioning, SRs of exposed animals were found to be consistently higher than those from their age-matched controls. The levels of SRs of control and exposed animals after efferent sectioning were comparable to those obtained previously from control and exposed animals in which no efferent sectioning was performed. This suggests that maintenance of tone exposure induced hyperactivity is independent of central inputs to the DCN.

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142 Relationship between Tinnitus and Hyperactivity in the Dorsal Cochlear Nucleus following Intense Sound Exposure

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Chronic increases in spontaneous multiunit activity (MUA) can be induced in the dorsal cochlear nucleus (DCN) of hamsters by intense sound exposure (Kaltenbach and McCaslin, 1996). This hyperactivity has a similar pattern as activity evoked in the normal DCN by stimulation with sound (Kaltenbach and Afman, 2000). This suggests that hyperactivity after intense sound exposure represents a neural code that could cause animals to hear sound in the absence of an acoustic stimulus. Indeed, recent behavioral studies have revealed that hamsters develop tinnitus-like percepts following exposure to the same sound conditions that cause hyperactivity in the DCN (Heffner and Harrington, 2002). The goal of the present study was to examine the degree to which the level of activity in the DCN correlates with the behavioral evidence for tinnitus. We recorded the level of spontaneous MUA in the DCN of animals that had previously been tested for tinnitus using the behavioral methods of Heffner and Harrington, (2002). The experiments were conducted in two groups of animals, those which had previously been exposed to intense sound (n=28; 10 kHz, 2-4 hours, 125-130 dB SPL) and those which had not (n=28). The presence of tinnitus was indicated by low behavioral performance scores and the absence of tinnitus by high scores. Electrophysiological recordings of spontaneous MUA were then carried out on the surface of the left DCN as described previously (Kaltenbach and Afman, 2000). The results revealed that a) animals testing positive for tinnitus had higher levels of spontaneous MUA than animals testing negative for tinnitus, and b) the level of peak activity in the DCN was moderately correlated with the behavioral performance score on the tinnitus test. These observations point to an important relationship between hyperactivity in the DCN following intense sound exposure and the presence of tinnitus.

143 Response Properties of Neurons in the Frog Auditory Midbrain During Reversible Unilateral Hearing Loss

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In a previous study response properties of frog auditory midbrain neurons were evaluated as a function of acutely reversible, unilateral conductive hearing loss. Attenuating the input to the ear ipsilateral to the recording site produced temporary changes in frequency selectivity and modified binaural response properties. The present study addressed the effect of unilateral sensorineural hearing loss on the response properties of neurons in the auditory midbrain. For this purpose, sensorineural hearing loss was induced unilaterally using a cryoprobe to cool the auditory nerve and produce a reversible block of neural conduction. The advantage of this technique is that response properties of individual central auditory neurons may be evaluated before, during, and after temporary hearing loss.

The cryoprobe was constructed from two different sized syringe needles such that the barrel of one was placed coaxially inside the other with the tip of the outer sealed. Cooled ethanol was pumped through the central barrel and exited through a port in the luer region of the outer barrel. Through a dorsal surgical approach the cryoprobe was positioned just above the auditory nerve and neural activity of auditory midbrain neurons was recorded before, during, and after reversible unilateral hearing loss.

The results of this study indicated that the inactivation of the auditory nerve was reversible and repeatable. Central auditory neurons revealed changes in response properties as a result of temporary unilateral hearing loss and these often reflected differences from properties determined under monaural intact stimulation.

144 Effects of Sodium Salicylate on Spontaneous Activity of Dorsal Cochlear Nucleus Neurons

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Tinnitus affects millions of people worldwide. The treatments for tinnitus have not been very effective because there is a lack of understanding of its underlying mechanisms. Our previous work found that, after intense sound exposure (a common tinnitus inducer), there was an increase of a bursting type of neural activity in the dorsal cochlear nucleus (DCN) and a decrease of regular activity. Also after intense sound exposure, the bursting neurons became more sensitive to carbachol, an acetylcholine agonist.

This study tested another common tinnitus inducer, sodium salicylate, in an attempt to find a common underlying mechanism related to central tinnitus.

Young adult Sprague Dawley rats were treated with sodium salicylate via their drinking water (8 mg/ml), and brain slices were made after exposure times of 2 days to 5 weeks. The control group consisted of age-matched rats given normal water. Three types of neuronal spontaneous activity were recorded from DCN slices: regular, irregular, and bursting. Comparisons of neuronal activity were made between the control and salicylate-treated rats. The overall neuronal densities (units/penetration) were similar between the two groups. However, the density of bursting activity increased by about 19 % ($p<0.03$) in the treated group. Within the treated group, the amount of bursting activity increased with longer exposure times. The proportion of bursting neurons, among the three types of neurons, was higher in the treated group (64 % vs 56 %). The mean firing rate of bursting neurons in the treated group was 11 % higher than that of controls ($p<0.04$).

In conclusion, changes of spontaneous activity in the DCN may be a common underlying mechanism of central tinnitus resulting from a variety of factors. Further study is underway to test effects of other tinnitus inducers.

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145 Is The Dorsal Cochlear Nucleus a Central Source of Trauma-Induced Tinnitus?

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A growing body of evidence implies that the dorsal cochlear nucleus (DCN) plays an important role in tinnitus. We have shown that DCN fusiform cells, in chinchillas with psychophysical evidence of acoustic-trauma-induced tinnitus, have elevated levels of spontaneous activity

and elevated stimulus-driven activity that parallels the psychophysical features of their tinnitus (Brozoski, Bauer & Caspary, 2002). Kaltenbach, et al. (2000) have shown elevated DCN multi-unit activity in hamsters following acoustic trauma. The DCN however, has not been shown to play a necessary role in causing tinnitus, since the evidence to date is associational.

To test the hypothesis that elevated DCN activity causes or significantly contributes to tinnitus, the DCN of rats with psychophysical evidence of tinnitus, was lesioned. If the DCN plays a necessary role in tinnitus, lesioning the DCN should decrease tinnitus in subjects previously shown to have tinnitus.

To induce tinnitus, rats were unilaterally exposed once, for 60 min, to octave-band noise, 110 db (SPL) peak, centered at 16 kHz. After the exposed rats were shown to have psychophysical evidence of tinnitus, using the method of Bauer & Brozoski (2001), rats with and without evidence of tinnitus were given either ipsilateral or bilateral DCN lesions, sham lesions, or control lesions of equal volume in a non-auditory area.

Ipsilateral DCN lesions did NOT significantly decrease the psychophysical evidence of tinnitus, compared to appropriate controls, and bilateral DCN lesions appeared to INCREASE the evidence of tinnitus. Although the DCN may play an important role in tinnitus, that role has yet to be defined and may be one of active suppression of tinnitus arising from a source other than the DCN

146 Effects of Conductive Hearing Loss on Unit Responses in the Ventral Cochlear Nucleus.

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This study investigated physiologic changes in the ventral cochlear nucleus (VCN) of guinea pigs resulting from conductive hearing loss (CHL). Based on findings of changes in oxidative metabolism in the CN contralateral to ossicular disruption (Tucci et al., J Assoc. Res. Otolaryngol. 2002:89-106), alterations in the function of the commissural CN pathway were anticipated.

Unit responses were recorded from the ipsilateral VCN after conductive hearing loss (CHL) was induced by ossicular disruption. Responses were obtained from animals immediately (control), 1 day, 1 week and 2 weeks post surgery. Multichannel electrodes enabled data collection from a large number of units in response to ipsilateral and contralateral sound stimulation.

Thresholds to ipsilateral sound stimulation were elevated 40-50 dB in CHL animals. In response to contralateral broadband noise (BBN), CHL units showed inhibitory responses similar to those in normal control animals (Shore et al., In: Central Auditory Processing – Integration with other systems. Ascona, 2002). Thresholds for inhibition ranged from 40-65 dB SPL. In contrast, in CHL animals at 1 day and greater, excitatory responses to contralateral BBN that could not be acoustic crossover were more common than in normal animals. Thresholds were as much as 30 dB below ipsilateral thresholds. In normal hearing animals excitation usually occurred at higher levels. However, a few high-threshold units in normal animals also exhibited contralateral excitation at levels below their ipsilateral threshold. These preliminary data suggest that CHL may result in alterations in the sensitivity of excitatory synapses and/or dysinhibition of VCN neurons.

147 Tonotopic Nucleus Magnocellularis Neuron Loss Following Cochlear Ablation

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Previous studies have shown that neurons in chicken Nucleus Magnocellularis (NM) are heavily dependent on excitatory afferent input from cochlear hair cells. These neurons suffer from deafferentation and 20-40% die following cochlear ablation. The goal of the current study is to examine whether tonotopic differences exist in the percentage of NM neurons that die following cochlear ablation.

Chickens (P14) received unilateral cochlear removals and survived for 7 days. At sacrifice, chickens were perfused and the brains removed and frozen. Coronal sections (16 microns) were cut through NM and stained for cytochrome oxidase (1 in 2 series). Ipsilateral and contralateral profile counts of NM neurons were performed and NM neuronal percent loss (ipsilateral vs. contralateral) was calculated for each tonotopic quartile (rostral to caudal through NM).

For the entire nucleus, results reveal similar percent loss (35%) as previous studies report. However, when NM is divided into tonotopic quartiles, the percent loss differs. There is greater percent loss in the rostral, high frequency quartiles of NM compared to the caudal, low frequency quartiles. These data indicate that removing all excitatory afferent input affects the neurons differently depending on their tonotopic location within Nucleus Magnocellularis. Rostral, high frequency NM neurons suffer more neuronal death than caudal, low frequency NM neurons following cochlear ablation.

148 Active Caspase-9 Immunoreactivity in the Avian Cochlear Nucleus Following Deafferentaton

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Afferent activity has been shown to be crucial for neuronal survival in a variety of developing sensory systems. For example, elimination of auditory nerve activity in the chick results in the death of approximately 30% of the neurons in the cochlear nucleus, nucleus magnocelluaris (NM). Several morphological and metabolic correlates of cell death have been identified in dying NM neurons within hours after deafferentation, but the molecular cascade of events leading towards the ultimate degeneration of deprived NM neurons are unknown. A molecular event that commonly occurs during cell death in other model systems is the activation of proteolytic enzymes known as caspases. The present study examined possible caspase activation in NM neurons following deafferentation.

Activation of caspase-9 was examined at various times following cochlea ablation using an antibody specific to the cleaved (active) form of this enzyme. Cochlea ablation surgery was performed unilaterally in order to compare intact and deafferented NM neurons in the same tissue sections. Immunoreactivity for active caspase-9 was observed in the ipsilateral NM neurons within 3h following cochlea ablation. This immunolabeling was observed up to 24h following surgery, but was not observed 4 days following surgery, a time point after a subpopulation of NM neurons have degenerated. Unexpectedly, the increased immunolabeling for active caspase-9 did not appear to be restricted to a sub-population of deafferented neurons. This suggests that competing cell death and cell survival mechanisms must work downstream of caspase activation to regulate cell death in NM neurons.

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149 Cochlear Ablation Induced Signaling Through ERK And SAPK Transduction Pathways In Auditory Nuclei

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In adult brain auditory pathways, unilateral cochlear ablation (UCA) induced degeneration of the cochlear nerve, rearrangements of synaptic contacts, changes in transmitter release and regulation of synaptic receptor activity. These changes may reflect altered regulatory mechanisms and/or gene expression stemming from transduction of signals that emerge after UCA. To test this idea, we determined if UCA in adult guinea pigs induced signal transduction through the extracellular signal-regulated kinase (ERK) and the stress-activated protein kinase (SAPK) pathways. Using Western blots, we measured phosphorylated ERK1 (ERK1-P), ERK2 (ERK2-P), p46 and p54 SAPK

(SAPK-P) and c-Jun (c-Jun-P) in tissue lysates of the anteroventral (AVCN), posteroventral (PVCN) and dorsal (DCN) cochlear nucleus, lateral and medial superior olive, medial nucleus of the trapezoid body and inferior colliculus for up to 145 postablation days. Since cytosolic ERK1-P, ERK2-P and SAPK-P can enter the nucleus to activate transcription factors, we determined the cellular localization of these proteins immunohistochemically. In most tissues, ERK1-P and ERK2-P were elevated at 7 and 145 postablation days and depressed at 30 and/or 60 days. Also, ERK1-P and ERK2-P were elevated at 3 days in the AVCN and PVCN. After 5 days, most ERK1-P and ERK2-P in the CN was mainly in neuronal nuclei. Only minor changes were evident in total ERK1 and ERK2 at all postablation times. Several correlations were evident between previously observed postablation plasticities and the changes in ERK1-P and ERK2-P. These findings suggest that signaling through the ERK pathway may effect changes in regulatory mechanisms and/or gene expression that contribute to the plasticities induced by UCA. In contrast, concomitant elevation of SAPK-P and c-Jun-P was not evident, except in the DCN at 3 postablation days, where most SAPK-P was localized in the molecular layer. Since few, if any, correlations could be made to previously observed postablation plasticities, such changes were unlikely to have been induced by transduction through the SAPK pathway.

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150 PKA And CaMKII Regulate D-[^{3}H]Aspartate Release In The Auditory Brain Stem Nuclei After Unilateral Cochlear Ablation

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Unilateral cochlear ablation (UCA) in adult guinea pigs altered transmitter release from glutamatergic terminals in auditory nuclei (Exp Neurol 148: 222, 1997). This may reflect altered excitation or trophic support transduced in neurons by signaling cascades that use protein kinases to regulate transmitter release. Since protein kinase C (Exp Neurol 175: 245, 2002) and protein kinase A (PKA) (ARO Abs 25: 207, 2002) regulate transmitter release in auditory pathways of intact animals, we determined if regulation by PKA and calcium/calmodulin-dependent protein kinase II (CaMKII) influenced release from glutamatergic terminals after UCA. The evoked release of D-[^{3}H]aspartate was quantified 7 and 145 days after UCA in samples of the dorsal (DCN), posteroventral (PVCN) and anteroventral (AVCN) cochlear nucleus, lateral (LSO) and medial (MSO) superior olive, and medial nucleus of the trapezoid body (MNTB). One week after UCA, 0.2 mM dibutyryl-cAMP, a PKA activator, elevated the evoked release. Thus, after UCA, PKA still regulated glutamatergic release. Twenty weeks after UCA, 2 μM H-89, a PKA inihibitor, depressed the evoked release in the LSO and the ipsilateral AVCN, PVCN and MSO. Similarly, 4 μM KN-93, a CaMKII inhibitor, depressed the release in the LSO and the ipsilateral MSO. These findings suggest that both PKA and CaMKII maintain upregulation of the postablation release in the LSO and the ipsilateral MSO, while PKA also maintains upregulation of the postablation release in the ipsilateral AVCN and PVCN. Both H-89 and KN-93 also elevated the postablation release in the MNTB and the contralateral MSO, implying that both PKA and CaMKII exert downregulatory influences on the postablation release in these nuclei. These findings imply that after UCA, regulation of PKA and CaMKII helps to control the level of release from several glutamatergic pathways in the auditory brain stem.

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151 Effects of Hearing Loss on the Large End Bulb of Held Synapse in the Ventral Cochlear Nucleus of the Mouse

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The synaptic property of neurons in the cochlear nucleus in response to ongoing hearing loss has not been well studied. In this report, we used mouse strains that either show early onset of hearing loss (DBA/2J), or show no hearing loss in their adult life (CBA/CaJ) to address this issue. We took advantage of the fact that "naturally" occurring presbycusus in DBA mice shows a pattern of progressive worsening from high to low frequencies, thus allowing direct comparison of neuronal properties from "deafened" high frequency regions to those from "hearing" low frequency regions. Cochlear nucleus slices were prepared from 18 to 69 day old DBA and CBA mice. Whole cell patch clamp recordings were made in visually identified anterior ventral cochlear nucleus (AVCN) neurons from both high and low frequency regions.

Two populations of cells were distinguished: those with repetitive firing of action potentials with regular interspike intervals (type I) and those with rectifying subthreshold current-voltage relationships and phasic firing of 1-2 action potentials (type II). Type II responses in AVCN are derived from bushy cells. We found that miniEPSCs from type II cells in high frequency regions of older DBA mice have a significantly smaller amplitude and slower decay time constant compared to those miniEPSCs from cells in the same frequency regions of young DBA. Furthermore, mEPSC event frequency was significantly decreased in old DBA mice. In contrast, miniEPSC analysis from healthy hearing CBA mice (either old or young) showed no statistically significant difference in mEPSC properties. Interestingly, the measurements from CBA type II cells were largely comparable to those of high frequency cells in young DBA as well as to those cells from low frequency regions in old DBA mice. These results suggest that there may be a systematic change of AMPA receptor composition that is associated with age-related hearing loss. Our preliminary data of RT-PCR analysis has indicated that there is an elevated expression of GlutR2 in the hearing-impaired old DBA mice.

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152 Deafness Related Changes in Glycine-Immunoreactive Staining in the Rat Cochlear Nucleus

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There is increasing evidence that prolonged loss of activity in auditory brainstem pathways results in decreased inhibition and/or increased spontaneous activity in auditory brainstem neurons. Glycine is a major auditory brainstem inhibitory neurotransmitter and can exert strong control over the discharge of many auditory neurons through numerous axo-somatic endings. We used postembedding immunocytochemistry (ICC) to determine if there are changes in glycine immunoreactive (IR) puncta in the cochlear nucleus (CN) 14 days following deafness. Deafness was achieved by intrascalar injection of neomycin through the round window. An ABR threshold shift of greater than 80 dB across three frequencies was necessary for inclusion in the study. Deafened animals (14 days post-deafening) and age-matched normal control animals were perfused with fixative and sections through the CN were embedded in plastic. Comparable regions through the ventral and dorsal cochlear nucleus were identified based on anatomical landmarks. Post-embedding immunoperoxidase staining using an antibody to glycine was carried out with one micron sections from control and experimental animals processed together. Digital images of regions of interest, defined by pre-determined anatomical criteria, were acquired from each immunostained section using a Spot camera with MetaMorph (Universal Imaging) image acquisition software. Metamorph image analysis software was then used to determine the number and density of glycine IR puncta on the somata of defined cell types. The CN from

normal animals contained a large number of glycine IR axosomatic puncta on many cell types including bushy cells, multipolar stellate cells and fusiform cells. A significant decrease in the total number and density of glycine IR puncta on bushy, multipolar stellate and fusiform cell somata was found in the CN of deafened animals when compared to normal hearing controls.

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153 Deafness Related Changes in Glycine Immunoreactivity in the Rat Superior Paraolivary Nucleus

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Inhibitory neurotransmitters undergo substantial changes in the auditory pathways following elimination of ascending excitatory input. In the current study, we used immunocytochemistry (ICC) to examine deafness-induced changes in glycine immunoreactive (IR) staining in the rat superior paraolivary nucleus (SPON). Rats were deafened by intrascalar infusion of neomycin with an 80dB shift in ABR necessary for inclusion in the study. Deafened animals (14 days post deafening) and normal-hearing, age-matched controls were perfused with fixative and vibratome sections through the auditory brain stem were embedded in plastic. Using an antibody to glycine, ICC was performed on 1 micron plastic sections from deafened and normal hearing animals. Regions of interest were defined using anatomical landmarks and digital images of these regions were acquired and assessed for the number, shape and optical density of glycine IR somata and puncta, using MetaMorph software. The number of glycine IR axosomatic puncta on SPON principal neurons was found to decrease 30% with respect to normal hearing controls. The decrease in puncta number is likely the result of decreased glycine production in one or more of the nuclei that project to the SPON. There could be a partial decrease in the major glycinergic input, the medial nucleus of the trapezoid body (MNTB), or a more substantial decrease in a smaller glycinergic input, such as the lateral nucleus of the trapezoid body (LNTB). Given that there was an almost numerically identical decrease in GABA-IR puncta on SPON principal cells and the LNTB output is believed to be GABA/glycine while the MNTB is glycine only, we examined changes in the intensity of glycine IR somata in the MNTB and LNTB. As expected, densitometric evaluation indicated a much more substantial change in LNTB, suggesting this may make an important contribution to the deafness related decrease observed in SPON puncta.

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154 Novel Gene Expressed in the Auditory Brainstem of Mice with Hearing Deficit: a Study with cDNA Subtractive Hybridization

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Hearing deficit by mechanical cochlear damage, intense noise or ototoxic drugs produces a variety of structural and functional changes in the auditory brainstem, and also these changes may involve expression of novel genes in the auditory pathway. In the present study, we identified novel gene expression in the auditory brainstem of mice with hearing deficit using cDNA subtractive hybridization. Hearing deficit was achieved by malleus removal bilaterally through ear canals. A total of 100 forward subtracted clones were obtained from hearing deficit cDNAs subtracted by normal hearing ability cDNAs, and 80 reverse subtracted clones were also obtained. These 180 clones were screened with dot hybridization to identify differentially expressing clones. A total of 27 clones were identified as differentially expressing, and all of them were sequenced. We identified 13 unknown cDNA fragments by the BLAST search. In situ hybridization of brainstem slices between mice with hearing dificit and those with normal hearing ability was carried out using Digoxigenin-labeled RNA probes for these 13 unknown cDNAs. These hybridized slices were compared for differentially expressing mRNA in the auditory brainstem. We obtained a novel gene which shows differential expression of mRNA in the lateral superior olive. The results suggest the presence of a novel gene in the auditory brainstem closely associated with the alteration of hearing ability.

155 Changes In Calretinin-Immunostaining In The Superior Olivary Complex In Ferrets Reared With Unilateral Cochlear Ablation

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Calretinin (CR) expression has been described in specific cell types in auditory nuclei. In this study, we used semiquantitative image analysis to assess changes in CR-immunostained circuits in the lateral (LSO) and medial (MSO) superior olivary nuclei after ferrets were reared for two months with unilateral cochlear ablation. Cochlear ablation was performed in ferrets 30-40 days of age, soon after hearing onset, to avoid direct effects on developing synaptic circuits. There was a decrease in the mean optical density of CR-immunostaining throughout the entire LSO ipsilaterally and MSO bilaterally compared to unoperated controls. In addition, cochlear ablation resulted in a significant decrease in the mean cross-sectional area of CR-immunoreactivity within neurons in the lateral but not medial limb of the ipsilateral LSO. This decrease was present in comparison to both the contralateral LSO and the LSO in controls. However, the mean optical density of CR-immunoreactive cells in the medial limb of ipsilateral LSO was significantly less in the cochlear ablated than in the control animals. In MSO, there was no difference in either the mean optical density of CR- immunostained cells or in the mean cross-sectional area of CR-immunoreactivity within those neurons between operated and unoperated groups. Finally, using synaptophysin as a synaptic marker, there were no differences in the mean optical density of labeled terminals for either the LSO or the MSO as a function of cochlear ablation. Thus, cochlear ablation appears to result in a decrease in CR expression in the superior olivary complex in the absence of a decrease in synaptic innervation.

156 Changes In Calretinin-Immunostaining In The Cochlear Nuclei Complex In Ferrets Reared With Unilateral Cochlear Ablation

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Calretinin (CR) is a calcium-binding protein expressed in specific cell types of mammalian auditory brainstem nuclei. The mature distribution of this protein is present by hearing onset. To test if early hearing loss results in changes in CR expression in the cochlear nuclei, unilateral cochlear ablations were performed in ferrets soon after hearing onset (P30-P40) and CR-immunoreactivity was analyzed in the cochlear nucleus two months later. In comparison with control animals and contralateral cochlear nucleus, cochlear ablation resulted in a significant decrease in the mean optical density of CR-immunostaining throughout the ipsilateral anteroventral (AVCN) and posteroventral (PVCN) cochlear nuclei but not in the dorsal cochlear nucleus (DCN). Further analysis revealed that this decrease is due to a significant decrease in the mean cross-sectional area of CR-immunostaining within AVCN and PVCN neurons. However, the mean optical density within neurons is unchanged in operated animals suggesting that the decrease in overall optical density is due to a decline in CR in the neuropil of AVCN and PVCN. To further assess changes in the neuropil due to the cochlear ablation, synaptophysin-immunoreactivity was quantified. Results using this synaptic marker revealed a significant increase in the mean optical

density of labeled terminals in both the AVCN and PVCN ipsilateral to the cochlear ablation. These results are consistent with a decrease in the CR-expression and a new growth of synaptic endings.

157 Opportunistic Infections in Vestibulo-Auditory Pathways in AIDS

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The incidence of hearing and vestibular disease is higher in HIV+ patients than in an age-matched, general population. The etiology of the symptoms is unknown, but may be related to opportunistic infections.

We evaluated 16 temporal bones and 9 brain stems collected at autopsy from AIDS patients. Tissues were paraffin embedded and immuno-stained to detect viral proteins and inflammatory cells using antibodies against herpes simplex virus (HSV) types 1 and 2, cytomegalovirus (CMV) and leukocyte common antigen (CD-45). Positive control tissue included samples with known infection and lymph nodes. Negative control tissue included 8 temporal bones and 7 brainstems from non-HIV infected individuals. Sections of each were run with each assay.

No virus was detected in any temporal bones from HIV+ individuals. Inflammatory (LCA+) cells, however, were present in the scala tympani and spiral ligament of 2 cochleas. Leukocytic infiltration was present (abundant to moderate) in the modiolus of 10 cochleas. Inflammatory cells were also present in the VIIIth nerve in 9 of the HIV+ temporal bones, being severe in 2 cases. A few isolated leukocytes were present in the modiolus in 2 of the control cochleas, but no inflammatory cells were seen in the scalae or spiral ligament. HSV infection of cells in the floor of the fourth ventricle, including the vestibular nuclei was seen in 1 HIV+ brainstem. Another had CMV+ cells scattered in the periphery of the brainstem. LCA+ cells were present in both HIV+ and control brainstems.

The incidence of CMV and HSV infection of the temporal bone is not higher in our sample of AIDS patients. However, 63% of the temporal bones showed inflammatory infiltration suggesting the presence of other pathogens in these tissues. CMV and HSV infection were more frequent in brain stems of HIV patients than controls.

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158 Effect of Tea on Acoustic Reflex Thresholds

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Consumption of black tea has been found to cause inhibition of iron absorption. Some investigators have suggested that consumption of tea may constitute an important factor for the development of iron deficiency anemia in some countries. Other experiments have suggested that sensorineural hearing loss in some cases may be related to iron deficiencies. The reported cochlear histopathological changes induced by iron deficiency are strial atrophy and reduction of spiral ganglion cells. In addition, it appears that noise induced hearing loss may be worse in iron deficient animals. The average consumption of tannin is 400 mg/d in an American diet and is estimated to be about 2500 mg/d in the diet of individuals from India. Asian Indian Americans are known to consume black tea on a daily basis. The population included in this study consisted of Asian Indian Americans with normal middle ear status and hearing within normal limits. Acoustic reflex thresholds were monitored to track changes that may occur following the consumption of black tea. Ipsilateral acoustic reflex thresholds were obtained from 15 male and 15 female participants before and 30 minutes after the consumption of warm black tea. The tea was prepared by boiling 4 ml of black tea in 300 ml of boiled water. 4 ml of sugar was added to the tea for test. The thresholds were obtained bilaterally; with 0.5 and 1 kHz activator tones and a 226 Hz probe tone. The MANOVA showed a significant ($p = 0.007$) difference between pre and post tea acoustic reflex thresholds. The acoustic reflex thresholds were slightly elevated following the consumption of black tea. Possible implications of these findings will be discussed.

159 Binaural Interaction Components. Their Possible Origin in Rat Auditory Brainstem Response

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Binaural interaction component of the auditory brainstem evoked response originally described as the difference waveform elicited by subsequently subtracting the sum of the two monaurally evoked potentials from the binaurally evoked potentials of the auditory brain stem response. The amplitude of the summed monaural potential is usually found to be larger than the binaural response, whereas the binaural interaction component has an inverted polarity compared to the binaural response. The binaural interaction component is thought to reflect the activity of neural units in auditory pathway responding specifically to binaural stimulation. It has been demonstrated that binaural interaction in the brainstem nuclei is reflected in certain waves of the ABR. In this study, binaural interaction component was recorded between the skull and non-cephalic reference from Wister rats before and after making discrete lesions of the auditory pathway in the midbrain and pons. The control binaural interaction component waveform was shown with six negative peaks n1~n6. In mid collicular section there was amplitude decrease of n6. Also 0.4 n4, 0.5 n5 & 0.5 n6 were latent. In bilateral collicular resection, there was amplitude decrease for n5 & n6. Also peak latencies of 0.6 n4, 0.8 n5, 1.1 n6 were latent. In mid olivary complex section n5 & n6 were abolished and n4 was 1.0 ms latent. In resection of lateral superior olive the n4 was abolished and the amplitude of n3 decreased. Also 0.6 n2 & 1.1 n3 were latent. The result interpreting that each part of the binaural interaction component arises from a focal region of the brain stem auditory pathway and that the peaks n4, n5 & n6 are dependent upon the crossing fibers, but peaks n1, n2 & n3 are not dependent on these projections. Also the result indicates that binaural interaction component is not totally dependent on Superior olivary complex and supra olivary portions of the auditory pathway.

160 Quantification of Binaural Interaction by Behavioral and Auditoray Brainstem Response

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Binaural hearing is advantageous for sound localization, speech recognition and binaural summation for pure tone and speech. However, the point at which individuals lose binaural interaction has not been well quantified. In this study, we examined the auditory brainstem evoked responses (ABRs) in individuals with simulated hearing loss as a means to determine the threshold at which binaural interaction is lost. A baseline physical examination, tympanogram, audiogram, otoacoustic emission and ABRs were obtained in 22 normal hearing individuals. A gradually increasing conductive hearing loss of 10 db, 15 db, 20 db and 30 db were created by placement of biologically calibrated packing material in the external auditory canal. ABRs will be used to establish when binaural interaction is lost. The ABR waveforms were added to determine when binaural interaction is lost and has implications in the surgical decision making for patients with hearing loss. The Results will be discussed.

161 The Human Frequency-Following Response: Correlates of the Binaural Masking Level Difference

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The masking level difference (MLD) is the threshold difference between in-phase, low frequency binaural tones and masker (SoNo) to threshold for an antiphasic condition with a 180 degree interaural phase delay (IPD) of either the signal (SpiNo) or the masker (SoNpi). Threshold disparity is partially related to 'coincidence-detecting' units in the medial superior olive (MSO) in the caudal brainstem that integrates monaural input. MSO units have a characteristic delay whereby discharge rate is modulated by the IPD of the stimulus. Recently, Jiang et al (1997) recorded masking release in discharge patterns of low frequency inferior colliculus units with characteristic delays equal to 0 or 180 degrees IPD for 500 Hz stimuli. Kevanishvilli et al (1987) recorded the auditory brainstem response (ABR), middle latency, and late latency response (LLR) with 580 Hz SoNo and SpiNo stimuli. Significant latency and amplitude differences were found for only the LLR. One explanation for insignificant ABR results might be that onset activity mediates physiologic release at the caudal brainstem level to a lesser extent than phase locked activity. The human frequency-following response (FFR) is an early-latency evoked potential representing the activity of phase locking neural units in the brainstem to low frequency stimuli. FFRs were recorded from ten normal hearing subjects with 500 Hz tone bursts using a 1.5 kHz low-pass masker. Most subjects showed no FFR masking when the perceptual masker level was used even though perceptual release was present. At higher masker levels that reduced the unmasked FFR amplitude by approximately 50%, release was present in the antiphasic conditions by an increase in the 500 Hz FFR amplitude re: SoNo. These results support that phase locked neural activity mediates the MLD at lower brainstem levels.

Jiang et al. (1997) J. Neurophysiol. 77: 3085

Kevanishvili (1987) Scan Audiol. 16: 3

162 Binaural Auditory Evoked Potentials With Virtual Acoustics

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The binaural difference potential (BD) is commonly regarded as neural correlate for binaural processing. It is calculated as the difference between binaural and summed monaural auditory brain stem responses (ABRs). Previous studies used click stimuli with "artificial" binaural differences, i.e., fixed interaural time and level differences. In this work, instead of the click an optimized chirp was used, designed to compensate for basilar membrane dispersion resulting in a significantly larger ABR wave-V amplitude (Dau et al., 2000, J. Acoust. Soc. Am. 107(3) 1530-1540). The chirp was presented with "natural" binaural differences, i.e., convolved with individual head- related transfer functions (HRTFs). Eight virtual directions in the horizontal plane with 45° angular spacing were chosen for this study. In addition, the unconvolved "pure" chirp was presented diotically as a reference condition. ABR were recorded from 32 scalp electrodes. In contrast to stimuli with artificial binaural differences, the "natural" spatial stimuli result in a perceived object outside the subject's head (externalization). The BD shows a systematic dependence on the laterality of the virtual sound source. The latency of BD wave DN1 increases monotically with laterality. The BD is largest for central (front, back, diotic) and smaller for lateral directions. This is in accordance with results from click stimuli with "artificial" binaural differences (Riedel et al., 2002, Hear. Res. 163 (1-2), 12-26). Differences between frontal and backward directions are mostly not significant. The results indicate that at the level of the brain stem mainly lateralization is extracted. Spectral cues are presumably processed on cortical levels of the auditory pathway.

163 Evaluation of the Input/ Output Function and the Effect of Contralateral Acoustic Stimulation on the Frequency Following Response- Distortion Product at 2f1-f2

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Distortion product otoacoustic emissions (DPOAE) measured clinically in the ear canal present a non-invasive tool to assess the functional integrity of the cochlea. However, recording DPOAE at frequencies below 1000 Hz is difficult technically due to the effect of physiological and ambient noise. The aim of this study is to evaluate the brainstem frequency following response ?distortion products (FFR-DP) measured with scalp electrodes, as a non-invasive technique to overcome the DPOAE test limitations. The effect of changing the stimulus level on the amplitudes of the cubic difference tone (2f1-f2) was examined by simultaneously recording the DPOAE and the FFR-DP to a low frequency stimulus complex (F1= 500 Hz, F2= 610 Hz) in 10 normal hearing adults. The stimulus levels ranged between L1= 40 ?70 dB nHL with L2 = L1-5 dB, and the F2/F1 ratio= 1.22 The effect of contralateral acoustic stimulation on the FFR-DP was also investigated and compared to that of the simultaneously recorded DPOAE in 10 normal hearing adults. The stimulus complex (F1= 500 Hz, F2= 610 Hz) was used at L1= 50, L2= 45 dBnHL. Three different types of noise were used for contralateral stimulation; a broad band noise (50-8000 Hz) and two narrow band noises centered around frequencies equal to 2f1-f2 and f2 respectively. The results revealed differences in the input/ output functions for the DPOAE and the FFR-DP responses, presumably reflecting different degrees of contributions from the two sources generating distortion products in the cochlea. Also, the FFR-DP was consistently more repeatable and less variable than the DPOAE at all stimulus levels. These findings suggest that the FFR technique can be useful in evaluating cochlear function at low stimulus frequencies.

164 Searching for the Optimal Stimulus Eliciting Auditory Brainstem Responses in Humans.

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This study examines auditory brainstem responses (ABR) elicited by rising frequency chirps. A new chirp stimulus was developed that is based on estimates of human basilar membrane (BM) group delay derived from stimulus-frequency otoacoustic emissions (SFOAE) at a level of 40 dB SPL (Shera and Guinan, 2000). The chirp was designed such as to compensate for BM group-delay differences across frequency, in order to maximize neural synchrony at cochlear level. Chirp-evoked responses were compared with click responses and with responses to the "original" chirp stimulus as defined by Dau *et al.* (2000) on the basis of a (linear) cochlea model. Our hypothesis was that, at low stimulation levels, the new chirp should produce a better synchronization than the original chirp, while at high levels, the original chirp should be more efficient since it is based on high-level BM data. In an additional experiment, ABR were also recorded for a set of level-dependent chirps that were developed on the basis of wave-V latency data by Neely *et al.* (1988) obtained for tone pulse stimuli. Overall, wave-V amplitude for the various chirp stimuli was always larger than that for the click. However, there was surprisingly little difference between the results for the different chirps. It seems sufficient to only roughly match the (inverse) delay-line characteristic of the cochlea; further "fine tuning" of the stimulus parameters does not have much effect, at least as long as average data are considered.

165 Auditory Brainstem and Frequency Following Response measurements in children with Specific Language Impairment

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Specific language impairment (SLI) is a developmental language disorder in which children demonstrate varying degrees of difficulties in acquiring spoken language. Numerous hypotheses have been postulated to explain the cause of specific language impairment. One such hypothesis is that SLI results from inefficient processing of rapidly presented auditory signals. The aim of this study is to evaluate the physiological responses to rapidly presented auditory stimuli in children with SLI and compare these responses to those obtained in children with normal language development. Auditory brainstem responses were measured to clicks presented at low and high rates (21.1/sec and 71.1/sec). Frequency-following responses (FFR) were obtained to tone bursts presented at the same low and high rates and also to tonal glides with different sweep rate (1333.33, 2666.67, 3999.99, 5333.32 and 6666.65 Hz/s). The stimulus levels ranged between 40-60 dBnHL. Preliminary results indicates that the adaptive effect (latency prolongation of wave V) is more pronounced in children with SLI, compared to children with normal language development, with complete deterioration in the ABR waveform morphology. FFRs to tonal glides were also poorer for the SLI group children with no discernible spectral following of the frequency change at faster stimulus sweep rates. These results seem to suggest that the deficit in processing rapidly changing auditory stimuli in children with SLI might be due, in part, to a degradation of phase-locked encoding of frequency change in the auditory brainstem.

166 Sources of Functional Auditory Brainstem Maturation in the Human Neonate

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The magnitude of the auditory brainstem response (Fsp) recorded from surface electrodes in full term human neonates increases in direct proportion to the number of hours since birth over the first 36 hours of life. Near simultaneous additional measures indicated that any changes in the external ear, middle ear or cochlea did not contribute to the effect. Further, the same trend also was observed longitudinally in individual neonates. Therefore, the phenomenon was attributed to maturation of auditory brainstem function. However, because the magnitude of the auditory brainstem response was quantified as a signal-to-ratio, it could not be determined if the response increase was due to an increase in the brainstem response itself, a concomitant decrease in the noise, or a combination of both. It is possible that measurement noise from neuromuscular function or other entities also changes systematically after birth. The purpose of this study was to investigate functional maturation changes in the human neonatal auditory brainstem response using more advanced analysis to further isolate maturational effects from entities other than the auditory brainstem. The variances of the auditory brainstem response and the variances of the concomitant noise were analyzed separately in a group of full-term neonates. The variances of the noise did not change significantly across neonates in the presence of increases in the variances of the auditory brainstem response. The results suggest that the observed auditory brainstem functional maturation in full term neonates is independent of any measurement noise as well as middle ear or cochlear effects.

167 The Effects of Diabetes on the Auditory Brainstem Response: An Epidemiological Study

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The effects of diabetes on auditory function are controversial and are not well understood. Audiometric test results are often confounded by age, by noise exposure and by comorbidity. An epidemiological study is underway at the National Center for Rehabilitative Auditory Research that will involve 400 diabetic patients and 400 age- and gender-matched control patients from the veteran population between the ages of 25 and 85 years. One primary objective is to determine whether there is a significant association between diabetes and auditory function. Audiometric testing includes pure tone thresholds, speech recognition, immittance testing, auditory brainstem responses, evoked otoacoustic emissions and tinnitus evaluations. An extensive questionnaire obtains data regarding noise exposure, comorbid conditions, self-perception of overall health, and other relevant information. This is the largest and most comprehensive study of diabetes and auditory function reported to date. We report here on auditory brainstem response (ABR) findings to date from over 400 diabetic and control patients. Age and noise exposure account for a significant amount of hearing loss found in this sample population, but auditory brainstem abnormalities are seen disproportionately in diabetic patients.

168 Latency-Intensity Functions of Brainstem Auditory Evoked Responses Explained by Synaptic Processes

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Brainstem Auditory Evoked Potentials (BAEP) are elicited by brief acoustic stimuli and recorded using scalp-electrodes. A typical response consists of five consecutive waves, characterized by their latency, the most prominent one being wave V. The latency-intensity function (LIF), derived by plotting the latency versus intensity of stimulation, is used as one important non invasive measure of the peripheral auditory pathway's functional integrity (Vannier et al., 2001). On average, wave V latency in response to clicks increases from about 5.6 ms at 80 dB nHL to 8.2 ms at 10 dB nHL in the normal system. This latency increase has been interpreted to be due to a more apical spread of cochlear excitation and increased cochlear travel time as intensity decreases (Picton et al., 1981; Jacobson, 1994).

Here we present a novel analysis of LIFs based on a microscopic model of the inner hair cell to auditory-nerve fiber synapse. Stimuli were non-filtered condensation clicks of 0.1 ms duration, presented at 18 Hz. BAEPs were recorded at sound pressure levels (SPL) from 90 to 15 dB SPL in 5-dB steps. The obtained LIFs of 20 normal adult human subjects were fitted by three-parametric model curves. The model accurately describes the latency of wave V as a function of SPL (unexplained variance < 5% for each subject). The obtained parameters are in excellent agreement with predictions from analyses of first-spike timing of cat auditory-nerve fibers and cortical neurons and of tone detection thresholds as a function of duration in cats and humans (Heil & Neubauer, 2001, 2002).

169 Short-Term Adaptation of Auditory-Evoked Brainstem Responses in Mice

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Recovery of neural responses following a prior auditory event shapes the physiological processing of subsequent acoustic stimuli and, therefore, is an important feature in sound detection. In previous studies assessing the adaptation of single auditory nerve fibers, several recovery periods were reported, each attributed to a different physiological process. Although time constants for recovery were similar among some species, they differed for others. The purpose of this study was to examine adaptation in mice because of the prominence of this species in studies of genetically induced hearing loss. Auditory-evoked brainstem responses (ABRs) were recorded from adult BALB/c mice in response to probe stimuli alone and following a non-simultaneous (forward) masking stimulus. Characteristics of the forward masking stimulus, including its level and duration, were altered parametrically, in addition to the interval between masker offset and probe onset ("delta t"). For a given forward masking stimulus, amplitudes of ABR component peaks decreased as "delta t" was reduced. This effect was more pronounced as masker level was increased, and saturated at higher levels where additional masking was not observed with further increases in masker level. Conversely, latencies of ABR peaks were prolonged as "delta t" decreased, with greater latency shifts observed at higher masker levels. Masker duration had an effect that was similar to masker level: as masker duration was increased, the response to the probe stimulus declined in amplitude and peak responses occurred later relative to probe onset. The effects of masker duration were greater as the interval between masker and probe was reduced, and saturated at longer durations. These data may be useful to optimize stimulus parameters for acquiring ABR-derived tuning curves from mice, as well as for evaluating adaptive processes in mice with genetically induced hearing loss.

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170 Hearing in Exotic Felids

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At last year's midwinter meeting, we reported that tigers - and an African lion - respond preferentially to low-frequency tone bursts and are relatively insensitive to middle and high frequency acoustic signals. This finding is consistent with expectations based on the middle ear studies of Huang et al. (2000) and the notion that the relative size of the middle ears of felids determines, at least partially, the bandwidth of acoustic energy to which individuals are responsive. Since then, the opportunity to record auditory brainstem responses (ABRs) from a jaguar and a clouded leopard arose and preliminary findings resulting from that effort suggest that the audiological range of smaller felids, like the leopard, is similar to those reported for larger felids; e.g., tigers, lions and jaguars. This unexpected preliminary finding will be explored more thoroughly in the upcoming months to further test the hypothesis that body size, and thereby middle ear dimensions, partially determine the shape of threshold-frequency curves observed within the cat family. Felids studied as a part of this investigation were initially anesthetized with ketamine and xylazine or medetomidine, and were subsequently maintained on gaseous anesthesia throughout the recording session. ABRs were recorded differentially to both clicks and tone bursts between 32 kHz and 0.5 kHz, sampled in one-half octave steps. Stimulus levels were varied from near threshold to at least 90 dB SPL in 10 dB steps. Response waveforms recorded from each species studied were similar to those observed in domestic cats and response magnitude and recording quality were directly correlated with head size. Other response features were similar to those observed in other mammals. Acknowledgements: We would like to acknowledge the patience of Sarah Dankof, the Supervisor of the Hospital, and all the caretakers who have helped with the investigation.

171 Far-field Neural Population Responses in Belgian Waterslager (BWS) Canaries

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The Belgian Waterslager (BWS) canary has a behavioral high-frequency hearing loss, even though hair cell loss is distributed throughout the basilar papilla. Auditory brainstem response (ABR) recordings from sedated BWS canaries show elevated ABR audiograms compared to non-BWS canaries. Here we examine other aspects of ABR measurements in BWS canaries that may differ from other birds.

Preliminary results show that the shape of the ABR audiogram in BWS canaries is similar to what might be expected based on ABR thresholds in humans with high frequency sensorineural hearing loss (Stapells and Oates 1997). Increasing the presentation rate of high intensity clicks showed that latency measures for wave I were similar but slightly longer than non-BWS canaries at high presentation rates. Wave I and II peak amplitudes in BWS canaries were 2-3 times smaller than amplitudes of non-BWS canaries regardless of presentation rate.

Pathology at the level of the hair cell and auditory nerve may help explain some of these differences. The ratio of hair cells to afferent nerve fibers is about 0.5 in non-BWS canary (Gleich et al 1998). Since BWS canaries have an approximate 30% reduction in hair cell number and of these remaining hair cells an average of 30% have abnormal stereovilli bundles (Ryals et al 2002) the total number of hair cells likely to respond normally is reduced by as much as 60%. Further, the total number of auditory nerve fibers is reduced by approximately 12% in BWS. Systematic investigation of hair cell innervation patterns in BWS should be useful in interpreting differences in neural population responses both in adulthood and during the progression of hearing loss in this strain during early development.

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172 New Methodology for Acquisition of High Stimulation Rate Evoked Responses: Continuous Loop Averaging Deconvolution (CLAD)

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A new method of acquiring Evoked Responses using high stimulation rates is developed and implemented. The technique allows users to generate a wide range of complex stimulus sequence patterns and deconvolves the resulting signals to reveal the overlapping EP morphology. The theory, mathematics and implementation principles are developed and explained. This technique works in the time domain by solving a set of simultaneous equations generated by a particular stimulus sequence and finds the unique response provided a solution exits. A computer algorithm is developed and implemented in an evoked potential averaging system to test the deconvolution theory. The system consists of two modules: a stimulus sequence generation module and a stimulation and acquisition module. The stimulus sequence generation module allows users to design custom stimulation sequences and generates a deconvolution solution matrix. A wide variety of sequences are easily generated and tested to determine if the resulting signal can be deconvolved. The stimulation and acquisition module uses two continuous averaging loops to acquire signals while measuring signal-to-noise ratio and residual noise in real-time. The deconvolution process can be conducted in real-time while averaging. Until recently, only a limited number of very specialized stimulus sequences generated by the Maximum Length Sequences (MLS) or

Legendre sequences (LGS) were capable of deconvolution of such responses. In this method many arbitrary stimulus sequences can be generated and averaged responses can be deconvolved. The MLS method is shown to be a special case of this theory with unique integer coefficients. Computer simulations with synthetic responses and real Auditory Brainstem Responses (ABR) and Middle Latency responses (MLR) obtained from normal subjects at high stimulus rates (up to 800 per second) verify the theory and the algorithm.

173 Acquisition of ABRs at Very High Stimulation Rate Using CLAD (Continuous Loop Algorithm Deconvolution)

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Auditory Brainstem Responses (ABR) are usually recorded using stimulation rates no faster then 50-100 per second due to overlap of the responses. Special methods such as Maximum Length Sequences (MLS), or Legendre Sequences (LGS) are needed for recording responses at higher rates. Such specialized methods have several limitations and problems. A new method for high rate recording, Continuous Loop Averaging Deconvolution (CLAD) was proposed by Delgado and Ozdamar. CLAD enables the deconvolution of overlapping responses and extracting the underlying response to a stimulus.

Recordings were acquired at 7 stimulation rates ranging from 58.6 to 800.8 per seconds. Click levels from 0 to 70 dB nHL were used. Similar recordings were obtained using MLS and LGS as well. ABR wave V was observable at all rates up to and including 800.8 per second. Response amplitudes were shown to decrease and latencies to increase with increasing stimulus rates. Earlier ABR waves also disappeared at increasing rates and thresholds were somewhat elevated due to diminishing signal amplitudes. Results showed that CLAD recordings were similar to responses obtained by MLS and LGS. CLAD recordings, however, provided a wider range of stimulus patterns and provided better control of the inter stimulus intervals with less jitter. Due to a wide selection of stimulus sequences, CLAD provides a suitable methodology to study adaptation effects at very high stimulus rates. This technique may enable us to study the early effects of demylinating diseases on evoked responses and differentiation of adaptation along the auditory pathway which may be useful as a diagnostic tool.

174 Functional Characterization of a Newly Developed Ossicular Chain Replacement Prothesis in the Rabbit

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The purpose of this interdisciplinary experimental study was the functional characterization of a newly developed ossicular chain replacement prosthesis. The aptness of this prothesis composed of titanium-dioxide was examined by reconctructive surgery of the sound conducting ossicle found within the middle ear of rabbits. A total of 35 rabbits implanted with titanium-dioxide prosthesis in the way of total ossicular replacement prosthesis. The thresholds, latencies and interpeak-latencies by means of auditory evoked potentials (ABR) and bone conduction were established in both ears of all of the animals. In addition tympanograms of all rabbits ears were determined. The measurements took place directly before surgical intervention as well as28,84 and 300 days post op.

The relatively minor increase in the potential threshold of 19,85 dB with the click impulse and 28,19 with the pip-impulse can be assessed as rather positive exeprimental outcome.

The potential thresholds that eveluated by bone conduction remained in the normal range on both ears during th entire study. By establishing a tympanogram, defects in the tympanic membrane as well as any tympanic effusions that my occured postsurgically, could be ruled out. A correlation between the tympanogram and the severity of the conductive hearing loss could not be determined. The reference value stablished in thes study however, can serve as guidelines for further experimental trials.

175 Cochlear Physiology in Mice

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Although the ability to create targeted mutations in mice makes this species a valuable animal model, it is important to determine if mice process acoustic inputs as efficiently as do other species. Hence, this work compares cochlear responses from the CBA/CaJ strain, which does not demonstrate age-related hearing loss, with those from the mongolian gerbil. Round window measurements from CBA/CaJ mice indicate that compound action potential (CAP) thresholds are sensitive out to 50 kHz, the highest frequency tested. This performance is similar to that reported in gerbil (Overstreet et al., Audio&Neuro-Otol., 2002). In addition, CAP tuning curves at 12 kHz show similar Q10s and tip-to-tail ratios in both species. Hair cell activity was also assessed indirectly using the cochlear microphonic (CM) and summating potentials (SP). CM iso-response functions indicate that criterion responses are measured at approximately the same sound pressure level from 5-50 kHz in CBA/CaJ mice. This result suggests that the CM, which reflects hair cell receptor currents, is not attenuated at high frequencies in contrast to ac receptor potentials, which are filtered by resistances and capacitances associated with the hair cell's basolateral membrane. The robust nature of CM responses is consistent with the idea that extracellular potentials may be able to drive OHC motility, as suggested by Dallos and Evans (Science 267, 2006, 1995). Finally, iso-response functions for the SP are V-shaped with the tip of the function occurring at ~45 kHz. Similar functions in the gerbil reveal a tip at ~33 kHz, suggesting that round window recordings in this species are dominated by hair cells with somewhat lower best frequencies.

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176 Response Properties of Single Auditory Nerve Fibers in CBA/CaJ Mice

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The availability of transgenic lines with interesting auditory phenotypes makes the mouse a valuable model to study the auditory system, and recordings from single auditory nerve (AN) fibers can provide a powerful window into cochlear function. To establish baseline data for the mouse, we chose the CBA/CaJ strain for initial study.

Single-unit recordings were made from the AN in CBA/CaJ mice, aged 8-17 weeks, using high impedance glass microelectrodes. Mice were anesthetized with xylazine and urethane, paralyzed with tubocurarine and artificially respirated. Sound stimuli were delivered with a closed acoustic system, and distortion product otoacoustic emissions were monitored to assess cochlear condition. The AN was reached via a posterior fossa approach after cerebellar aspiration. Because the AN is difficult to expose directly without compromising cochlear function, electrodes were directed through the posteroventral cochlear nucleus, ventrally and rostrally into the AN itself. To differentiate AN fibers from cochlear nucleus cells, electrode depth and PST histograms of tone-burst responses at the characteristic frequency (CF) were analyzed: measures of the coefficient of variance of the interspike intervals and first spike latency were extracted, as described by Young et. al. [1988, J. Neurophys. 60: 1-29].

Tuning curves, spontaneous discharge rates (SRs), PST histograms, and rate-versus-level functions for CF tone bursts were obtained whenever contact time permitted. AN fibers had CFs between 3kHz and 55kHz and SRs varying from 0 to 52 sp/sec. Basic features of AN response in the mouse were similar to other mammalian species studied: 1) sharpness of tuning (Q10dB) increased with increasing CF, 2) response threshold was inversely proportional to SR, 3) onset peak to steady state response ratio was a function of SR (low SR < high SR) and CF (low CF < high CF), and 4) the dynamic range of response to CF tone bursts was 15-30dB.

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177 Dual Representation of the Pitch of Complex Tones in the Auditory Nerve

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Previous auditory-nerve studies of the coding of the pitch of complex tones have documented a temporal representation based on interspike intervals, but have largely neglected possible rate-place cues to pitch available when the individual harmonics are resolved by the peripheral auditory system. We investigated the resolvability of harmonics of complex tones in the cat, and compared the effectiveness of rate-place and interval codes over a wide range (110-3520 Hz) of fundamental frequencies (F0).

We recorded responses of single AN fibers in anesthetized cats to periodic complex tones with a missing fundamental at moderate sound levels (10-50 dB SPL per component). The harmonics were all of equal amplitude and spanned a spectral region of 2.2 octaves around a fiber's CF. We used three phase conditions: cosine-phase, Schroeder-phase and alternated sine-cosine phases.

The average discharge rate of a fiber was greater when the CF was a small integer multiple of F0 than when the CF fell between two harmonics. In general, harmonics up to the 5th were thus resolved in rate responses, although this number decreased at higher stimulus levels. Using data from 12 to 47 single units, we could estimate F0 from rate-vs-CF profiles with errors smaller than 2-3%. However, few reliable estimates were obtained below 400 Hz, due to the broad cochlear tuning at low frequencies in the cat.

We generated "pooled" interspike interval distributions by summing all-order interval histograms from all the sampled fibers. By finding the best fitting periodic template to the pooled distributions, we were able to estimate F0 with errors smaller than 1%, up to 1200 Hz. Phase had no obvious effect on either rate-CF profiles or pooled interval distributions.

In summary, both rate-place and interspike-interval codes are viable over a wide range of F0 in the cat, but the intervals code is more useful at lower F0s, and the rate-place code at higher F0s.

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178 Temporal Properties of Cochlear Suppression Derived From Auditory Nerve Responses

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We previously presented a novel method to examine, from auditory nerve data of the cat, cochlear phase and amplitude transfer at arbitrary frequencies [van der Heijden and Joris, ARO 2002]. The method is based on an analysis of the interaction among components of an irregularly spaced tone complex. Among other things, the method measures, with sub-ms accuracy, group delays over a fiber's entire response area.

We adapted the method to enable study of cochlear suppression by using a tone complex as a suppressor. The interactions among the suppressor's components modulate the neural response evoked by the probe signal. A similar analysis as in the excitatory case yields phase and amplitude characteristics of the suppression process.

We were able to examine suppression at many different frequencies in a single animal, both for suppressor frequencies above or below the probe frequency. Comparisons between the phase characteristics of suppression and excitation yielded information on their different latencies. For all frequency configurations tested, we found that suppression mediated by a a tone complex is consistently slower than excitation evoked by the the same tone complex. The exact latency of suppression depends on the relative frequencies of probe and suppressor and on their intensities. The data suggest that suppression is mediated at a cochlear site slightly basal to the probe's characteristic location. Our findings also impose constraints on the speed of the suppression process itself.

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179 Auditory Nerve and Inferior Colliculus Responses to 22 kHz Air- and Bone-Conducted Tonebursts t Ipsi/Contra Stimulation

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Ultrahigh frequency (UHF) stimuli can be heard by humans via bone conduction (BC), but not via air conduction (AC). Possible mechanisms to explain this include non-cochlear origin of these percepts, distortion of the bone-conducted signal leading to audiofrequency stimulation, or the inefficiency of the middle ear transfer function at these frequencies. In this study, we evaluate responses from an animal model (chinchilla) capable of hearing via AC above 20 kHz, in order to compare AC and BC responses.

Recording electrodes were implanted in the inferior colliculus (IC) and auditory nerve (AN). Following recovery, animals were stimulated with a 22 kHz toneburst intensity series via AC and BC. Stimuli were presented to both the right and left ears (for AC) and right and left sides of the skull (for BC). Animals were then given carboplatin, and 4-5 weeks later, responses again recorded, the animal sacrificed and cochleae harvested.

BC stimuli presented to the same (ipsi) and opposite (contra) side of the skull produced AN responses with similar amplitudes and latencies, with the latencies slightly prolonged at a given stimulus level for the contra placement. For AC stimuli, AN threshold was substantially higher, amplitude was smaller and latency was longer for the contra stimuli. Caboplatin produced a moderate inner hair cell loss, and reduced AN response amplitudes and increased latencies. From IC, contra AC and BC responses were larger than ipsi responses, and all responses decreased post-carboplatin. For AC tones, IC latency was dependent on stimulus level, stimulated ear and pre- versus post-carboplatin. For BC tones, contra latency was shorter than ipsi latency; post-carboplatin, a small latency increase was seen for the contra placement.

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180 Gap Detection From the Auditory Nerve and Inferior Colliciulus of the Chinchilla

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Guo and Burkard (2002) reported gap threshold from the inferior colliculus to paired noisebursts to be on the order of 0.5-1 ms for onset responses, while offset responses showed gap thresholds on the order of 8 ms. Similarly prolonged offset response thresholds compared to onset response thresholds were also reported in the auditory cortex. The present investigation compared gap detection in the auditory nerve (AN) and IC, to determine if the difference in gap threshold for offset

responses as compared to onset responses is seen in the auditory nerve response.

Nine chinchillas had electrodes chronically implanted in the left IC and the right AN. Following a recovery period, recordings were made in the unanesthetized chinchilla. Paired, 50-ms, 80 dB SPL noisebursts were presented to the right ear. Noiseburst gaps ranged from .5 to 64 ms. After the first recording, the chinchillas were given carboplatin (75 mg/kg); 4-5 weeks later, the gap protocol was rerun, and the cochleas harvested.

Cochleograms showed a moderate inner hair cell loss, with a minimal outer hair cell loss. For both AN and IC, the onset response to the second noiseburst (NB2) increased in latency and decreased in amplitude with decreasing gap. Gap threshold from both the AN and IC for the NB2 onset response was on the order of 1 ms, while the offset response was typically only seen for gaps of 8 ms and above from the IC, but for gaps of 1-2 ms and above from the AN. Following carboplatin, response amplitudes decreased and latencies increased for onset responses, with offset responses typically missing from the AN, but present in the IC.

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181 Activity Growth Rates in Auditory-Nerve Fibers Following Noise-Induced Hearing Loss

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Loudness recruitment imposes a significant constraint on hearing-aid algorithms; however, the physiological correlates of loudness and recruitment are still not well understood. The growth of auditory-nerve (AN) activity with sound level was measured for simple and complex stimuli to directly test the hypothesis that AN-fiber rate-level functions (RLFs) are steeper in an impaired ear.

RLFs were measured in both normal-hearing cats and in cats with a noise-induced high-frequency hearing loss. Stimuli included BF and fixed-frequency tones, broadband noise, and a brief speech token. Two-line fits to sloping-saturation RLFs characterized low-level and high-level slopes; one-line fits were made for sharply saturating responses.

Normal-hearing RLFs showed typical variations across stimuli and BFs that can be accounted for by compression and suppression associated with outer-hair-cell (OHC) function. Three types of impaired responses were observed: (1) Fibers with similar RLFs across all stimuli had broad tuning and lacked sloping saturation, consistent with OHC loss. (2) Fibers with a wide dynamic range and shallow slopes above threshold often retained sharp tuning, consistent with primarily IHC damage. (3) Fibers with very steep RLFs for all stimuli had thresholds above ~80 dB SPL and very broad tuning, consistent with severe IHC and OHC damage.

While recruitment is often assumed to result from steeper basilar-membrane (BM) responses associated with OHC damage, AN fibers do not provide a simple representation of the BM I/O function. These results demonstrate that impaired AN rate-level slopes are not consistently steeper than normal low-level slopes, and suggest that both OHC and IHC damage can affect AN response growth. The similarity between normal and impaired AN growth near threshold is consistent with recent psychophysical evidence that loudness grows normally near threshold in impaired listeners (Buus and Florentine, 2001).

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182 Discriminability Curves For Primary Auditory Afferents From Their Rate-Level Curves

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The ability of auditory neurons to signal changes in sound pressure level has typically been discussed in terms of the neuron's dynamic range. The literature contains many schemes for dynamic range, all based on firing-rate criteria. These schemes result in a plethora of possible dynamic ranges for any given primary auditory afferent (Nizami, Hear. Res. 167, 2002). This happens because, although dynamic range is imagined as the contiguous range of intensity over which the neuron can indicate some criterion change in level, the published schemes do not specify that criterion change. A new method is introduced in which a Signal Detection model provides an equation for the intensity-difference limen (DL). This DL equation was applied to rate-level plots for primary afferents in the cat, plots that were replaced for computational purposes by smooth functions that were obtained by fitting each rate-level plot to a double-logistic proven to fit well to such rate-level plots (Nizami and Schneider, Math. Biosci. 141, 1997; Nizami, 2002). The model yields generally U-shaped discriminability curves, indicating that the afferents are more sensitive around their mid-points and less sensitive at their extremes. Each curve's minimum can be obtained by computation. If a horizontal line is drawn above the minimum, at some criterion discriminability (in dB), that horizontal line will generally cut the DL curve in two places. The separation between those 2 points corresponds to the range in dB over which the afferent can signal the given criterion change in level. Plotting each such range vs. its DL criterion yields a neuronal discriminability curve that replaces the plethora of possible dynamic ranges obtained using contemporary methods. Examples will be shown of neuronal discriminability curves for both sigmoidal and sloping-saturating rate-level plots, taken from the literature.

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183 Recovery From Short-Term Adaptation in the Cochlear Nerve of the Chick.

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The release of neurotransmitter vesicles at the base of the hair cell is a prerequisite for discharge activity in auditory nerve units. The depletion of the vesicle pool has been associated with the adaptation of auditory nerve discharge rates. The replenishment of these vesicle pools, as seen in the recovery from adaptation, is examined here. Chicks 6 to 10 days old were anesthetized and prepared for a trans scala tympani approach to the cochlear (auditory) nerve. The ear was stimulated with 100 ms tone bursts (2.5ms rise/decay time) at the characteristic frequency of the unit. Pairs of these tone bursts, separated by 6, 11, 20, 39, 80, 158, or 355 ms (with inter-pair intervals of 340 ms) were used to construct peri-stimulus-time (PST) histograms summed over approximately 100 pair presentations at each interval. Each unit was tested at all 6 intervals. The PST histograms for the first and second stimulus presentation were fit to a three parameter, single exponential decay function. From the fitted function, the peak discharge rate in spikes per second, the time constant, the percent of adaptation, and the adapted discharge rate were determined. These parameters were compared between the first and second PST histograms for each unit at each interval. The peak response and percent adaptation of the second PST histogram are substantially depressed with a 6-ms separation. With increasing durations the values of the first and second histogram begin to approximate each other. Complete recovery is achieved between 40 and 80 ms after the first tone. The time constants, however, show no significant differences between the first and second PST histograms at all intervals. The results suggest that total vesicle pool replenishment in the chick cochlear nerve occurs between 40 to 80 ms after acoustic stimulation.

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184 When a "Primitive" Fish listens to Tones: Encoding of Sound in the Auditory Periphery of the Shortnose Sturgeon, Acipenser brevirostrum

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We investigated frequency response properties of peripheral units encoding pure tones in the sturgeon, a primitive fish. Our goal was to find out to what extent these responses resemble those of teleost fishes ("advanced" bony fishes) and other vertebrates.

Shortnose sturgeons were presented with pure tone stimuli from 50 - 1000 Hz and intensities from 120 - 160 dB (re 1 µPa) using a tank with a submerged loudspeaker. Fifty-three units were recorded in the auditory periphery of five sturgeons. Only 17% of the units showed spontaneous activity, most of them with a low firing rate (< 15 spikes/s, 80%), and 20% fired with moderate activity (15-50 spikes/s). Three patterns of spontaneous activity could be found: regular firing (30%), bursting (11%), and irregular firing (59%). Most irregular units responded to sound, whereas most of the regular firing units did not respond. This is consistent with recordings from the 8th nerve in teleost species. The large number of units with zero spontaneous activity recorded in the 8th nerve in sturgeon is not characteristic of teleosts.

Units showed different response pattern categorized as tonic (73%), phasic (23%), and phasic-tonic (11%) firing. Some units (15%) showed sharp ON and OFF responses (8th nerve units) or inhibition. The most sensitive units responding to 100 Hz and 400 Hz respectively had their lowest thresholds at 120 dB. Units in the lower frequency range (100 to 600 Hz) were tested at 140 dB for isolevel frequency response functions. Units responding to higher frequencies (1 kHz, 7 units) had thresholds at 160 dB.

The results for sturgeon, a "primitive" fish, are consistent with general physiological features found in the periphery of other vertebrates with regard to different patterns of spontaneous activity, zero spontaneous activity, different response patterns, phase-coupling, inhibition, and tuning.

185 Two Point Process Modeling Approaches to Generating Auditory Nerve Spikes

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The generation of action potentials or spikes is a process common to all sensory systems, but it is of particular interest in relation to the auditory system where the temporal aspect of the encoding process may play a crucial role in information processing. In previous work (Deligeorges 2001, ARO Abstract #22064), a test regimen was developed to examine the performance of instantaneous rate models for the auditory nerve using a standardized set of test. This analysis of performance has now been extended to models which simulate the generation of spikes. In this work two stochastic point process models for spike generation are examined, both models explicitly account for absolute and relative refractory periods, but differ in the method by which the refractory period is implemented. The first model is based on the work of Gray (Biophys J 1967 7:759-777) using a modified implementation developed by Carney (J Acoust Soc Am 1993 Jan;93(1):401-17), this model uses spike history in a multiplicative process to create absolute and relative refractory effects. The second is a model based on the work of Weiss (Annu Rev Physiol 1984;46:247-59) using a new implementation, this model applies spike history in an additive way to create refractory effects. Both models used the Dual AGC adaptation model (Deligeorges and Mountain 1997, Computational Neuroscience, Plenum Press) for the auditory nerve as an excitation function to drive the point process models. Biologically relevant values for absolute and relative refractory period for an auditory nerve fiber were chosen for the models, all other parameters were fixed to published values or pre-existing parameter sets. Results show that the multiplicative approach functioned well over a wide dynamic range, but did not have temporal synchronization properties as good as those seen in the experimental data. The additive approach produced better synchronization characteristics, but performance was more sensitive to changes in level.

186 Coding of Auditory Information into Nerve-Action Potentials.

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Neuronal sound processing requires compression of the dynamic range of acoustical signals to the limited dynamic range which can be coded by neurons. Here I present a phenomenological model of a compressive inner ear model followed by a realistic inner hair cell model. Frequency analysis was achieved using a wave-digital filter model of cochlear hydrodynamics (Strube, 1988). Numerically stable dynamic compression was realized by adding second-order resonators at the cochlea's outputs and modulating their quality factors (Q) using a Boltzmann function. Cascading multiple (4) resonators and modulating their Q-values from 10 to 1 accomplished up to 80 dB compression together with reasonably broad response curves. Stereociliary bundles of the inner hair cells were stimulated by fluid forces derived from basilar-membrane displacement. Transduction currents caused an inner hair cell receptor potential, which in turn activated voltage dependent Ca-channels. Elevated Ca-levels caused fusion of synaptic vesicles (Beutner et al., 2001) and finally elicited nerve action potentials. The kinetics of vesicle fusion was modelled according to Moser and Beutner (2000).

The model reveals that the dynamic range problem can not be solved by mechanical compression in the cochlea alone. In addition, neuronal feed-back mechanisms have to be introduced, which finely control BM-vibration by efferent feedback to cochlear mechanics and to the afferent synapses terminating of the inner hair cells. The spike trains transmitted by the auditory nerve fibres to the cochlear nuclei and further to the midbrain allow for both spectral and temporal information processing, which is required for robust speech recognition especially in noisy environments.

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187 Comparison of Two Non-linear Auditory Nerve Models using a DSAM Computer Application and Matlab/Octave

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We present a computational methodology for investigating the characteristics of two non-linear auditory nerve models. Many animal-based models of the auditory nerve have been used in auditory investigations. Computational models can also used for such investigations. It is the aim of this presentation to promote the merits of using computational modelling.

Popular visualisation tools are used in conjunction with the Auditory Model Simulator (AMS); an application built using the development system for auditory modelling (DSAM) computer library. DSAM is a computing library designed as a standard platform for producing applications to create and evaluate auditory models. It brings together many published auditory models (produced by different research groups), analysis functions and general utility functions. All these features are available within a flexible programming platform.

The AMS application inherits the interface and all of the other features of DSAM. Like other DSAM applications AMS allows a variety of interface options. There is a graphical user interface (GUI) that provides comprehensive access to model and application parameters. AMS accepts command-line options giving access to all parameters, so it can be employed to produce quite complex analysis runs using scripting tools. AMS also has a command-line only version, for fast runs.

In this presentation, the Xhang et al. (2001) and Sumner et al. (2002) auditory nerve models will be compared. The models are set up using DSAM simulation scripts within AMS. The AMS simulations are then run and the results plotted using Octave, the freeware Matlab clone. The scripts have also been tested using Matlab.

AMS is available as an "out of the box" Windows installation (95/98/2000 and NT) for PC's, Linux RPM's and can be installed on UNIX machines using its auto-configuration system. It is available for download from our WWW site. The Matlab/Octave scripts are also available within the AMS installation.

188 BCL-2 Overexpression Delays Age-Related Spiral Ganglion Cell Degeneration in a Mouse Model of Presbyacusis.

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The anti-apoptotic protein, BCL-2, has been shown to prevent programmed cell death in many biological systems. The aim of the present study was to determine whether transgenic expression of the human *bcl-2* gene in C57BL/6J mice could prevent the age-related degeneration of the auditory nerve typical of these mice. Transgenic mice and wildtype littermate controls (genotyped by DNA analysis using polymerase chain reaction) were examined at 2, 10, 15 and 20 months of age. Auditory brainstem response (ABR) thresholds increased with age, such that by 15 months of age animals exhibited a profound hearing loss across all frequencies tested. There were no significant differences in ABR thresholds between transgenic and wildtype animals at any age. Immunohistological analysis of the cochleas verified expression of the transgene, under control of the neuron-specific enolase promoter in these mice, in the inner hair cells and spiral ganglion cells of all transgenic animals. Spiral ganglion cell (SGC) density in the basal cochlear turn was measured from toluidine blue-stained thin sections of plastic-embedded cochleas. In both wildtype and transgenic animals, SGC density decreased with age; however the rate of degeneration in transgenic animals was reduced compared to wildtypes. The greatest difference was seen in the 15-month-old animals, where mean (± SEM) SGC density in the transgenic animals was 696.13 (±56.05) cells/mm^2, compared to 203.61 (±44.47) in wildtypes (p<0.001, t-test). Analyses of inner hair cell (IHC) survival correlated positively with SGC survival across all animals (r=0.84), suggesting that the increased survival of SGCs in transgenic animals may partly reflect prolonged survival of IHCs. These results indicate that BCL-2 overexpression may delay the age-related degenerative changes seen in presbyacusis.

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189 Molecular Basis for Age-related Loss of Spiral Ganglion Neurons

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Age-related hearing loss (Presbycusis) is a major health concern for the elderly population. Loss of spiral ganglion neurons is consistently associated with Presbycusis. The sequence of molecular events for age-related loss of spiral ganglion neurons is unknown. We investigated whether molecular pathways initiated by neuregulin-1 contributes to age-related loss of spiral ganglion neurons.

The protein products of neuregulin-1 constitute a family of isoforms mostly with an extracellular domain, a transmembrane domain, and a well-conserved cytoplasmic domain. Interactions between the extracellular domain of neuregulin-1 and erbB receptor tyrosine kinases can occur after release of the extracellular domain of neuregulin-1, either directly following synthesis of isoforms lacking transmembrane domains, or following proteolysis of the transmembrane precursor form. Genetic analyses have demonstrated that both the extracelluar and cytoplasmic domains of neuregulin-1 are essential for synaptic formation. We found that the cytoplasmic domain of neuregulin-1 is present in the nuclei of spiral ganglion neurons. The nuclear level of the neuregulin-1 is up regulated during aging in spiral ganglion neurons. Nuclear translocation of the cytoplasmic domain of neuregulin-1 regulated expression of several downstream genes. One of them is involved in synaptic formation, other are apoptotic genes. These findings imply that one of molecular mechanisms underlying age-related loss of spiral ganglion neurons is initiated by the cytoplasmic domain of neuregulin-1.

190 Contributions of Nicotinic Acetycholine Receptors to Age-related Loss of Spiral Ganglion Neurons

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One major health concern for the elderly population is age-related hearing loss (Presbycusis), which is consistently associated with age-related loss of hair cells and spiral ganglion neurons. The sequence of causative molecular events for age-related loss of spiral ganglion neurons is unknown. Certain nicotinic acetylcholine receptors are highly expressed in spiral ganglion neurons. Recently, some nicotinic acetylcholine receptors are found to contribute to age-related neuronal degeneration in central nervous system, and it is unknown whether these receptors also contribute to age-related loss of spiral ganglion neurons.

We first determined that there are changes in the expression of nicotinic acetylcholine receptor subunits in spiral ganglion neurons during aging. Comparisons of spiral ganglia extirpated from four vs. eight month-old C57BL/6J mice revealed increased levels of alpha 4 subunit mRNA and significantly decreased levels of both alpha 5 and beta 2 subunits mRNAs when total neuronal mRNAs were kept constant. To address whether the down-regulation of alpha 5 and beta 2 subunits in spiral ganglion neurons during aging might be directly related to auditory function, we measured hearing loss in eight month-old alpha 5 or beta 2 null mice. Assessment of the threshold of auditory brainstem responses to tone stimuli revealed significant deficits in beta 2 null mice, while alpha 5 null mice appear normal compared to their age and genetic background matched controls. There are also significantly fewer spiral ganglion neurons in beta 2 null mice, but not in alpha 5 null mice. Thus, age-related down regulation of beta 2 acetylcholine receptor subunit may contribute to age-related loss of spiral ganglion neurons.

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191 Cu/Zn Superoxide Dismutase and Age-Related Hearing Loss

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Presbycusis results from degenerative cochlear changes. Mice, in which the genetics can be manipulated and the life span is relatively short, enable evaluation of the effects of genetics on cochlear degeneration over time. Antioxidant enzymes such as Cu/Zn superoxide dismutase (Sod1) protect cells from toxic, reactive oxygen species and are hypothesized to be involved in age-related cell degeneration.

The effect of Sod1 on cochlear function and structure was examined in Sod1-null mice (-/-) (B6;129S-Sod1tm1Leb/tm1Leb) and in age- and genetics-matched (+/-, +/+) controls (n=156) at 9-10, 12, 15 and 18 months of age. Auditory brainstem responses (ABR) to clicks were measured. Mice were perfused with paraformaldehyde and cochleas prepared for frozen or paraffin sectioning. Sections were stained with cresyl violet or H&E.

ABR thresholds increased with age in all genotypes. At 9 months the magnitude of the loss was greatest in the -/- mice (30 dB loss, ANOVA $p < 0.001$). By 15 months the mean loss was 40dB greater than the loss in the 15 month-old +/+ or +/- animals. The ABR wave I was significantly smaller in the -/- mice. The cochleas of the -/- mice had degeneration in the organ of Corti and spiral ganglion, but not the stria vascularis. The loss of ganglion cells was the largest degenerative change and was greatest at the basal and apical ends. In contrast, the +/+ and +/- mice had much less cochlear degeneration. The variability among individual mice was large in spite of genetic and environmental similarity.

In conclusion, the absence of Sod1 makes the mouse cochlea more vulnerable to age-related sensori-neural degeneration.

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192 Effect of Overexpression of Cu/Zn Superoxide Dismutase on Noise- and Age-Related Hearing Loss

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Reactive oxygen species (ROS) have been implicated in hearing loss associated with aging and noise exposure. Superoxide dismutases (SODs) form a first line of defense against damage mediated by the superoxide anion, the most common ROS. Absence of Cu/Zn SOD (SOD1) has been shown to potentiate hearing loss related to noise exposure and age. Conversely, overexpression of SOD1 may be hypothesized to afford a protection from age and noise related hearing loss. This hypothesis was tested using a transgenic mouse model carrying the human SOD1 gene. Progressive age-related hearing loss was observed in both transgenic and non-transgenic littermates using auditory brainstem response (ABR) audiometry. However, no protection was observed in mice up to 20 weeks of age. Further, we observed no protection from noise induced hearing loss when 8 week old mice were exposed to broadband noise (4-45 kHz, 110 dB for 1 h) and ABRs recorded 1, 7, 14, and 30 days after exposure. SOD1 overexpression was not associated with reduction in mitochondrial DNA deletions, considered to be an index of aging. The present results do not support the hypothesis that overexpression of SOD1 confers protection from noise and aging in the peripheral auditory system.

193 Peripheral and Central Interaction During Aging in the CBA and C57 mouse. A calcium binding protein study.

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The quantitative stereological method was used for determining the total number of neurons and calcium binding immunopositive neurons during aging in the posteroventral- and dorsal cochlear nucleus (PVCN and DCN) in the CBA and C57 mice. Correlations were made between peripheral pathology (spiral ganglion loss, inner and outer hair cell loss) and calcium binding immunoreactivity in the cochlear nucleus. These two mouse strains have illustrated different patterns of calcium binding protein expression in the PVCN during aging. In particular, the C57 mouse demonstrated percent increases in parvalbumin and calbindin in the PVCN, while the CBA did not show any change with increasing age in the PVCN. However, the CBA and the C57 mice showed similar age-related percent increase in parvalbumin, calbindin, and calretinin in the DCN. Thus, a strain difference was particularly evident in the PVCN for the C57 mice. Strain differences were also found when correlations were made between periphery pathology and calcium binding protein expression. The percentage of parvalbumin and calretinin in the DCN showed a statistically significant correlation with peripheral pathology in both CBA and C57 mice. The C57 mice also showed significant correlations between peripheral pathology and the parvalbumin and calbindin in the PVCN. In summary, the findings imply that degenerative changes in the auditory periphery can modulate neuronal homeostasis by increasing calcium binding proteins in the PVCN and DCN during aging. In the C57 mouse the PVCN is particularly sensitive to peripheral damage compared to the CBA mouse. These findings suggest a role for calcium binding proteins in protecting against age-induced calcium toxicity.

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194 Age Related Declines in Calbindin Expression in the Ventral Cochlear Nucleus of Deafened and Hearing CBA/CaJ and C57Bl/6 Mice

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Hearing deficits in aging humans occur in both peripheral and central auditory systems, and intracellular calcium imbalances have been implicated in age related hearing loss, presbycusis. Calcium binding proteins are regulatory proteins that act as a buffering mechanism for intracellular calcium. The expression of one of these proteins, calbindin, was examined in the anterior and posterior divisions of the ventral cochlear nucleus (AVCN, PVCN) in CBA and C57 mice. CBA mice, like humans, have functional hearing until a very late age, while the C57 strain exhibits a high frequency sensorineural hearing loss at a very early age and is deaf later in life. A subset of the young adult CBA mice were deafened and then aged to determine if changes with age were strain related. In the PVCN calbindin staining in hearing 24 month-old CBA mice exhibited a 45% decrease in the number of labeled neurons relative to young CBA mice, while the deafened CBAs had a 73% decrease. In the AVCN, the old CBA mice had a 17% decline while the deafened CBAs had a 57% decline in CB immunoreactivity. Nissl counts did not show significant declines with age or deafening in either region. The C57 mouse strain did not exhibit any significant changes in calbindin staining with age. In summary, old hearing CBA mice demonstrate a decrease in calbindin immunoreactivity with age and an even greater decrease with age and deafening, while in the C57 strain, early onset hearing loss does not induce such a decline. Thus the decrease appears to be strain specific, and is exacerbated by deafening.

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195 KV3.1 Channel Expression in the MNTB of Old CBA Mice.

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The neurons of the medial nucleus of the trapezoid body (MNTB) are known for their ability to transmit high frequency phase locked information very precisely. Voltage-gated potassium channels are thought to be important in regulating their temporal properties. As part of a project examining age-related temporal processing deficits, we examined the expression of Kv3.1 channel proteins in the MNTB of 4 month-old and 24 month-old CBA/CaJ mice. Brain sections from old and young mice were reacted identically with an antibody against Kv3.1 (Alomone Labs) and images of the MNTB were digitally captured and analyzed using Image Pro4. Because Kv3.1 is expressed in neurons and neuropil we manually outlined the MNTB and calculated the mean optical density for the entire region.

The density of Kv3.1 staining was 76% less in the MNTB of old mice as compared to young mice. We are currently imaging individual MNTB neurons to determine whether there are age-related differences in the neuronal compartments expressing this channel. Because Kv3.1 has been shown to regulate spike duration in vitro, our results suggest that old mice may have longer spike durations and consequently lower maximum firing rates in the MNTB than young mice.

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196 Contralateral Suppression of Distortion Product Otoacoustic Emissions Declines with Age in CBA Mice

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The auditory efferent medial olivocochlear (MOC) system plays a critical role in modulating cochlear function at the outer hair cell (OHC) level. Prior researchers have shown that activation of the MOC system leads to suppression of cochlear activity. We previously described an age related decrease in MOC inhibitory function for distortion product otoacoustic emissions (DPOAE) in humans. We wish to extend this finding by studying aging effects on the MOC in CBA mice, a strain known to maintain auditory function into old age. The objective of this study is to measure age-related changes in the MOC efferent system in CBA mice by comparing DPOAE's generated with and without contralateral white noise stimulation. This contralateral noise activates the MOC and suppresses the magnitude of cochlear output. By studying contralateral suppression on cochlear output in mice of different ages, it is possible to describe aging effects on the MOC. Young, middle-aged, and old CBA mice were tested. DPOAE-grams were obtained with L1/L2=65/50 dB SPL, f1/f2=1.25, using 8 points per octave covering a frequency range from 5.6-44.8 kHz. Wide band noise (3-30 kHz bandwidth) was applied contralaterally at 55 dB SPL. Analysis revealed that DPOAE levels decreased with age, and that contralateral suppression declined in middle-aged and old groups relative to young subjects. This reduced MOC activity preceded measured declines in DPOAE and ABR levels. We conclude that these CBA mice findings parallel those of our previous human study. Namely, functional decline of the MOC-efferent system with age precedes OHC degeneration. Loss of MOC suppressive function may play an important role in the development of presbycusis. Contrasting these findings to mouse strains possessing distinct time courses of deafness (i.e., C57) should provide additional insights into age-related characteristics of the efferent system.

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197 Effects of Age and Sensorineural Hearing Loss on Contralateral Suppression of Distortion Product Otoacoustic Emissions in C57 Mice

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Previous studies have shown that contralateral noise activates the medial olivocochlear (MOC) efferent system and leads to suppression of outer hair cell (OHC) activity as measured by Distortion Product Otoacoustic Emissions (DPOAE). We have previously described functional declines with age of the MOC efferent system preceding OHC degeneration in humans and the CBA mouse model. The purpose of this study was to evaluate the effects of age on contralateral suppression of DPOAEs in early onset, age-related hearing loss using a C57Bl/6J (C57) mouse models. Starting in young adulthood, the C57 mouse displays an age-related hearing loss and decline in DPOAE activity, beginning in the high frequencies and progressing toward lower frequencies. This reflects age-related changes in cochlear function, especially the OHC system. Young adult and middle-aged C57 mice were tested. DPOAE-grams were obtained with L1=65 and L2=50 dB SPL, f1/f2=1.25, using 8 points per octave covering a frequency range from 5.6-44.8 kHz. Wide band noise (3-30 k Hz bandwidth) was applied to the contralateral ear at 55 dB SPL. DPOAE levels declined with hearing loss. Both young adult and middle-aged group exhibited little contralateral suppression of DPOAEs. We conclude that low levels of contralateral suppression of DPOAEs in young adult and middle-aged C57 mice may indicate dysfunction of the MOC system. Even young adult mice with normal hearing showed poor MOC function. Poor functional status of the MOC system in young adult C57 mice may be a precursor of early-onset age-related hearing loss. Further study is needed to elucidate the role of MOC dysfunction in human presbycusis.

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198 Age-Related Decrements in the Integrative Action of the Auditory Nervous System seen in the Acoustic Startle Reflex of the CBA Mouse

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We measured the time course of excitability in the auditory brainstem of mice by examining the strength of temporal integration of the acoustic startle reflex (ASR) to tone pips, S1 and S2, with varied stimulus onset asynchrony (SOA). As SOA increases ASR output increases to a maximum and then declines (Marsh et al., JCPP, 1973). Summation is initially offset by the refractory effect of action potentials (AP) in some cells activated by S1 and first increases as this effect decays, then later declines with the decay of subthreshold excitatory potentials (EPSP) in other cells. The duration of these decaying processes is thought to be determined by different families of outwardly rectifying potassium ion channels that return the cell to its resting potential following an EPSP or an AP: deficits in the former should lengthen the period of temporal summation, deficits in the latter delay its peak. Here the ASR was elicited by pairs of 12 kHz tone pips: 1 ms duration including 0.25 ms RT/FT, SOA from 0 to 10 ms (0.5 ms steps between 1 and 6 ms), and levels of 100 dB to 130 dB (SPL); in Young (2 mo., n =7), Middle aged (7 mo., n =12), and Old (24 mo., n =15) CBA/6J mice. Peak summation occurred at a longer SOA in old mice (median = 3.5 ms in O, 2.5 ms in Y and M, p =.002), and was less strong (at high levels, about 200% vs. 400%, p<.001). Post-peak summation persisted longer in old mice, the decay curve of younger mice dropping away from that of the old mice 2 ms after the peak (p<.003). These data indicate that the post-AP refractory effect persists longer in the old mouse (accounting for the later peak of two pulse summation), as does the subthreshold EPSP (this accounting for its

greater persistence). Both effects agree with our working hypothesis that ion channels responsible for restoring the resting potential following a momentary perturbation in acoustic input are less effective in the auditory brainstem of old mice.

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199 Hair Cell Loss and ABR Thresholds in Muted Mice

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Muted mice have missing otoconia in the saccule and utricle. This vestibular disorder is a recessive trait. Last year at ARO, Burkard and colleagues reported that these mice, at seven months of age, showed a selective inner hair cell (IHC) loss, with auditory brainstem response (ABR) thresholds that were ~10 dB higher than those of a group of age-matched CBA/CaJ mice. Interestingly, ABR thresholds and IHC loss were similar for the heterozygotes (mu/+) and the homozygotes (mu/mu). The present investigation evaluated ABR thresholds and hair cell loss in a group of muted mice across a wide range of ages.

Twelve 2-2.5, six 12 and two 18 month old muted mice served as subjects. ABRs were obtained to clicks and 3, 6, 12, 24 and 36 kHz tonebursts. For all stimuli, level decreased from 90 to 0 dB pSPL in 10-dB steps. Upon completion of data collection, mice were sacrificed, cochleas were harvested, and hair cell counts made.

One 2-2.5 month old animal showed elevated ABR thresholds and will not be considered further. The remaining mice (5 mu/mu, 6 mu/+) showed mean ABR thresholds that were quite similar to each other (mu/mu versus mu/+), and similar to ABR thresholds seen previously in the 7 month old mice. Cochleas showed little IHC or outer hair cell (OHC) loss. For the 12 and 18 month (all mu/mu) mice, ABR thresholds were substantially higher than seen for the younger mice. The cochleograms from the 18 month old mice (and informal inspection of some cochleas of the 12 month old mice) show moderate IHC loss in the basal half of the cochlea. These data demonstrate that IHC loss is progressive in muted mice, beginning sometime after several months of age.

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200 Expression by Acoustic Stimulation of Immediate-early Genes, *c-Fos* and *Arc*, in the Gerbil Auditory System

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Immediate-early genes represent the first wave of gene expression triggered by cellular stimulation and their protein's immunoreactivity can be used as an index of functional activity in the brain. Here we examined Fos- and Arc-like immunoreactivity elicited in the central auditory system of young, adult gerbils in response to acoustic stimulation. The stimuli used were 1 and/or 16 kHz pure tone bursts, 200 ms in duration, presented at a rate of 1 Hz. We used sound levels of 50 and 70 dB SPL, with stimulation times of 2, 10 and 15 minutes. Animals were maintained in silence in a sound-proof chamber for 1 hour before and after sound stimulation. Control animals were maintained in silence but not tone stimulated. Some brains were cut in the coronal or horizontal plane. Others were dissected and flattened-cortex sections were prepared. The sections were processed using Fos and Arc antibodies from Santa-Cruz Biotechnology. Fos-like immunoreactivity (FLI) was found in neurons at every level of the auditory pathway, from the cochlear nucleus to the auditory cortex. The number of positive cells was directly related to the intensity and duration of sound stimulation. The distribution of FLI cells also varied with sound frequency. In the auditory cortex, FLI cells were found mainly in the infragranular layers. Arc-like immunoreactivity (ALI) in the cytoplasm had a more restricted distribution than FLI and was observed only in the auditory cortex when high sound levels and long stimulation times were used. ALI cells were also found mainly in the infragranular layers, but were less numerous than FLI neurons. These differences in protein expression suggest that Arc may provide a powerful tool for investigating the role of the auditory cortex.

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201 Establishing Cytoarchitectronic Divisions in Ferret Auditory Cortex

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We aimed to establish cytoarchitectonic sub-divisions between primary and non-primary auditory cortex in the ferret using histochemical markers and tracer injections. Combining cytochrome oxidase (CO), neurofilament protein of medium and high molecular weight (SMI-32) staining and Nissl staining, it was possible to differentiate primary and non-primary fields in both coronal sections and a flattened cortex preparation. Heavy CO labelling was observed in primary areas, in the middle and anterior ectosylvian gyri (MEG, AEG), which was restricted mainly to layer IV. CO labelling in secondary areas, including posterior ectosylvian gyrus, was less intense and uniform across the layers. SMI-32 immunoreactivity showed a distinct bilaminar pattern in the MEG and AEG, with strong labelling of pyramidal neurons in layers III and V. In secondary auditory areas, only layer V pyramidal neurons had SMI-32 immunoreactivity. SMI-32 staining therefore appeared to distinguish clearly between primary and secondary auditory fields.

Injections of biotinylated dextran amine (BDA) in the primary fields resulted in terminal labelling with small boutons in the medial geniculate body, which was found mainly in topographically appropriate regions of the ventral division. Giant terminals were also observed in the dorsal division, homologous to those previously described in the cat (Bajo et al 1995). Labelled terminal fields were also present in the inferior colliculus (IC), mainly in its dorsomedial part. Retrograde tracer injections in this IC region labelled pyramidal cells in layer V of every auditory field. In conclusion, tracer injections show that areas delineated as primary cortex using histochemical markers make appropriate connections with sub-cortical structures.

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202 The Columnar Organization of Ferret Primary Auditory Cortex

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Using tungsten electrodes, extracellular field potential recordings were obtained within neuronal columns of the Primary Auditory Cortex (AI) of barbiturate-anaesthetized ferrets (Mustela Putorius), in response to monaurally presented acoustic clicks and simple tones. Starting from the pial surface the electrode tip was advanced downward in increments of 100 um to 1000-1500 um.

The analysis uses Current Source Density (CSD) methods to estimate the underlying laminar current sources, and Independent Component Analysis (ICA) to remove neural noise and to separate different sources and their independent time activation waveforms. Extended ICA compensates for super- and sub- gaussian waveform distributions by using natural gradient extensions, and thus appropriate for neural statistics.

The current-density components obtained suggest the existence of two distinct functional populations of columnar cortical neurons: One supra-granular, concentrated in the superficial layers I/III that gives rise to a strong polarity reversal at those layers, and another granular/infra-

granular residing in the middle cortical layers III/IV. The supra-granular population is characterized by short-latency/small-duration biphasic activation, whereas the granular/infra-granular one exhibits a mix of short/small-latency and slow/long duration excitation activations. The first activation pattern is typical of thalamic afferents, suggesting direct thalamocortical projections to the superficial cortical layers, whereas the latter suggests a mix of direct thalamocortical projections and slower polysynaptic cortico-cortical projections from the supra-granular neurons, as well as possibly within the granular/infra-granular population itself. These results indicate a vertical cascade of excitatory activation at the evoked potential onset.

203 Response Characteristics of Layer V Neurons in Rat Primary Auditory Cortex

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Layer V pyramidal cells comprise the major output of primary auditory cortex (A1). Two distinct pyramidal cell types can be distinguished by their morphology, connectivity, and in vitro physiology. One cell type is characterized by robust excitatory responses and is a primary source of corticofugal projections. The second type is characterized by strong inhibitory influences (which can be released by intracellular application of chloride channel blockers) and corticocortical and corticocaudate projections. It is presently unclear whether these two cell types can be distinguished by their extracellular responses to acoustic stimuli in vivo. Preliminary single unit data (N=46) from A1 in young adult anesthetized rats revealed two distinct layer V response types. Approximately 1/3 of the neurons demonstrated strong monotonic responses that resembled a slightly higher threshold pattern of response map observed for lower auditory structures. Response maps for these neurons (discharge-rate as a function of 40 stimulus frequencies and 18 intensities) showed low-side sloping V-shaped excitability, and were relatively unchanged across the three sequential response map repetitions run on each neuron. The remaining 2/3 of the neurons demonstrated a variety of response map patterns characterized by strong inhibition, including nonmonotonicity and sideband inhibition. In addition, best frequency for these units tended to vary from one repetition to the next and in some cases even changed from strong excitation to strong inhibition on successive response maps. The two general categories of response maps discussed here appear congruent with the two types of layer V neurons described in slice preparations.

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204 Response Properties of Neurons in Core and Medial Belt Auditory Cortex of Marmoset Monkeys

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The functional organization of the belt areas caudal and medial to the primary (core) region of auditory cortex in monkeys has not been the subject of intensive study. In a series of experiments to explore the architecture and connections of the medial belt areas in the common marmoset (*Callithrix jacchus jacchus*) (de la Mothe et al, *Soc. Neurosci. Abstr., 2002*), the response properties of neurons in these areas were also studied. Multiunit recordings were obtained under light isoflurane (4 cases) or intravenous ketamine infusion (3 cases). The stimulus battery consisted of the following: 1) pure tones to obtain frequency response area (FRA) (29 frequencies, 0.3 to 40 kHz; 8 intensities, -10 to 60 dB SPL, 50 ms duration); 2) broad band noise (BBN; -20 to 60 dB SPL, 10 dB steps; 50 ms duration); 3) FM sweep trains modeled after twitter calls (1, 2, 4, 6…24 Hz; 2.0 or 6.0 kHz start frequency, 267 kHz/s; 50 or 30 ms duration); 4) twitter calls (3 exemplars, courtesy X. Wang). Neurons in the core and belt areas were responsive to all stimulus categories. Tonotopic gradients in the caudal belt mirrored AI. Tuning bandwidth and response thresholds for tones were typically higher in the belt. Suppression of activity after the onset response was more often observed for belt neurons, especially for BBN and higher intensity pure tones. This suppression tended to reduce response synchrony to FM sweep trains and twitter calls, as did spectral mismatch between the FM stimulus and the FRA. Most clusters in the core exhibited little suppression, maintaining periodic responses for FM sweep trains through 24 Hz. The spectrotemporal response profiles of core and medial belt neurons were consistent with anatomical distinctions between these fields.

205 Functional Organization of Cortical Field AI in the Common Marmoset (*Callithrix jacchus*)

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The common marmoset is an attractive model for studies on the role of the primary auditory cortex (AI) in auditory perception and learning. The representation of sound frequency from about 1 -12 kHz is accessible on the lateral surface of the temporal gyrus, and marmoset vocalizations contain energy within that frequency range. Moreover, this species can be trained to discriminate auditory cues. To investigate sound processing in the marmoset AI, our approach in this study used pure tones to determine the topographical representations of the characteristic frequencies (CFs), the response thresholds at CF, the minimum latencies at CF and the bandwidths (BWs) of spectral receptive fields (SRFs). Tone pips (50 ms duration; 675 intensity-frequency combinations) were delivered free-field, and seven hemispheres in four monkeys were densely mapped using metal microelectrodes. Preliminary results showed a clearly defined tonotopy in each hemisphere, with low to high frequencies represented rostroventrally to caudodorsally on the lateral surface of the temporal gyrus. Topographies for response thresholds and excitatory bandwidths at 30 dB above threshold covaried with the CF gradient: Low CFs were associated with higher thresholds and broader BWs; high CFs were associated with lower thresholds and narrower BWs. The representations of minimum latencies varied across monkeys and apparently were independent of the other response parameters. Our results are consistent with findings observed in the cat, the squirrel monkey and the owl monkey, which suggest that a graded, distributed representation of important SRF characteristics is a basic organizing principle in the mammalian AI.

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206 Spatial Organisation of Response Properties in the Primary and the Caudomedial Field of Monkey's Auditory Cortex

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The response properties of neurons and their spatial organisation in the auditory cortex of monkeys are only partly known. For example it is well established that the best frequency (BF) is topographically distributed in primary auditory cortex (A1), while this is less clear for the caudomedial field (CM). This study aimed at finding differences between A1 and CM of primates.

We used a 7-electrode array and recorded at many sites multiunit activity and field potentials from upper layers of the auditory cortex in 4 anesthetised monkeys (Macaca fascicularis). At each site, response properties were measured by presenting pure tone bursts with frequencies from 0.1 to 32.0 kHz. The BF of a site was determined by finding the tone that elicited the maximal amplitude of the field

potential and the tone that elicited the maximal number of spikes. At most recording sites the BF of the field potential and the BF of the multiunit response were similar. In A1 and CM amplitude and latency of responses were in largely overlapping ranges. In A1 the BF was quite orderly distributed with a monotonic gradient that changed from lower values (0.1 kHz) to higher values (24 kHz) in rostrolateral direction. In CM the gradient was inverted and the distribution was less orderly. These findings suggest that different auditory fields are functionally specialised with respect to the topographic arrangement of response properties rather than by the range of response properties.

207 Tissue Reaction to Chronic Cortical Placements of University of Michigan Neuroprobes

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Silicon substrate, University of Michigan neuroprobes are being increasingly utilized for multichannel recording in the auditory pathways. There are still many challenges, however, for their use in chronic placements to enable recording the same neuronal populations over time. We have therefore examined tissue reaction at different times following a chronic placement of these probes in the guinea pig auditory or visual cortex. Recordings were made to verify placements and characterize site function over time. Histological assessments were made using plastic sections through the probe tract as well as immunocytochemistry on cryostat sections. Only a small rim of tissue reaction, typical of wound healing, was observed around the probe tracts. Normal appearing neurons were seen adjacent to the tracts. Vimentin (an intermediate filament marker for epithelial cells) immunoreactive (IR) staining was first to appear around the probe tract, forming the innermost layer of the tissue envelope around the probe shanks. This suggests that epithelial cells are either pushed down during the placement or migrate down from the meninges shortly after placement. A second layer of glia, marked by IR staining for glial fibrillary acidic protein (GFAP) forms shortly after and increased over the early course of placement, forming the second layer of the tissue envelope. A neuronal contribution, as marked by neurofilament IR staining, was an inconsistent feature of the tissue envelope, around less than 50% of the probe shanks assessed, also varying along the length of the probe shank. IR staining for the extracellular matrix protein, fibronectin was often seen in association with the probe tract. This is likely a product of the epithelial cells of the tissue envelope surrounding the probe. In some case it formed a "path" suggesting there was migration of the probe through the tissue.

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208 Imaging the Internal Architecture of Auditory Cortical Gray Matter in Living Humans

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An obstacle in relating structure to function in human auditory cortex has been the inability to anatomically delineate cortical areas in vivo, in individual subjects. Here, we are exploring ways to differentiate anatomical areas in living subjects using submillimeter MRI and 3D structural analysis techniques. Specifically, we examined several features of gray matter architecture (thickness, myelination and laminar structure) that are known to distinguish major divisions of human auditory cortex, but have previously been resolved only in postmortem tissue. Our results showed that gray matter thickness is low (~2.2 mm) on the posteromedial aspect of the temporal lobe (vicinity of primary auditory cortex), and increases laterally and anteriorly within non-primary areas (to ~2.6 mm; 9 subj.). Additional analyses of the gray matter in one subject showed changes in MR parameter T1 consistent with greater myelin content in the vicinity of primary auditory cortex than in more lateral non-primary areas. Examinations of gray matter laminar structure revealed a dense band of myelination (which likely corresponds to layer IV) in non-primary cortex of the superior-temporal gyrus. These patterns of gray matter thickness, myelination and laminar structure are broadly consistent with descriptions of primary and non-primary areas based on histological material. This suggests that it may be possible to delineate auditory cortical areas in vivo using classical anatomical criteria. Combined with functional neuroimaging, such delineations would enable cortical neuroanatomy and function to be related directly in individual humans in ways that have so far only been possible in animals.

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209 Is it Tonotopy after All?

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We report experiments on the functional organization of the human auditory cortex using functional magnetic resonance imaging. With an epoch-related design, consisting of alternating periods of auditory stimulation at four different frequencies and resting periods, we were able to demonstrate several frequency-dependent activation sites on the surface of the auditory cortex in individual subjects. We propose an interpretation of this activation pattern that differs from that of other recent fMRI studies. It is argued with regard to the results of electrophysiological and cytoarchitectonic studies in humans and in non-human primates,that the multiple frequency-dependent activation sites found in the present study as well as in other recent fMRI investigations are no direct indication of tonotopic organization of cytoarchitectonically defined areas. Differences in the response properties of medial compared to lateral and frontal compared to occipital portions of Heschl's gyrus (HG) strongly support this notion. The second aspect of the present study is a methodological and anatomical consideration. The individual gyral pattern of the superior temporal plane, especially the anatomy of Heschl's gyrus (HG), was found to be the major source of inter-individual variability. This variability represents a severe problem in studies of subject groups because one can neither get over it by the ubiquitous talairach transform nor by brain warping. We propose a new second-level analysis to account for the anatomical variability based on the idea that gradients of cortical activation strength are likely to follow individual anatomical landmarks. We tracked the frequency responsiveness to the four stimulus frequencies along individual Heschl's gyri. This procedure yielded medio-lateral gradients of responsiveness (which we termed frequency profiles) to high frequencies medially and low frequencies laterally.

210 Simultaneous High Density Evoked Potential Recordings and 3T fMRI Acquisition.

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The purpose of this investigation was to determine the feasibility of recording evoked potentials from a high density electrode montage while simultaneously performing functional magnetic imaging at 3 Tesla. Nine normal hearing adults participated in this study. Data were collected using a sparse sampling sequence. Thus, auditory stimuli were presented to one ear and evoked potentials were recorded from 128 scalp electrodes. Functional MR images were collected at a time empirically determined to correspond to the peak of the hemodynamic

response to the stimuli, and again once the response had dissipated. Results demonstrated that sufficient signal-to-noise is maintained in the functional images to localize activations produced by unilateral acoustic stimulation to the contralateral superior temporal plane. Analyses of the evoked potentials revealed both consistencies and differences with the fMRI data. Correspondence was observed in the location of activation for the hemisphere contralateral to the stimulated ear. However, the evoked potential data also showed evidence of a significant activation of auditory cortex ipsilateral to the stimulated ear, which was not observed in the fMRI data. These findings indicate that co-registered high-field fMRI and high density evoked potential recordings are possible and achievable. The data collected suggest that evoked potentials and fMRI are complimentary brain imaging techniques that when used in combination, improve our understanding of both timing and location of activity in the central auditory system.

211 Tonotopic Organization of The Auditory Cortex for High Frequency Tones

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It has been known that sounds activate specific areas of the cochlea according to their frequency. This tonotopy also exists throughout the auditory pathway up to the primary auditory cortex (PAC). In human beings, investigation was hindered by the fact that the techniques used to record the cortical activity were invasive. A breakthrough came with the development of non-invasive measurement techniques. Most of these techniques, however, only study the tonotopic organization of the PAC up to 6 kHz.

In this study we examine whether high frequency sounds (i.e higher than 6kHz and up to 12 kHz) are also organised tonotopically in 11 normal-hearing subjects using a 151-channel-whole-head magnometer. For each frequency, the dipole location, latency and amplitude of the N100m wave according to the side of stimulation and the hemisphere studied were studied.

We observed that various parts of the PAC do specifically encode low and high frequency sounds. The organization, however, differs according to the hemisphere studied and the kind of stimulation applied. Thus, following contralateral stimulation, the generators of the N100 m wave in the left hemisphere are located both more posteriorly and more medially with increasing stimulus frequency, whereas in the right hemisphere the generators are located more posteriorly but more superiorly with increasing stimulus fequency. Following ipsilateral stimulation, no clear tonotopic organisation could be observed in either hemisphere.

Furthermore, the amplitude of the N100m wave also varied according to stimulus frequency,

and the location of the N100n generators in both hemipheres differing slightly according to the side of stimulation.

Our findings suggest that the PAC is tonotopically organised from low to high frequency sounds in both hemispheres. N100m wave generators also seem to differ according to the kind of stimulation, which may indicate the existence of several tonotopic maps in the PAC.

212 Distinct Representations of Pitch Chroma and Pitch Height in the Human Brain

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Pitch is a complex percept that can be modelled as having two distinct dimensions: chroma and height. Pitch chroma (note letter in music) is a basis for acoustic patterns that do not depend on the particular sound source. In contrast, pitch height is a basis for the segregation of acoustic patterns early in the analysis of distinct sound sources. In this functional magnetic resonance (fMRI) experiment, we demonstrate distinct mapping of these two types of pitch change in the human brain: chroma change is specifically represented anterior to primary auditory cortex (PAC), while height change is specifically represented posterior to PAC.

213 Different Lateralization of Spectral and Virtual Pitch Processing in Human Auditory Cortex

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Complex harmonic sounds elicit a percept with a pitch that corresponds to the periodicity of the sound. This is still the case when a number of lower harmonics are not contained within the signal (= pitch of the missing fundamental, periodicity pitch, virtual pitch). However, the pitch percept may be ambiguous: depending on the subjects attention it may correspond either to the periodicity or the spectral content of the sound. Using fMRI we investigated the differences between spectral and virtual pitch processing in human auditory cortex (AC).

Sequences of three amplitude modulated tones (AM), where the spectral content was changed either in the same or the opposite direction as the periodicity, were presented to a total of 10 subjects. Subjects were asked to focus their attention either on the spectral or virtual pitch and had to report whether the pitch change of the AM sequence was upwards or downwards. Behavioral performance of all subjects was above 90% correct responses for both attentional conditions. Although the AC showed strong activations in both conditions, the spectral pitch condition showed significantly stronger activations in right secondary AC (Gyrus temporalis superior, Area 42, 22) and right Area 40, whereas the virtual pitch condition gave significantly stronger activations in left secondary AC (Area 42) and left Areas 20 and 21 (Gyrus temporalis medius and inferior). Note that the stimulus material was identical in both conditions. Therefore it seems that a top-down process, which may be reflected in additional activations we observed in several locations of left prefrontal and left limbic association cortex, selectively activates cortical areas responsible for spectral and virtual pitch processing, respectively. These activations are not only located in different hemispheres (spectal pitch: right, virtual pitch: left), but also include different cortical areas.

214 Representation of Complex Sounds in Auditory Thalamus, Primary and Non-Primary Cortex

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Little data exists on how spatiotemporal inputs engage the central auditory system particularly in the thalamus and cortex of non-specialist mammals. In this study, we characterized neural representations of the thalamus, primary auditory cortex (A1), and posterior auditory field (PAF) to broad classes of spatially and temporally distributed sensory inputs.

We systematically mapped thalamic and auditory cortical areas using extracellular recording technique under barbiturate anesthesia in adult rats to study the robustness of distributed responses to complex input signals. Our stimulus repertoire included a wide range of sounds with varying degrees of spectral and temporal complexity (e.g. tones, modulated trains of tones and noise, monkey vocalizations, & human speech sounds). In addition to traditional frequency tuning curve analysis, population PSTHs and neurograms were computed and compared.

The spatiotemporal discharge patterns of both thalamic and A1 neurons was related to the spectrotemporal acoustic input pattern. In contrast, PAF neurons exhibit a degraded representation of vocalization sounds and their variants. Response strength to vocalization stimuli was strongest in the thalamus, followed by A1 and then PAF. These experiments reveal the transformation of the spectral and temporal precision of MGB, A1, and PAF neurons responding complex sounds. These results will serve as control data for neural plasticity studies designed to examine how long-term sensory experience with spatiotemporal inputs alters thalamic and cortical responses.

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215 The Effects of Various Periodic Stimuli on the Envelope Following Response (EFR) from the Inferior Colliculus and Auditory Cortex of the Chinchilla

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Adult chinchillas were used to examine the effects of periodic stimuli on the Envelope Following Response (EFR) in the inferior colliculus (IC) and auditory cortex (AC) of the chinchilla. Ketamine/acepromazine was used to anesthetize the animals while tungsten electrodes were chronically implanted. After a recovery period, the chinchillas were put in a restraining device and placed in a sound-attenuating chamber. Stimuli were presented to the left ear, while recordings were made from the right IC and AC. Stimuli consisted of noiseburst (0 ms risetime; 2 ms plateau), 2kHz tonebursts (2 ms cosine-squared risetime; 1 ms plateau), and two-tone stimuli with F1 remaining constant at 2000 Hz and F2 varying between 2019.5 and 2312 Hz. Presentation rates of the noiseburst and toneburst stimuli varied between 19.5 and 312 Hz. Stimuli decreased from 80 to 40 dB SPL in 20 dB steps. Data was analyzed by recording both the overall RMS amplitude, as well as the spectral peak at the modulation frequency, and constructing a modulation rate transfer function (MRTF) for both of these measures.

In the IC and AC, overall RMS and spectral peak amplitude decreased as the intensity level decreased for all of the stimulus conditions. In addition, the greatest EFR amplitude was found for the noiseburst condition, followed by the 2 kHz toneburst condition, with the two-tone stimuli producing the smallest EFR amplitude. IC responses produced a bandpass MRTF, with a peak occurring at either 78 or 156 Hz. In the AC, using RMS amplitude, noiseburst and toneburst stimuli produced a low-pass function, while the two-tone stimuli produced a peak at 78 Hz for all stimulus levels. In the AC, spectral analysis revealed a clear peak in the MRTFs at 78 Hz for all of the stimulus conditions.

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216 The Effects of Nembutal Anesthesia on the Auditory Steady State Response (ASSR) from the Chinchilla Inferior Colliculus and Auditory Cortex

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In the present study, adult chinchillas were used to examine the effects of sodium pentobarbital on the amplitude of the auditory steady state response (ASSR) in the inferior colliculus (IC) and auditory cortex (AC) of the chinchilla. The ASSR is a response that follows the envelope of the stimulus. Tungsten electrodes were chronically implanted following anesthesia with ketamine/acepromazine. After a recovery period of approximately 1 week, the chinchillas were placed in a passive restraining device and put in a sound-attenuating booth. Recordings were made from the right IC and AC simultaneously, while a two-tone stimulus was presented to the left ear. The stimuli consisted of two equal-level tones (F1 and F2) that were mixed acoustically; F1 remained constant at 2000 Hz, while F2 varied between 2029 and 2249 Hz, in steps of ~20 Hz. The stimuli decreased in 10 dB steps from 80 to 30 dB SPL.

In the IC, sodium pentobarbital caused an increase in ASSR amplitude at difference tone (DT) frequencies at or below 90 Hz, while a decrease was seen at DT frequencies at or above 90 Hz. For any given stimulus level, modulation-rate transfer functions (MRTFs) showed a bandpass function. Statistical analysis revealed a significant increase at two of the DT frequencies below 90 Hz, and a significant decrease at several of the DT frequencies above 90 Hz. In the AC, a decrease in amplitude was seen for all of the DTs and at all stimulus levels. Generally, the MRTFs showed a low-pass function. Statistical analysis revealed a significant decrease at all of the intensity levels and several of the frequencies.

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217 Modulation Spectra of Natural Sounds.

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We analyzed the joint temporal and spectral statistics of natural sounds by calculating their modulation spectra. These modulation spectra are obtained by calculating the two-dimensional Fourier transform of the auto-correlation matrix of the sound stimulus in its spectrographic representation. Since time and frequency are conjugate variables in a spectrographic representation of sound, we show that that joint modulation spectrum of sound occupies a restricted space and sounds cannot have rapid temporal and spectral modulations simultaneously. Within this restricted space, we show that natural sounds have a characteristic signature. Natural sounds in general, are low-passed showing most of their modulation energy for low temporal and spectral modulations. Animal vocalizations and human speech are further characterized by the fact that most of the spectral modulation power is found only for low temporal modulation. We postulate that the auditory system could exploit this statistical structure to obtain an efficient neural representation of sound. To test such a hypothesis we create synthetic sounds with identical first and second order statistics as natural sounds.

218 Tuning for the Spectral and Temporal Modulations of Song in Male Zebra Finches: Responses of Single Auditory Neurons in the MLd, Field L and cHV to Complex Natural and Synthetic Stimuli.

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In songbirds, specialized forebrain areas that control song learning and production contain auditory neurons that respond preferentially to a bird s own song. Lower in the auditory processing stream, neurons in the forebrain region, Field L, show stronger responses to conspecific song than to simple synthetic sounds with matched power spectra. We studied the auditory response properties of neurons in the mesencephalicus lateralis dorsalis (MLd), Field L and the caudal ventral hyperstriatum (cHV) to determine if selectivity for the spectral and temporal modulations of song exists and if it develops hierarchically from midbrain to higher forebrain. In male zebra finches, we recorded the extracellular responses of single neurons in MLd, Field L and cHV to conspecific song, dynamic ripples containing only the spectro-temporal modulations found in song but no phase information (song ripples) and dynamic ripples containing all spectro-temporal modulations (flat ripples). Responses were analyzed by calculating spike rates and Spectro-Temporal Receptive Fields (STRFs). Results suggest that, across all cells, mean spike rates do not differ to the 3 stimulus types. Analysis of STRFs for individual cells suggests that a subset of neurons in all 3 auditory regions shows similar Receptive Fields in response to song and song ripples but different Receptive Fields in response to flat ripples. Responses to these complex stimuli become progressively more nonlinear, more shaped by inhibition and less temporally precise through the hierarchy. Findings suggest that some neurons in all 3 auditory regions MLd, Field L and cHV are selective for the spectro-temporal modulations found in conspecific song.

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219 What does Precise Spiking in AI tell us about the Structure of its Receptive Fields?

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Several recent investigations of cortical response characteristics have demonstrated that most cells are unable to follow rapidly changing stimuli (> 30 Hz) that evoke strong responses at all pre-cortical stages. Yet, these same cells are capable of producing precisely timed spikes (with millisecond accuracy) at stimulus onset and other instants throughout the stimulus. In this study, we explored this paradox using specially designed ripple stimuli that resemble broadband frozen noise with a spectro-temporally modulated envelope. The stimuli are constructed by adding hundreds of closely-spaced carrier tones with a slowly drifting spectro-temporal profile. Using such ripples (or combinations of ripples), it is possible to characterize the modulation transfer functions of auditory cortical cells, and to measure their Spectro-Temporal Response Fields (STRFs). Using these same stimuli, it is also possible to observe the "fine structure" of the responses due to the carrier tones or their interactions. We shall demonstrate in this report the existence of this "fine structure". Specifically, we demonstrate that some AI responses are locked to the stimulus carrier with millisecond accuracy. This is likely to reflect auditory-nerve responses to the interactions among the carrier tones of the ripples. The results also suggest that the slow dynamics of the STRFs are an emergent property of cortical circuits, especially its inhibitory inter-neurons.

220 On-Line Spectro-Temporal Receptive Fields in the Primary Auditory Cortex of the Behaving Ferret

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The auditory system naturally performs a wide variety of analyses on incoming sound from the acoustic scene, and depending upon the behavioral context or salient cues in an auditory task, can adapt cortical neuronal response properties to optimize its performance. In order to understand the role of dynamic change in auditory cortical function, and its contribution to behavioral adaptive plasticity, it is important to describe the magnitude and timecourse of these adaptive changes. We designed a behavioral physiology paradigm to capture possible changes in response properties by creating tasks in which the measurement of the receptive field properties of the neuron occurs simultaneously with task performance. We compared the cortical spectro-temporal receptive fields (STRFs) in the awake but non-behaving (passive) ferret versus STRFs measured while the ferret performed either a "temporal" or a "spectral" auditory discrimination task. In our physiological experiments, following isolation of an auditory responsive cortical neuron, information was initially gathered in a passive stimulus presentation condition and an STRF was derived using ripple stimuli. Neuronal responses to the same stimuli were then measured in an active behavioral condition. In the spectral discrimination task, ferrets were trained to detect a tonal target against a background of reference ripple sounds. In the temporal discrimination task, ferrets were trained to discriminate between reference ripples and ripples with silent gaps. We shall illustrate the shapes of the STRFs derived under the two behavioral conditions and discuss their possible functional significance.

221 Onset and Steady-State Spectrotemporal Sensitivity of Neurons in Cat Primary Auditory Cortex.

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The frequency-tuning curve is a static representation of the neuron's sensitivity to stimulus frequency. Excitation and inhibition, which determine the tuning properties, are dynamic activities, whose onset and duration depend on synaptic properties. The temporal aspects of the frequency sensitivity can be captured in the spectro-temporal sensitivity (STS) for discrete single- or multi-frequency stimuli. STSs can equally well be presented as the average PSTH following a specific tone pip or as the average spectrogram of tone pip preceding a spike. The temporal envelope of the stimulus ensemble tends to produce smoothing, as a consequence the PSTH representation is more fine-grained than the spectrogram representation. Here we compare STSs for 1/s and 20/s single-frequency stimuli and for on average 20/s steady-state multi-frequency stimuli (randomized chords) for 87 recording sites in primary auditory cortex of cats, and assemble these in frequency-time tuning curves. In 35% of the recordings the excitatory frequency-tuning curves were very similar for single and multi-frequency stimuli, in the remaining 65% the most common finding was an intensity independent bandwidth for the multi-frequency stimuli. Part of this did result from the higher stimulus repetition rate for these stimuli. The minimum response latencies increased by less than 5 ms for repetition rates from 1/s to 20/s single frequency stimuli and considerably more for the multifrequency stimuli (5-15 ms). In 672 estimated STSs, for multi-frequency stimuli mostly presented at 55-65 dB SPL, we found lateral inhibition combined with post activation suppression in 24% of the cases, post-activation suppression only in 51 % , and in 25% only excitation. We use this comparison to estimate the roles of adaptation and lateral inhibition in the responses of cortical neurons, and to classify cortical neurons into distinct groups.

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222 Spectro-Temporal Features of the Auditory Cortex: the Activation in response to Dynamic Rippled Noise

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The human brain is well trained to process speech, containing broad spectro-temporal modulations. Some studies indicate that characteristics of spectro-temporally modulated noise are topographically mapped onto the cerebral cortex. Yet little is known about the function of the different fields in the auditory cortex. In this experiment functional MRI was used to investigate the activation induced in the human brain by dynamic rippled noise.

Nine subjects with normal hearing were included. Auditory stimuli consisted of 3.5 s rippled noise bursts with a dynamic spectral profile. Four stimulus parameters were varied systematically (modulation frequency, density and amplitude; and drift direction), and responses in the auditory cortex were measured. Per subject a total of 240 MRI scans was acquired using a sparse T_2* EPI-sequence. *T*-test statistics were performed to test whether rippled noise led to more activation than the pink noise baseline condition ($p < 0.001$).

fMRI is able to discriminate between the activation caused by pink noise and rippled noise stimuli in the perceptually important range. All subjects showed clear activation bilaterally in the superior temporal cortex, showing multiple fields of activation that possibly correspond to the location of functional fields. The extent and level of the activation strongly depended upon the ripple parameters, in agreement with psychoacoustical measurements. Furthermore, we found topographic mappings of the optimal modulation density and frequency onto the auditory cortex, indicating a functional hierarchical organization of the

auditory cortex; phonetic spectral features being mainly extracted in secondary cortex, and acoustic features in primary cortex.

223 Auditory Cortical Processing of Complex Time-Varying Stimuli

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A novel acoustic stimulus with parametrically varying temporal structure is introduced to study time-dependent auditory cortical processing. Stimuli were nine seconds in duration, and comprised of segments consisting of a sum of sinusoids with randomized amplitude, phase, and frequency components drawn from a Gaussian distribution. Six conditions were created by choosing Gaussian distributed segment duration means of 12, 25, 45, 85, 160, and 300 ms. Manipulating the starting (f1) and ending (f2) frequency of each segment, and the segment 'offset' relative to its neighbor within a half-octave range (1000-1500 Hz), produced three qualitatively different types of stimuli: 1) 'Constant;' f1 = f2 with no offset between segments, 2) 'Tonal;' f1 = f2 with Gaussian distributed offsets, and 3) 'FM;' f1 swept linearly (and randomly) upwards/downwards to f2 over the entire half-octave range. All three stimulus types possess the same RMS power, and power spectral density (over the nine-second stimulus duration), permitting analysis of explicitly temporal processing. A single-trial event-related functional magnetic resonance imagining (fMRI) design was employed. Analysis consisted of categorical contrasts between each of the FM and Tonal conditions and the Constant condition. Results show that 1) activation increases with increasing segment duration, 2) there were no significant differences between FM and Tonal responses, 3) areas STG and superior aspect of MTG (STS) were the primary contributors to the activation, and 4) different patterns of responses were exhibited in STG and MTG.

224 Models of Population Dependence in Auditory Cortex

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We have shown previously the importance of considering the impact of correlated noise on the location of sound sources by an ensemble of AI cortical neurons (Jenison, JASA, 107: 414-421, 2000). Generally, the concept of correlation has become almost synonymous with the concept of dependence. Indeed, correlation does provide a correct and complete picture of the underlying dependence structure for multivariate Gaussian models. However, for non-Gaussian models (i.e. non-elliptical distributions), correlation only conveys partial and often misleading information on the actual underlying dependencies. It is often the case that probability distributions other than the Gaussian are necessary and appropriate to realistically capture the stochastic nature of single neuron behaviors. In such cases, the construction of a flexible model of covariance is typically not straightforward. In order to solve this problem, we show here how appropriate probability densities can be coupled to allow greater flexibility in the construction of multivariate neural population models. Dependence relationships between cortical neurons can be factorized into so-called "copulas", whose impact on neural coding of spatial location can be directly analyzed using mutual information.

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225 A Biologically-Plausible Code for Auditory Space Using Relative Spike Times in Cat Auditory Cortex.

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In cat auditory cortex, response latencies relative to stimulus onset convey significant amounts of information about the locations of sound sources. Recent work in our laboratory shows that latencies in cortical field PAF are particularly sensitive to stimulus location−varying by 20 ms or more across space, compared to ~4 ms in A1 and ~9 ms in A2. Nevertheless, latencies relative to stimulus onset would be of no behavioral or perceptual value without an external reference to the time of stimulus onset. Here, we test a more biologically-plausible hypothesis, that stimulus-related information is conveyed by the relative timing of spikes between neurons.

We recorded simultaneously from pairs of neurons in PAF and/or A1. 80-ms noise bursts were presented from loudspeakers arranged in azimuth and elevation; responses were converted to distributions of between-unit interspike intervals (ISIs). We assessed the mutual information of ISI and stimulus location using a pattern-recognition algorithm, and found that pairs of units within PAF transmitted significantly more information than pairs of A1 units. Between-field pairs (i.e., one neuron in PAF and one in A1) transmitted significantly more information than pairs within either field alone. The results can be described using a simple model in which PAF, but not A1, latencies are magnified by early inhibitory processes. Although latencies in both areas are modulated by stimulus location, the smaller range of A1 latencies provides a relatively stable reference to which the magnified latencies of PAF can be compared. The model is general and predicts similarly effective latency codes for other parameters related to stimulus effectiveness, such as frequency and level.

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226 Responses to Binaural Beat Stimuli in the Primary Auditory Cortex

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Binaural beats, generated by presenting tones of different frequency to the two ears (e.g. 800 and 801 Hz), can be used for investigating sensitivity to the full range of interaural phase differences (IPDs). The frequency difference corresponds to the beat frequency and below 4-6 Hz can evoke the sensation of sound movement in humans. Here binaural beats were presented in a closed sound system to guinea pigs anaesthetised with a ketamine/xylazine mixture. In the primary auditory area (A1) 152 multi-unit clusters with characteristic frequencies of < 1.2 kHz responded to binaural beats with a rhythmic firing pattern synchronised to the beat. For 89 of these units the mean IPD was measured for a range of different tone frequencies. The resulting phase plots were similar to those recorded at the midbrain and included both linear (66) and non-linear (23) examples. The linear units had characteristic delays of -850 to 1210 μs (mean 160 μs) with half (33/66) within the physiological range (<300 μs). There was no evidence of units with a similar characteristic delay being located in cortical columns. Inverting the frequency difference at the ears reverses the direction of the apparent motion. For most units (96/141) reversing the direction made little difference to the vector strength, but for others (45/141) there was at least a two-fold change. The asymmetries in the responses to the two directions of beats appear to represent adaptation effects and do not necessarily imply that the units are involved in motion detection. There was no evidence of direction-sensitive units being located in a specific layer or within columns.

227 Binaural Interaction in the Amplitude Modulation Following Response (AMFR)

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Amplitudes of auditory evoked responses typically differ for monaural and binaural stimulation. These differences range from inhibition of the amplitude of a response by addition of a contralateral stimulus to a larger amplitude for binaural stimulation than that for the sum of two monaural stimuli (i.e., the binaural interaction component or BIC).

Responses generated in the brainstem tend to show a BIC whereas inconsistent results have been reported for more centrally-generated resposes such as the middle latency and late auditory responsese. The amplitude modulation following response (AMFR) is a steady-state response elicited with amplitude-modulated tones or transients presented at specific rates. Here, we examine the effects of binaural stimulation on the AMFR recorded from human subjects with normal hearing. Stimuli were clicks (1-ms duration) presented at rates of 20 or 40/second. Stimuli were presented monaurally or binaurally. Binaural stimuli were presented either simultaneously to the two ears (diotic) or with a delay of between the ears (dichotic). The delay was 25 ms for the presentation rate of 20 clicks/s and 12.5 ms for the rate of 40 clicks/s. For monaural presentations, the largest responses occurred for presentation rates of 40 clicks/s. A small 40-Hz response component was observed in the monaural presentation of 20 clicks/s. No BIC was observed for diotic stimuli presented at 20 or 40 clicks/s. Dichotic presentation at 20 clicks/s elicited a 40-Hz response which was equal in amplitude to that elicited by monaural presentation of 40 clicks/s. Thus, the auditory cortex, the probable generator of the 40-Hz AMFR, is capable of producing a robust 40-Hz response with either monaural stimulation or binaural stimulation which adds to 40 Hz.

228 Binaural Interaction in the Human Auditory Cortex Revealed by Neuromagnetic Frequency-Tagging: Effects of Stimulus Intensity

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Frequency-tagging associated with magnetoencephalographic (MEG) recordings has been recently introduced as a new tool to study contributions of each ear to binaural interaction in the human auditory cortex (Fujiki et al., J Neurosci 2002, 22, RC205: 1-4). As the method has potential for assessing plastic changes in patients with unilateral hearing deficits, we studied 10 healthy adults to find out effects of stimulus intensity on binaural interaction. Cortical auditory steady-state fields (SSFs) were measured with a 306-channel whole-scalp neuromagnetmeter to stimuli presented monaurally or binaurally at 45, 60 and 75 dBSL. Amplitude-modulated sounds (carrier frequency 1 kHz) were presented with modulation frequencies of 39.1 Hz to the right ear and 41.1 Hz to the left. During binaural listening, SSFs of both hemispheres were suppressed more to ipsilateral than contralateral input. Compared with monaural stimulation, the contra/ipsilateral-hemisphere ratio of SSFs increased to both inputs ($p < 0.01$ to the left-ear input, $p < 0.001$ to the right). Thus, the hemispheric contralaterality was enhanced during binaural listening to inputs from both ears. Similar patterns of binaural interaction were observed at all 3 stimulus intensities. We hope these data to be useful in examining patients who suffer from auditory disorders as well as in revealing basic mechanism of human auditory processing.

229 Spatial Hearing in Patients with Acquired Brain Lesions affecting Primary Auditory Cortex

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Different studies yielded controversial results regarding the principle role of primary auditory cortex (PAC) in spatial hearing. In animals, cortical single units with panoramic response characteristics and peak responses located in the contralateral hemifield were recorded. In neuropsychology, the two prevailing hypotheses on auditory space perception suggest dominant representations of the contralateral hemifields or a general right-hemisphere dominance. However, most of the evidence stems from lateralization, i.e. headphone experiments, or from studies in patients with lesions of higher cortical areas. In this study, we aimed at the functional significance of PAC in sound localization. We investigated the ability to spatially segregate auditory events in patients with acquired brain lesions by measuring minimal audible angles in the horizontal plane. We applied an adaptive three-alternative forced-choice paradigm, proven suitable for working with neurological patients. Patients with lesions affecting left or right PAC were tested with different reference directions and using stimuli of different spectral composition. The patients' thresholds critically depended on the site of lesion. Compared to data from control subjects, spatial resolution was severely impaired in patients with lesions affecting right PAC, while the performance of patients with left-hemispheric lesions tended to be poorer in the right hemifield, if impaired at all. These results support the functional importance of the right PAC in human spatial hearing, and at the same time give evidence for the hypothesis of contralaterally dominant representations at PAC level.

230 Gradual Adaptation to Shifts in the Peripheral Acoustic Frequency Map

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It is generally accepted that cochlear implants (CI's) impose a basalward shift to the acoustic input, that is, sounds stimulate neurons with higher characteristic frequency than the acoustic frequency of the original stimulus. This frequency misalignment may have a negative influence on speech perception by CI users. However, a perfect frequency-place alignment may result in the loss of important low-frequency speech information. A trade-off may involve a gradual approach: start with correct frequency-place alignment to allow listeners to adapt to the spectrally degraded signal first, and then gradually increase the basalward shift to allow them to adapt to it over time.

Two pairs of normal hearing listeners underwent 15 hours of speech perception training and testing using a real-time acoustic model of a CI. This 8-channel model simulated different amounts of basalward shift. Subjects were randomized either to the “gradual” group (who were gradually exposed to a 6.5 mm basalward shift over the course of ten sessions) or to the “fixed” group, who were exposed to the full 6.5 mm shift since the beginning. Both groups underwent 15 1-hour sessions using audiovisual speechtracking as well as vowel, consonant and sentence recognition tests. For the second pair of subjects, three fMRI recordings were conducted at the beginning, middle and end of the study to assess changes in cortical activation in response to the CI acoustic simulations with 6.5 mm shift.

Speech perception scores were initially much higher for the “gradual” group, but by the end of the 15 sessions the “fixed” group had almost caught up with them. Imaging results suggest that the behavioral adaptation shown by increasing speech perception scores was paralleled by increases in cortical activation of language areas. These results suggest that the "gradual" method may result in faster speech perception improvement by CI users than the "fixed" method currently used in clinical practice.

231 Acoustic Simulation of Binaural Cochlear Implants: Effects of Spectral Mismatch and Speech Levels

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While normal-hearing listeners may take advantage of binaural cues for recognition of speech in noise, cochlear implant listeners may be unable to do so because of differences between electrode locations, auditory nerve survival and signal processing in each ear. The present study explores the effects of spectral mismatch and signal level difference on speech recognition by normal-hearing subjects listening to a simulation of binaural cochlear implants. For each ear, speech and speech-shaped

noise were mixed and vocoder-processed into 4, 8, 16 or 20 channels to simulate various degrees of spectral resolution. Sinusoidal carriers were used and assigned to simulate basally-shifted, matched or apically-shifted electrode locations, relative to the normal frequency-to-place mapping. Baseline speech recognition thresholds (SRTs) were measured for monaural and binaural presentations of all multi-channel processors at the matched cochlear location. Binaural SRTs were slightly better than monaural SRTs for the 8-, 16- and 20-channel processors. Binaural SRTs were then measured by varying the relative cochlear location of stimulation, while matching the presentation level between the two ears. Scores were similar for all conditions, except when basally-shifted speech was presented to both ears. Subjects attended to the ear with the least shift in spectral information. Binaural SRTs were also measured for basally-shifted speech in one ear, and apically-shifted speech in the other; however, the presentation level of the apically-shifted speech was varied relative to that of the basally-shifted speech. Even when the presentation level of the apically-shifted speech was far below that of the basally-shifted speech, listeners continued to attend to the apically-shifted speech. These results suggest that spectral mismatches between two cochlear implants may reduce any binaural advantages, as listeners may attend only to the best-matched implant.

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232 Some Effects of Electrode Location on Cochlear Implant Function

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A principal strategy for improving function of cochlear implants involves positioning electrodes near the modiolar wall and thus, presumably, as close as possible to auditory neurons. Beneficial effects of this strategy have not been completely defined or clearly demonstrated. This study is a part of a series aimed at understanding the functional effects of electrode location using psychophysical and neurophysiological studies in guinea pigs. The current experiment used six-electrode banded animal implants (Nucleus, Ltd.). When inserted in the basil turn of the scala tympani through a cochleostomy, the apical end of this implant tended to lie near the modiolus while the basil end lay near the lateral wall of the scala tympani. The current study assessed psychophysical detection thresholds for stimulation at the apical and basal ends of the implant under various stimulation parameters. For short-duration pulses, thresholds for stimulation at the apical end of the implant were consistently lower than those for stimulation at the basal end. Magnitudes of the apical-basal difference were similar to those for across-site variation in human subjects. The magnitude and pattern of across-site variation observed depended on stimulus parameters. The apical-basal threshold difference was greater for tripolar and bipolar stimulation than for monopolar stimulation in most cases. In addition the pattern for 100 Hz sinusoids was different from that for short-duration pulses. These data are consistent with models that account for across-site variability of thresholds in terms of distance between the electrodes and the site of neural excitation, but the site of excitation may depend on stimulation parameters. Future studies will assess other functional correlates of these across-site differences.

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233 Electrode Placement Affects Current Paths in the Implanted Cochlea

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This study explored the influence of reference electrode placement on the current and potential-field distributions generated by a cochlear implant. A Clarion HiFocus electrode was studied under different conditions. First, the effects of different reference electrode placements on potential field and current distributions were evaluated in cochlear models. Then, the HiFocus electrode was implanted in a human temporal bone. The reference electrode was placed either outside of the temporal bone, simulating a clinical cochlear-implant configuration, or within the modiolus.

Cochlear-model results show clearly that voltage and current distributions are greatly influenced by reference-electrode placement. Temporal-bone data demonstrate that when the reference electrode is placed within the modiolus, current flow is facilitated through the modiolus, where the target auditory neurons are located, rather than shunted through scala tympani. Voltages recorded in the modiolus are 3-5 times higher than those recorded with the reference electrode placed remotely outside of the temporal bone. These findings suggest that if the reference electrode is located in the modiolus, the power required to activate auditory neurons may be significantly lower compared to the power requirement with the reference electrode located remotely. Reduction of power consumption may increase battery life, and minimize cochlear damage resulting from prolonged electrical stimulation.

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234 Electrode Discrimination of „Place-Pitch“ with Deeply Inserted Electrode Arrays and Its Relation to Speech Recognition

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Electrode discrimination ability (DA) was measured in twelve cochlear implant patients implanted with the MED-EL Combi 40+ implant device. The MED-EL Combi 40+ electrode array provides an especially deep insertion in the cochlea and a wide spatial separation of the stimulating electrodes of 2.4 mm. In a 2 AFC procedure three reference electrodes located in the basal, middle and apical region of the cochlea were compared with four respective surrounding electrodes. Each condition was tested at three different pulse rates, 1515, 500 and 250 pps. The influence of electrode region in the cochlea, spatial separation of the electrodes and stimulating pulse rate on DA was investigated. The DA varied considerably among listeners. However, the overall DA revealed that the position of the electrodes in the cochlea did not influence the subjects' performance. Further, DA increased with increasing spatial separation. The DA results show no dependency on stimulation pulse rate. DA was correlated with several parameters as speech recognition scores, the duration of implant use, auditory deprivation, range of the electrical stimulation level between hearing threshold and maximum comfortable level (dynamic range), maximum comfortable level and the pulse duration. Correlation of DA was found with three different speech recognition scores and as high as 0.89 for speech understanding in noise. A correlation of DA with dynamic range was also shown. The results of this study indicate that residual spiral ganglion cells in the apical region of the cochlea even close to the helicotrema are sensitive for pitch changes provided by different places of electrical stimulation. The ability of MED-EL Combi 40+ users to differentiate among stimulation to different electrodes in terms of the perception of the evoked pitch is a prerequisite to perceive the spectral fine structure of speech.

235 Intensity Discrimination Using Bipolar and Monopolar Electrode Configurations in Nucleus Contour® Cochlear Implants

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Intensity discrimination is vital for perceiving amplitude modulations in critical environmental stimuli such as speech. Previous investigations have shown that the ability of implant users to discriminate changes in

current level improves with the increasing level. Broader electric fields, which occur at higher current levels, activate more auditory neurons. This might account for listeners' abilities to discriminate smaller changes in current level. It is hypothesized that monopolar (MP1+2) electrode configuration will excite more neurons and therefore yield smaller intensity discrimination thresholds than bipolar (BP) configurations at a given loudness level. The ability of listeners using Nucleus CI 24RCS contour implants to discriminate differences in current level was investigated using the following independent variables: a) electrode configuration (BP and MP1+2), b) longitudinal electrode placement (sites 8 and 16) and c) reference stimulus level (5, 15, 25, 50 and 80% of the dynamic range). The ability to discriminate changes in current level improved as a function of reference level except for cases in which performance was exceptionally good. In this case, a floor effect was observed due to the minimum current step in the prostheses. No consistent effect of electrode configuration or longitudinal electrode placement was found. Extensive differences were observed within listeners relative to specific sites and configurations. In future studies, these data will be compared to human and guinea pig psychophysical measures and physiological measures in guinea pigs in an effort to determine the mechanisms underlying the effects of current level and electrode configuration.

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236 Channel-Interaction With Dynamic Stimuli in Cochlear Implant Listeners

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A modulation-masking paradigm is being used to measure interactions between dynamic signals on two channels of a cochlear implant. Carrier stimuli on each channel are 500-Hz trains of biphasic current pulses at 50% of the dynamic range. The "signal" stimulates a fixed middle electrode location. The masker is presented to different electrodes in turn. Masker and signal pulses are interleaved in time. The subject's task is to detect SAM at 50 Hz in the signal carrier, in the presence of a competing masker. The masker envelopes are (all dynamic envelopes have a fixed 20% fluctuation depth): SAM at 20, 50, or 125 Hz; SAM at 50 Hz but out-of-phase with the signal SAM; Noise; Steady-state (SS); Steady-state at the peak amplitude of the dynamic envelopes (SSpeak). We find that modulation thresholds can be significantly higher with the dynamic maskers than with the SS and SSpeak maskers. We define the dB difference between modulation thresholds obtained with the dynamic maskers and the SSpeak masker as the "envelope" component of channel-interaction. This component can be as large as 10 dB and does not necessarily peak at the signal electrode location: in several cases, it reaches a minimum at the signal location. There is a strong dependence on the masker envelope. The 20 Hz modulated masker is very effective at masking the 50 Hz modulation, often more so than the 50 or 125 Hz modulated masker: possibly this dependence is related to the lowpass modulation transfer function. When the masker and signal are modulated at the same rate, their phase relationship has a strong effect on the results. Compared with forward-masking patterns obtained in the same subjects, these results suggest that channel-interaction can be significantly altered by introducing dynamic elements into the stimuli.

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237 The Relationship between Electrophysiologic and Psychophysical Measures of Spatial Spread in Cochlear Implants

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Recent electrode designs have focused on placing the electrode array closer to the modiolus in an attempt to reduce overlap of spatial excitation patterns that result in channel interaction. Spatial excitation patterns can be measured both electrophysiologically and psychophysically; therefore these measures should be related. The goal of this study was to examine the relationship between electrophysiologic measures of spatial selectivity and the ability to discriminate between electrodes in a psychophysical task.

Adult subjects implanted with either the Nucleus 24M straight array or the Nucleus 24R(CS) Contour array participated in this study. For the electrophysiologic portion of the study, the electrically evoked compound action potential (ECAP) was measured using Neural Response Telemetry (NRT). ECAPs were measured with a probe pulse fixed on a given electrode while a masker pulse was sequentially applied to all 22 intracochlear electrodes. The resulting function provided a measure of spatial selectivity in the cochlea. For the psychophysical portion of this study, subjects were asked to discriminate between two electrodes on the basis of pitch. The gradient of the psychometric function was then compared with the amount of channel interaction as measured using the ECAP.

Data collected to date show a moderate to weak correlation between the slope of the psychometric function and the amount of spatial selectivity in the cochlea. Electrodes that demonstrated more restricted spatial selectivity tended to yield better discriminability relative to adjacent electrodes. In addition, electrode discrimination ability and amount of spatial selectivity varied across electrodes within the array.

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238 Prospective Evaluation of an Actuarial Procedure for Determining Criteria of Candidature for Unilateral Cochlear Implantation in Post-lingually Deafened Adults

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Some candidates for cochlear implantation (CI) obtain benefit from acoustic hearing aids. They need to know the likelihood of achieving a better outcome with an implant. We developed an actuarial procedure to make such predictions. Patients (n=376) were recruited who had used an implant for >9 months. Their accuracy of identifying spoken words was measured with audio recordings of BKB sentences. Patients were ordered by duration of profound deafness before CI *(D)*. A window was placed to include the first 150 patients and their median value of *D* was noted. These patients were re-ordered by BKB score and labelled with their percentile rank *(r)*. The data were re-plotted as the relationship between each patient's score *(S)* and the odds *(O)* that a randomly selected patient would exceed that score, calculated as $O=(100-r)/r$. The relationship was described with the function $O=x_0((100/S)-1)^{1/b}$ (Eqn. 1). The window was advanced in steps of 37 patients and the analysis was repeated to form a sequence of values of D, x_0 and b. Polynomial functions in D were fitted to x_0 and b, and were substituted in Eqn. 1, creating a formula which calculates the odds of exceeding a pre-operative score, given the duration of deafness of the ear to be implanted. The formula was evaluated prospectively with 84 new patients, each of whom scored above zero on the BKB test before CI. *Monte Carlo* simulations predicted the proportion of patients who would exceed their pre-operative score by amounts ranging from 0% to 50%. Predicted and observed proportions did not differ significantly.

Benefit from CI was quantified as the gain in BKB Score and in health-related quality of life. Patients with odds worse than 2:1 displayed little benefit in either domain. Restricting CI to ears with odds of at least 2:1 would help maintain effectiveness. Specifying the criterion of candidature in this way would be informative for patients.

239 Relationship Between ECAP and High Resolution™ Program Settings in Patients Using the Clarion® CII Bionic Ear™

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The CII Bionic Ear™ is equipped with a differential amplifier that allows the clinician to record electrically evoked compound action potentials (ECAP). The utility of ECAP measurements for fitting cochlear implants

implementing conventional low-rate sound processing strategies has been studied extensively, and the relationship between perceptual loudness and neural response magnitude is not straightforward. It is unknown how HiResolution™ sound processing, designed to deliver high-rate stimuli in order to desynchronize neural responses to the carrier, will relate to ECAP measurements generated by single pulse stimuli that produce high neural

synchrony. This poster presents data comparing ECAP measures to HiResolution™ program settings in CII users. Because HiResolution™ programs are created using an adaptive protocol that customizes individual-patient program parameters, in most cases, CII users will have HiResolution™ stimulating pulse widths that are different from the pulse widths used to elicit the ECAP. In order to explore the relationships between these stimuli, we also studied the ECAP and loudness percepts elicited by constant charge pulses ranging in pulse width from 11 to 75 microseconds. As a first approximation, the data indicate that constant-charge pulses give rise to similar perceptions of loudness and similar ECAP magnitudes. Thus, normalizing to constant charge provides a way to compare various HiResolution™ program levels to the ECAP.

240 Comparison of Stapedial Reflex and Neural Response Telemetry -Programming Cochlear Implants

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The goal of this study was to find the relative efficacy of two objective measures that are available for use in programming the Nucleus 24 cochlear implant, and how these measures compare to the standard behavioral programming technique. The objective was to determine the relationships among Compound Action Potential (CAP) thresholds, electrically elicited Stapedial Reflex thresholds (eSRT), behaviorally determined thresholds (T) and maximum comfortable levels (C).

Twenty-five experienced subjects with the Nucleus 24 participated in this study. Fourteen subjects used SPEAK and 11 subjects used ACE coding strategy. CAP thresholds were recorded using the Neural Response Telemetry (NRT) software (Cochlear Corporation, Denver CO) and were compared with eSRT, C and T levels obtained for each subject. These measures were compared for 5 or 6 pre-selected electrodes that were evenly distributed across the electrode array. To determine potential effect of speech-coding strategy, subjects were grouped by strategy and compared.

Among subjects there were relatively small differences in correlation between levels based on objective measurements (NRT thresholds or eSRTs) and C levels. The overall correlation between C levels and eSRTs was high (0.92.) The overall correlations for NRT thresholds with C levels (0.77) and NRT thresholds with eSRTs (0.76) were moderate. NRT thresholds showed the strongest correlation with C levels and eSRTs for the mid-array located electrodes. The relationship between C levels and eSRTs was the strongest and showed the least variation for the more apical electrodes. The correlation between NRT thresholds and T levels was 0.69, whereas eSRT with T levels showed the lowest correlation of 0.57. The mean eSRT was approximately 17.7 Programming Units (PU) higher than mean NRT threshold.

241 Relationship between Loudness, Electrical Stimulus Charge, and Electrophysiological Measures in Med-El Cochlear Implant Users

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Loudness with cochlear implants using pulsatile stimulation is dependent on stimulus amplitude, pulse rate, and pulse duration. Studies suggest that loudness corresponds to the product of stimulus amplitude and pulse duration (electrical charge) within certain ranges of pulse rate and pulse-to-gap ratio. The present study investigates a) the relationship between loudness, stimulus amplitude, and pulse duration for different pulse rates, and b) the correlation of behavioral loudness measurements with electrophysiological recordings.

Subjects were 12 adults implanted with the Combi40+ cochlear implant (Med-El) who had used their implant for more than six months. Behavioral loudness growth functions for different pulse durations and rates were measured on several implant electrodes. Electrically evoked auditory brainstem responses (EABR) were recorded and analyzed for wave V amplitude and latency. The relationship between behavioral loudness measurements and EABR wave V amplitude was investigated. Furthermore, we hypothesized that a linear inverse relationship between pulse duration and amplitude exists to evoke a constant loudness.

In this study, behavioral data supported the tested hypothesis. This indicates that both a proportional change of either stimulus level or pulse duration has a similar effect on changes in loudness. In general, the same electrical stimulus charge on different implant electrodes resulted in the same loudness, whereas wave V amplitude varied. The correlation of behavioral results with electrophysiological results was non-significant and considerable inter-subject variability was present.

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242 Voice Gender Discrimination and Vowel Recognition in Normal-Hearing and Cochlear Implant Users

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The perception of voice gender is dependent upon a number of acoustic factors, including fundamental frequency, formant frequencies, and breathiness while the recognition of vowels is mainly dependent on the spectral cues. The present study investigated the relative importance of temporal and spectral cues in voice gender discrimination and vowel recognition in cochlear implant users and normal hearing listeners. A simulation of cochlear implants using a noise-band vocoder created the conditions for normal hearers whereas a normal voice condition was used for cochlear implant users. The number of channels in the noise-band processors ranged from 4 to 32 to systematically alter the spectral cues. Two cutoff frequencies (50 Hz and 500 Hz) of the envelope filters in the noise-band speech processors were used to determine the temporal cues. Gender discrimination and vowel recognition were measured as a function of the number of channels and the cutoff frequency in both quiet and noise. For normal-hearing subjects, results showed voice gender discrimination and vowel recognition scores increased gradually when the number of channels increased at all conditions. A significant improvement was observed when the cutoff frequency changed from 50 Hz to 500 Hz. However, the difference became smaller when the number of channels increased. There is huge variability of voice gender discrimination and vowel recognition scores among cochlear implant users. The relationship between vowel

recognition scores and voice gender discrimination scores in both cochlear implant users and normal-hearing subjects was also explored.

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243 Talker Discrimination by Adults with Cochlear Implants

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Speech simultaneously conveys linguistic information and talker-specific or indexical information (e.g., age, gender, emotional state, etc). Research with normal hearing (NH) listeners suggests that encoding talker-specific speech information is important for interpreting the linguistic content. Although cochlear implant (CI) recipients' word and sentence recognition skills have been studied extensively, little is known about their ability to extract talker-specific information from speech. This investigation examined cochlear implant users' ability to discriminate talker identity as a function of the linguistic content of the stimuli and the talker gender.

21 CI users and 24 NH adults participated. They were presented with pairs of words or sentences produced by 10 different talkers. CI participants heard natural speech. The NH control group heard stimuli that were processed using a 6 of 20 channel-picking strategy similar to the SPEAK CI processing strategy. The participants were tested under two linguistic conditions. In one, the linguistic content of each stimulus pair was identical (e.g., cat-cat); in the other, the linguistic content of each pair differed (e.g., cat-dog). Within each linguistic condition, the same talker produced half of the stimulus pairs. In the remaining stimulus pairs the talkers differed. Participants indicated whether the pairs of stimuli were produced by the same or by different talkers.

Results revealed a similar pattern of performance for both groups. Discrimination accuracy was significantly better when the same talker produced both tokens in the stimulus pair. When the talkers differed, performance was significantly higher for male-female talker pairs than within-gender stimulus pairs. There also was a significant effect of linguistic condition. That is, discrimination accuracy was better in stimulus pairs where the linguistic content was held constant rather than differed. Like NH listeners, CI users do not independently process indexical and speech information.

244 The Investigation of the Binaural Effect in Postlingually Deafened Bilateral Cochlear Implant Users: Results of a Multi-Center Study

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Research within hearing aids and normal hearing subjects has shown that binaural hearing offers additional advantages over monaural hearing. A multi-center study (BilCIA) was initiated in April 2000 to investigate into the binaural effects in post-lingually deafened cochlear implant users. All patients included in the core group were bilaterally implanted with a standard MED-EL COMBI 40+ electrode array, which was fully inserted into the cochlea. Furthermore, a multi-center study in the United States was initiated in September 2001. Currently, more than 240 patients (September 2002) were implanted bilaterally with the MED-EL COMBI 40+ implant.

Speech testing within the BilCIA study was performed in quiet and in noise with respectively CD recorded monosyllable and sentence materials. Within the noise condition, the signal was presented at +10 dB SNR. The test set-up consisted of 3 loudspeakers, one presenting speech at 0 degrees azimuth (1 meter distance) and two presenting speech weighted noise at +/- 90 degrees azimuth (1 meter distance).

Preliminary analysis of the data reveals the presence of a head shadow effect ($p<0.001$) in all patients. A squelch effect and diotic summation effect was present in some patients.

Localization testing was performed in an anechoic chamber in 25 subjects. The test set- up consisted of an array of 7 loudspeakers within 180 degrees. The majority of the patients tested were able to localize sounds.

Further data collection and analysis is ongoing and will be presented.

245 Audiovisual Speech Perception in Adult Cochlear Implant Users: Effects of Sudden vs. Progressive Hearing Loss

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Hearing-impaired adults with an early onset of deafness are better lipreaders than those with a late onset of deafness (Tillberg et al., 1996). Does the rate of onset of a hearing loss (sudden vs. progressive) affect lipreading ability in postlingually deaf adults? To investigate the effects of the time-course of hearing loss on audiovisual speech perception in postlingually deaf adults following cochlear implantation, we conducted a retrospective analysis of a set of patient data from the clinical records of the Department of Otolaryngology-Head and Neck Surgery at Indiana University. Adult cochlear implant (CI) users in this study experienced either a sudden hearing loss (N = 13), defined as the onset of a profound hearing loss within one month, or a progressive hearing loss (N = 32), defined as the gradual onset of a profound hearing loss over a period of time greater than one month (range: 1 – 45 years). These adults were given the City University of New York sentence recognition test (Boothroyd et al., 1985), in which they were asked to repeat aloud meaningful English sentences presented under auditory-alone (A), visual-alone (V), and audiovisual (AV) conditions. Listeners' responses were scored in terms of correctly identified key words in each sentence. We found that postlingually deaf adult CI users with sudden and progressive hearing loss performed best in the AV condition, followed by the A condition, and finally the V condition.

There were no differences in performance across the two groups in A and AV conditions. However, adult CI users with progressive hearing loss performed better than those with sudden hearing loss in the V condition. Thus, rate of hearing loss influences lipreading abilities in postlingually deaf adult CI users.

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246 Facial Stimulation in Multichannel Cochlear Implant Users

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The objective of this study was to investigate optimization of programming strategies and stimulating parameters in our population of patients who developed facial stimulation following cochlear implantation.

This study included several components: 1. a chart review of four hundred and ninety multichannel cochlear implant users to identify subjects who experienced facial stimulation was undertaken; 2. for identified subjects, charts were examined to identify device type, CT and/or MRI findings, etiology of deafness, and surgical procedures; 3. depending on device-specific capabilities, subjects were programmed to maximize their hearing while minimizing facial stimulation.

Subjects included in this study were cochlear implant patients who experienced facial nerve stimulation on at least one intracochlear electrode at levels between not heard or very soft up to maximum comfortable level.

Included patients were implanted with various versions of Clarion, Nucleus and Med-El multichannel cochlear implants with different kinds of electrode arrays and positioners. Thirty one (6.3%) were found to experience facial stimulation upon activation of one or more electrodes. Facial nerve stimulation could not be controlled in three cases. Causes of facial stimulation were categorized as related to etiology, symptoms, location of electrodes causing facial stimulation, type of cochlear implant, optimal programming strategy, stimulation mode and stimulation parameters.

247 Henry Puharich and the Miniature Tooth Radio; a Historical Perspective

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Just one year after Djourno & Eyries described the first neural prosthesis to restore hearing, an application was sent to the U.S. patent office in 1958 describing a neural prosthesis for hearing very different from the cochlear implant. Its inventors, Henry Puharich and Joseph Lawrence, proposed a radiofrequency receiver implanted in the tooth that would utilize an alternative neural pathway, *the facial system,* to bypass the cochlea and stimulate the auditory brainstem in order to restore hearing. This historical note describes the patents related to this invention and explores the possible validity of such a device. Despite its challenge to traditional thought, there is both anatomic and clinical data suggesting such an auditory prosthesis may function. We also profile the primary inventor, Henry Puharich, and address the circumstances that likely influenced his invention. Although the miniature tooth radio did not achieve significant clinical applicability, Puharich·s patents continue to be cited in the patent literature, a mark that his inventions have had a lasting impact.

248 Modeling Laryngeal Protective Mechanisms After Mammalian Anatomy to Develop a Surgical Technique to Help Patients With Supraesophageal Symptoms of GERD

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Patients with supraesophageal complications of Gastroesophageal Reflux Disease (GERD) are difficult to treat. To investigate possible treatments for reflux, we studied laryngeal anatomy in 70 mammalian genera including ruminants (which regurgitate cud) and aquatic mammals (which have constant laryngeal exposure to water). Morphologic conditions that could prevent reflux were identified as possible models to develop a technique for surgical intervention. In six human cadavers, a prosthetic implant was inserted under the posterior pharyngeal mucosal layer and positioned to oppose the cricoid cartilage or the edge of the laryngeal aditus.

Results showed all mammals have a higher laryngeal position compared to humans where the epiglottis overlaps the soft palate. Four possibly protective features around the laryngeal opening were observed. 1) A pronounced aryepiglottic fold forms a lateral wall. 2) A prominent palatopharyngeal fold surrounds the opening. 3) Arytenoid and corniculate cartilages curve posteriorly and interlock with the palatopharyngeal fold. 4) A thick soft tissue connection is found between the corniculate cartilages. The latter three features form a posterior wall for the larynx.

In the cadavers, we found that a prosthetic implant inserted under the mucosal layer of the posterior pharynx created a protective prominence (like the ruminant palatopharyngeal fold) that may resist the passage of refluxed material. Future studies will propose to augment this procedure by removing tissue under the pharyngoepiglottic fold. This may widen the vertical diameter of the piriform sinuses and raise the apparent height of the aryepiglottic fold, protecting against lateral incursions of refluxed material. These interventions could supplement fundoplication and improve long-term results in patients with poor surgical outcomes.

249 Is Human Papillomavirus Type 11 a High Risk Virus in Laryngeal Papillomatosis?

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Recurrent respiratory papillomatosis (RRP) is a serious illness caused by the human papillomavirus (HPV). HPV types 6 and 11 are the most frequently detected genotypes. While these HPV types are thought to be "low risk" in that their association with malignancies is infrequent, patients with RRP develop laryngeal carcinoma in 1% of cases. We have previously reported that children with lesions containing HPV-11 have a more aggressive course than patients with HPV-6 (Rabah et al. 2001). These patients required more surgical procedures, and were less likely to go into remission than HPV-6 infected patients. In addition, three children with HPV-11 infection developed squamous cell carcinomas. These cases, as well as others like it in the literature, suggest that HPV type 11 may play a more significant role in carcinogenesis in the larynx than previously thought, especially with regards to those patients with a history of RRP.

We have obtained specimens from nine patients with a history of RRP who have developed squamous cell carcinoma of the larynx. RNA and DNA extraction revealed the presence of HPV type 11 in the malignancies of all nine patients. As we have previously demonstrated that 50% (29/61) of RRP patients are infected with HPV type 6, and 50% (32/61) with HPV type 11, this data shows a statistically significant relationship between the presence of HPV-11 and the progression to malignancy ($p<0.0001$).

Our hypothesis is that lesions containing HPV type 11 become malignant after the oncogenic genes E7 and E6 are integrated into the host genome. This confers a growth advantage to the infected cells resulting in an invasive carcinoma. Using PCR techniques, we will demonstrate integration of the HPV-11 genome into the host genome. Furthermore, we will sequence the p53 genes to monitor for mutations in this proto-oncogene thought to play a large role in the malignant transformation of HPV-infected cells.

250 Capillary Length Density in the Aging Human Thyroarytenoid Muscle: A Stereological Study using Confocal Laser Scanning Microscopy

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The aging process in the human thyroarytenoid (TA) muscle differs from that in limb muscles. However, in spite of high blood flow requirements in this highly oxidative muscle, it is not known if changes in capillary density play a role in the pathogenic mechanism underlying age-related TA dysfunction. Since the maximal oxygen flux in muscle is limited by a sharp diffusion gradient that is localized between the red cell and the sarcolemma, maximal rates of oxygen diffusion are largely a function of the amount of contact between capillaries and muscle fibers. In the present study, design-based stereological techniques have been used to provide an unbiased, quantitative, three-dimensional estimate of relative and absolute capillary lengths in the aging TA.

The results indicate a significant age-related increase in the length density of capillaries in contact with muscle fibers referenced to the fiber volume. The mechanism underlying this increase in the capillary supply to muscle fibers is unclear. However, there was a trend toward an age-related increase in the length of capillaries in contact with type 2 fibers referenced to the volume of type 2 fibers. Since this would

support an increase in the maximal oxygen flux to the volume of these fibers, it may be related to an increase in the their oxidative capacity. This change in the adaptation for fatigue resistance in type 2 fibers may be the result of an increased recruitment of type 2 fibers due to the reported age-related loss of type 1 fibers in the human TA.

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251 Is Film In Situ Zymography Useful Exammination With Pathological Diagnosis During Operation of The Thyroid Gland Tumor ?

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[Introduction] We showed an overexpression and an activation of MMP-7, -2 and MT1-MMP in human papillary carcinoma (PC) of the thyroid gland.This result indicated the involvement of these MMPs in destructive growth and metastatic spreading by means of both tumor and stroma cell-interaction and active proteases-network. In those study, we used gelatin film in situ zymography method□iFIZ method) in order to demonstrate the localization of gelatinolytic activities. It was consisted of GN membrane and SN mebrane. GN membrane is coated gelatin, and was reduced by gelatinase which is included in the frozen tumor tissue section of which picked up GN membrane. SN membrane coating with colloidal silver reacted and discolorationed with free sulfhydryl groups which released from pre-MMPs during the latent forms transform into the active forms. In this study, we could determine the quantity of discoloration of PC , follicular carcinoma (FC) and follicular adenoma (FA). And we suppose to be able to utilize to distinguish benign or malignant during the operation of the thyroid tumor.

[Method and Result] The frozen section was sampled in the operation, picked up SN membrane, a few minute later, discolorized resions were taken a photomicrograph and changed RGB channel with computed analysis and estimated discolorational socore. More than half of PC rised score in red channel and green channel. Although, more than half of FA not even discolorized. Other samples only rised score in green channel.

[Conclusion] These results showed that more than half of PC and FA will be assumed during an operation with FIZ method. Especially, this method can will discrimination a number of FC and FA. At present, pathological feature of FC is peripheral or vascular invasion and distant metastasis. Although during operation, these observations almost can not be detected. In the future. FA and FC may be diagunosed and over-resection of the normal thyroid gland may be avoidable.

252 Prospective Phenotypic Analysis of Turner Syndrome (TS): Clinical Markers for Initial Detection of Mild TS

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Turner syndrome (TS) affects up to 1/2000 live female births and is characterized by the total or partial loss of one X chromosome in females with short stature and gonadal dysgenesis. Less penetrant features include sensorineural hearing loss (SNHL), chronic otitis media (OM), pterygium colli, and craniofacial dysmorphism. Previous otolaryngologic reports on TS originating from specialty clinics likely reflected ascertainment bias toward more severely affected individuals.

Here we report the otolaryngologic features observed in a TS cohort with a wide spectrum of phenotypic severity that has been prospectively ascertained through a multidisciplinary study of genotype-phenotype correlation in TS at the National Institutes of Health. The cohort was comprised of 28 TS patients (range = 7-53 years, average = 30.4) with an average age of diagnosis of 10.5 years (range = prenatal-28.5 years). Subjects were evaluated by otolaryngologic physical examination, pure tone and speech audiometry, tympanometry and stapedial reflex testing. At least 12 subjects (39%) had a high-arched, distinctively angulated (ogival) bony secondary palate and nearly all of the patients had minimally or mildly dysmorphic pinnae. Sixteen subjects (56%) had SNHL, primarily affecting high frequencies, and 14 (50%) had a past or current history of chronic or recurrent OM.

We conclude that chronic or recurrent OM is common in TS patients, even those with absent or minimally dysmorphic features. TS is likely to be a commonly missed diagnosis in female children presenting with otitis media, short stature, and minimally dysmorphic pinnae. The ogival palate serves as a distinctive phenotypic marker for TS and, in association with chronic or recurrent OM, may facilitate the initial detection, early diagnosis, and treatment of TS females.

253 Inhibition of Influenza Viral Neuraminidase by Elderberry Lectin

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Elderberry trees contain lectins in almost all tissues. These are either type 2 RIP, lectins derived from type 2 RIP precursors, or lectins encoded by truncated Type 2 RIP genes. One of these lectins, SNA-I, exhibits specificity to a specific sialic acid residue, Neu5Acα(2,6)Gal/GalNAC. This is the same terminal sialic acid residue that is cleaved from glycoconjugates by influenza neuraminidase. It is theorized that the elderberry lectin SNA-I competitively inhibits influenza neuraminidase by competitively binding to this specific sialic acid moiety. SNA-I isolated from a partially purified bark extract using a combination of routine protein purification techniques and affinity chromatography. This lectin is shown to inhibit several strains of influenza virus *in vitro*. The possible utility of this lectin in the treatment of influenza and other viral illnesses is discussed.

254 Carcinoma Ex Pleomorphic Adenoma of the Palatal Minor Salivary Gland with Extension into the Nasopharynx

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Carcinoma ex pleomorphic adenoma presenting in the nasopharynx is extremely rare. We present a case of carcinoma ex pleomorphic adenoma occupying the nasopharynx and the soft palatein a 51-year-old woman. To the best of our knowledge, this is the first reported case of carcinoma ex pleomorphic adenoma in the nasopharynx.

255 Phase Contrast Microscopy In Allergic Nasal Smears

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Background:A new technique termed, Phase contrast microscopy (PM) with modified Hansel's staining (employed with bright field microscopy), was developed to identify mast cells (MC) and granulocytes (eosinophil/neutrophil/basophil(E/N/B).

Methods:Nasal scratching smears from 618 patients with Japanese cedar pollinosis were examined using this new technique.

Results:This technique permitted accurate morphological identification. MCs can be discriminated from E/N/Bs. The surface of the cell membrane appeared as low refractile (lr-), moderately refractile (mr-), or high refractile (hr-). This was caused by the light, which is not related to the phase difference, but rather originated from the difference in the refraction of the direct light. In specimens from the onset stage (i.e., 1-3 days after onset), lr-MC and mr-/hr-E/N/B were dominant. In

specimens from the early stage (i.e., 4-7 days after onset), lr-E/N/B significantly increased in number.

Conclusions:The phospholipid bilayers of the cell membrane exhibit a phase transition after the onset, and phase refractivity of the cell membrane is closely related to the activity of the cell. This indicates that in the onset stage, MCs are already activated, while most of the E/N/Bs are not. In contrast, the latter cell types become activated subsequently in the early stage.

256 The Expression of Phospholipase A2 in the Guinea Pig Nose

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When the cell membrane receives mechanical or chemical stimulation, phospholipase A2 (PLA2) existing on the membrane is activated and the arachidonic acid in phospholipid is isolated. Subsequent conversion of isolated arachidonic acid into prostaglandins and leukotrienes is mediated by cyclooxygenase and 5-lypoxygenase, respectively. These substances function as active substances and contribute to various physiological roles. Therefore, it is possible to say that PLA2 is the key enzyme on considering arachidonic acid metabolism. However, localization of PLA2 in the nose has not been reported to date, though positive association between nasal allergy and the metabolites of arachidonic acid has been indicated. The purpose of the present study was to clarify intranasal PLA2 location and its metabolic mechanism. The guinea pig nose was perfused with 0.5%zinc-10%formalin and decalcified with 5%EDTA. After dehydration, the specimens were embedded in paraffin and cut into 20mm thickness for immunohistochemistry by using antibodies against secretary PLA2 (sPLA2) and cytosolic PLA2 (cPLA2) which are classified on the basis of the similarities in structure and properties. The stomachs and kidneys of the same animals were used as controls. The nasal glands showed both cPLA2 and sPLA2 activities. However, no specific reaction was observed on the epithelium, olfactory glands, nerves and vessels. The chief cells of the gastric fundus showed sPLA2 but not cPLA2. On the other hand, the kidney tubules presented cPLA2 but not sPLA2. Our results revealed the possibilities that cPLA2 regulates the secretion from the nasal glands and sPLA2 is secreted from nasal glands into the nasal mucus.

257 Localization of 5-Lipoxygenase in the Nasal Cavity of Guinea Pig

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Lipoxygenase (LOX) is known to react with arachidonic acid that has been activated and freed by phospholipase A2 on cell membranes, and to be involved in the synthesis of various leukotrienes. In addition, these synthesized compounds have been confirmed to play a role in maintaining the biological functions of the body. LOX is involved in the onset of nasal allegy, a condition that is becoming more prevalent. However, the role of LOX in the nasal cavity remains largely unknown. As a result, clarification of the localization of LOX in the nasal cavity could assist our understanding of the role of LOX in the nasal cavity and the mechanisms of allergic rhinitis. In the present study, localization of 5-LOX was investigated immunohistochemically in the nasal cavity of a guinea pig. The guinea pig was perfused using zinc-formalin solution and then immersed and fixed in the same solution. The nose was then removed, decalcified using 5% EDTA solution, dehydrated, embedded in paraffin, and sliced into 20_m sections. After deparaffinization, specific antibodies against 5-LOX were used for immunostaining. The results revealed that 5-LOX localized specifically in the nasal gland tissue, but not in the vascular endothelial cells nor the surrounding tissues, olfactory gland, respiratory epithelium, olfactory sensory epithelium, submucosal tissue or nerves. In addition, localization of 5-LOX was confirmed in the renal tubules and gastric fundal glands of the same animal. The results of the present study may suggest that 5-LOX is involved in ion exchange to control nasal discharge from the nasal gland, and that 5-LOX is secreted from the nasal gland into the nasal cavity.

258 The Postnatal Changes In The Neural Excitation Of The Rat Accessory Olfactory Bulb.

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We have reported that, in the matured rat accessory olfactory bulb (AOB) slice, there are at least three subdivisions, and an oscillatory excitation in the external plexiform and mitral cell layers (EPL/MCL) is not elicited by electrical stimulation of vomeronasal nerve layer, unlike the guinea-pig AOB. We examined the postnatal changes in the synaptic transmission and signal propagation using electrophysiological and optical recordings. At early postnatal days (P4-11), although three subdivisions were already present, oscillatory field potentials, which were superimposed on slow potentials lasting more than 200 msec, were observed. These oscillatory and slow responses disappeared at P18-30 (the matured type). In the matured type, the application of non-NMDA glutamate receptor antagonist, CNQX suppressed mostly the field potentials, indicating that non-NMDA receptors predominate in synaptic transmission between vomeronasal nerve fibers and mitral cell dendrites in the glomeruli. On the other hand, at early postnatal days, CNQX reduced the oscillatory field potentials and left the slow response unblocked. The slow response was blocked by NMDA glutamate receptor antagonist, APV. Further, the removal of Mg2+ from the perfusate weakened the oscillation, and the application of APV restored the oscillation.

These results suggest that, at early postnatal days, NMDA receptors not only participate in synaptic transmission in the glomeruli, but also mediate inhibition of the oscillation in the EPL/MCL.

259 Effect of p14ARF Gene on Cell Growth of Human Laryngeal Tumor Cells

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Objective: To explore the inhibitory effect of p14ARF on cell growth of laryngeal carcinoma and the expression on endogenous p53. Methods: p14ARFcDNA was transferred to the cell line Hep2 of squamous cell carcinoma of the larynx by gene transfer to study the cell cycles and the expression of endogenous wild type p53 by using flow cytometry, RT-PCR and Western-blotting. Results: Expression of p14ARF significantly affected the Hep2 cell growth. The clone-forming efficiency of the Hep2 cells transferred p14ARF was 57%,compared with empty vector pcDNA3. The numbers of cells transferred p14ARF at the G0/G1,G2/M phase as twice as the control after 48 hours transferred with p14ARF cDNA . The expression of endogenous wild type p53 significantly enhanced. Conclusions: Expression of p14ARF can up-regulate the expression of endogenous wild type p53 and arrest the Hep2 cell growth of human laryngeal squamous cell carcinoma at the G0/G1,G2/M phase.

260 The Informative Value of Contact Endoscopy in Otorhinolaryngology

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Contact endoscopy enables the investigator to differentiate intraoperatively between carcinomas and other mucosa lesions. The contact endoscopy was performed on 42 patients to confirm or exclude malignant transformation of the mucosa.

After the mucosa had been stained with methylene blue, a contact endoscope was placed directly onto the stained area (contact procedure) and an assessment of the cells was performed under magnification. Documentation was subsequently carried out using video or, with the aid of the Storz AIDA system, using digital photography. At the same time, samples for histological diagnosis were taken from the examined areas and evaluated by a pathologist.

The epithelia examined were located in the area of the larynx (29), hypopharynx (5), oropharynx (7) and nose (1). In a blind trial the documented images were then independently evaluated by two ENT physicians and a cytologist. The results thus obtained were compared with the final histological findings.

In the histological evaluation one of the following diagnoses was made: negative (1), chronic inflammation (24), dysplasia (7) and carcinoma (10).

Overall, the evaluation of images obtained via contact endoscopy matched the histological diagnosis by the ENT physicians in 88.1 % (37) and the cytologist in 83.3 % (35) of cases respectively.

A carcinoma was correctly detected by the ENT physician in nine cases. This corresponds to a correct assessment in 90 % of cases.

The contact endoscopy procedure is a helpful but somewhat limited diagnostic technique, as there is no means of determining the depth to which carcinomas extend. Further studies are required to show whether this problem can be solved, and the informative value can be enhanced still further, through combination with other methods such as optical coherence tomography.

Overall, the results clearly demonstrated that contact endoscopy can make a useful contribution to rapid intraoperative evaluation of mucousa alterations.

261 One Hundred Years of Inner Ear Induction

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The development of the inner ear has been studied actively for over a hundred years. On a purely practical level, the anlagen of the inner ear - the otic placode - is readily visible from an early age in most vertebrate embryos, making it an attractive tissue for developmental biologists to study. However, part of the historical motivation to study inner ear development undoubtedly arose from the fascination in seeing a highly complicated sensory organ produced from a simple patch of ectoderm. It is this transformation - from a very simple tissue to a Darwinian "organ of extreme perfection" - that modern researchers seek to understand. We will first review some of the historical work on otic placode induction, and argue that the concept of inner ear induction has changed dramatically with the advent of molecular markers.

The hindbrain and cranial mesoderm have both been proposed to induce the inner ear. We are examining this question by examining the necessity and sufficiency of these two tissues, both in normal embryos, and in vitamin A-deficient quail embryos, in which the posterior half of hindbrain (which is normally adjacent to the otic placode) fails to form. Our results suggests that the ear is induced in the absence of the posterior hindbrain. Both FGF and Wnt family members expressed in the hindbrain and cranial mesoderm have been proposed to act in ear induction. Our preliminary results suggests that FGF, but not Wnt signaling is necessary for otic placode induction.

262 Fgfs in Mouse Inner Ear Development

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Expression analysis of Fibroblast growth factors (Fgfs) and their receptors suggests roles for a number of these genes in otic development and genetic disruption has defined the roles of individual genes. For example, Fgf3 is expressed in the neurectoderm adjacent to the developing placode, in the placode itself, in the neurogenic region of the otic vesicle and later in the developing sensory epithelium. Mice lacking Fgf3 fail to induce the endolymphatic duct and have a small otic ganglion. The penetrance and expressivity of this phenotype varies, suggesting that other Fgfs and/or their receptors might also contribute to the early stages of otic development. Indeed, Fgf10 is expressed in mesenchyme underlying the prospective otic placode and also in the otic cup and ganglion at later stages. Fgf10 mutants have small inner ears with severe disturbances of vestibular development and innervation. Also, mice lacking Fgfr2IIIb, which encodes the high-affinity receptor for Fgfs3 and 10, have an otic phenotype that is similar to, but more severe and penetrant than that of either Fgf3 or Fgf10 mutants. Surprisingly, Fgf3/10 double mutants lack otic vesicles. Expression of otic marker genes is disturbed, but hindbrain patterning is normal and proliferation of otic ectoderm is not blocked, suggesting that these Fgfs act directly on the ectoderm to induce otic placode. Studies of embryos with 3 mutant alleles reveal quantitative and unequal contributions of these Fgfs to otic development.

Otic induction in zebrafish requires fgf3 and fgf8 expression in the hindbrain. Mouse Fgf8 is not seen in the hindbrain or in known otic-inducing tissues, but is found in pharyngeal endoderm. To determine whether Fgf8 plays a role in mouse otic development, we intercrossed Fgf3+/-;Fgf8+/- animals. At E9.5, the otic phenotype of Fgf3-/-;Fgf8+/- embryos is more severe than that of Fgf3-/- embryos. These data suggest an early role for Fgf8 in mouse otic development.

263 Determination of Cell Fate and Patterning in the Organ of Corti

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The sensory epithelium of the mammalian cochlea (the organ of Corti) is characterized by a highly regular pattern of sensory hair cells and non-sensory supporting cells. Analysis of auditory sensitivity in several strains of mutant animals has demonstrated that the appropriate formation of this pattern is required for normal hearing. Although our understanding of the cellular and molecular factors that regulate cell fate and pattern formation in the organ of Corti is still quite limited, the results of recent studies have provided valuable insights regarding how this structure develops from an un-patterned population of precursors into a regular mosaic of differentiated cell types. In particular, results from a number of laboratories have demonstrated that signaling through the neurogenic pathway regulates the number of cells that will develop as hair cells. In addition, recent results from our laboratory suggest that the determination of individual cell fates may also be regulated through phosphorylation of specific families of kinases. All of these results can be incorporated into the formation of a model for the development of the organ of Corti.

264 Differentiation of Sensory Hair Cells

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For hair cells to function as efficient mechanotransducers and transmit sensory information to the CNS they have to acquire a number of important features during their development. These features include, amongst others, a directionally-sensitive, apically-located hair bundle complete with functional mechanotransducer channels, and a repertoire of basolateral potassium and calcium conductances with appropriate kinetics. For mammalian outer hair cells, the acquisition of electromotility is an additional event of importance. The manner and time course over which these characteristics appear once a hair cell has been specified will be described, and the current evidence for the potential molecular mechanisms that may underlie these aspects of hair-cell differentiation will be discussed.

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265 Wiring the Ear to the Brain: the Molecular Basis of Formation, Guidance, and Survival of Sensory Neurons.

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An overview will be presented of the molecular and cellular origin of the sensory neurons of the inner ear, molecular biology of connection establishment to specific sensory epithelia and the molecular nature of their survival. Sensory neurons of the ear depend on a single basic Helix-Loop-Helix protein (bHLH) for their formation, neurogenin 1 (ngn-1). An immediate downstream gene is the bHLH gene neuronal differentiation (NeuroD). Targeted null mutations of ngn-1 results in absence of sensory neuron formation, targeted null mutation of NeuroD results in loss of almost all spiral and many vestibular sensory neurons. NeuroD and a downstream gene, the Pou domain factor Brn3a, play a role in pathfinding to and within sensory epithelia. The molecular nature of these pathfinding properties is unknown but may be related to semaphorin receptors Npn-1 and 2. Quantitative effects of ngn-1 null mutations on hair cells suggest a clonal relationship that may play some role in the acquisition of the pathfinding properties of sensory neurons. Sensory neuron neurite growth to sensory epithelia is initially independent of trophic factors released from developing sensory epithelia, but becomes rapidly dependent on those factors. In null mutations of a given neurotrophic factors, specific sensory populations undergo rapid cell death whereas misexpression of neutrophins results in massive rerouting of fibers.

For example transgenic expression of BDNF under NT-3 promoter control causes vestibular sensory neurons to project to the cochlea.

266 Developmental Genes Associated with Human Hearing Loss

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There are a multitude of proteins expressed in the inner ear during development, and a subset of these are invovled with human hereditary hearing loss. To date, there are 70 loci mapped to human chromosomes that are associated with hereditary hearing loss in extended families; 29 of these genes have been cloned. A few of these have clear roles in the development of the inner ear; for others, their role in development, if at all, is still unknown. The genes related to hair cell specification during development, including NeuroD, Fgf19, Jagged 1, Hes1, Hes5, Ser1, and Math1, are not yet known to be associated with human hearing loss. Although null mutations in most of these genes would not be expected to form a viable organism due to their role in other developmental processes, point mutations would presumably lead to loss- or gain-of-function viable phenotypes in heterozygote form. Other genes, such as the POU4F3 transcription factor, known to be involved in terminal differentiation of hair cells, is associated with human progressive hearing loss. The EYA4 transcription activator, associated with human hearing loss, is involved in embryonic development as well. Mutations in myosin VI and myosin VIIA, both expressed early in hair cell development, lead to human dominant and recessive hearing loss. While a mutation leading to human hearing loss is significant, more of our knowledge about the gene in question comes from research performed on the mouse. A number of genes have an important role in mouse inner ear development, discovered from the study of mouse mutants, including AP2α for middle ear conductive hearing loss, Pou3f4 for cochlear and temporal bone abnormalities, and Foxi1 for inner ear defects. These and other examples will be presented.

267 Overview and Clinical Trials

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Tinnitus is a common sensation that sometimes causes suffering. Clinical management relies mostly on evaluation for serious or treatable causes, followed by non-specific counselling and reassurance when no such cause is found. Many randomized clinical trials (RCTs) have been completed, but these have failed to establish the superiority of specific treatments, either in eliminating tinnitus sensation or in long-term reduction of the attendant suffering. Problems in studying tinnitus treatment include (1) the difficulty of designing placebo arms for non-drug therapies, (2) the lack of consensus in outcome measurement, (3) our ignorance of prognostic factors that, if understood, might allow more powerful stratified study design, and (4) the likelihood that tinnitus patients are a heterogeneous group and that different treatments would be best for different patients. Strategies for overcoming these problems include cost-effectiveness studies, open trials prior to RCTs, and more studies of untreated tinnitus patients.

268 Animal Models of Tinnitus

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Although tinnitus is a prevalent neuro-otologic complaint, both its etiology and a universally effective treatment have proven elusive. One factor impeding progress on understanding the phenomenon has been the difficulty of determining when an experimental animal is experiencing tinnitus. Moreover, quantification of the amount and quality of the perceived tinnitus, normally obtained from the verbal reports of patients experiencing the problem, has not been available from experimental animals. The highly variable nature of the auditory perceptions associated with tinnitus make such data, obtained from individual subjects, critical in understanding the relationship between the perceptions being experienced and the physiological events in the auditory system that give rise to those perceptions.

An early series of studies by Jastreboff and colleagues (Behav. Neurosci., 6:811-822, 1988) demonstrated that tinnitus could be detected in a behavioral animal model. That procedure relied on the assumption that an animal trained to respond in a particular way to silence; that is, to the offset of an ongoing auditory stimulus, would fail to do so when experiencing tinnitus. More recently, several laboratories have successfully applied variants of this procedure to detect tinnitus. This presentation will review the existing animal models for tinnitus, as well as some new approaches, and will evaluate each in terms of several criteria including ability to exclude alternative explanations for the obtained results, ability to determine when tinnitus is occurring in an individual subject, and ability to estimate the perceived level and quality of the tinnitus.

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269 Neural Correlates of Tinnitus

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There is growing evidence that some forms of tinnitus involve abnormal levels of spontaneous activity in the central auditory system. Human imaging studies demonstrate that tinnitus is associated with increased activity of cortical and subcortical auditory structures. Studies in various animal models have revealed areas of increased activity following exposure to tinnitus inducing agents such as salicylate, quinine, noise and cisplatin. The areas include, but are not necessarily limited to, the cochlear nucleus, inferior colliculus, and auditory cortex. In this presentation, we will focus on work in our laboratory, which has shown that when animals are exposed to high levels of sound, a condition of chronic hyperactivity, which is manifest as an increase in the level of spontaneous multiunit activity, can be induced in the dorsal cochlear nucleus (DCN). This same abnormality can also be induced in the DCN by cisplatin treatment. This hyperactivity is not a direct correlate of hearing loss as its onset follows a different time course from noise-induced hearing loss, developing slowly over a period of days, but lasting for at least 6 months. There are several lines of evidence supporting the view that the observed hyperactivity in the DCN may be an important component of tinnitus. For example, recent behavioral studies have shown that the same exposure conditions that lead to the development of hyperactivity in the DCN also cause animals to

experience tinnitus-like percepts. Indeed, when electrophysiological recordings were carried out in animals that had been tested behaviorally for tinnitus, the presence of hyperactivity in the DCN was found to correlate with the presence of tinnitus. Some clues are beginning to emerge concerning the mechanisms by which hyperactivity in the DCN (and by implication, tinnitus) can be triggered. This evidence will be reviewed and the implications for the development of anti-tinnitus strategies will be discussed.

270 Sensory Nuclei in Tinnitus.

*Susan E. Shore Otolaryngology, U of Michigan, Kresge Hearing Research Inst., Ann Arbor, MI

Patients with "somatic" tinnitus can modulate its loudness or frequency by clenching the jaw or touching the face, regions innervated by the trigeminal nerve. If a physiological correlate of tinnitus is increased spontaneous rate of auditory neurons (Kaltenbach et al., J. Neurophysiol. 88:699-714), then somatosensory input to auditory nuclei is necessary for modulation and perhaps generation of somatic tinnitus. We have demonstrated that the trigeminal ganglion send a projection to the cochlear vasculature (Vass et al., Neuroscience 84: 559-567) and, together with the trigeminal nucleus, to the ventral cochlear nucleus (VCN) of the guinea pig (Shore et al., J. Comp. Neurol. 419:271-285; present findings).

The function of these pathways was examined by electrically stimulating the trigeminal ganglion/nucleus while recording VCN unit responses using multichannel electrodes. Units showed complex responses to trigeminal ganglion stimulation consisting of one or more peaks of excitation sometmes followed by a longer, inhibitory phase. Applications of kanamycin to the round window to eliminate the cochlear contribution, diminished but did not eliminate the excitation. Cochlear destruction showed similar effects, suggesting that trigeminal innervation of the cochlea facilitated the VCN responses. Stimulating the spinal trigeminal nucleus similarly resulted in a combination of excitatory and inhibitory responses in VCN neurons.

These results demonstrate that projections from sensory neurons can influence the activity of second-order auditory neurons, and may play a role in integration mechanisms involving the cochlea and its central targets. If increased firing rate in the absence of sound ("phantom firing rate") is a necessary condition for tinnitus, then sensory input to peripheral auditory structures may play a role in generating the perception of "phantom sounds".

271 Imaging Human Tinnitus

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Functional neuroimaging provides a way to probe brain activity directly in people with tinnitus. In recent years, two main neuroimaging techniques have been applied to tinnitus: functional magnetic resonance imaging (fMRI) and positron emission tomography (PET). Both provide an indicator of population neural activity on a spatial scale of millimeters. In this talk, we will describe three main ways that functional imaging has been applied to tinnitus. One approach has examined the level of steady-state brain activity. Another involves modulating the tinnitus percept through oral-facial movements, deviations in eye position, or cutaneous stimulation and examining the brain for associated changes in activity. A third approach involves modulating the tinnitus percept using an acoustic masker and localizing correlated activity changes. Findings obtained with these various approaches will be reviewed and their implications for the neural substrate of tinnitus will be discussed.

Supported by the American Tinnitus Association, Tinnitus Research Consortium and Royal National Institute for Deaf People.

272 The Cortex and Tinnitus

*Jos J. Eggermont

In ketamine anesthetized cats we investigated changes in spontaneous firing activity (putative correlates of tinnitus) in up to three auditory cortical fields after administering a tinnitus-inducing agent. We used sodium salicylate (200 mg/kg; 5 cats), quinine hydrochloride (100-200 mg/kg; 7 cats) and transient pure tone trauma (29 cats) in acute experiments and compared pre and post trauma data for the same neural units. We also investigated long-standing pure tone trauma (10 cats) as a function of time (> 3 weeks) after trauma onset in comparison to age-matched littermate controls. Significant changes in spontaneous activity for the CF regions corresponding to the hearing loss suggest multiple potential correlates of tinnitus. After salicylate and quinine no significant changes in spontaneous firing rates were found in AI and AAF. However in AII spontaneous activity increased by 30% after salicylate and by 130% after quinine. Neural synchrony (only investigated in AI) did not change after salicylate but increased by 40% after quinine. After transient noise trauma, spontaneous firing rates doubled in AI, did not change in AAF and decreased by 40% in AII. Neural synchrony in AI increased by 50%. Permanent noise trauma resulted in a reorganization of the tonotopic map accompanied by a doubling of spontaneous firing rate in AI and an increase of neural synchrony with 50%. No changes in spontaneous burst firing were observed. If these findings are correlates for tinnitus they appear to differ for drug or noise trauma induced tinnitus.

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273 Electrical Suppression of Tinnitus

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There is a long history of attempts to suppress tinnitus with electrical stimulation. While these attempts have been emprical, with minimal knowledge regarding either the pathophysiology of tinnitus or the specific effects of electrical stimulation on the auditory nerve, a number of these studies have suggested promising results in some tinnitus sufferers. A variety of data suggest that tinnitus may represent an abnormal response of the central auditory pathways to deafferentation associated with cochlear injury. One of the prominent effects of such injury is the loss of peripheral spontaneous activity and it was suggested over thirty years ago that this may be a significant etiology for tinnitus.

In our studies of cochlear implant signal processing, we have determined that electrical stimulation of the deaf cochlea with 5000 pps pulse trains can produce patterns of auditory nerve firing similar to normal spontaneous activity. We are investigating the hypothesis that such stimuli, applied either to the round window in mild hearing loss, or intracochear for profound hearing loss, may suppress tinnitus without producing a sustained percept.

We have studied fourteen subjects with severe tinnitus complaints. Eleven of these have mild hearing loss and underwent round window stimulation via a transtympanic electrode. Three of these had cochlear implants which were reprogrammed to produce the desired stimuli. With partial blinding of the subjects it appears that somewhere between a third to a half of them achieve clinically significant tinnitus suppression without a sustained percept when a 5000 pps pulse train is applied for several minutes at an appropriate stimulus amplitude. Based on these findings, implantation of a chronic electrode in such subjects is anticipated in the near future. Double-blinded evaluation of tinnitus suppression should be possible with such subjects.

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274 Tinnitus Retraining Therapy

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The neurophysiological model of tinnitus [Jastreboff, P.J. Phantom auditory perception (Tinnitus): mechanisms of generation and perception. Neuroscience Research, 8:221-254, 1990], applied the principles of neuroscience into studies of mechanisms of tinnitus and methods of its alleviation. In cases of clinically-significant tinnitus, the focus is placed on the neurophysiology of non-auditory systems in the brain, particularly on the limbic and autonomic nervous systems, with the auditory pathways playing a secondary role. Once the links are created between the tinnitus-related neuronal activity in the auditory system, and activation of these non-auditory systems, tinnitus evokes negative emotions, stress, anxiety. These functional connections are governed by the principles of conditioned reflexes. According to the model, it is not the perception of tinnitus, but the presence of inappropriate functional links between the auditory, and the limbic and autonomic nervous systems that is responsible for tinnitus distress. Weakening, and finally breaking of these functional connections, through proper retraining techniques, results in habituation of tinnitus-induced reactions and tinnitus perception, which are the goals of Tinnitus Retraining Therapy (TRT). Consequently, TRT is a practical implementation of the neurophysiological model of tinnitus.

Teaching / counseling and sound therapy are the inherent parts of TRT. Their particular implementation vary dependant on a patient category. TRT also helps patients with decreased sound tolerance (working on both components - hyperacusis and misophonia), by gradual desensitization of the auditory system and retraining functional connection linking the auditory system to the the limbic and autonomic nervous systems. While work is in progress to validate the effectiveness of TRT in a formal manner, currently available data from a number of centers support the usefulness of TRT in clinical practice.

275 Cochlear Afferent Response Types: Synaptic Mechanisms, Efferent Control and Functional Significance.

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Each inner hair cell communicates with the auditory brainstem via a population of 5 - 30 myelinated cochlear nerve fibers, depending on species and cochlear location. Individual fibers from a hair cell's afferent innervation can differ in threshold sensitivity by as much as 40 dB and in spontaneous discharge from rates of essentially 0, to over 100 sp/sec. The close relation between sensitivity and spontaneous rate (SR) in all mammalian ears investigated has suggested a functional subdivision of cochlear nerve fibers into high-, medium- and low-SR groups. Intracellular labeling experiments have shown that the peripheral terminals of these SR groups are spatially segregated around the inner hair cell, differ in synaptic morphology, and have different central projection patterns in the cochlear nucleus. There is also evidence for differential innervation of the different SR groups by terminals of the lateral olivocochlear efferent system. Recent work in our laboratory suggests that activation of the lateral olivocochlear system via electrical stimulation of the inferior colliculus can both increase and decrease the excitability of cochlear nerve fibers, depending on stimulation site. This overview presentation will summarize these new data on efferent control of neural processing as well as current understanding of the peripheral mechanisms generating the SR-based heterogeneity of cochlear nerve response properties, the functional significance of the SR continuum, and its generality across non-mammalian vertebrates.

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276 The Synaptic Vesicle Cycle at Hair Cell Afferent Synapses

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We used electron tomography of frog saccular hair cells to reconstruct the ultrastructure of afferent synapses under three stimulus conditions. Prolonged depolarization caused a massive depletion of synaptic vesicles, compared to either resting or inhibited synapses. The decreased surface area of the synaptic vesicle pool was offset by a nearly equal increase in surface area of irregularly shaped cisterns and plasmalemmal infoldings, suggesting that these structures are involved in vesicle recycling, and that reformation of synaptic vesicles from cisternal membrane is the rate limiting step in the cycle during intense prolonged depolarization. Depolarization also depleted docked vesicles, including those that were located hundreds of nanometers from the active zone, and created a gradient of vesicles on the synaptic body. These results will be interpreted in light of current models of the vesicle cycle at ribbon-class synapses.

277 Calcium-Dependence, Kinetics, and Ontogeny of Membrane Exocytosis and Endocytosis in Inner Hair Cells

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Inner hair cells (IHCs) of the cochlea convert mechanical signals into patterns of neurotransmitter release. Their afferent synapses present synaptic ribbons, which most likely enable them to signal over prolonged periods of time. We study the regulation of presynaptic exocytosis and endocytosis using capacitance measurements. Exocytosis of mouse IHCs before and after the onset of hearing was stimulated by voltage-gated calcium entry or uncaging of caged Ca^{2+}.

We have obtained estimates for the sizes of synaptic vesicle pools and the Ca^{2+} dependence of exocytosis and endocytosis at these ribbon type synapses. Furthermore, we studied exocytosis of IHCs during pharmacological and genetic manipulations of their Ca^{2+} channels. Thereby, we worked out the impact of different Ca^{2+} channel subtypes on exocytosis and addressed the channel-vesicle topography at the afferent synapse.

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278 Transmitter Release from the Hair Cell Ribbon Synapse

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Neurotransmitter is released continuously at the inner hair cell (IHC) ribbon synapse. We investigate hair cell transmitter release in the postnatal rat cochlea by recording excitatory postsynaptic currents (EPSCs) from afferent boutons directly abutting the IHC ribbon synapse. EPSCs are carried by rapidly-gating AMPA receptors. Spontaneous EPSCs are clustered in time and have highly skewed amplitude distributions. We propose that the ribbon synapse operates by multivesicular release, possibly to achieve continuous as well as high frequency neurotransmission. Three questions will be addressed: First, do the quantitative details of EPSCs recorded at the ribbon synapse correspond to the predictions for transmitter release based on capacitance measurements of vesicular fusion in the IHC? Second, are EPSC recordings at the onset of hearing an adequate representation of synaptic function at the fully adult ribbon synapse? Third, do glutamate transporters play a role in shaping EPSCs at the IHC ribbon synapse?

279 Fast Excitatory Synaptic Transmission: From Molecular Mechanisms to Therapy

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The cochlear inner hair cells use glutamate (Glu) or a related congener at synapses with the auditory nerve dendrites. Fast excitatory transmission was investigated by comparing the actions of a Glu antagonists on compound and single unit activity from the auditory nerve action potentials when applied into the perilymphatic space of the guinea pig cochlea. In our hand, the selective AMPA antagonist GYKI 53784 had the same potency as the AMPA/kainate antagonist DNQX and the NMDA antagonist D-AP5 had no effect on auditory nerve activity. When single-fiber activity was blocked with GYKI 53784, the effects of AMPA or kainate were also antagonized. GYKI 53784 completely blocked excitotoxicity (i.e. destruction of the afferent nerve endings) induced by AMPA and kainate. This suggests that AMPA-preferring receptors are functionally located at the sensory cell-afferent synapse whereas NMDA and kainate receptors are not. Besides its fast excitatory properties, glutamate is known to have neurotoxic properties when released in large amounts or when incompletely recycled. In the cochlea, pharmacological blockade of Glu transporter GLAST, but not GLT-1, leads to the development of excitotoxicity. The capacity of the afferent dendrites to form new synapses was investigated on an in vivo model of AMPA perfusion -induced excitotoxicity. Synaptic repair and a functional recovery were observed within 5 days. At the molecular level, the synaptic repair was correlated to an increase in the expression of mRNAs coding for NMDAR1 receptors. The blockage of the NMDA receptors delayed the synaptic repair, suggesting that NMDA receptors have an important neurotrophic function in the neuronal regeneration and synapse formation in response to excitotoxic injury. Tracking the molecular basis of excitotoxicity presages the development of new pharmacological strategies in human for excitotoxic related pathologies (ischemic- or noise-induced deafness), as well as the related tinnitus.

280 Ontogeny of Auditory Primary Afferent Activity.

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Activity begins in auditory primary afferents well before the onset of hearing. Spontaneous rhythmic discharge patterns that are unique to this period will be described. The first primitive response to sound occurs one or more days after the onset of spontaneous rhythmic activity but before frequency selectivity appears. The emergence of frequency tuning and spatial coding (tonotopic map) continues as the cochlea reaches a relatively mature status. We will emphasize two major periods of development: 1) those that support central circuit building and pre-hearing refinements; and 2) those concerned with acquisition of frequency tuning after hearing begins. Factors influencing the temporal progression of functional maturation (e.g., middle ear mechanics) and hypothetical mechanisms responsible for rhythmic discharge patterns will be discussed. Special attention will be given to the question of the functional status of the cochlear base in the late embryo (E19). A number of recent findings in the avian embryo have transformed our thinking regarding the cochlear base and these will be featured. The new insights will be cast in the context of historical perspectives for both birds and mammals.

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281 Combined Electrical and Acoustical Stimulation: A New Cochlear Implant Strategy

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The performance of the present generation of cochlear implants in postlingually deafened adults and prelingually deafened children have redefined the clinical management of profound deafness. Electrical signal processing has been able to improve discrimination while conventional or implantable amplification has not been able to provide similar speech perception enhancement. Recent studies by have demonstrated that acoustic amplification of hearing loss above 60 dB HL for frequencies greater than 2500 Hz provides no enhancement of speech recognition (Hogan and Turner, 1998; Ching et al.,1998; and Turner and Cummings, 1999). Electrical signal processing may be able enhance speech discrimination in these individuals. Studies have shown that a short electrode can be placed in experimental mammalian cochleae resulting in preservation of hair cells and auditory sensitivity distal to the electrode (Ni, et.al.,1992; Xu, et.al.,1997).

We have developed a novel cochlear implant that allows preservation of residual low frequency acoustic hearing and at the same time provide electrical signal processing. Six devices have been implanted. Residual hearing has been preserved in all six. A change in insertion depth to 10 mm has provided significant speech perception enhancement. Complex musical tone pitch discrimination is also preserved in this population. The results combining acoustic and electrical signal processing in the same ear are encouraging. In general, speech recognition scores have the potential to be quite high if low-frequency (acoustic) information (below 500 or 1000 Hz) is combined with electric speech for frequencies of 3000 Hz and above. Speech perception, psychoacoustics, and music testing will be described.

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282 Neuronal Responses in Cat Inferior Colliculus to Combined Electrical and Acoustical Stimulation of the Cochlea

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Combined electrical and acoustical stimulation (EAS) of the cochlea is successfully implemented in the rehabilitation of cochlear implant subjects with residual low frequency hearing. The present study examines the influence of EAS on neuronal responses in the inferior colliculus (IC). In acute experiments normal hearing cats were unilaterally implanted with scala tympani electrodes stimulated as bipolar pairs. An earphone was sealed to the auditory meatus for acoustic stimulation. Electrodes were placed at the round window for recording of frequency specific compound action potential (CAP) audiograms. After cochlear implantation, increases in thresholds mainly in the high frequencies were observed.

The IC was exposed, and acoustical tuning curves were obtained for single neurons or multineuronal clusters. Neuronal responses in the IC were recorded separately to acoustical stimulation (50 ms tone burst at characteristic frequency, CF), electrical stimulation (0.2 ms/phase, biphasic pulses, 30 pps) and combined EAS. For EAS recordings a broad range of intensity combinations were used for the two stimulus components.

At electrical stimulus levels near threshold, the simultaneous presentation of suprathreshold acoustical stimuli suppressed and desynchronized the responses to electrical stimuli. With increasing electrical stimulus intensities the responses to the acoustical stimuli showed an increase in thresholds and a suppression of the sustained and

offset responses, while the onset responses remained virtually unchanged. These results were independent of the neurons' CF.

The present data suggest that combined EAS leads to complex interactions in the central auditory system that are strongly dependent on the relative intensity of the electrical and acoustical stimulus components.

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283 Spectral Shape Perception and Speech Recognition in Normal Hearing, Hearing Impaired and Cochlear Implant Listeners

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The distribution of energy across frequency, or the shape of the spectral envelope, is one important feature of complex acoustic signals. Speech recognition relies in part on the ability of the listener to perceive the shape of the spectral envelope, and to discriminate between different spectral shapes. In this study, spectral shape perception was investigated in normal hearing (NH), hearing impaired (HI) and cochlear implant (CI) listeners. The stimuli were rippled noise signals with a logarithmic spacing of spectral peaks. A forced-choice adaptive procedure, in which the spectral envelope frequency was varied from 0.125 to 10 ripples/octave (rpl/oct), was used to determine the closest ripple spacing at which an interchange in the peak and trough positions in the rippled spectrum could be discriminated for each listener. This test is hypothesized to provide a measure of the listeners' ability to perceive the frequency locations of the peaks in a generic, speech-like acoustic signal. The results showed poorer spectral peak perception in CI listeners (average 0.6 rpl/oct, range 0.4 - 0.8 rpl/oct) than in NH listeners (average 4.3 rpl/oct, range 2.4 - 5.5 rpl/oct), and a wide range of spectral peak perception in the HI listeners (average 1.9 rpl/oct, range 0.7 - 5.5 rpl/oct). There was a significant relationship between spectral peak perception and both vowel ($r=0.66$, $p<0.0001$) and consonant ($r=0.80$, $p<0.0001$) recognition. In general, accurate speech recognition (>90% correct) requires that a listener be able to perceive at least 3 rpl/oct. These results suggest that the spectral peak perception test used in this study is predictive of speech recognition across the wide range of listener abilities, and that efforts to improve spectral peak perception for CI users, via for example improved electrode arrays and speech processing strategies, may lead to improved speech perception ability.

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284 Noise Susceptibility of Cochlear Implant Users: The Role of Spectral Resolution and Smearing

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Current multi-channel cochlear implant devices provide high levels of speech recognition in quiet. However, performance deteriorates rapidly with increasing levels of background noise. Previous studies have shown that this noise susceptibility may be partly due to the loss of fine spectral information. The present study evaluated the effects of modulated speech-shaped noise on sentence recognition by cochlear implant listeners and normal-hearing subjects listening to a noise-band implant simulation. Implant listeners wore their everyday speech processors; for the noise-band processors, the spectral resolution was varied according to the number of channels, while spectral smearing was varied according to the slope of the carrier filters. Speech recognition thresholds (SRTs) were measured for one steady-state and six modulated noise conditions (modulation frequencies between 1 and 32 Hz). Results showed that implant users did not experienced any release from masking for any of the modulated noise conditions. However, for all modulation rates, normal-hearing listeners experienced significantly more release from masking as the spectral resolution was increased. This release from masking was reduced as the amount of spectral smearing increased. Interestingly, for all modulation rates, the SRTs of implant listeners were very close to those of normal-hearing subjects listening to 4-channel noise-band with large amounts of spectral smearing. These findings suggest that the noise susceptibility of cochlear implant users is primarily due to the reduced spectral resolution and increased spectral smearing caused by electrode interaction. Efforts to improve the spectral resolution and reduce electrode interaction should improve implant performance in noise, especially temporally modulated noise.

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285 Speech Recognition in Noise: Comparison of Two Acoustic Cochlear Implant Models Using Normal Hearing Subjects

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Some cochlear implant patients perform very well in laboratory tests of speech recognition. In noisy conditions, as routinely encountered in daily life, the task of recognizing speech becomes considerably more difficult. This study measures speech recognition for normal-hearing listeners using two acoustic cochlear implant models at eight signal-to-noise levels, ranging from +10 dB to –2 dB. The extensive range of signal-to-noise levels and the measurement of performance using both acoustic models for every subject distinguish this study from previous work. Sentences from the Hearing In Noise Test (HINT) combined with long-term speech average spectrum shaped noise were processed using an eight analysis, eight presentation filter (8F) model analogous to the Continuous Interleaved Sampling (CIS) speech processor, and a twenty analysis, six presentation filter (6/20F) model similar to the Spectral Peak (SPEAK) speech processor. Preliminary results indicate higher speech recognition scores on within-subject sentence sets using the 6/20F model. Overall group performance also indicates better performance using the 6/20F model versus the 8/F model in noisy conditions.

286 Threshold Prediction for Noise-Modulated Pulse Trains

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The incorporation of low levels of noise into an electrical stimulus has been shown to improve frequency representation in a single sciatic nerve [Morse R. and Evans F. "Enhancement of vowel coding for cochlear implants by addition of noise." Nature Medicine, 2(8): 928-32, 1996] and thresholds in human subjects [Zeng FG. Fu QJ. Morse R. "Human hearing enhanced by noise." Brain Research. 869 (1-2): 251-5, 2000]. These studies utilized physiological or psychophysical procedures and data.

Theoretical neural responses to noise-modulated single-pulse stimuli have also been studied previously [Xu YF and Collins L.M. "Threshold prediction for a noise-modulated electrical stimulus using a stochastic auditory nerve model: implications for cochlear implants", ARO2002]. The addition of noise did not enhance threshold or intensity discrimination with single-pulse stimuli. In this work, thresholds for noise-modulated pulse train stimuli are predicted by utilizing a neural-behavioral model of ensemble fiber responses to bi-phasic stimuli. A count comparison rule has been presented for both threshold and intensity discrimination under the assumption that loudness is a monotonic function of the number of neuron spikes [Bruce I. et. al., "The effects of stochastic neural activity in a model predicting intensity perception with cochlear implants: low-rate stimulation", IEEE Trans. Biomed. Eng. 46(12): 1393-404, 1999]. An alternative approach involves analyzing the neural response to each pulse within the pulse train in order to investigate threshold performance. We first derive the stochastic properties of the auditory nerve response to each pulse within

the pulse train. Then, the probability of detecting portions of the total stimulus is studied (N of M test) and a logarithmic rule is hypothesized for the pulse train threshold. The predictions from this rule are shown to match psychophysical data not only for the noise-free stimuli but also for the noise-modulated stimuli.

287 Influence of Pulse Rate on Channel Interaction in a Cochlear Implant

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We measured the effect of sub-threshold stimulation of one cochlear-implant channel on the threshold of a second channel. Two-channel electrode arrays were implanted acutely in the scala tympani of anesthetized guinea pigs. Stimuli were trains of biphasic electrical pulses, 20 microsec per phase, presented from both channels either simultaneously or interleaved in time. Neural spike activity was recorded simultaneously from 16 sites in the primary auditory cortex. Channel interaction was quantified as the shift in the cortical threshold for apical-channel stimulation that was produced by sub-threshold stimulation of the basal channel.

In every case, threshold shifts were substantially greater when pulses from two channels were simultaneous than when they were interleaved; simultaneous basal-channel stimuli at >12 dB below the basal-channel threshold produced appreciable apical-channel threshold shifts. In interleaved conditions, threshold shifts consistently were greater at pulse rates of 4069 pulses per second (pps) than at 254 pps. For instance, 4069-pps trains at >3 dB below the basal-channel threshold produced appreciable threshold shifts whereas 254-pps trains produced threshold shifts only when the basal channel stimulus was within 1 dB of its threshold. Threshold shifts were insensitive to the duration of inter-pulse delays in the range of 40 to 123 microsec. In contrast, delays of 1966 microsec abolished threshold shifts in the 254-pps condition. We also tested the influence of pulse rate on thresholds in a one-channel condition. Thresholds were constant for pulse rates of 250, 500, and 1000 pps but decreased at a rate of ~2 dB per doubling of rate from 1000 to 8000 pps. Increased channel interaction at high pulse rates is of concern because of the trend toward use of higher pulse rates in speech processors for cochlear prostheses.

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288 Effects of High-Rate Pulse Trains on Frequency Discrimination by Cochlear Implant Users.

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It has previously been demonstrated that the addition of a high-rate "conditioning" or "desynchronizing" pulse train produces spontaneous-like activity in a computational model of the deafened auditory nerve [Rubinstein et. al., Hear Res, 1999]. Animal studies support these predictions. We have recently demonstrated gains in dynamic range on individual electrodes by users of the Clarion C-II cochlear implant [Hong et al., Otol & Neurotol, (in press)]. A 5000-pps biphasic pulse train was used to generate spontaneous-like activity in the neurons stimulated by the particular electrode pair, resulting in increases in dynamic range consistent with stochastic resonance (mean largest increase ~7 dB). In the present study, we examined the effects of the conditioning pulse train on frequency discrimination on a single electrode pair. Subjects had participated in the dynamic range study and were familiar with the psychophysical testing. The electrode pair demonstrating the greatest increase in dynamic range was selected for the present study. Using a 3-interval forced-choice procedure, the chosen electrode pair was stimulated with three 500-ms sinusoid bursts. The reference stimuli were two 202-Hz sinusoids, while the test signal was a sinusoid higher in frequency than the references. The subjects were asked to identify the test signal. The procedures were carried out with and without the conditioning pulse train. Similar results were obtained whether the conditioner was present or not suggesting that at least in this preliminary study, the addition of a 5000-pps pulse train does not improve a CI user's ability to make fine discriminations along the frequency domain on a single electrode pair.

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289 Perception of Sinusoidal Modulations of High-Rate Electric Pulse Trains by Cochlear Implant Listeners

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In continuous interleaved sampling strategies (CIS) for cochlear implants, sound is represented as modulations of electric pulse trains. The HiRes strategy from Advanced Bionics can accurately represent fluctuations as fast as 4000 Hz in the modulation envelope. Although HiRes represents more of the sound as compared to earlier CIS, it is unclear whether cochlear implant listeners can use the additional information contained in these higher-frequency envelope fluctuations. In this study, subjects were asked to judge relative closeness (in pitch) of an electric, sinusoidally modulated pulse train (test sound), to two standards: one a modulated pulse train (modulation frequency 100 Hz), and another, an unmodulated pulse train. All three sounds had the same carrier rate (either 3 or 10 kHz). Modulation frequency of the test sound was varied from 100 to 1000 Hz. Because perceived loudness may depend on modulation frequency, amplitude of the test sound was varied from presentation to presentation.

For all subjects except one (who reported no difference in pitch between standards), perceived pitch of the test sound changed methodically with modulation frequency from pitch of first to pitch of the second standard. Perceived pitch depended weakly on amplitude of the test sound, suggesting that it is based primarily on modulation frequency. The lowest modulation frequency for which perceived pitch was near that of the second standard varied from 300 to 800 Hz across subjects. Subjects also differed in qualitative description of the differences between the two standards. One subject described low-rate modulated pulse trains as complex, and unmodulated pulse trains as pure, while another described both as pure sounds. These results suggest that most subjects should benefit from the additional temporal information in the HiRes strategy.

290 The Time Course of "Simultaneous" Masking in Cochlear Implant Listeners: an "Overshoot" in Electrical Stimulation?

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The present study was aimed at obtaining a further understanding of the temporal properties of the masking caused by background sounds in cochlear implant listeners. The detectability of a 20-ms-long probe was measured as a function of time delay (TD) from the onset of a 300-ms-long background to the onset of the probe. Probe and masker signals were both 500-Hz pulse trains with their pulses interleaved in time. Subjects were three adult users of the Nucleus-22 cochlear implant. Signals were presented in bipolar+2 mode with the background always on electrode pair (10, 13) and the probe on either (10, 13), or applied remotely at (4,7) or at (16, 19). Thresholds for the probe were determined using a 3-interval forced-choice paradigm with feedback. Backgrounds were applied at various sensation levels ranging from that just producing masking of a probe on (10, 13) to an upper level at approximately 70% of the listener's dynamic range.

The backgrounds almost always produced masking that was a function of TD, first increasing and then reaching a maximum by a TD of 21 ms. Masking then decreased to a quasi steady-state (SS) value at TDs in excess of 121 ms. Hence these data exhibited an "overshoot" in masking analogous to that seen psychoacoustically. The maximum and SS threshold shifts increased as a function of masker level, as did the overshoot as measured by the difference between them in microamps. The dB overshoot either increased or remained constant as masker level increased. Masking and overshoot decreased for probes applied at the

remote electrodes, however, similar amounts of SS threshold shift generally corresponded to more overshoot for the remote electrodes. The maximum overshoot observed ranged from 1.5 to 2.0 dB across subjects, which is significant considering the limited dynamic range in cochlear implants.

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291 Binaural Interaction with Bilateral Cochlear Implants in Cats

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Since some years children and adults were implanted with Cochlear Implants bilaterally. But there exist no or poor physiological data till yet analysing binaural interaction during electrical stimulation during maturation. Therefore binaural interactions in brain-stem auditory evoked potentials were studied in normal hearing kittens and adult cats, neonatally and adult deafened cats. The stimuli were binaurally symmetrical and isochronical presented at 40 dB SL (sensation level) or with interaural time disparities (ITD) of +/- 0,1 to +/- 2,5 ms, or intensity disparities (IID) of +/- 2 to +/- 14 dB. At the age of 9 to 30 days the recordings took place every 4 days, later once a week.

The binaural interaction component (BIC) could be clearly identified at day 17 in four kittens. All cats showed a BIC at day 20.There were great differences in the amplitudes and latencies of wave 4 of the ABR. Wave 5 first appears at day 17. From day 17 to day 60 the BIC became more prominent and the latency of the BIC-components (DP1, DN1, DP2 and DN2) decreased. After day 60 the BIC had adult form. The findings of this study were compared with adult cats that were implanted with intracochlear electrodes. During the recording the electrical thresholds for apical, basal and electrode pairs 1-8 were evaluated for both implants. Bilateral stimuli at about 3 to 4 dB-μA above the thresholds were presented to both ears. In all electrode pairs the amplitude of the sum of the monotic stimulation was larger than the recordings with diotic stimulation so that a BIC could clearly be identified.

These results showed that the advantages of binaural hearing such as binaural fusion, localisation and detection of signals in noise, could also be achieved by a bilateral cochlear implant stimulation.

292 Psychophysical Studies on Sound Localization and Speech Intelligibility in Noise in Bilaterally Implanted Cochlear Implant Users

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In recent years, there have been improvements in speech processing strategies used in CIs, which are particularly evident in speech understanding in quiet. However, for most CI users speech reception in a noisy or complex environment is still very challenging. To address this problem, a limited number of CI patients have received two implants. Most patients' anecdotal reports are extremely positive; they much prefer the use of both CIs together and report that auditory images are significantly more externalized and localizable. However, objective measures of these reports are rather sparse. As part of a multi-site bilateral clinical trial, 45 patients, , received simultaneous, bilateral cochlear implants (Nucleus 24 Contour). All patients use the same speech processor and coding strategy for both ears. Testing was conducted on a subset of 17 patients, at least 3 months after activation of the two processors. All tests were repeated with both implants activated or with each implant separately. Tests included sound localization with an 8-speaker array, and speech intelligibility in the presence of competing babble. Preliminary results suggest that bilateral devices generally have an advantage compared with the "better ear" device. With one device most users are unable to identify source locations, or even the correct hemifield. With bilateral implants, the majority (88%; 15/17) of patients show improvement in location identification. In addition, speech intelligibility in the presence of competing babble is better with bilateral devices than with either ear alone, especially when the target and interfering signals are spatially separated.

293 Click Train Encoding in a Song Bird; Evidence For Two Mechanisms of Pitch Perception

*Jeffrey A. Cynx

In humans, the pitch of click trains composed of pulses with the same and alternating polarity can vary depending on whether the pulse sequences are below 100 Hz or above 200 Hz. Below 100 Hz, the pitch is equal to the pulse rate regardless of polarity. Above 200 Hz, the pitch is determined by the fundamental frequency and the pattern of click polarity (J.L. Flanagan and N. Guttman, J. Acoust. Soc. Am., 1308-1319,1960). European starlings, *Sturnus vulgaris*, a species of songbird, were trained on an operant procedure with bipolar stimuli on a two tone discrimination, then tested with unipolar stimuli. We then built a psychometric function, especially covering the ambigous region between 100 and 300 Hz, and the upper frequency limits.

294 Effects of Pitch-Altered Auditory Feedback on Budgerigar Vocal Production

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Birds rely on auditory feedback (AF) to develop and maintain a normal vocal repertoire. Most of what is known about this phenomenon comes from deafening studies in young and adult birds. Recent work, however, has uncovered intricate effects of AF on vocal production. For instance, altering or disrupting AF in zebra finches generally results in the production of abnormal songs in which new syllables are created and existing syllables are multiplied, distorted, or deleted entirely. Because of the obvious parallels between birdsong and human speech and language, there is considerable interest in understanding the mechanisms whereby AF allows a bird to learn and produce stable song throughout its life. Previous work has shown that budgerigars increase the intensity of their contact calls in the presence of increasing levels of background noise, a phenomenon known in humans as the Lombard effect (Manabe et al., 1997). Here we extend these experiments to the pitch-shift reflex, an involuntary raising or lowering of a speaker's voice pitch in response to a reciprocal raising or lowering of the AF pitch. We trained budgerigars to produce contact calls for food reinforcement in the presence of pitch-shifted AF. Results suggest that these birds, like humans, can adjust the pitch of their calls to compensate for the presence of altered feedback.

295 Discrimination of Pitch Direction by Macaque Monkeys

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Behaviorally significant information is contained in the spectral and temporal relationships between the individual elements of acoustic signals. For example, humans recognize melodies widely independent of the spectral composition, pitch, and intensity of the notes and of the tempo of the tune. After an extensive research, D'Amato (Music Perc., 5: 452-480, 1988) concluded that animals cannot hum a tune because they do not recognize relationships between tones. Recent studies, however, have found evidence that monkeys can extract tone relationships (Wright, AA et al., 2000; Hauser, MD et al., 2001). The goal of the present study was to explore whether monkeys have the capacity to recognize melodies.

The most salient feature for the recognition and identification of melodies is the pitch direction, which is characterized by the sequential up- and-down patterning of the pitches of adjacent tones in a tune. Therefore, we trained two Macaca fascicularis to discriminate the direction of a pitch change. As this task was quite difficult, task

complexity was progessively increased during training. Initially, subjects were trained with sequences of repeating tones with two different frequencies, in which they had to signal when the frequency of the tones changed. Subsequently, sequences of repeating tones with 3 different frequency were used. Subjects were now required to respond when the frequency of the tones changed in a downward direction and to refrain from responding when the frequency remained constant or increased. After several ten thousands of trials, subjects categorized pitch directions well above chance level, over a 4.5-octave range of frequencies and largely independent of the ordinal position of the downward pitch direction within the sequence. This indicates that monkeys can recognize pitch relationships and suggests that monkeys have the ability to identify melodies.

296 Fusion of Harmonic Partials by Ferrets

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Human listeners automatically fuse harmonically-related spectral components of sound into a unitary perceived entity, a phenomenon that is important for the ability to parse a sound field into its many constituent sources. In order to determine whether harmonicity is a similarly potent grouping cue in animals, we tested if ferrets can distinguish between harmonic and inharmonic complex tones.

To test if harmonic fusion occurs automatically (rather than with practice) animals were trained to discriminate pure tones from inharmonic complex tones, and then the learned categorization was probed with harmonic complex tones. Water-deprived animals drank from a spout while listening to ongoing inharmonic-complex-tone reference sounds. They were trained to withdraw from the spout for pure-tone targets by means of a mild shock immediately after failed withdrawals for the target. The unitary quality of pure tones, as opposed to the disjointed quality of inharmonic complex tones, was the main cue available for distinguishing between reference and target sounds. After proficiency was reached at the task (> 80% targets detected), 10% of the reference sounds were replaced by harmonic-complex-tone probes to test if they, like the pure tones, elicited withdrawal. The probes were detected at a four times greater rate than the false-alarm rate in one of two ferrets tested, which suggests that harmonic complex tones were distinguished from inharmonic complex tones and that they were confused with the pure tone. In the other ferret, the probes were not detected at a significantly different rate than the false-alarm rate, probably because the animal cued on the timbre difference between pure tones and complex tones.

The finding that at least one animal distinguished harmonic complex tones from inharmonic complex tones suggests that ferrets can automatically fuse harmonically-related spectral components of sound.

297 Evidence that Musical Universals are Determined by the Statistical Characteristics of Human Voices

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Listeners of all ages and societies perceive tones separated by an octave interval as musically equivalent and produce a similar consonance ordering of chromatic scale tone combinations. The apparent universality of these perceptual phenomena suggests a basis in some fundamental, species-wide property of human audition. This hypothetical property, however, has remained elusive, for despite intense interest in these perceptual phenomena over several millennia, they have no generally accepted explanation in physical, psychological or biological terms. Here we show that both octave equivalence and consonance ordering can be understood in terms of the statistical relationship between a pattern of sound pressure at the ear and the possible generative sources of the acoustic energy pattern. Since conspecifc vocalizations are the primary source of the tone-evoking (i.e., periodic) sound energy to which humans are exposed, we obtained normalized spectra from >100,000 recorded speech segments. The probability distribution of amplitude/frequency combinations derived from these spectra predicts the perception of octave equivalence, the fundamental frequency ratios that define the chromatic scale intervals and the consonance ordering of chromatic scale tone combinations. We suggest that these observations reveal the statistical character of the underlying process by which the auditory system guides biologically successful behavior in response to inherently ambiguous sound stimuli.

298 Infants' Perceptions of Transposed Melodies

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In music, pitches are defined by height and chroma. It has been suggested that infants are similar to adults in their perception of tone chroma (Demany and Armand, 1984), in that they perceive two notes an octave apart as having the same chroma. This is called "octave-equivalence". In previous reports, using simple tonal melodies, we have shown that infants learn to treat a transposition as equivalent to a base melody faster for transpositions of the 4th and 5th than for the octave. This is opposite of what would be predicted if infants recognized the common chroma of the base melody and octave transposition. In the present study, infants' and adults' perceptions of the 7th and 9th transpositions were tested. These intervals bracket the octave in tone height, but do not share common chroma with the base melody. Subjects were tested using an observer-based procedure. After having heard a standard background melody, subjects were trained to respond to a training melody of a different contour and key. Once that discrimination had been learned, transpositions of the background melody were introduced. Each test condition contained the original background melody and one type of transposition of the background melody. Both adult and infant subjects were then trained to continue to respond to the training melody, but not to the transposition of the background melody. If infants are similar to adults in that they recognize the shared chroma of the background melody and its octave transposition, they should learn to ignore the octave transposition more quickly than other transpositions. The results showed that infants treated the test transpositions of the 7th and 9th more like they had treated the octave, and not like transpositions of the 4th or 5th. It still took more trials for infants to learn that test transpositions of the 7th and 9th were simply transpositions of the background melody, suggesting a frequency distance effect rather than supporting octave-equivalence.

299 Pitch Perception of Transposed Stimuli

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This study examines our ability to use high-frequency envelope cues, normally only available in the low-frequency fine structure, to perceive pitch. By multiplying a high-frequency carrier with a halfwave-rectified low-frequency modulator, van de Par and Kohlrausch [J Acoust Soc Am 101:1671-80 (1997)] found that these "transposed" stimuli could produce very good performance in binaural tasks. We tested whether this conclusion was also true for monaural temporal processing, in particular for frequency discrimination. Frequency difference limens (FDLs) were measured for sinusoids with frequencies from 55 to 320 Hz and for transposed stimuli with carrier frequencies between 4000 and 10000 Hz. Binaural lateralization performance was studied in the same subjects by measuring just-noticeable differences in interaural time differences (ITDs) using the same tones and transposed stimuli. Replicating Bernstein [J Neurosci Res 66:1035-46 (2001)], ITD performance for the transposed stimuli matched or exceeded that for the low-frequency tones for frequencies up to 150 Hz. In contrast, FDL performance was considerably worse for transposed stimuli than for pure tones in all conditions. Complex pitch perception was tested by modulating three high-frequency carriers with the second, third, and fourth harmonics of a low-frequency fundamental, and requiring listeners to judge whether the pitch of the resulting complex was higher or lower than the pitch of a transposed fundamental alone. Despite some training and continuous feedback, no listener could perform the task,

suggesting that they were unable to extract the fundamental from the complex. The results suggest that temporal information alone is not sufficient to produce strong pitch salience. Instead, and in contrast to most current temporal models of pitch, stimulation at the "correct" place along the cochlear partition may be crucial for good pure-tone and complex pitch perception.

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300 Pitch Shifts For Unresolved Complex Tones And The Implications For Models Of Pitch Perception

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This experiment compared the pitches of complex tones consisting of unresolved harmonics with fundamental frequencies (F0s) of 100, 125, 166.67, and 250 Hz. The complexes were bandpass-filtered between the 22nd and the 30th harmonic to produce a set of unresolved harmonics with distinct envelope peaks ("pitch pulses"). Each tone burst had a duration of 5 waveform cycles and two tone bursts were presented consecutively, separated by a brief gap of either 0, 1, or 2 waveform periods. The envelope phase of the second tone burst in each pair was advanced or delayed by 0.25, 0.5, or 0.75 periods. Effectively, this resulted in a variation in the inter-pulse interval (IPI) between the two tone bursts. A no-shift control was also included, in which the IPI was fixed at an integer number of periods. Pitch matches were obtained by varying the F0 of a comparison complex tone with the same temporal parameters as the standard, but without the phase shift. Relative to the no-shift control, the variations in IPI produced substantial pitch shifts when there was no gap between the bursts, but no effect was seen for gaps of 1 or 2 periods. This is consistent with a pitch mechanism employing a long integration time for continuous stimuli that is reset in response to temporal discontinuities of greater than 1 period of the waveform. The results were inconsistent with the autocorrelation model of Meddis and O'Mard (1997), but a modification of the weighted mean-rate model of Carlyon et al. (2002) could account for the data.

301 Effects of Harmonic Resolvability on the Cortical Activity Produced by Complex Tones

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Activity in certain areas of human auditory cortex increases with temporal regularity in iterated rippled noise (IRN; Griffiths et al., Nat. Neurosci. 1:422-7, 1998). Because temporal structure and pitch salience co-vary with IRN, it is not clear whether the changes in cortical activity reflect pitch salience or stimulus regularity. Complex tones comprising peripherally resolved harmonics evoke a stronger pitch than unresolved harmonics, despite both being perfectly periodic. Here we used functional magnetic resonance imaging (fMRI) to investigate whether harmonic resolvability can affect human cortical evoked activity. Four conditions were used, comprising complex tones with either low (80 to 95 Hz) or high (240 to 285 Hz) fundamental frequencies (F0), bandpass filtered into either low (320 - 1150 Hz) or high (1200 - 2000 Hz) spectral regions. Both low-F0 and high-F0 complexes contained resolved harmonics in the low spectral region, but only the high-F0 complex contained resolved harmonics in the upper spectral region. The brains of normal-hearing subjects were scanned while listening passively to the stimuli. A comparison of the activation across the different conditions revealed a difference between resolved and unresolved harmonics that could not be attributed to F0 or spectral region. These results suggest that the changes in cortical activity may reflect pitch salience rather than regularity in the temporal waveform.

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302 Population-interval Models for Pitch Masking and Harmonic Resolvability

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Population-wide all-order interspike interval distributions at the level the auditory nerve constitute a temporal, autocorrelation-like neural representation of the stimulus spectrum for periodicities below ~5 kHz. Features of these representations successfully predict the low pitches of complex tones. Recently auditory nerve simulations were used to assess whether these representations might also account for pitch masking and harmonic resolvability.

Computer simulations incorporated an array of 24 logarithmically spaced gammatone filters, half-wave rectification, low-pass filtering (2-5 kHz rolloff), rate-level compression (3 fiber classes per CF), and spontaneous activity. Global population-interval distributions were computed by pooling autocorrelations of all fiber responses. Distributions were then weighted using a tapering window (0-33 ms). Estimated pitch salience was the ratio of pitch-related intervals/bin (in a subharmonic interval bin sieve (1/f, 2/f, 3/f …n/f, ± 100 us) to the mean intervals/bin in the distribution. Pitches with saliences > 1.3 were assumed to be audible.

Pitch masking patterns for two simultaneous pure tones predicted from simulated population-interval distributions qualitatively replicate basic features of pure tone masking audiograms, such as upward spread of masking and competition for cochlear territory. Predicted pitch saliences of AM tones depended on both fc and harmonic number n (ceteris paribus, higher n produced weaker pitches). Predictions were generally consistent with the existence region for F0-pitch of AM tones. For harmonic complexes (F0=100-400 Hz; n=1-12) saliences of individual harmonics declined with increasing n. The model predicted that the pitches associated with only the first 4 harmonics should be audible/resolvable (saliences > 1.3). Other strengths and weaknesses of these models are discussed.

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303 Auditory Evoked Fields Reflect Minimum Duration Thresholds in Temporal Pitch Processing

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In the Auditory Image Model (AIM), pitch corresponds to a stable pattern of phase-locked time-intervals in the "Auditory Image". The current studies were conducted to determine the time constants involved in temporal pitch extraction and to relate these parameters to the auditory evoked field (AEF). Psychoacoustic thresholds were determined for a) the minimum duration required to detect a short segment of a Regular Interval (RI) sound embedded in noise, and b) for the detection of a short segment of noise in a RI sound. Experimental variables were the delay and the number of iterations for RI generation. There were four listeners. As expected from AIM, threshold duration was found to increase with the delay of the RI sound and to decrease with its temporal regularity, both for RI sounds in noise and vice versa. However, thresholds for the detection of RI sounds were significantly lower than for the reverse. The AEF-experiment had eight conditions: four RI sounds with one of two delays (8 or 20 ms) and one of two degrees of regularity (low and high) were presented in noise and vice versa. Responses from seven subjects were recorded using magnetoencephalography. Source waveforms derived from one equivalent dipole located in Heschl's gyrus in each hemisphere revealed marked differences between conditions. RI sounds evoked a prominent anterior N1m that was found to increase with the number of iterations, length of the RI sound and decreasing delay. In contrast, noise bursts evoked an early positive response. The correspondence between the

asymmetry of masking in the perceptual data and the N1m asymmetry suggest that the source is a pitch-sensitive generator.

304 Different Effects of Overnight Consolidation on Three Types of Learning in Interaural-Time-Difference Discrimination

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Training on perceptual discrimination often yields improvements after a period of consolidation, during which the new skills and memories are stabilized. We investigated whether consolidation could be used to differentiate three types of learning proposed to occur early in training: learning of (1) the general methodology, (2) the discrimination task, and (3) the specific stimulus. We tested listeners on a target interaural-time-difference (ITD)-discrimination condition following 20 min. of training on a condition that shared in common with the target condition (1) only the methodology, (2) the task and methodology, or (3) the stimulus, task, and methodology (the target ITD condition itself). ITD testing occurred either immediately after training (presumably before consolidation) or on the day after training (presumably after consolidation). Methodology-trained listeners had slightly lower ITD thresholds than 67 untrained controls, both before (n=10) and after (n=9) consolidation, revealing methodology learning that was not affected by consolidation. Both 14 task/methodology-trained listeners and 20 target ITD-trained listeners had ITD thresholds significantly lower than controls before consolidation, revealing general task learning but no apparent stimulus-specific learning. However, after consolidation, ITD thresholds were significantly lower than controls for 20 ITD-trained listeners but not for 15 task/methodology-trained listeners, revealing stimulus-specific learning and a seeming loss of general task learning. Consolidation of specific learning likely occurred overnight, as 16 task/methodology-trained listeners who tested 10 hours after training on the same day still had ITD thresholds significantly lower than controls. Thus, consolidation differentiated three types of learning, having no effect on methodology learning, and masking task learning by enhancing stimulus learning.

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305 Learning of Interaural-Time-Difference Discrimination with High-Frequency Complex Tones

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Interaural time differences (ITDs) and interaural level differences (ILDs) are the two primary cues used to localize sound sources on the horizontal plane. Previously we reported that, following a stage of rapid learning, multi-hour practice induced further learning on human discrimination of pure-tone ILDs at 4 kHz but not ITDs at 0.5 kHz [1]. Here we investigated whether these different learning patterns resulted from greater plasticity in the binaural processing of (1) stimuli with high than low center frequencies or (2) ILD than ITD cues. Toward this end, we trained 7 normal-hearing listeners 1 hr/day for 9 days to discriminate the lateral position of a 4-kHz tone that was sinusoidally amplitude modulated at 0.3 kHz, based on on-going ITDs in both the envelope and fine structure of the sound. Before and after training, we tested the trained listeners and 8 untrained controls on the trained condition. The final average threshold of all listeners was 217 μs, markedly higher than the 33 μs previously obtained with pure tones. Nevertheless, similar to our previous pure-tone results, the thresholds of both trained and control listeners decreased significantly and equally, and none of the trained listeners learned over the training sessions, indicating that learning occurred rapidly and that further practice had no effect. Thus, taken together with our previous results, we observed (1) the same learning pattern for ITD discrimination of sounds with both high and low center frequencies, and (2) different learning patterns for ILD and ITD discrimination of sounds with a high center frequency. These results suggest that the different learning patterns of ITD and ILD discrimination reflect differences in the plasticity of ILD and ITD processing.

Supported by NIH

[1]Wright BA, Fitzgerald MB (2001). Different patterns of human discrimination learning for two interaural cues to sound-source location. PNAS 98, 12307-12312.

306 Binaural Training Effects on Spatial Release from Masking in Children Following Otitis Media with Effusion (OME)

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The long-term effects of OME on hearing are not well understood. Children who have chronic OME can spend much of the first few years of life with disrupted interaural level difference (ILD) and interaural time difference (ITD) cues that vary as a function of the pathology. Their fluctuating conductive hearing loss might impair their ability to utilize binaural cues. Reduced binaural unmasking has been reported, and it is possible that chronic OME might also impair the ability to interpret speech materials in the presence of spatially separated interfering signals. Finally, recovery from OME-induced impairments may be accelerated with auditory training. We studied 6 children aged 9-11, who had OME for the majority of their lives and had been treated with ventilation tubes. They had reduced ILD and ITD discrimination ability. Binaural training in a pure tone, 2AFC task in ILD or ITD discrimination, or both, was undertaken for 30 minutes/day for 6 days. An adaptive staircase was used for threshold estimation and feedback was given on discrimination performance. Speech Reception Thresholds (SRTs) were obtained PRE and POST training using a 4AFC task, with targets presented in front and interfering speech from 0º front or 90º right. Results suggest that SRTs are lower in the POST-training conditions, especially in the most difficult condition, with both target and interferer at 0º front. While binaural abilities may not be permanently compromised as a result of OME, it is possible that binaural training facilitates the recovery of children's ability to extract signals in noisy environments.

307 Predicting Masked Detection and Speech Recognition in Reverberant Rooms

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The purpose of this research is to develop a model to account for masked detection of signals in reverberant rooms and to use that understanding to predict speech reception in reverberation. In particular, the goal is to predict detection and speech recognition as functions of room variables (size, acoustic absorption), source variables (spectral content, location, and directivity index of the signal and masker sources), and listener variables (left-ear, right-ear, or binaural listening). To enable initial measurements of signal detection in well-controlled and manipulable conditions, an image-method simulation of a rectangular room was used, with a sphere modeling the head. Signals and maskers created with this simulation were presented to listeners over headphones. Monaural and binaural masked detection thresholds were obtained for one-third-octave noise signals in a broadband masker. Primary stimulus variations included signal frequency, room absorption, and source direction and distance. Monaural detection results were modeled using general room acoustics equations combined with sphere diffraction to estimate the signal-to-masker ratio at each ear. Predictions of binaural detection were based on Durlach's EC model. Among the predictions tested in this study were that reductions in binaural advantages with increasing reverberation occur because of increasing reverberation of the masker, not the signal. Also explored was the nature of the interaural correlation of early and later reflections and how these affect predictions of binaural release from masking. The

predictions based on the combination of room-acoustics equations and the EC model provide an acceptable account of the monaural and binaural detection data examined thus far. Such detection predictions allow binaural advantages and source-direction effects to be incorporated into methods for predicting speech intelligibility in reverberation.

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308 Enhancing ITD-Based Extents of Laterality at High Frequencies by Using "Transposed Stimuli"

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An acoustic pointing task was used to determine whether interaural temporal disparities (ITDs) conveyed by high-frequency "transposed" stimuli, having envelopes designed to provide high-frequency channels with information similar to that available in low-frequency channels, would produce larger *extents of laterality* than ITDs conveyed by bands of high-frequency Gaussian noise. Lateralization was measured for low-frequency Gaussian noises, the same noises transposed to 4 kHz, and high-frequency Gaussian bands of noise centered at 4 kHz. Extents of laterality obtained with the transposed stimuli were greater than those obtained with bands of Gaussian noise centered at 4 kHz and, in some cases, were equivalent to those obtained with low-frequency stimuli. Additionally, the general effects on lateral position produced by imposed combinations of bandwidth, ITD, and interaural phase disparities (IPDs) on low-frequency stimuli remained when those stimuli were transposed to 4 kHz. Overall, the data were fairly well accounted for by a model that computes the cross-correlation subsequent to known stages of peripheral auditory processing augmented by low-pass filtering of the envelopes within the high-frequency channels of each ear. These outcomes provide further support for Colburn and Esquissaud's (1976) hypothesis [H. S. Colburn and P. Esquissaud, J. Acoust. Soc. Am. 59, S23 (1976)] that frequency-related differences in sensitivity to ongoing ITDs stem from frequency-related differences in the properties of the temporal signatures of the "internal" stimuli, rather than from frequency-related differences at the level of binaural interaction, per se.

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309 Free-Field Binaural Unmasking in Rats: Effects of Masker Level and Signal Frequency

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The detection of acoustic signals in noise is important for understanding both, the mechanisms of hearing and how the auditory system functions under natural conditions. In the presence of a masking noise, detection threshold is increased. Binaural unmasking quantifies the improvement in threshold when signal and masker are presented with different interaural properties. In the free-field condition used in our experiments, spatially segregated sources for signal and masker created interaural cues as they occur in the real world. These cues probably provide an important basis for the segregation of complex auditory scenes and to separate signals of behavioral relevance from background noise.

We investigated the effects of spatial release from masking in a small mammal, the laboratory rat, with good high-frequency hearing ability. Three well trained rats were tested in a 2AFC-procedure (constant stimuli and adaptive testing) for the effects of masker level, signal frequency, and the angle of separation between signal and masker source. Animals had to detect the side of signal presentation in the presence of background noise offered from the same or a loudspeaker located at a different direction. In most cases, the effect of a parameter was tested very efficiently in a single session with the procedures using different test values randomly interleaved.

Spatial release from masking was between 10 and 20 dB for the one signal/one noise source situation depending on separation (90°, 180°) and signal frequency (5, 10, 20, 30 kHz). Effects increased with signal frequency, but were very similar for the 90° and 180° situation. Masker level had only minor influences on masking thresholds. Therefore, binaural unmasking is also present in the rat under various stimulus conditions and these effects are especially pronounced in the high frequency range.

310 The Effect of Recurrent Otitis Media with Effusion on Binaural Hearing in Pre-Teen Children

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Otitis media with effusion (OME) is a common childhood condition. It causes a fluctuating conductive hearing loss, disrupting interaural level differences (ILD) and interaural time differences (ITD), and reducing masking level difference (MLD), another measure of binaural processing ability. MLD remains reduced after resolution of OME but returns to normal by the teenage years. We determined how children with a history of recurrent OME performed in ILD and ITD discrimination tasks and we measured their MLDs. Three groups were tested: 9-11 year old children with a history of recurrent OME treated with ventilation tubes (C1), age-matched children, prospectively studied by regular tympanometry in their first six years of life, known to have had minimal or no OME (C2), and audiometrically normal adults (A). All subjects were tested on pure tone ILD and ITD discrimination using a 2 alternative forced choice task. An adaptive staircase was used for threshold estimation. MLD was derived from the difference between thresholds in (i) a binaural condition of tone signal and noise, interaurally in phase, and (ii) a condition in which the signal was inverted by 180° in one ear. In ILD discrimination, C1 performed significantly worse than C2. A similar trend was seen in ITD discrimination. In both ILD and ITD, A performed significantly better than C1 and C2. MLDs in C1 were not significantly smaller than in the other groups, but showed greater variability. We conclude that children with a history of recurrent OME perform worse in ILD and, to a lesser extent, ITD discrimination tasks than age-matched controls. We suggest that ILD may be relatively more disrupted by OME, or due to the fluctuating nature of OME may be the less reliable cue, leading to a slower recovery. Increased variability in MLDs may be due to the range in time since recovery from OME.

311 Is There a Role for Sound Localisation in Speech Intelligibility in Noise?

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When a target voice is spatially separated from a concurrent interferer, focusing attention on the target voice's location is often thought to aid intelligibility. As the direction of a sound is determined by interaural time difference (ITD) and interaural level difference (ILD), we tested the importance of sound localisation for speech intelligibility in noise by introducing inconsistencies between different frequency regions in each of these binaural cues. Target sentences were presented as a pair of high- and low-pass bands separated by a 1-ERB notch with each band having its own ITD or ILD. Speech reception thresholds (SRTs) of normally hearing participants were measured for target sentences against a background of either brown noise or a speech interferer. SRTs for consistent and inconsistent target ITDs were significantly lower than those measured for a baseline condition (target and interferer share a common ITD), but indistinguishable from each other. SRTs for consistent and inconsistent ILDs were significantly different. Here, listeners appeared to be able to attend to the 'best' ear, but unable to

exploit ILDs that favoured different ears at different frequencies. The results of these experiments indicate that for the intelligibility of speech in the presence of an interferer ITDs only have to differ between target and interferer within each frequency band, while ILDs are processed monaurally. Speech intelligibility in noise appears not to be dependent on sound localisation.

312 Differential Effects of Fluoxetine on Auditory and Frontal Cortex Networks Growing on Multielectrode Arrays

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Serotonin is a neurotransmitter involved in stimulus reactivity and sensory reception. Low levels of serotonin is linked to abnormal conditions such as depression, hyperacusis, migraine headaches and decreased auditory processing. Fluoxetine (prozac), a selective serotonin reuptake inhibitor (SSRI) is widely prescribed to facilitate serotonergic transmission and reduce symptoms of depression. In this research, spontaneously active neuronal networks growing in culture on multielectrode arrays were acutely exposed to various concentrations of fluoxetine. The networks showed repeatable, quantifiable and tissue-specific electrophysiologic responses to fluoxetine. Auditory cortex networks showed excitatory responses to fluoxetine at concentrations of 1-10 μM, followed by inhibitory responses around 15 μM, and complete cessation of activity at 20-25 μM. Fluoxetine had only inhibitory effects on frontal cortex networks, and terminated all activity at concentrations of 10-16 μM. The IC50 mean ± S.E. for spike rates was 15.9 ± 1.0 μM for auditory networks, and 5.4 ± 0.7 μM for frontal networks. The effects of fluoxetine were, however, reversible with a complete medium change.

313 Dopamine Blocks Acetylcholine-induced Reduction of Glutamate Currents in the Auditory Cortex

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Dopaminergic fibers project to all cortical areas including the primary auditory cortex AI. The role of dopamine (DA) has been extensively investigated in the prefrontal cortex, where DAergic activity has been correlated with various human pathologies. Less attention has been devoted to the role of DA in primary sensory cortices. Cortical layer 2/3 neurons receive abundant excitatory inputs from non thalamic sources, that is selectively depressed in the presence of acetylcholine (ACh). We tested the hypothesis that DA interferes with ACh in the modulation of glutamate-mediated currents in A1 by in vitro recordings. Excitatory Post Synaptic Currents (EPSCs) were evoked by extracellular stimulation of layer 4 and recorded in layer 2/3 in the presence of the GABAA-R antagonist bicuculline (10μM). Evoked EPSCs, which were blocked by the AMPA-R antagonist DNQX (10μM), were reduced to 48±4% of control after bath application of the muscarinic agonist oxotremorine (10 μM). Bath application of DA did not change EPSC amplitude nor kinetics, but prevented the ACh-induced EPSC amplitude decrease (87±5% of control, n.s.). DA failed to block the effect of the muscarinic agent oxotremorine in the presence of the D1-like antagonist SCH23390 (10 μM) or of the D2-like antagonist spiperone (10 μM). Bath-application of the D1-like agonist SKF38393 (50-200 μM) failed to mimick DA effect, while application of the D2-like agonist quinpirole (10 μM) only partially prevented the ACh-induced EPSC amplitude decrease. On the contrary, joint application of SKF38393 and quinpirole mimicked DA in preventing the ACh-induced reduction in eEPSCs amplitude (86±6% of control), suggesting a cooperative effect of D1R and D2R activation.

We speculate that this cellular mechanism can enhance cortical response following reward-driven attention.

314 Synaptic Processing in Auditory Cortex: Data from In Vivo Intracellular Recordings and the Auditory Thalamocortical Slice.

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Lemniscal auditory information is relayed from the ventral division of the medial geniculate body (MGv) to layers 3-4 of primary auditory cortex (ACx). However, it is not clear how this information is processed intracortically to create auditory receptive fields (RFs). Intracellular whole-cell recordings (WCRs) from ACx layers 2-4 were performed in anesthetized rats to determine tone-evoked synaptic responses. Subthreshold synaptic RFs spanned up to 5 octaves at 70 dB SPL. This RF is broader than previously-shown RFs based on spike counts in both ACx and MGv, implying convergence at the level of ACx. To investigate the synaptic circuitry underlying broad RFs in vivo we turned to the auditory thalamocortical brain slice. Electrical MGv stimulation in 14 – 17 day old mice resulted in a focused, short latency field potential (FP) in layer 4 and longer latency FPs at more distant cortical sites (also recording within layer 4 but across presumed isofrequency bands). Laminar recordings revealed a current sink in layer 4 at the site of the shortest latency FP, and current sinks outside of layer 4 at the more distant cortical sites. These data predict that the characteristic frequency (CF) for a given cortical location will result in a short latency layer 4 current sink, whereas spectrally distant non-CF stimuli will result in longer latency sinks in supragranular or infragranular layers. Preliminary data in vivo have shown that CF elicited the shortest onset EPSP, while non-CF tones resulted in progressively increased onset latencies with spectral distance from CF. We hypothesize that CF information from MGv is relayed through layer 4 to other regions of primary ACx, contributing to the synaptic convergence underlying broad (subthreshold) spectral RFs.

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315 Intracortical Inhibitory Synapses Are Altered Following Sensorineural Hearing Loss

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Inhibitory synapses in the auditory brain stem display activity-dependent plasticity during development. Early deafening prevents the anatomical restriction of inhibitory terminals within the LSO or MSO, and weakens synaptic strength, including depolarization of E_{IPSP} within the IC (Sanes and Takács, 1993; Kotak and Sanes, 1996; Vale and Sanes, 2000; Kapfer et al., 2002). To explore whether auditory cortex exhibits such plasticity, whole cell recordings were obtained from neurons in layers 2-4 of AI in 500 μM horizontal brain slices at 31°C. Bilateral cochlear ablation (BCA) was performed at postnatal day (P) 9 and synaptic properties were examined following hearing onset (P14-P17). The location of AI was verified anatomically with DiI labeling of medial geniculate nucleus (MG) afferents in fixed slices, and with biocytin labeling of recorded neurons. The AI region was also characterized by MG-evoked field potentials (Cruikshank and Metherate, 2001). Evoked potentials varied from 100 to 600 μV, had a latency of 14 to 20 ms, and decayed within 15 to 90 ms (N=3 each for control & BCA). Whole cell recordings from AI neurons displayed MG-evoked EPSPs in both control and BCA neurons. Intracortical stimulation 1-2 mm rostral to AI evoked IPSPs in the presence of ionotropic GluR antagonists. The mean amplitude of cortex-evoked IPSPs was significantly smaller in neurons from BCA animals as compared to age-matched control neurons (Control: 6.4 ± 0.9 mV; BCA: 3.3 ± 0.8 mV; p=0.02, df=16). The mean duration of IPSPs was also smaller in BCA neurons (Control: 719 ± 213 ms; BCA: 212 ± 70 ms; p=0.04, df=16). These findings are consistent with a disuse-dependent decline in inhibitory synaptic strength described previously in the auditory brain stem, and suggest that cochlear activity is essential for the maturation of inhibitory synapses in AI.

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316 Role of Inhibition in Temporal Processing in the Primary Auditory Cortex of the Unanaesthetized Mongolian Gerbil

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The temporal structure of the envelope of speech sounds plays an important role in speech segmentation. Neurons in the primary auditory cortex (AI) have been shown to code for such slow amplitude modulations by phase-locking their responses to the envelope of the sound, thereby representing the temporal structure of the sound by the temporal structure of their neuronal discharges. Here we investigate the role of intracortical inhibition in such temporal stimulus processing. Single and multi-unit responses to pure tones and sinusoidally amplitude modulated (AM) tones were recorded from the left AI of unanaesthetized Mongolian Gerbils before, during and after microiontophoretic application of the $GABA_A$-receptor antagonist bicuculline (BIC). We used 3-barrel glass pipettes (total tip diameter 10-18 µm), with one barrel containg BIC, and the other two NaCl for recording of neuronal activity and for current compensation. Prior to the application of BIC, most units showed phase-locking to the AM envelope at periodicities below 30 Hz, although in some units we observed phase-locking up to 60 Hz. When $GABA_A$-mediated inhibition was blocked by iontophoresis of BIC (20-40 nA; 5-10 min), we observed an increase in spontaneous activity and in the duration of onset responses to pure tones, increased discharge rates to both pure and AM tones, and a broadening of the frequency response range for pure tones. Moreover, any phase-locked discharges that were seen in the AM tone responses before the application of BIC were eliminated during BIC application. All these effects were reversible and they recovered within approximately 20 to 30 min of the termination of BIC application. We conclude from these results that $GABA_A$-mediated inhibition plays a crucial role in the temporal processing of sounds in AI.

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317 Tinnitus-Related Neuronal Plasticity in Auditory Cortex and Central Amygdala of Gerbils (Meriones unguiculatus) Evidenced by Arc Expression

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Subjective tinnitus is a phantom auditory percept, which can be induced by high doses of salicylate. Systemic salicylate application reduces activity in auditory brainstem, but increases it in auditory cortex (AC) and amygdala (Am) (Wallhäusser-Franke, Langner, Proc.6th Int. Tinnitusseminar 1999). Here we investigated if neuronal plasticity is involved in tinnitus formation examining the expression of Arc (activity regulated cytoskeleton associated protein) in the gerbil. Arc is induced via an NMDA-receptor dependent mechanism and plays an important role in memory related processes (Lyford et al, Neuron 1995). One group of animals (n=6) received an i.p. injection of sodium salicylate (350 mg/kg bw), whereas a second group was exposed to narrow band white noise centered at 8 or 1 kHz with an intensity of 80 dB(n=6). Arc protein was detected 5 and 3 hours after treatment. To allow comparison with previous results c-fos immunohistochemistry was performed in addition. Arc expression was restricted to the forebrain. In AC noise exposure evoked Arc expression bilaterally mainly in areas representing the frequency content of the stimulus. After salicylate injections labeled neurons were organized chiefly in clusters along isofrequency contours uni- or bilaterally in AC. In Am labeled neurons were found with both treatments, however, only with salicylate Arc-positive neurons were present in its central nucleus (CeA). C-fos immunohistochemistry revealed an activation of the auditory brainstem and midbrain after noise exposure but not after salicylate. These results support previous findings of a strong correlation between activity in CeA and AC after treatments that elicit tinnitus in gerbils. Moreover our results indicate that synaptic plasticity may occur after experimentally induced tinnitus in AC and CeA.

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318 Interpretation of an Intracortical Electrical Stimulus Depends on Preceding Brain States in the Ongoing Activity

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We have evidence, that the lack of success in the development of sensory cortex prostheses is based on a naive transfer of unidirectional stimulation procedures operative in peripheral neuroprosthesis (Brindley & Lewin 1968; Schmidt et al. 1996). In our preliminary work we applied intracortical microstimulation to the auditory cortex of the Mongolian gerbil via a simple unidirectionally operating cortical implant. In parallel we recorded an epidural multichannel electrocorticogram. Animals were trained in a GO/(NO-GO)-paradigm to discriminate stimulation sites in the low and high frequency region of the tonotopic map of the primary auditory cortex (Deliano et al. 2002, Scheich & Breindl 2002). We analyzed the timing relations between the occurrence of discernible spatial patterns of activity distribution, so-called *marked states* (Ohl et al., 2001), and the electrical stimulation in a trial-by-trial fashion. Thereby we could demonstrate that the success of the animal to correctly interpret the GO and NO GO stimuli behaviourally was dependent on the cortical activity state that existed just *prior* to the stimulation. In a unidirectional mode of operation of the prosthesis such temporal relationships can only occur by chance. We therefore aim at constructing a novel type of *interactive cortex prosthesis* which controls its operation interactively with the recorded brain state to ensure optimized communication between prosthesis and the nervous system.

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319 Cholinergic Modulation of Auditory Evoked Potentials

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Optimal sound processing depends on a delicate balance of inhibition and excitation. We have previously demonstrated that housing conditions have a significant impact on the auditory evoked potential. Diffuse neuromodulatory systems, such as acetylcholine, likely play an important role in modulating responses in the central auditory system. To determine whether differences in cortical acetylcholine levels could explain our earlier enrichment effects, we compare auditory evoked responses from rats with lesions of the nucleus basalis and sham controls in two environments.

Adult Sprague Dawley rats (230-270 g) were chronically implanted with an epidural recording electrode over auditory cortex. The immunotoxin 192 IgG-Saporin (or an inactive control) was injected into the ventricles. After recovery, noise bursts and tones were delivered 125 times in random order with a 10s ISI and middle latency auditory evoked responses were recorded twice a week for 9 weeks. The Pa component (25 ms latency) of the auditory evoked potential is typically small in uninjected and sham lesioned controls. After lesion of the cholinergic nucleus basalis, the amplitude of the Pa component was

substantially increased. These changes may be related to cholinergic deficits in autism and other neurological disorders, characterized by abnormal filtering of sensory stimuli. Damage to the cholinergic system will be confirmed with post-mortem acetylcholinesterase histochemistry.

320 Dynamic Filtering in Rat Auditory Cortex

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Understanding how experience modifies cortical response properties lends insight into auditory information processing and learning. It has been demonstrated that associative learning can result in stimulus specific plasticity of frequency receptive fields in the auditory cortex. Condon and Weinberger (1991) demonstrated that habituation also induces a specific change in the frequency response of neurons. Dynamic changes in receptive field organization may increase the robustness of the cortical representation of auditory scenes. In addition, adaptation may contribute to perceptual illusions and priming phenomena. This study expands our understanding of habituation's effect on cortical responses by systematically studying the effect of habituation duration and the presentation rate. Cortical responses are monitored using chronic multi-channel neural recording from up to 15 locations in rat primary auditory cortex simultaneously. This experimental paradigm allows us to study the effect of habituation on multiple cells across the auditory cortex. Our results verify that habituation can result in frequency specific modifications of the receptive fields. Understanding the normal function of sensory adaptation may shed light on a number of neurological disorders characterized by reduced adaptation (including tinnitus, schizophrenia, and autism).

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321 The Auditory Cortex in Macaque Monkeys: Context Dependent Activity and Pattern of Corticothalamic Projection

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Neuronal activity was recorded in the auditory cortex of two monkeys performing an acoustico-motor task, where pure tones are associated by trial and error to a pointing movement to different targets using the ipsilateral arm. We sought to establish whether different behavioral contexts influence the response to a tone with invariant physical properties. Some neurons exhibited an influence of the behavioral context on their discharge rate. For instance, the response to a tone instructing the monkey a given movement was different from the response to the same tone delivered when the target was touched, confirming that the behavioral response was correct. The anatomical support for such behavioral context effects in the auditory cortex is unknown, although reciprocal loops between the auditory thalamus and cortex may be involved. To establish the properties of the corticothalamic projection originating from the auditory cortex in the monkey, the anterograde tracer BDA was injected in the primary auditory cortex of one monkey. Anterogradely labeled terminal fields were observed mainly in the ventral and dorsal divisions of the medial geniculate body and, to a lesser extent, in the medial division of the medial geniculate body, the posterior thalamic nucleus and the suprageniculate nucleus. Most terminal boutons were of small size except some of them in the posterior thalamic nucleus which were of large size (giant endings). This observation extends to primates the dual pattern of termination of the corticothalamic projection (small and giant endings) previously reported for the auditory system of the cat and rat. Furthermore, the present data in monkeys extend to the auditory system a mode of dual pattern of corticothalamic projection previously described in primates for the visual, somatosensory and motor systems.

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322 Postnatal Maturation in the Auditory Cortex of the Mustached Bat

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The mustached bat uses CF-FM echolocation signals with the second harmonic constant frequency component (CF2) at about 61 kHz. To resolve Dopplershifts in returning echoes, the bat employs exceptional fine frequency tuning. In the primary auditory cortex there are sharply tuned neurons whose responses are enhanced by combinations of the first harmonic CF-component of the emitted call and higher harmonic CF-components of the echo (CF/CF region, Suga et al., 1978, Science 200:778-781).

Young mustached bats require a time span of 4-6 weeks to be able to leave their maternity colonies and to hunt insects. During this time the CF2 frequency of the echolocation calls and the frequency of a cochlear resonator involved in sharp tuning shift from about 48 to 61 kHz. We mapped the primary auditory cortex of young bats at two developmental stages (younger stage: age of about 6-12 days; older stage: 20-26 days). There were sharply tuned responses to the individuals' CF2-frequency range in both groups (younger stage: 52-58 kHz, older stage: 58-63 kHz). In addition, in the older animals some neurons were already CF/CF combination sensitive. None of the animals tested was yet capable of active flight. This implies that some aspects of cortical circuitry specifically important for echolocation can develop intrinsically without the animal being involved in active prey pursuit. It remains to be tested to what degree learning during during the first hunting flights will shape and modify cortical properties in the mustached bat.

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323 Post-Natal Development of Receptive Fields and Tonotopic Maps in Auditory Cortex of Chinchilla.

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In early development of most sensory pathways, axonal growth cones extend towards their targets in rough topographic order, under genetic control. Subsequent to synaptogenesis, this initial projection appears to be made more precise, as inappropriately-placed synapses, axon collaterals, and even entire neurons are eliminated. Less clear is the role of stimulus-driven activity in this process: normal activity patterns have been shown essential for map refinement in several systems, but apparently unnecessary in several others, in which refinement was completed prior to the onset of sensation. We are comparing cortical single-unit responses, and their tonotopic organization, in the normal-hearing newborn and adult chinchilla, whose developmental timeline parallels that of man in many respects.

Our primary objective is to quantify the degree of order in the adult and newborn mapping. An important methodological issue and source of potential ambiguity is the definition of "best frequency", usually a nonconstant function of tone intensity and post-onset latency. A case in point is the considerable fraction of neurons that is selective for more than one distinct frequency range. It appears that the shortest-latency component (its instantaneous rate of firing notwithstanding), which

presumably corresponds to the most direct path to cortex, is the one that best fits into the prevailing tonotopic arrangement. This leaves the question of the longer-latency component(s): do they reflect functional specializations, or initial errors in wiring that have not been corrected? Early data supports both possibilities.

324 Development of Refractoriness in the Central Auditory System

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We are examining aspects of development of the central auditory pathways. One measure of functional development in the central auditory system is the refractoriness of the cortical auditory evoked potential (CAEP). Refractoriness, or recovery cycle may affect the transmission and temporal neural encoding of complex acoustic signals and speech. Therefore, it is of interest to examine the changes in refractoriness of the CAEP as they relate to maturational changes in the central nervous system. In this study we recorded CAEPs in children ranging in age from 4 to 12 years in response to a speech sound "uh". The stimuli were presented in a sequence consisting of four presentations of the vowel sound separated by decreasing interstimulus intervals (ISIs) of 2000, 1000, 560, and 360 ms. Morphology, latency, and amplitude of the CAEP were analyzed to examine the effects of age and ISI on the CAEP. Information about the waveform morphology was extracted by evaluating the instantaneous rate of change in the evoked potentials. Preliminary results showed distinct changes in CAEP waveform morphology with increasing age. In older children, a double-peaked positive component (P1) of the CAEP was observed at all ISIs. In the youngest children, there was no evidence of a double-peaked component at any of the ISIs. Additionally, the negativity corresponding to the N1 was larger and more salient across ISIs with increasing age.

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325 Neural Changes in Cat Auditory Cortex after a Transient Pure-Tone Trauma

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Here we present the changes in cortical activity occurring within a few hours after a 1-h exposure to a loud (125 dB SPL) pure-tone (5 or 6 kHz). The changes were assessed by recording, with 8 or 16 micro-electrode arrays, the same multi-unit clusters (MU) before and after the trauma in 16 ketamine-anesthetized cats. The MUs were grouped into 3 frequency bands: those with a CF below the trauma tone frequency (TF) were labeled as "Be", those with a CF less than 1 octave above the TF were labeled "Ab1", and those with a CF >1 oct. higher than the TF were labeled "Ab2". The trauma induced a significant increase in CF threshold in all groups (5, 10 and 20 dB on average for Be, Ab1 and Ab2, respectively). After the trauma, the tuning curves of MUs in the Ab2 group were shifted considerably toward lower frequencies. Specifically, the CF and best frequency were significantly decreased in the Ab2 group (0.64 and 0.74 oct. in average, respectively). The index of monotonicity increased in Be, and so did the maximum driven firing rate (FR) in Be and Ab2. At medium intensity in Be and Ab1 groups, the onset response after the trauma lasted on average shorter than that before the trauma. The spontaneous activity (SA) measured over the 15 minutes following the exposure was significantly increased when all the data were combined. On the other hand, when the data were divided into the 3 frequency bands, the increase in SA was not significant. However, the cross-correlation coefficient (r) was significantly increased in Ab1-Ab2 and Ab2-Ab2 groups (by a factor of 1.3 and 1.5, respectively). In contrast, a few hours after the trauma, SA was significantly increased for Be and Ab2 groups (by a factor of 2.5) as was r in Ab1-Ab2, Ab2-Ab2 and Be-Ab2 groups. The results are discussed in terms of unmasking of cortical excitatory connections and implications for plasticity of tonotopic maps.

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326 Maturation of field AI in congenitally deaf cats

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One consequence of auditory deprivation in adult congenitally deaf cats (CDC) are significant deficits in functional organization of the auditory cortex (Kral et al., Cereb Cortex 2000, 10:714). Developmental plasticity in A1 field of CDCs shows a sensitive period (Kral et al., Cereb Cortex 2002, 12: 797). Are the deficits found in adult CDCs the consequence of an abnormal maturation or of a degenerative process superimposed on more or less normal maturation? Seven CDCs and 7 hearing cats (ages 1-6 months) were investigated. Hearing controls were pharmacologically deafened at the beginning of the experiment. Stimulation with biphasic pulses (200 µs/phase) was applied through an intracochlear electrode (monopolar configuration). Local field potentials (LFPs) were recorded at 100-170 positions in the primary auditory cortex. Activated cortical areas (cortical area with LFPs > 300 µV) were significantly larger at 8-12 weeks p.n. in CDCs, possibly indicating a cortical disinhibition at this age in deprived animals. The comparison of the shape of LFPs within activated areas showed no differences in maturation of Pa waves between CDCs and hearing cats. However, the maturation of Nb waves was delayed by 2 months in CDCs. Additionally, differences in Pb waves were observed. Long-latency P1 waves were found in CDCs below 3 months age with similar shape and relative amplitude. In contrast to hearing cats, P1 amplitudes substantially decreased with further age in CDCs. P1 latency in young CDCs was smaller than in hearing cats.

The data demonstrate that cortical auditory maturation is substantially altered and delayed. In addition, degenerative processes set in at around 4 months p.n. Comparison with chronically stimulated CDCs shows that the maturation can be caught up and the degenerative processes can be reversed in early implanted CDCs (Kral et al., Cereb Cortex 2002, 12: 797).

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327 Changes of 2-Deoxyglucose Acitivity in Auditory Brain Cortex of Deafened Rat

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In the previous study with preoperative FDG-PET in prelingually deafened pediatric cochlear implant candidates, decreased glucose uptakes were observed in auditory cortex and associated area when compared with normal PET. These hypometabolic areas were shrunk as the age of FDG-PET study increases. But it was impossible to compare with FDG-PET of age-matched normal children because of ethical problem. This study was designed to understand the changes of glucose uptake according to time in deaf animal model using age matched control. SD rats of postnatal 10 days were operated to destroy the both cochlea. Three deafened and three normal rats were sacrificed in 1 week, 2 weeks, 4 weeks, 7 months after operation respectively. 16ug/100g 2-deoxyglucose(DG) were injected 1 hour before sacrifice. The brain was sectioned in 20 um thickness and exposed to BAS image plate. In the two representative section of each sample, the relative 2-DG uptake of auditory cortex, delineated using rat brain atlas, were compared. There was no difference between normal and deaf group (both P-value=0.7) in postoperatively 1 week. As the duration of deaf increases, the 2-DG uptake was significantly higher in normal group

than in deaf group.(at 2 weeks, p-value was 0.09 in right side, 0.42 in left side, at 4 weeks, 0.002 in right side, 0.02 in left side) The difference was most prominent in 4 weeks after operation. At 7 months after operation, the difference in right side has become insignificant (p=0.07) but in left side, the uptake in deaf rat was still significantly lower than in normal. (p=0.024) In summary, the decreased uptake of 2-DG and asymmetric development of hypometabolism could be observed in rat deaf model with age-matched control and the hypometabolic change of auditory cortex was most prominent in 4 weeks after the operation, and at 7 months, the recovery of metabolism could be observed in right auditory cortex.

328 Short Term Metabolic Changes in Avian Tangential Nucleus After Vestibular End-Organ Damage

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The avian vestibular system has the capacity for morphologic and functional recovery after damage to the inner ear. The effects of peripheral end-organ damage on the central nervous system are not fully understood. Previous work investigating metabolic changes in the Superior Vestibular Nucleus (SVN) using 2-deoxyglucose (2DG) uptake and cytochrome oxidase (CO) staining showed very little change in activity after damage to the peripheral end organs. Here we examined whether short-term metabolic changes occur in the Tangential Nucleus (TA) after permanent or reversible end-organ damage. Transient damage was produced by unilateral topical application of a streptomycin-soaked Gelfoam pledget in the inner ear. The contralateral ear was treated with a water-soaked pledget. Inter-animal controls were treated bilaterally with water-soaked pledgets. Permanent damage to the inner ear was created via a unilateral labyrinthectomy. The contralateral ear was not treated. Inter-animal controls received only general anesthesia. Animals survived for 6 hours, 1 day or 4 days after treatment and then received an IP injection of 2DG. After 45 minutes of vestibular stimulation, animals were sacrificed and brains were frozen. Alternate coronal sections were stained for CO or exposed to x-ray film. Optical density measurements of CO staining or 2DG film labeling were taken bilaterally. The ipsilateral/contralateral ratio of TA labeling was compared among experimental groups.

Results suggest that the ipsilateral/ contralateral ratio for 2DG labeling in TA decreases following labyrinthectomy compared to controls. The CO activity of ipsilateral TA neurons increases slightly after labyrinthectomy. Streptomycin-treated birds showed minimal changes in either 2DG or CO labeling, similar to that seen previously in SVN. These results suggest that TA may be more susceptible to damage by changes in peripheral end organ input than other vestibular nuclei previously examined.

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329 Differential Recovery of VOR Function Following Unilateral Canal Plugging and Unilateral Labyrinthectomy

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Following unilateral plugging of all 3 semicircular canals or unilateral labyrinthectomy there is a reduction in both the ipsilesional and contralesional VOR to steps of acceleration (3000°/s^2 - 150°/s). With time there is improvement in the contralesional gain measured during the acceleration (acceleration gain) portion of the stimulus. There is a similar improvement in both the ipsilesional and contralesional gains measured during the constant velocity (velocity gain) portion of the stimulus. This increase in the contralesional acceleration gain produces an asymmetry in the response to ipsilesional and contralesional rotations. We measured the change in this asymmetry over time (weekly intervals) in 2 squirrel monkeys that had undergone unilateral plugging of all 3 semicircular canals and 2 squirrel monkeys that had undergone unilateral labyrinthectomy. During the first week following surgery there was a 46% asymmetry for the canal-plugged monkeys and a 38% asymmetry for the labyrinthectomized monkeys. These asymmetry values were constant for the first several weeks following surgery. By the 6th week, the asymmetry in the canal-plugged monkeys had decreased to 24%, and by the 9th week, the asymmetry had decreased to 17%. In comparison, there was little change in the asymmetry values over time for the labyrinthectomized monkeys. At 98 weeks post-labyrinthectomy, the asymmetry value was 30%.

This difference in recovery is felt to be due to preserved tonic function in the ipsilesional vestibular nerve following canal plugging. The improved response in the canal plugged animals appears to be due to central compensatory process utilizing the contralesional vestibular input, rather than preserved rotational sensitivity on the ipsilesional side, since plugging of the semicircular canals on the previously intact side abolishes the angular VOR.

330 Linear and Nonlinear Components of the 3-Dimensional Vor Evoked by High-Frequency, High-Acceleration Rotations in the Squirrel Monkey.

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We have identified linear and non-linear pathways mediating the horizontal angular VOR evoked by high-frequency, high-acceleration rotations (Minor et al. J. Neurophysiol. 82:1254, 1999). The linear pathway has a relatively constant gain and phase across frequencies and velocities. The non-linear pathway has a gain that rises with velocity at higher frequencies and is more modifiable than is the linear pathway following vestibular lesions. We sought to determine if these pathways were also evident in the VOR evoked by rotations in the pitch and roll plane as well as in the planes of the vertical canals (LARP and RALP).

In three squirrel monkeys we measured binocular 3-dimensional eye position using the magnetic search coil technique. We analyzed the 3-dimensional VOR evoked by acceleration steps (500–6000 deg/s/s to a peak velocity of 50–300 deg/s) and by sinusoidal rotations (0.5-15 Hz, 20-100 deg/s) delivered about the earth-vertical axis while each animal was positioned; upright (YAW), right-ear-down (PITCH), supine (ROLL), supine-left-ear-down-45deg (LARP) and supine-right-ear-down-45deg (RALP).

Linear and non-linear components were identified in the responses. The data at 8 Hz provide an example of these responses. For sinuosoidal rotations in the yaw, pitch, roll, LARP and RALP planes there is a 20-30 % rise in VOR gain as peak velocity increases from 20 deg/s to 100 deg/s. The VOR phase remained compensatory (175-185 deg phase lag relative to head velocity) across this range of stimuli with a tendency to increase with velocity at higher frequencies. For the steps of acceleration the gain during the acceleration portion of the step (GA) was 25-50 % higher than the gain during the plateau portion of the stimulus (GV). These findings indicate that the angular response evoked by activation of the vertical semi-circular canals show similar linear and non-linear components as previously shown in the horizontal VOR.

331 Tilt Perception and Horizontal Eye Movement Responses during Tilt and Translation

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We investigated how the nervous system processes ambiguous cues from the otolith organs as a function of frequency by providing

sinusoidal roll tilt and translational stimuli to 8 healthy subjects. Stimulus frequency ranged from 0.005 Hz to 0.7 Hz. All stimuli yielded peak inter-aural otolith stimulation of 0.34 G. Roll-tilt stimuli were provided using an earth-horizontal axis rotational device with the rotational axis near the center of the head. The peak roll tilt was 20 deg and the inter-aural otolith stimulus was simply the sine component of gravity. All translational stimuli, except 0.7 Hz, were provided using centrifugation. The subject was rotated at 250 deg/s for 5 minutes before initiating sinusoidal translations with peak displacement between 0.115 m and 0.175m. During these trials, the inter-aural otolith stimulus consisted of the centrifugal force plus the actual linear acceleration. For the 0.7Hz trial, the subject was simply translated from side-to-side (no rotational motion). We measured eye movements and perceptual roll tilt responses. During roll tilt trials, the perceived roll-tilt was nearly constant at all frequencies. During translational trials, the perceived roll-tilt showed characteristics consistent with a low-pass filter; tilt response amplitude was roughly constant at low-frequencies and substantially diminished at frequencies above around 0.05 Hz. Qualitatively, these data are consistent with previously published modeling predictions. One important conclusion derived from these perceptual data is that the roll rotation cue, provided primarily by the semicircular canals, has a large influence on perceived tilt. Horizontal eye movements were very small at low frequencies for all stimuli and began to grow substantially at frequencies above around 0.1 Hz. As suggested previously, it appears that reflexive eye movement responses include a component that is due to direct otolith stimulation and another component that involves central processes of sensory integration.

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332 Vestibular-induced Eye Movements While Performing an Information Processing Task in Young and Older Humans

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The purpose of this study was to investigate the influence of a concurrent information processing task on eye movements induced by earth-vertical axis rotation (EVAR) in young and older humans. Twenty older subjects (10 F, 10 M, 69.3 +/- 3.2 years) and 20 young subjects (10 F, 10 M, 23.5 +/- 2.9 years) performed three different tasks during sinusoidal EVAR in darkness at 0.05 Hz, 60 deg/sec peak velocity: a Simple Reaction Time, a Disjunctive Reaction Time, and a Choice Reaction Time task. Tones were presented to the subject through headphones and they were instructed to react accurately and as quickly as possible. Eye movements were recorded with electro-oculography or video-oculography. We observed a small but consistent increase in VOR phase lead while subjects performed a concurrent information processing task. Also, the pattern of most subjects' eye movements during EVAR were unaffected by a concurrent task. However, some subjects exhibited nystagmus dysrhythmia and in some subjects nystagmus actually ceased. Results were neither different between young and older subjects nor influenced by which information processing task was performed. These results suggest that attentional processes, which are primarily dependent upon the cerebrum, influence the velocity storage mechanism of the VOR, which is dependent primarily on the brain stem and cerebellum.

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333 Vestibular Nucleus Projections to the Ventrolateral and Lateral Columns of the Periaqueductal Gray (vlPAG and lPAG): Potential Links Between Balance, Nociception and 'Coping Responses'

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Recent studies have indicated that the vlPAG and lPAG are important for coordinating emotional responses to physical stressors. These regions receive somatic, visceral and rostral prelimbic cortex inputs related to stressors. This study provides the first documentation of projections from the vestibular nuclei to PAG. Adult male albino rabbits and received iontophoretic injections of biotinylated dextran amine or Phaseolus vulgaris leucoagglutinin into the vestibular nuclei. Immunocytochemical methods were used to visualize PHAL; ABC-peroxidase (Vector Laboratories) was used to identify BDA. Anterogradely labeled axons could be traced to varicose terminations in the caudal half of PAG in seven rabbits with injections centered in the superior vestibular nucleus (SVN). The fibers were predominantly ipsilateral and predominantly within the vlPAG, with fewer fibers within the lPAG. These projections were not observed in rabbits with tracer injections centered in the medial, lateral or inferior vestibular nuclei. As reviewed by Bandler et al. (Prog. Brain Res. 122 (2000) 331-349), vlPAG and lPAG are a component of 'emotional motor' pathways that mediate passive emotional coping in response to deep or chronic pain or traumatic injury. These responses include hyporeactivity, quiescence and central antinociception, which resemble prodromal components of motion sickness such as the 'sopite syndrome'. Thus, SVN-PAG projections may contribute to emotional responses to motion transduced by vertical semicircular canals and otolith organs.

334 Vestibulo-ocular Zones Controlling the Activity of Lateral and Medial Recti in the Rat Using Recombinant Strains of Pseudorabies Virus

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Prior studies of angular vestibulo-ocular reflexes provided evidence that the cerebellar flocculus was organized in longitudinal zones, which were suggested to modulate vestibulo-ocular reflex eye movements arising from individual semicircular canals and affecting individual extraocular muscles. In particular, Purkinje cells of the central zone of the floccular region were hypothesized to modulate the activity of lateral and medial recti in the plane of horizontal semicircular canals. However, the organization of vestibulo-ocular pathways controlling both muscles has not yet been elucidated. For this, two recombinant strains of pseudorabies virus expressing unique proteins, either β-galactosidase or green fluorescence protein, were injected into the medial rectus of one eye and lateral rectus of the other eye. Transynaptically-labeled neurons were mapped in the vestibular nuclei and floccular region at a survival time of about 84 hours. Transynaptically-labeled neurons were distributed bilaterally in the rostral part of medial vestibular nuclei, superior vestibular nuclei, and the dorsal aspect of y-group. In the floccular region, labeled Purkinje cells were detected in the rostral half of both sides. Dual-labeling immunofluorescence revealed three populations of neurons: two groups of neurons with only one strain of virus and a third group with both viruses. Dual-labeled neurons were primarily concentrated in the rostral part of the medial vestibular nuclei and the dorsal flocculus and adjacent ventral paraflocculus. This study provides the first evidence that a subset of neurons in the vestibulo-ocular pathways send collateralized projections to motoneurons innervating the lateral and medial recti, known to receive their main signals from horizontal semicircular canals. These dual-labeled neurons are responsible for coordinating conjugate angular vestibulo-ocular reflexes in the plane of horizontal semicircular canals.

335 Pronase as a Suitable Enzyme to Optimize the Acute Dissociation of Inferior Colliculus Neurons for Patch-Clamp Recordings

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The extensive branching of inferior colliculus (IC)-neurons is a problem for a detailed electrophysiological analysis of membrane properties in slice preparations. After acute experimental dissociation the branching is reduced to the main dendritic shafts. So that patch-clamp recordings can be performed under controlled conditions. The aim of the present study was to adapt dissociations methods to the highly sensitive mature IC-neurons of mice. For these purposes, pronase was successfully choosen as a tool.

Transverse slices of the IC of different thickness' (150, 300, 400 µm) were prepared from NMRI-mice aged 20-50 days. Different concentrations of pronase (0.1, 0.3, 0.5, 1, 2, 2.5 mg/ml) for different periods of time (15, 20, 30 min) were used. Subsequently, the slice was triturated with fire-polished Pasteur pipettes of different apertures (1.1, 0.9, 0.7 mm).

Optimal dissociation was achieved using a 300 µm thick slice at 2 mg/ml pronase over 20 min which led to a yield of 15-20 vital IC-neurons. The cells showed a smooth surface, a sharp contour and homogenous cytoplasm, also a phase-bright nature and some processes. This is clearly indicative of the cellular integrity as confirmed by patch-clamping.

Several experimental conditions (concentration of pronase, incubation time, temperature, thickness of the slice and apertures of the Pasteur pipettes) had to be optimized to be able to reliably and repeatedly dissociate vital IC-neurons with cellular integrity. Pronase was more suitable for the dissociation of IC-neurons than papain. The increasing myelinisation of the IC during development could play a crucial role to select the appropriate enzyme for dissociation of adult IC-neurons.

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336 Salicylate Affects Spontaneous Activity of Mouse Inferior Colliculus Brain Slices

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Salicylate is well known to produce tinnitus in humans and animals. It has been shown that systemic application of salicylate primarily changes outer hair cell electromotility, but that it can also influence neuronal activity in several parts of the auditory system. Because a direct action of salicylate on neurons of the auditory pathway cannot exclude the present study investigated the in-vitro-effect of salicylate application on the spontaneous activity of mouse inferior colliculus neurons in brain slices.

Single unit responses were extracellularly recorded in 300 µm thick slices of the deafferented inferior colliculus. During the measurement of spontaneous activity, 1.4 mM sodium salicylate (corresponding to tinnitus related serum levels in rats (Cazals, 2000, Prog. Neurobiol. 62, 583-631)) were added by superfusion. The frequency- specific location of investigated neurons within the tonotopic structure of the inferior colliculus was determined according to established maps published elsewhere (Romand and Ehret, 1990, Dev. Brain Res. 54, 221-234).

From a total of 51 investigated neurons, 44 demonstrated a significant and reversible change in firing rate during superfusion with salicylate (37 increased and 7 decreased the firing rate). The mean value of absolute changes in neuronal firing rate was significantly lower in the 10-20 kHz area. This frequency range includes the perceived tinnitus frequency in rats determined in behavioural experiments after salicylate application (Bauer et al., 1999, Otolaryngol. Head Neck Surg. 121, 457-462).

The evident effects during salicylate application on the isolated network of the mouse inferior colliculus suggest that this brain region plays a key role in salicylate-induced tinnitus generation.

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337 Ih Improves Temporal Processing in the Inferior Colliculus

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Temporal and non-temporal information from all parts of the auditory brainstem converges in the inferior colliculus (IC). IC neurons can be classified according to their firing pattern to positive current injections (onset, adapting, sustained). We were interested whether specific neuron types in the IC express the hyperpolarizing activated cation current (Ih) that has been described in a number of auditory brainstem neurons, especially those that are involved in the processing of temporal information. We also investigated whether Ih properties differ among these cell types and how Ih contributes to the analysis of input patterns.

To test this we recorded voltage changes and whole cell currents from neurons in acute IC slices of P17-P19 rats using standard patch-clamp techniques. Neurons were characterized upon their response to 500 ms long current injections ranging from –800 to 800 pA. All onset and adapting and most sustained firing neurons showed a depolarizing sag upon hyperpolarization that was blocked by the Ih-channel blocker ZD7288. This sag was significantly larger and faster in onset and adapting compared to sustained cells. Voltage-clamp recordings confirmed that Ih activation was significantly faster in onset compared to sustained neurons. Functional analysis of Ih in onset and adapting cells, using the Ih blocker ZD7288, provided evidence that Ih reduces temporal summation for both depolarizing and hyperpolarizing current wave injections (simulated after EPSC and IPSC recordings). Ih also induces after-hyperpolarization and rebound spiking which is dependent on the duration of the hyperpolarizing current injection.

We provide evidence that Ih improves the analysis of the temporal input pattern in a specific subset of IC neurons namely the onset and adapting cells.

338 Contribution of AMPA, NMDA and $GABA_A$ Receptors to Responses of Neurons in the Inferior Colliculus to Repetitive Stimulation of Synaptic Inputs

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To understand how AMPA, NMDA and $GABA_A$ receptors process temporal information in the central nucleus of the inferior colliculus (ICC), we examined synaptic responses evoked by repetitive stimulation of the lateral lemniscus in brain slices perfused with receptor specific antagonists. Brain slices (400 µm) were obtained from Wistar rats (9-13 days old). Excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) evoked by a single electrical pulse (0.1 ms) and 10 pulses at frequencies between 10-100 Hz were recorded by whole-cell patch clamp methods. Most EPSCs evoked by a single pulse had two distinct components with different time courses. CNQX blocked a fast component, and APV blocked a slower component, indicating that the excitatory responses in ICC are mediated by glutamate through AMPA and NMDA receptors. Most IPSCs were greatly reduced by bicuculline, suggesting that synaptic inhibition in ICC was predominantly mediated by $GABA_A$ receptors. With repetitive pulses at rates of 20-100 Hz the AMPA and $GABA_A$ receptor mediated responses to each pulse could be distinguished, but NMDA responses merged together and became one prolonged inward current. These results suggest that excitatory responses mediated by AMPA receptors convey temporal information, but that NMDA receptors do not. With repetitive stimulation the amplitude of AMPA receptor mediated EPSCs

was progressively reduced (i.e. showed response suppression), but NMDA receptor mediated EPSCs summed temporally. Activation of $GABA_A$ receptors could reduce the suppression of AMPA responses and the extent of temporal summation of NMDA responses. These results suggest that GABAergic inhibition shapes excitatory responses to repetitive stimulation in ICC neurons and allows them to preserve and transmit temporal information more precisely.

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339 Physiological Properties of Neurons in the External Cortex of the Inferior Colliculus Studied *In Vitro* Brain Slice

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The inferior colliculus (IC) is a principal component of the auditory midbrain and serves as a significant integrative center within the central auditory pathway. In mammals the anatomical subdivisions of the IC include the central nucleus (ICc), the dorsal cortex (ICd) and the external cortex (ICx). Unlike the ICc, the ICx receives its primary inputs from the contralateral and ipsilateral ICc, auditory cerebral cortical areas and from regions not directly associated with auditory function. To better understand the auditory function of the ICx, intrinsic membrane properties and synaptic responses of ICx neurons were examined.

Visual whole-cell patch clamp recordings were taken from ICx neurons in coronal slices from rats at age of 9-12 days. The current-voltage relationship for all of the neurons tested was linear. The resting potential of the neurons was –59.5 ± 3.0 mV (n=8). The input resistance and time constant were 31.3 ± 1.6 MΩ and 21.4 ± 1.1 ms respectively (n=8). The threshold of current injection for eliciting action potentials (APs) was 590.0 ± 29.5 pA (n=8). The APs had an average amplitude of 55.9 ± 2.8 mV (n=8) and displayed a sustained "regular-type" of firing pattern in response to positive current injection. The AP firing rate did not exceed 50 Hz in response to a maximum positive current injection of 1900 pA. Furthermore, the APs of the ICx neurons exhibited an undershoot with two components. A small and short component was followed by a large and long one with the amplitude of 26.5 ± 1.3 mV and duration of 93.7 ± 4.7 ms (n=8). To study the synaptic responses, a stimulating electrode was placed in the ICc approximately 1 mm from the recording site. The resultant responses displayed a combination of excitatory/inhibitory and excitatory only potentials with an average response latency of 2.2 ± 0.1 ms (n=7).

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340 NBQX and Memantine Counteract Salicylate-Induced Suppression in Inferior Colliculus of Gerbils: A 14C-2-Deoxyglucose-Study

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Subjective tinnitus is a phantom auditory percept. It can be evoked by salicylate that causes elevation of hearing thresholds and altered activity throughout the auditory system. The 14C-2-deoxyglucose (2DG)-method revealed suppressed activity in ventral cochlear nucleus (VCN) and inferior colliculus (IC) of the gerbil together with enhanced activity in auditory cortex (AC) the latter interpreted as tinnitus-related activation (Wallhäusser-Franke et al, NeuroRep 7, 1996). Suppression in IC was ameliorated by the NMDA-antagonist memantine (Braun et. al., ARO 2002). Here effects of memantine and the AMPA-antagonist NBQX on tinnitus-related activity were investigated. Gerbils received i.p. injections of salicylate (350mg/kg bw) plus an i.p. injection of memantine (1mg/kg bw), NBQX (1mg/kg bw) or saline 1h later, while two groups were injected only with memantine or NBQX. After an i.p. injection of 2DG 30min later they were placed into a soundproof chamber in silence for 1h. Differences between treatments were found in the IC: Salicylate with saline produced weak 2DG uptake as seen after salicylate alone. Salicylate with memantine led to enhanced and diffuse uptake, higher uptake was seen after memantine alone.Salicylate plus NBQX induced elevated activity only in the low-frequency region of IC whereas NBQX alone led to enhanced and diffuse 2DG labeling throughout this area, although less pronounced than after memantine alone. No changes were observed in cochlear nucleus and AC in comparison to salicylate alone. We conclude that memantine as well as NBQX counteract the salicylate-induced suppression in IC but not in VCN. The elevated activity of AC was not reduced by these antagonists suggesting that it is not sufficient to restore activation in IC to reduce tinnitus-related cortical activity.

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341 Developmental Expression of KCC2 in the Inferior Colliculus of the Rat

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To get morphological substrates on time-sensitive interactions (inhibitory and excitatory) mediated by glycine and gamma-aminobutyric acid (GABA), the expression of KCC2 was examined in the inferior colliculus (IC) of postnatal rats. KCC2 has been reported to be a neuron-specific K-Cl co-transporter (Payne et al., 1996) functioning in creating both inhibitory and excitatory interactions (Williams et al., 1999). The fact that many auditory neurons contain glycine and/or GABA and other much physiological and pharmacological evidence lead us to think that these neurotransmitters play important roles in the central auditory system. These amino acids are too simple in structure to analyze directly. We then prepared mRNA probes which recognize c-terminus of KCC2 for in situ hybridization (ISH) study, and we raised a poly-clonal antibody against KCC2 in the rabbit for immunohistochemistry (IHC) study. The ISH and IHC studies were undertaken using developing rats, i.e. new born (P0), postnatal 7 days (P7), postnatal 14 days (P14) and as adults. As a control, the cerebellar cortex (CB) was used. On P0, no KCC2 signal above background was detected in either the IC or the CB. On P7, a moderate number of KCC2 positive neurons were appeared in both the IC and the CB. On P14, the number of KCC2 positive cells doubly increased without significant difference between the IC and the CB. In adult rats, the number of KCC2 positive cells in the IC were found to slightly decrease, whereas the number in the CB decreased down to the level on P7. These results indicate that: 1) Glycine and/or GABA play important roles in the auditory processing. 2) In the IC, the expression of KCC2 is not transient but persistent. 3) KCC2 may be involved in the time-sensitive interactions, a key mechanism in the auditory brain.

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342 Intrinsic and Commissural Connections of the Rat Inferior Colliculus

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We examined the patterns of ipsilateral and commissural convergence between inferior colliculus (IC) subdivisions. Small deposits of gold conjugated to cholera toxin beta subunit in each IC subdivision in adult rats labeled ipsi- and contralateral projection cells.

Six IC subdivisions were studied using Nissl preparations, histochemistry, angiography, and immunocytochemistry. They include central nucleus (CN), external cortex (EC), dorsal cortex (DC), rostral pole (RP), nucleus of the commissure (CO) and nucleus of the brachium (NBIC).

There is ipsilateral convergence onto each subdivision from at least four others. The strongest input to EC, RP, and CO is from EC. EC has strong reciprocal relations with DC and RP. While CN is the strongest

input to CN and DC, CN has only weak reciprocal projections with other subdivisions. CO has the fewest targets.

Much like the ipsilateral data, in 3/5 cases commissural EC input is strongest. In all but one case, the strongest ipsilateral subdivision was also the strongest contralateral input. For CN, EC and DC, the commissural pattern matches the ipsilateral pattern, which is not the case for RP and CO. CN, EC and RP receive only sparse contralateral input (18%, 5% and 2% of total IC input, respectively).

The IC is more than a relay station between the olive and thalamus. Its physiology and anatomy suggest roles as diverse as coordinating binaural signals, redistributing corticocollicular input, or integrating somatic and auditory information. Features useful for such processes are 1) specialization of shell nuclei for intrinsic processing, 2) reciprocal projections between subdivisions with somatic or cortical input and those whose input is mainly auditory, 3) convergence from multiple subdivisions, and 4) matching patterns of intrinsic and commissural projections.

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343 Organization of the Inferior Colliculus in the Gerbil: Projections from the Ipsilateral Cochlear Nucleus

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Our studies using retrograde tracers have shown that neurons in the most ventral parts of the dorsal and ventral cochlear nuclei and in the most dorsal part of the medial superior olivary nucleus project to the anterolateral inferior colliculus (IC) and to a continuation of this area on the dorsal surface. It is likely that these are the neurons that represent the lowest frequencies heard by the gerbil. To further characterize the projections to the anterolateral and dorsal IC, we made iontophoretic injections of anterograde tracers into restricted parts of the cochlear nucleus. Our results are in agreement with those of others, showing that projections from the ipsilateral dorsal and ventral cochlear nuclei terminate mainly in the low frequency parts of the IC. Injections into middle or dorsal (higher frequency) regions of the cochlear nucleus result in little or no ipsilateral labeling. Projections from the ipsilateral cochlear nucleus terminate non-uniformly; in some cases, most of the label in the anterolateral IC is confined to a dense patch of terminals. Contralateral projections also terminate non-uniformly in the anterolateral region. Comparison of the projections to this region of the IC on the two sides suggests that the ipsilateral and contralateral projections from restricted parts of the cochlear nucleus only partially overlap.

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344 Auditory Thalamus of the Gerbil. I. Cytoarchitecture.

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In order to better understand the organization of the auditory thalamus of the gerbil, we prepared tissue using standard cell and fiber stains, as well as cytochrome oxidase, parvalbumin and Wisteria floribunda agglutinin staining methods. The auditory thalamus is similar to that of other mammals, consisting of the three main divisions of the medial geniculate body (dorsal, MGd; medial, MGm; ventral, MGv) and adjacent structures. Distinct features in the gerbil thalamus include (1) a well organized caudal region in the MGd and (2) differential staining patterns that can be used to further subdivide the MGv. The caudal MGd is dominated by a cellular region which is fully encapsulated by myelinated fibers. This encapsulated region (ER) serves to divide the MGd roughly into dorsal, middle (ER), and ventral tiers. The dorsal tier is largest, occupying a position dorsal to both ER and MGv, and is divided into dorsal, deep dorsal, and suprageniculate subdivisions. The ventral tier lies in close association with the peripeduncular, subparafascicular, and posterior intralaminar nuclei. The ER is a cylindrically shaped cell mass stretching across the width of the MGd. It is embedded within the large myelinated fibers of the brachium of the inferior colliculus, giving the region a swirly appearance in all of the stains we used. Rostrally the ER abuts the MGv. In cell and fiber stained sections, the caudal part of MGv can be divided into the classically described medial (ovoidea) and lateral parts. In material prepared using the other methods, these parts are further divisible on the basis of differences in staining density. In these preparations, the MGv contains four distinct subregions arranged from medial to lateral. Experiments described in our companion abstract show that each of the subdivisions of MGd and MGv receives a distinct pattern of projections from the inferior colliculus and auditory cortex.

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345 Auditory Thalamus of the Gerbil. II. Projections from the Inferior Colliculus and Auditory Cortex.

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We are studying the afferent projections to the auditory thalamus by injecting an anterograde tracer, biotinylated dextran amine, into either the inferior colliculus (IC) or auditory cortex (AC). Projections from the central nucleus of the IC (ICC) to the ventral division of the medial geniculate body (MGv) demonstrate a distinct topographic pattern. The dorsal (presumed low-frequency) ICC sends both a large projection to the most lateral region of MGv and also a small projection to the medial MGv. Injections in the central ICC (presumed mid-frequency) also give rise to two terminal zones in the MGv, adjacent to the low-frequency zones. The ventral ICC (presumed high-frequency) gives rise to a single large projection to the middle of the MGv. Injections across the frequency axis of primary AC demonstrate projection patterns to MGv that appear virtually identical to, and overlap, those from the ICC. We predict that, in the MGv of the gerbil, there are at least two separate functional representations of middle and low frequencies surrounding an area that responds to high frequencies.

The medial (MGm) and dorsal (MGd) divisions of the medial geniculate body also receive inputs from both IC and AC, with varying degrees of convergence. Injections in the external nucleus of the IC show a robust projection to MGm, and a lesser one to MGd. IC regions caudal to ICC project to the ventral tier of MGd and the associated peripeduncular, subparafascicular, and posterior intralaminar nuclei. Regions dorsomedial to ICC project heavily to the middle tier (encapsulated region) of the MGd. Cortical regions dorsal and caudal to the primary AC project heavily to the dorsal tier of MGd, especially the suprageniculate nucleus.

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346 Descending Projections from Auditory Thalamus to Olivocochlear Neurons in the Gerbil

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Descending projections from the neurons in the medial geniculate body (MGB) to the olivocochlear (OC) neurons were examined. Olivocochlear neurons, with descending axons projecting to the cochlea, are known to receive descending projections from the inferior colliculus (IC)(Vetter et al., '93, Hear. Res. 70:173-186), which in turn receives projections from the upstream MGB (Kuwabara and Zook, '00, Brain Res. 878:79-87; Winer et al., '02, Hear. Res. 168:181-195). Little is known whether direct projections from MGB to OC neurons exist.

Olivocochlear neurons of the gerbil were prelabeled in vivo using a retrograde tracer (fast blue, Fluoro-Gold or HRP). Quasi-parasagittal brain tissue slices containing the ipsilateral auditory cell groups from the level of the thalamus to the lower brainstem were subsequently made and the anterograde marker Biocytin was injected into the MGB with visual guidance and incubated to allow for transport. After being processed differentially for the visualization of Biocytin and HRP, the

tissue was microscopically examined for projections of descending axons to the identified OC cells.

Biocytin labeled axons with terminals and en passant varicosities were found in close apposition with the cell bodies of labeled OC neurons, including large multipolar medial OC cells and small, round and ovoid lateral OC cells. Many of the observed axons were collaterals of those projecting to the IC.

Our results indicate that there are likely direct descending projections from the MGB in the auditory thalamus to OC neurons in the superior olivary complex, suggesting that this pathway may be parallel-hierarchical, like many other parts of the auditory pathway. Further study using finer tracing is underway to obtain a more detailed picture of point-to-point descending projections at this level of the auditory system.

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347 Carboplatin Results in Neuron Loss in the Cochlear Nucleus but not the Inferior Colliculus or Auditory Cortex of Chinchilla

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Carboplatin selectively destroys inner hair cells and type I spiral ganglion neurons in the chinchilla cochlea; however, its effects on the central auditory system are largely unknown. The aim of this study was to determine if carboplatin treatment affects neuron survival in the cochlear nucleus (CN), inferior colliculus (IC) or auditory cortex (AC) of the chinchilla. Chinchillas were treated with carboplatin (100 mg/kg IP). Three weeks later, they were sacrificed and perfused intracardially with fixative. Surface preparations of the organ Corti were analyzed for hair cell loss. Serial frozen sections taken from the CN, IC and AC were stained with toluidine blue, and the number of neurons were counted in representative sections from normal (control) and carboplatin-treated chinchillas. The results showed that carboplatin destroyed approximately 80% of the inner hair cells in the cochlea. In the central auditory system, carboplatin resulted in a significant loss of neurons in the anteroventral, posteroventral and dorsal divisions of the CN, but not in the IC or AC. Future studies will determine if the loss of neurons in the CN is a direct result of carboplatin neurotoxicity, or a secondary effect of inner hair cell and spiral ganglion neuron loss in the auditory periphery.

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348 Laminar Distribution and Projection Patterns of Corticocollicular Cells in Guinea Pig Auditory Cortex

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We used axonal tracing techniques to examine projections from auditory cortex (AC) to the inferior colliculus (IC) in guinea pigs. Injections of anterograde tracers into AC confirm reports of bilateral projections to dorsal cortex (ICd) and external cortex (ICx) of the IC (Druga et al., '87 In: Syka, Masterton, eds. Auditory pathway: Structure and function. NY: Plenum Press. p 293-298). In addition, we observed a projection to the ipsilateral central nucleus (ICc) that was less dense than that to the ICd or ICx. Injections of retrograde tracers into the IC confirmed the anterograde results, with many AC cells labeled by injections in ICd or ICx and a small number of cells labeled by injections in ICc.

The retrograde experiments confirmed a bilateral projection from layer V cells in the AC. In addition, we observed a previously unknown projection from layer VI cells to the ipsilateral IC. Following a large injection into the IC, we observed two bands of AC cells: the expected dense band in layer V and a thinner band in lower layer VI. Small injections restricted to the ICd or ICx labeled fewer cortical cells overall, but consistently labeled cells in layers V and VI. The number of layer VI cells averaged 12% of that in layer V (range 6-17%; 13 cases). Furthermore, the layer VI cells were distributed throughout temporal cortex, indicating projections from all auditory areas and suggesting that layer VI cells make a significant contribution to the corticocollicular pathway.

Our results demonstrate AC projections to all IC subdivisions in guinea pigs. The projections arise bilaterally from layer V cells. In addition, there is an ipsilateral projection from layer VI cells to the ICd and ICx. It will be important to include these newly found projections in future considerations of corticocollicular functions.

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349 The Beam Pattern of *Eptesicus fuscus* as an Index of Spatial Attention

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Echolocating bats produce directional vocalizations for spatial orientation by sonar. We have previously measured the beam pattern of free-flying bats (Ghose & Moss, JASA, in press). Here we describe two experiments where we use the direction of the sonar beam as an index of the bat's attentional state during obstacle avoidance and prey capture.

Both experiments were carried out in a large echo absorbent flightroom. Two highspeed cameras were used to record the bat flight path and positions of other objects in the room. A 16 microphone array arranged in a horizontal u-shape was used to record the beam pattern of the bat as it flew in the room.

In the first experiment, the bat performed an obstacle avoidance task to gain access to a tethered worm. It was trained to localize the insect behind one of two holes cut out of a mist net. The worm location was randomized across trials, and the bat could take the worm only if it flew through the correct hole. Some trials show the bat directing its beam at the edges of the hole and towards the target, before and after it has navigated the hole.

In the second experiment the bat flew from a fixed start point towards a reference marker. When the bat was within 2.5 m of the marker, a tethered mealworm was dropped from a trap door. The mealworm could be at two ranges from the marker (.27m or .67m) and either to the left or right of the bat. The bat's beam pattern was used to determine its response to the target presentation. The bat was tested on several sequential trials with the prey at one range, and then a "catch" trial was introduced with the prey at the alternative range. Initial results indicate that during catch trials the bat may miss the target altogether during the initial run, or may take longer to respond to it.

Together these results suggest that attentional processes contribute to spatial orientation by sonar.

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350 Spatial Orientation and Memory in the Echolocating Bat, Eptesicus fuscus

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We investigated obstacle avoidance, target tracking, and spatial memory in the echolocating bat, E. fuscus. Four bats were trained in a flight room to fly through a hole (35 cm in diameter) in a mist net and catch a tethered mealworm on the other side. Two high-speed video cameras (240 Hz) recorded the trials, and video data were used to reconstruct the bats' 3-D flight path. Two ultrasound microphones recorded simultaneously the acoustic behavior of the bats, and a 16-microphone array was used to calculate the aim of the bat's sonar beam. Thus, we were able to reconstruct the flight path and determine the aim of the sonar beam.

First, each bat was trained to fly through one of two holes to gain access to a tethered mealworm presented behind one of the holes. This task required the bat to negotiate obstacles as it localized and tracked its insect prey. The data show that the bat sequentially scanned the opening in the net and the tethered worm. After the bats performed this task for several weeks we began a study of spatial memory. We closed one hole and attached markers to the net that provided information about the position of the open hole. The hole and the landmarks were moved together every fourth trial to one of three positions in a random fashion. The bats crashed into the net where the hole was previously located on average 0.9 times/trial before finding the new location of the hole. Finally, we moved the hole and landmarks together after each trial, requiring the bat to use the landmarks to find the opening in the net. In preliminary trails, the bats crashed the net 0.3 times/trial and may have shifted from using spatial memory to active echolocation. Our data suggest that echolocating bats can alternate between the use of spatial memory and echolocation for spatial orientation. Using spatial memory about obstacles can free the bat to direct its attention to localizing insect prey.

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351 Hearing in a Small Megabat, *Cynopterus brachyotis*: Audiogram, Sound Localization, and Use of Binaural Locus Cues

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Although behavioral tests of hearing and sound localization have been carried out in several species of echolocating bats, including one megachiropteran, no information is yet available on the behavioral hearing abilities of a non-echolocating bat. Using a conditioned avoidance procedure, we determined the audiogram and sound-localization abilities for the short-nosed fruit bat, *Cynopterus brachyotis*, a small (35-45 g) non-echolocating bat.

Audiogram. At an intensity of 60 dB SPL, *C. brachyotis* hears from 2.8-70 kHz, with a best sensitivity (6.5 dB) at 10 kHz and a secondary region of good sensitivity (11 dB) at 25 kHz. Its high-frequency hearing is typical of similar-sized non-echolocating mammals.

Sound-localization acuity. The minimum audible angle for 100-ms noise bursts averaged 10.5°, comparable to the passive localization thresholds of echolocating bats.

Use of binaural locus cues. The use of binaural phase- and intensity-difference cues for localization was examined by determining the bat's ability to localize low- and high-frequency pure tones at 60° separation. *C. brachyotis* was able to localize frequencies of 12.5 kHz and higher, indicating that it can use the binaural intensity-difference cue. However, it performed at chance at 8 kHz and below, even when the pure tones were amplitude modulated, indicating the inability to use interaural time differences in either the carrier tone or its envelope. Such inability to use interaural time differences has also been found in other bats, hedgehogs, and some species of mice.

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352 Acoustical Cues for Sound Localization by Gerbils in an Ecologically Realistic Environment

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We explored cues for sound localization that are available to gerbils in an ecologically realistic environment. We placed surgically a probe-tube microphone into the ear, and measured acoustical transfer functions (TFs) from a sound source to a point near the tympanic membrane in an anechoic room. The sound source was varied in azimuth, elevation, and distance relative to the center of the animal's head. We measured the TFs with or without a round veneer-board floor to examine the effects of sound reflections by the ground. In addition, we examined the effects of the animal's posture: Measurements were made with the animal in a standing or reclining position, as often seen in gerbil behavior. The TF obtained in the free-field showed spectral peaks and notches over the range of 10-15 kHz, that varied somewhat systematically with the source azimuth and elevation, but not with the distance. These features were insensitive to animal posture. In the floor condition, distinct spectral features appeared over a wide range of frequencies (> 500 Hz), and varied systematically with sound source location even along the distance dimension. These features were particularly apparent for the standing position. The floor-related spectral cues could be accounted for simply by the interference of the direct and the reflected sound. The interaural level difference (ILD), measured in the free-field, was generally symmetrical between front and back locations for the standing position. For the reclining position, however, the ILD function was markedly asymmetrical, the greatest effect of the posture being seen for proximal (< 10 cm) and backward (> 120 °in azimuth) locations. In summary, the present study suggests that various kinds of information about sound source location are available for gerbils in a realistic environment, and that the floor- and the posture-related cues may override interaural and spectral cues that have been observed in traditional free-field conditions.

353 Minimum Audible Angle Measured in Young and Old CBA Mice Using Pre-Pulse Inhibition of Startle

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Startle response amplitude is modulated by prior perturbation of the acoustic environment. Here we demonstrate that swapping the source of continuous noise between two speakers causes pre-pulse inhibition of startle in mice, and since inhibition increases with angular separation, the technique can provide behavioral measures of minimum audible angle (MAA). 3 mo old CBA mice (N=13) were used in Experiment 1. They were placed in an acoustically transparent wire cage that restrained movement, and oriented with head facing the mid-line of 2 spectrally-matched high-frequency speakers (TDT ES1) located 45cm from the mouse, with angular separations of 180, 90, 45, 22.5, or 15°. Continuous broadband noise (1-50kHz) was presented from one speaker for a minimum of 15s then swapped to the other, 1 to 300 ms (ISI) priori to an overhead startle eliciting noise-burst. Inhibition was greatest for 180° separation, and increased with ISI to 20ms, then gradually decreased from 60 to 300ms. With decreasing angular separation, maximal inhibition was reduced and longer ISI was required for it to fully develop. Experiment 2 was similar, but the smallest angular separation was 7°, and three age groups of mice were used; 6, 12, and 24 mo. Preliminary results indicate that noise-swap over 7° is not inhibitory at any ISI for these mice, and that the functional dependence of inhibition on angle and ISI change with age.

The rapid onset of inhibition when angular separation is large, but not when it is small, indicates that large monaural cues at each ear are sufficient to alert the auditory system to a change in the acoustic environment, while the longer ISI required for inhibition when angular separation is reduced and such cues are small, suggests that binaural processing of the two inputs is needed to detect the change. Using this interpretation, these data indicate that the CBA mouse MAA at 0 degree azimuth is between 7 and 15°.

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354 Auditory and Visual Guided Head Turning Behavior in the Barn Owl

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The optic tectum, or superior colliculus, contains circuitry that controls the orienting movements of the head, eyes, or ears. In the barn owl (Tyto alba), it has a topographic map of auditory and visual space composed of bimodal (auditory/visual) neurons with spatial receptive fields (RFs). Although a tectal neuron's visual and auditory RFs are largely aligned, the visual RFs are considerably finer (Knudsen 1982, J Neurosci. 2: p 1177-1194). This discrepancy in the grain of visual and auditory RFs raises the possibility that the owl's natural head-turning behavior would be more accurate when directed at lights than at sound-sources. To test this idea, we compared the accuracy of head-turns directed toward auditory and visual stimuli in the free-field. Noise bursts or light-flashes were randomly presented from different locations along horizontal plane in a dark echo-attenuated room. The head-turns were measured using a search coil system. Contrary to our initial expectation, results showed that there was no difference in the accuracy of head-turns to light and sound. Maximum head-turning speed was positively correlated with angular distance between initial head position and target. This relationship was observed in both auditory and visual guided head-turns. Latency of auditory head-turn was shorter than that of visual head-turn. This latency difference in head-turns may be caused by the tectal neuron's response latency, since neuronal response latency to auditory stimulus is shorter (Knudsen 1982). Similar behavioral characteristics observed in auditory and visual head-turns could be due to a common motor command synthesized in the optic tectum.

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355 The Localization of Single and Multiple Source Stimuli by Cats

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Behavioral studies of sound localization in cats have shown that these nocturnal predators can be trained using operant conditioning methods to accurately saccade to broadband sounds delivered in anechoic spaces from single locations in space (e.g., Populin and Yin, 1998). Little is known, however, about localization accuracy in more natural, echoic environments. We have previously demonstrated that cats appear to experience both the precedence effect and summing localization: they saccade to the leading sound when two similar sounds are presented with a short (1-10 msec) interstimulus delay from two different locations in space and they saccade between the two sounds when the delays are short (<300 usec) . Here, we examine several factors thought to be important in the precedence effect. First, we show that the spatial locations of the leading and the lagging stimuli can affect the apparent location of the auditory image. Second, we show that when the stimuli are delivered with zero delay, intensity differences alone between two stimuli can elicit localization dominance. Third, conflicting intensity and time differences between the two stimuli can influence localization performance. Finally, paired stimuli known to elicit a phenomenon known as the Franssen Effect in humans are also successful in eliciting the illusion in cats, especially when the stimuli are at frequencies where cats are less able to accurately localize single sounds. As a whole, these results add to what is known about the localization of simple and complex stimuli in cats and provide the basis for future physiological studies of these sound localization phenomena.

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356 Joint Psychophysical and Physiological Investigations of the Precedence Effect and Echo Threshold in the Inferior Colliculus of the Behaving Cat

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The precedence effect (PE) is an auditory spatial illusion where the apparent location of two similar stimuli presented from two different spatial locations but separated by a brief delay is determined primarily by the spatial attributes of the leading stimulus. To study the mechanisms that produce the PE, we have recorded the responses of single units in the inferior colliculus (IC) of cats that were simultaneously engaged in a psychophysical sound localization task. Cats were trained to make saccadic eye movements to the apparent location of transient stimuli delivered from either single locations or pairs of transients delivered from two different locations but separated by a delay. Psychophysically, the cats exhibited the PE in that the apparent location of the paired stimuli with delays of 1-10 ms approximated the location of the leading sound only. For greater delays, the cats often saccaded to either the leading or lagging location, suggesting that these delays exceeded the echo threshold (ET). For the delays that the cats experienced the PE, the physiological responses of a population of IC cells to the lag stimulus were substantially reduced relative to their responses to stimuli presented from the lag location presented alone, but little effect was seen on the response to the lead. Hence, the responses of these cells were correlated with the cats' reports of apparent location in that, for delays encompassing the PE, the reported location was also similar in the presence or absence of the lag. At the psychophysically determined ET the IC responses to the lag were nearly fully recovered to the responses obtained to the lag presented in isolation. Together these results support the hypothesis that the IC is an important neural substrate of the PE and ET, and as such, a population of IC cells could account for many aspects of the PE and ET and potentially sound localization in general.

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357 Plasticity in Human Sound Localization Induced by Compressed Spatial Vision

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Auditory and visual target locations are encoded differently in the brain, but must be co-calibrated to maintain cross-sensory concordance. Mechanisms that adjust spatial calibration across modalities have been described (e.g. prism adaptation in owls), though rudimentarily in humans. We quantified adaptation of human sound localization in response to spatially compressed vision (0.5x lenses for 2-3 days). This induced a corresponding compression of auditory localization that was most pronounced for azimuth (minimal for elevation) and was restricted to the visual field of the lenses. Interestingly, sound localization was also affected outside the field of visual-auditory interaction (shifted centrally, not compressed). These results demonstrate that spatially modified vision induces adaptive changes in human sound localization, including novel mechanisms that account for spatial compression. Findings are consistent with a model in which the central processing of sound location is encoded by recruitment rather than by a place code.

358 Aftereffect in Sound Localization by Interaural Time Difference at High Frequency Region

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After a prolonged exposure to an adapting sound, test sound appears to be shifted laterally away from the adaptor (e.g. Carlile et al., 2001). To identify the localization cues related to this phenomenon, we examined whether exposures to sounds lateralized by interaural time difference (ITD) at high frequency region (over 3 kHz) induce a similar aftereffect. The adaptors and test sounds were sinusoidally amplitude modulated tones (SAMs) with different carriers in experiment 1. In experiment 2, they were tones modulated by half-wave rectified and low-pass filtered pure-tones (transposed stimuli) or tones of low frequencies. The carrier frequencies of SAMs and transposed stimuli were over 3.4 kHz in both experiments. The rates of modulation were 128 Hz in experiment 1, and 128 or 256 Hz in experiment 2. The adaptors were presented for 2 minutes at the start of each block, and for 10 seconds between each trial. Their interaural relationships (leading ear) were randomized between sessions. Subjects reported whether the positions of the test sounds were either to the right or to the left of the midlines. Psychometric functions relating ITD of the test sounds and proportion of "Right" responses were calculated. The functions systematically shifted relative to the no-adapting condition only when adaptors and test sounds were presented at the same frequency regions. The results suggest that adaptation for sound localization system occurs to ITD information conveyed via amplitude envelopes in a similar way to other localization cues such as interaural level difference (Thurlow et al., 1970, 1973) and ITD of fine structures (Kashino, 1996).

359 Precedence Effects for Varying Source and Echo Locations

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The precedence effect refers to a collection of auditory phenomena that are evoked when a sound source from one direction is presented along with a delayed echo of that sound from another direction. For delays on the order of a few milliseconds, experimental results suggest that only a single sound is perceived and the apparent direction of that sound is dominated by the direction of the first arriving sound. This "localization dominance" effect has been studied most frequently using two loudspeakers in a stereophonic configuration in front of the listener (roughly 60 to 120 degrees of horizontal separation symmetric about the midline): one to simulate the source and one to simulate the echo. Here we demonstrate, using virtual auditory space techniques, that this effect does not always generalize to arbitrary spatial configurations of source and echo. We examined 8 source/echo configurations: 6 on the horizontal plane, including a standard stereophonic configuration with 80 degrees of separation, and 2 on the median plane. The signal was a train of 50 ms flat-spectrum noise bursts presented at a rate of 5 Hz for 2 s. For each configuration, delays of 0, 0.1, 0.2, 0.5, 1, and 2 ms were tested. Anechoic virtual sources from 56 locations surrounding the listener were interleaved with precedence stimuli within a block of trials in order to encourage listeners' use of the entire range of possible apparent position responses and to assess baseline localization abilities. Although predicted localization dominance effects were observed for the stereophonic configuration, other configurations produced different effects. The largest difference was observed with the median plane configurations where apparent position depended almost entirely on delay rather than the spatial locations of source and echo.

360 The Effect of Cueing "Where" and "When" on Speech Intelligibility in Noise And The Influence of Attentional Context on Free-Field Presentation.

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In conventional laboratory procedures measuring speech intelligibility-in-noise, listeners often know both when and where the speech and noise will occur. Such situations are unlikely in every day listening. Here, we report some preliminary experiments measuring the effect of varying the attentional context by informing listeners when (or when and where) the speech would occur, compared to the baseline case with no information as to when or where. We measured single-word speech intelligibility using a four-alternative forced-choice task with normal hearing volunteers for free-field presentation. The target word could emanate from any one of nine loudspeakers in the frontal horizontal plane with azimuths between -72^{o} and $+72^{o}$. The masking noise consisted of a diffuse field. In the baseline condition the target and masker were gated simultaneously, with the inter-trial interval chosen randomly between 1 and 10 seconds; thus there was no cue as to when the target would occur or which loudspeaker it would emanate from. The attentional context was set in one condition ("when-not-where") by immediately preceding the target with a 500-ms noise burst from the 0^{o} loudspeaker. The attentional context was set in a second condition ("when-and-where") by presenting the alerting noise from the same loudspeaker as the target. The levels were set to give performance was in the middle of the psychometric function. The results showed a gain in performance of about 5% in the "when-not-where" condition compared to baseline. An additional gain, of 5-10%, was found in the "when-and-where" condition. These effects are substantial in comparison to the usual effect sizes observed in speech intelligibility-in-noise tasks. They point to the importance of attentional context in such tasks.

361 The Influence of High Frequencies on Speech Localisation

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Our work explores how the spatial perception of speech using realistic 3D auditory displays depends on the bandwidth of the presented stimuli. Traditional speech communication systems use band-limited speech and we show that this compromises at least its localisation.

A vocally trained actor was recorded in an anechoic environment reciting the Harvard list of phonetically-balanced monosyllabic words. Virtual auditory space localisation of these speech stimuli was tested for five human subjects using an accurate head-pointing task. Two band-pass conditions were compared: (i) 20 Hz – 16 kHz (broadband) and (ii) 20 Hz – 8 kHz (low-pass). The data showed that low-pass speech stimuli were localised poorly in comparison to broadband stimuli, indicating that high-frequency information is important for localisation. Furthermore, analysis revealed that the increase in error in the low-pass condition was evident for all words regardless of their high-frequency energy. This suggests that natural human speech contains useful localisation information above 8 kHz. We will discuss the influence of the nature and level of this high-frequency information on speech localisation, as well as possible effects on the ability of listeners to attend to talkers in 3D auditory environments.

362 Binaural Processing of Dynamic Interaural Time Differences

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When sinusoidally frequency modulated (FM) tones are presented to each ear with a 180-degree phase lag, sinusoidal modulation of the interaural time difference (ITD) occurs at the same rate. At slow modulation rates, dynamic ITDs provide acoustical cues to sound source movement. Human listeners are much more sensitive to dynamic ITDs than to diotic FM of the same form (1) and the dichotic advantage - greatest at low modulation rates - persists to high modulation frequencies where no movement is heard (60 Hz with 500-Hz carriers). This observation contradicts the hypothesis that the binaural auditory system is sluggish. Evidence for sluggishness is provided, in part, by observations that thresholds for discriminating dynamic ITDs from FM in a low-pass noise increase sharply as modulation rates are increased above about 10 Hz (2).

Three listeners participated in a psychophysical study to investigate sensitivity to dynamic ITDs with tonal carriers. Psychometric functions were constructed for the discrimination of dynamic ITDs from FM. For a 500-Hz carrier, subjects' sensitivity to the presence of dynamic ITDs did not decrease as modulation rates were increased from 2 to 300 Hz. This finding is not in accordance with previous observations for sounds with a broader bandwidth (2), and indicates that processing of dynamic ITDs for tonal carriers is not intrinsically sluggish. It may be that factors such as the bandwidth or the carrier frequency of the stimulus influence listeners' ability to perceive dynamic ITDs.

1) Witton et al., (2000). *J. Acoust. Soc. Am.*, 108, pp 1826-1833.

2) Grantham & Wightman (1978). *J. Acoust. Soc. Am.*, 63, pp 511-523.

363 Binaural and Monaural Contributions to Spatial Release from Masking in Anechoic and Reverberant Spaces

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Often, spatially separating a target from a masker improves a listener's ability to detect the target or identify its content due to differences in interaural cues in the target and masker and due to changes in the target-to-masker ratio (TMR) at the ears. This study investigates how the spatial locations of target and masker influence consonant identification in anechoic and reverberant space. Reverberation was expected to influence performance both directly by degrading acoustic cues important for consonant identification and indirectly by decreasing spatial unmasking (by altering interaural cues in T and M as well as the effective TMR at the ears).

Both target (nonsense syllables) and speech-shaped noise masker were simulated using KEMAR head-related impulse responses (HRIRs). Performance was measured as a function of TMR at the acoustically "better ear," to obtain multiple points along the psychometric function. Subjects were tested binaurally and monaurally (better ear only) for each spatial configuration.

Results show that separation of target and masker yields a modest spatial release from masking that decreases with reverberation. The remaining spatial release is equally large for monaural and binaural results, suggesting that interaural differences do not contribute to spatial unmasking in the current task. However, binaural performance is generally better than monaural performance in difficult listening conditions (i.e., in reverberant environments or at low TMRs in the anechoic condition), even when target and masker are spatially co-located.

These results suggest that for consonant identification, spatial unmasking arises from monaural, spectral differences, whereas binaural improvements in performance occur even when target and masker give rise to roughly the same interaural cues.

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364 Localization of Band-Limited Noise Related to Known Auditory Spatial Cues

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Sound localization requires central processing to transform auditory input into a spatial map. Cues used to do so include interaural time and intensity differences (ITD & IID) and directional spectral filtering. We examined horizontal and vertical sound localization in 10 normal human subjects (ages 20-38) using bandwidth-limited stimuli designed to emphasize cues related to localization. Subjects were tested in a darkened, echo-attenuated room. Their heads were centered and fixed, facing a cylindrical screen behind which an invisible speaker on a robotic arm randomly presented auditory targets in the frontal field (±50° H x ±25° V). Stimuli consisted of repeating 150 ms bursts of high-pass (HP: 3–10 kHz), low-pass (LP: 0.1–1 kHz), or broadband (BB: 0.1–10 kHz) noise designed to emphasize IID, ITD, or both cues, respectively. Targets were localized by manually guiding a joystick-mounted laser beam at perceived target locations.

Horizontal localization of HP noise was less accurate than that of LP and BB noise (which were the same), with an average overshoot (slope of response vs. actual location) across eccentricities of 45%, 16%, and 16%, respectively, but all centered around zero. In contrast, vertical localization of LP noise was less accurate than that of BB and HP noise (which were similar). Vertical localization was shifted generally upward, accompanied by an average undershoot of 35%, 62%, and 24% for HP, LP and BB noise, respectively. The precision (spatial scatter) of sound localization was poorest for HP noise in both planes, and was generally independent of target eccentricity. In conclusion, human subjects depend mostly upon low frequency cues for accurate horizontal localization, and high frequency cues for accurate vertical localization. They also tend to overestimate horizontal eccentricity and underestimate vertical eccentricity.

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365 Normal-hearing and Hearing-impaired Listeners' Sound Localization as a Function of Hearing Aid Microphone Directionality.

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Little is known about how microphone directionality affects hearing aid benefit. One of the known benefits of directional microphones is an improvement in signal-to-noise ratio for signals emanating from the frontal direction. However, we do not know how a hearing aid users' localization ability is affected for sounds at the side and the rear where directional microphones are less sensitive. In this study we tested the sound localization of 8 normal-hearing and 8 hearing-impaired persons with recordings made through hearing aids with adjustable directionality (omnidirectional, hypercardiod, supercardiod, and cardiod directivity patterns).

A Knowles Electronic Manikin for Acoustic Research (KEMAR) wearing custom in-the-ear hearing aids was placed in the center of an

array of 16 loudspeakers evenly spaced in a circle. The phrase, "Where am I now?" was played through each of 16 loudspeaker locations evenly spaced over a 360° range. The output of the ½" microphones located at the medial position in KEMAR's ear canal were recorded for each loudspeaker azimuth and with each of the four directional microphone settings.

Normal hearing subjects listened to the KEMAR recordings through insert earphones and hearing-impaired subjects listened to the signal through an appropriate linear hearing aid fitting (recordings sent via direct audio input). The subjects were seated in the center of the loudspeaker array and identified the number of the loudspeaker from which the speech emanated.

The patterns of performance with each of the directional microphones will be compared and discussed.

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366 Chirp Evoked Binaural Difference Potentials

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Binaural interaction in auditory brain stem responses (ABR) is commonly analyzed in terms of the binaural difference potential (BD), i.e., the difference between the evoked responses to binaural and summed monaural stimulation. The BD is thought to reflect the activity of neural units responding specifically to binaural stimulation. Rising frequency chirps compensating for the dispersion of the travelling wave on the basilar membrane evoke larger monaural responses than clicks (Dau et. al. (2000) J. Acoust. Soc. Am. 107(3) 1530-1540). In the first experiment binaural ABRs and BDs were compared for chirps and clicks. Binaurally evoked ABRs were recorded for clicks and flat spectrum chirps for levels from 10 to 60 dB nHL in steps of 10 dB. 10000 sweeps were collected for every stimulus condition for 10 subjects and 3 recording channels. For both stimuli the latency of DP1 is smaller than the latency of the binaural wave V, which in turn is smaller than the latency of DN1. For all stimulus levels wave V amplitudes are significantly larger for chirps than for clicks. The amplitude of the binaural difference potential, DP1-DN1, is significantly larger for chirps at the levels 30 and 40 dB nHL. Both, the binaurally evoked potential and the binaural difference potential, exhibit steeper growth functions for chirps than for clicks for levels up to 40 dB nHL. For higher stimulation levels the chirp responses saturate approaching the click evoked amplitude. The amplitude ratio of the BD and the binaural wave V was found to be independent from the stimulus level. The constancy of this ratio is well explained by a BD model assuming contralateral inhibitory and ipsilateral excitatory (IE) interaction. In the second experiment chirp evoked BDs were measured for 17 interaural time differences (ITDs) in the range from 0 to 2 ms at 40 dB nHL. In contrast to BD studies using the click considerable binaural interaction was found for ITDs larger than 1 ms.

367 Cochlear Efferents in Different Avian Species - A Way to Distinguish Between Auditory and Vestibular ?

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The anatomy of cochlear efferent neurons in the avian brainstem has been described using injections of different retrograde tracers into the inner ear, followed by light-microscopical detection of the marker in the brainstem. With this technique, it is not possible to differentiate between vestibular and acoustic efferents, since efferents to the lagenar macula, a statolith organ at the end of the avian cochlea are also labeled by tracers introduced into the cochlea.

Cochlear efferent somata can be found in two groups, a ventrolateral group medial to the superior olive, and a dorsomedial group near the abducens motor nucleus. While the ventrolateral group seems to be exclusively auditory, the area of the dorsomedial group contains auditory as well as vestibular efferents. Efferents projecting to the lagenar macula have been found only in the dorsomedial group of efferents.

While the number of auditory cochlear efferents is three times higher in the barn owl than in the chicken, the number of lagenar efferents is approximately the same in both species.

In our experiments, slow pressure injections of small amounts of Choleratoxin or Fluorogold, two potent retrograde tracers, were made via the round window into the scala tymani of anesthetized barn owls and chickens. After a survival time of 5 to 7 days, the birds were sacrificed with an overdose of pentobarbital and perfused with 4% paraformaldehyde in phosphate buffer. 50 µm cross-sections of the brainstem were cut on a cryostat and processed imunochemically to visualize the tracers.

While in the chicken cell numbers in the dorsomedial group (DM) were generally greater than in the ventrolateral group (VL), in the barn owl cell counts in the VL generally exceeded those in the DM. This suggests that the dorsomedial cell group is clearly dominated by non-auditory efferent somata.

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368 Single Superior Olivary Nucleus Neurons Provide Divergent Inhibitory Input to Parallel Auditory Pathways in the Avian Brainstem

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Parallel processing, a fundamental feature of vertebrate sensory systems, is exemplified by the avian auditory brainstem, in which a pathway specialized for temporal processing is largely separate from pathways that process other aspects of sound. One possible exception to this parallel organization is in the inhibitory input provided by the superior olivary nucleus (SON). Previous studies have shown that the SON project ipsilaterally to nucleus angularis (NA), nucleus magnocellularis (NM), and nucleus laminaris (NL), as well as higher order contralateral targets. We sought to address whether single SON neurons project to multiple targets, or alternatively, whether separate neuronal populations project independently to individual target nuclei. We developed an in vitro dye electroporation method for retrograde labeling of neurons. Two fluorescent tracers were injected into each preparation; one dye into NM, NL, or NA, and another dye into one of the other nuclei on the same side of the brain. A large number of double-labeled SON somata were observed in all cases where injections were made into pairs of ipsilateral targets in NA, NM, and NL (mean = 24.1% of all labeled cells were double-labeled), suggesting that individual SON neurons project to multiple targets. In contrast when injections involved the contralateral SON, double labeling was rare (mean = <0.5%) suggesting that contralateral and ipsilateral targets are innervated by distinct populations of SON neurons. These results suggest that at the earliest stages of auditory processing, there is interaction between pathways specialized to process temporal cues and those that process other acoustic features. Additionally, the finding that the contralateral projection of the SON is composed of a separate population of neurons suggests that this population is regulated independently from the ipsilaterally projecting neurons.

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369 Responses of Medial Olivocochlear (MOC) Neurons: Specifying the Central Pathways of the MOC Reflex

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Medial olivocochlear (MOC) neurons project to outer hair cells (OHC), forming the efferent arm of a reflex that affects sound processing and

offers protection from acoustic overstimulation. The central pathways that trigger the MOC reflex in response to sound are poorly understood. Insight into these pathways can be obtained by examining the responses of single MOC neurons recorded from anesthetized guinea pigs. Response latencies of MOC neurons are as short as 5 ms. This latency is consistent with the idea that type I, but not type II, auditory-nerve fibers provide the major inputs to the reflex interneurons in cochlear nucleus. This short latency also implies that the cochlear-nucleus interneurons have rapidly conducting axons. In the cochlear nucleus, lesions of the posteroventral subdivision (PVCN), but not the AVCN or DCN, produce permanent disruption of the MOC reflex, based on a metric of adaptation of the DPOAE (de Venecia et al. (2001) ARO Abstracts p. 46). This finding supports earlier anatomical results demonstrating that some PVCN neurons project to MOC neurons (Thompson and Thompson (1991) J. Comp. Neurol. 311: 495-506; Warr (1969) Exp. Neurol. 23: 140-155). Within the PVCN, there are two general types of units when classified according to post-stimulus time histograms: onset units and chopper units. The MOC response is very sustained (Brown (2001) J. Neurophysiol. 86:2381-2392), and cannot be produced by inputs with an onset pattern. The MOC reflex interneurons are thus likely to be chopper units of PVCN. Also supporting this conclusion, chopper units have sharp frequency tuning and large dynamic ranges similar to the responses of MOC neurons. Thus, the major MOC reflex pathway is likely to be:

IHC => Type I Auditory-Nerve Fiber => PVCN Chopper => MOC Neuron => OHC.

370 Do Cortical Projections Contact Both Ascending and Descending Projections from the Superior Olivary Complex?

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Projections from auditory cortex to the superior olivary complex (SOC) contact cells that project to the cochlear nucleus suggesting that cortical projections could modulate activity in descending auditory pathways (Coomes and Schofield, 2001, Assoc. Res. Otolaryngol. Abs.: 45.). The SOC also contains cells that project to the inferior colliculus (IC). These cells are different from those that project to the cochlear nucleus, raising the question of whether these cells are also targets of cortical projections. The present study addresses this question in guinea pigs.

We labeled cells that project to the ipsilateral or contralateral IC by injecting various fluorescent tracers (FluoroRuby, fluorescein dextran, or Fast Blue) into these targets. In the same animals, we injected a different tracer (fluorescein dextran or FluoroRuby) into temporal cortex to label cortico-olivary axons. We then examined the SOC both ipsilateral and contralateral to the cortical injection for apparent contacts between labeled cortical axons and labeled SOC cells. We observed contacts with both ipsilateral and contralateral SOC cells that project to the ipsilateral or contralateral IC. The contacts were observed frequently in the ventral nucleus of the trapezoid body and the superior paraolivary nucleus, and less often in other periolivary nuclei and the lateral superior olivary nucleus.

Our results suggest that cortical projections contact olivary cells with ascending projections. Combined with our previous findings, we conclude that cortical projections are in a position to modulate both ascending and descending pathways from the SOC.

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371 Immunogold Analysis of Excitatory and Inhibitory Amino Acid Terminals in the Lateral Superior Olive of the Rat.

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The lateral superior olive (LSO) performs a binaural signal analysis by integrating ipsilateral excitatory input and contralateral inhibitory input. In the present study, we investigated the synaptic distribution of glutamate (Glu) which is a major excitatory amino acid, and GABA and glycine (Gly) which are major inhibitory amino acids in the LSO of the P8 and P14 rats by use of post-embedding immunogold analysis. Immunogold labelling for Glu was observed within the nerve terminals projecting to LSO neurons. It is assumed that these glutamatergic terminals correspond to excitatory input from the ipsilateral cochlear nucleus. Immunogold double labelling for GABA and Gly identified three types of inhibitory terminals projecting to LSO neurons: Gly, GABA, and colocalized Gly and GABA (Gly/GABA). Although Gly terminals were predominant in both P8 and P14 rat, the combined ratio of GABA and Gly/GABA projecting to LSO neurons of P8 rats is lower than that of P14 rats. This result suggested that GABA plays an important role early in the development of the central auditory system.

372 Selective Effects of GABA and Glycine on the Response Properties of Neurons in the Superior Paraolivary Nucleus of the Rat

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The superior paraolivary nucleus (SPON), a prominent cell group of the superior olivary complex, is composed of a homogeneous population of GABAergic neurons that project to the inferior colliculus and receive abundant GABAergic and glycinergic inhibition. Single unit recordings indicate that the vast majority of SPON neurons in the rat respond only to contralateral stimulation with offset responses.

To examine the role of inhibition in the generation of offset responses, we made *in vivo* recordings from SPON neurons before, during and after iontophoretic application of bicuculline (a GABAa receptor antagonist) and/or strychnine (an antagonist of the glycine receptor). Stimuli were 50 ms tone bursts at the neurons BF and 20dB above threshold.

Application of bicuculline resulted in a significant increase in the number of spikes in all 12 neurons tested. Seven units (58%) continued to display offset responses, while the remaining five neurons (42%) responded during the stimulus and at the offset. Blockade of the glycine receptor (n=12) abolished the offset response in all neurons and resulted in transient responses at the stimulus onset. We also noted a significant broadening of response maps in the absence of glycinergic inhibition. When both receptors were blocked simultaneously (n=14), all units displayed sustained responses that lasted throughout the duration of the 50 ms tone.

Responses to sinusoidally amplitude modulated (SAM) stimuli were also examined. At all modulation frequencies tested (50 - 800 Hz), vector strengths were significantly decreased by the bic/strych cocktail.

These results demonstrate that GABAergic and glycinergic inhibition both play prominent roles in the formation of SPON offset responses to tone stimuli, and in the ability of SPON units to respond to SAM signals. The data also suggest that the offset responses observed in the SPON arise from a postinhibitory rebound mechanism.

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373 Increased Jitter in First-Spike Latency in MNTB Neurons of Mice Lacking the *Kcna1* Gene is Probably Caused by Increased Variability in Axonal and/or Calyceal Conduction.

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Voltage-gated potassium (Kv) channels are believed to play an important role in the temporal processing of auditory information. The Kv channel subunit Kv1.1 (encoded by the Kcna1 gene) is expressed strongly in auditory nuclei, including the ventral cochlear nucleus (VCN) and the medial nucleus of the trapezoid body (MNTB). We previously reported a larger variability in MNTB neurons' first-spike latency in Kcna1-null (-/-) mice than in wildtype (+/+) mice (Kopp-Scheinpflug et al., ARO 2001). The present study addresses the question whether this difference originates in the MNTB neuron or is already present in their inputs (VCN neurons and their terminals, the calyces of Held).

Single-unit recordings were made from VCN neurons, calyces and MNTB neurons of +/+ and -/- mice. At each recording site general physiological properties such as spontaneous rates, thresholds and the distribution of the characteristic frequencies (CFs) were not significantly different for +/+ and -/- mice. The latency of the first-spike was measured to tone bursts presented at the unit's CF and 80dB SPL. The mean first-spike latency of VCN neurons was only slightly shorter in +/+ mice (3.8ms, n=13) than in -/- mice (4.6ms; n=18). In contrast, first-spike latency in MNTB neurons was significantly shorter in +/+ mice (4.3ms; n=12) than in -/- mice (8.1ms; n=23). In addition, the standard deviation of first-spike latency (jitter) was significantly smaller in VCN (0.3ms) and MNTB (0.8ms) of +/+ mice compared to their -/- equivalents (VCN: 0.6ms, MNTB: 4.6 ms). Comparison of jitter within the -/- mice along the three different sites of ascending pathway showed that the jitter at the calyces (3.7ms; n=9) was significantly larger than in VCN and only slightly (not significantly) smaller than in MNTB. These results suggest that the jitter in MNTB arises mostly in the axons of VCN neurons, the calyces or both rather than in MNTB neurons.

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374 From CN to LNTB and Back

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The cat lateral nucleus of the trapezoid body (LNTB) receives excitatory input from the ipsilateral ventral cochlear nucleus (VCN) and in turn, projects back to all subdivisions of the cochlear nucleus (CN). By combining physiological recordings across experiments, a general tonotopic order was proposed for the LNTB (Tsuchitani, J Neurophys, 1977). In order to refine our knowledge of the tonotopy in the LNTB, we performed focal injections of biotinylated dextran amine (BDA) into identified frequency regions of the dorsal cochlear nucleus (DCN). Retrogradely labeled LNTB cells support the notion of the medial to lateral - high to low frequency arrangement of the LNTB. Furthermore, injections into the rostral DCN retrogradely labeled primarily rostral LNTB cells, while caudal DCN injections labeled primarily caudal LNTB cells, suggesting a rostro-caudal topography in the LNTB to DCN projection. Spirou and Berrebi (Adv Hear Res, 1994) presented anatomical evidence for feedback from LNTB to DCN. We further investigated this finding by BDA injections into separate subnuclei of the LNTB, which have recently been described by Spirou and Berrebi (J Comp Neurol, 1996, 1997, 1998). Anterogradely labeled fibers run dorsally from the LNTB into the intermediate acoustic stria and from there into the DCN. Large LNTB injections generate labeled fibers and endings on pyramidal cells across several isofrequency laminae in the DCN. A focal posteroventral LNTB injection anterogradely labeled fibers and endings in a smaller, medium to high frequency area in the DCN. Therefore, CN and LNTB may form a tonotopic feedback and feedforward circuit whose activation may modulate sound processing in the earliest stages of auditory activity.

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375 A Neural Model of an MSO Neuron

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A biophysical model of an MSO neuron including both excitation and inhibition is presented, and model responses are compared with MSO data from an extensively studied, low-frequency unit (unit 67-82-5 in Goldberg and Brown, 1969). Specifically, the effects of interaural time delay (ITD) are explored for different combinations of levels at the two ears. Output patterns, characterized in terms of discharge rates and period histograms, are compared with data from the MSO cell. Rate-ITD functions are generated at different sound levels and with different interaural intensity differences (IIDs) in order to study the roles of excitation and inhibition within the neuron. The model fits the non-monotonic rate-level functions for out-of-phase and monaural stimulation of the unit when level-dependent inhibitory inputs are included. Level-dependent inhibition shapes the rate-ITD curve by minimizing the out-of-phase rate within a narrow range across different levels. With an IID, the model simulates discharge rates and period histograms for the in-phase and out-of-phase conditions. The general shape of the rate-ITD functions of the model, with the maximum for in-phase excitatory inputs and the minimum for out-of-phase excitatory inputs, is not affected by IID. For both the data and the model, the combined temporal and rate information indicates that the relative strengths of excitation and inhibition vary with the sound level. Our conclusion is that inhibition is important for ITD tuning of this MSO cell, and the role of inhibition is to control the firing threshold to improve ITD sensitivities with increases in sound levels.

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376 A Model of the Physiological basis of Pitch Perception.

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Little is known about how pitch is processed by the auditory nervous system. Autocorrelation models of pitch extraction have been successful in simulating a large number of psychophysical results in this area but there is little support for the idea that the nervous system acts as an explicit autocorrelation device. To address this issue, this poster presents a design for a new model of pitch perception based upon known neural architecture and also presents some preliminary pitch analyses using the model. The model offers a physiologically plausible system for periodicity coding that avoids the need for long delay lines required by autocorrelation. The system incorporates a model of the human auditory periphery including outer/middle ear transfer characteristics, nonlinear frequency analysis and mechanical-electrical transduction by inner hair cells. The resulting 'auditory nerve' spike train is used as the input to three further stages of signal processing thought to be located in the cochlear nucleus, central nucleus and the external cortex of the inferior colliculus, respectively. The complete model is implemented using DSAM, a development system for auditory modelling. The output from the system is the activity of a single array of neurons each sensitive to different periodicities. The pattern of activity across this array is uniquely related to the fundamental frequency of a harmonic complex. The testing of the model is still in its early stages but has so far been successfully tested using a range of harmonic stimuli and iterated ripple noise stimuli. The poster will report on current progress in testing and refining the model

377 Simulations of Gerbil AVCN Responses Using a Cross-Frequency Coincidence-Detecting Model: Usefulness and Limitations.

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The cochlear nucleus represents the first stage of central processing in the auditory system. A complete understanding of how information regarding the auditory environment is encoded by cells of the cochlear nucleus requires understanding how the numerous synaptic inputs to these units are combined. The cells of the anteroventral cochlear nucleus (AVCN) have been well studied, in terms of their morphological properties and physiological responses. Studies have shown that bushy cells of the AVCN have both morphological and physiological properties consistent with a coincidence-detection mechanism across the excitatory inputs to these units. The objective of this project was to determine to what extent an excitatory coincidence-detection model could be utilized to explain the responses of gerbil AVCN bushy cells to various stimuli. In particular, the responses of model coincidence-detectors were fit to tone-in-noise rate-level functions and to noise-response PST histograms. Successful fits of model cells to gerbil data should provide predictions of both the change in rate with tone-level, as well as the fine-timing of the responses. Fits also provide a prediction of the set of excitatory inputs that converge on the gerbil unit. Initial efforts utilized a simple two-input coincidence-detection model based on a model of the psychophysical task of the detection of a tone in broadband noise. More recent work focused on more physiologically realistic models that received greater than two inputs. Results show that a coincidence-detecting model with multiple inputs can predict the fine-timing of AVCN bushy cells in response to noise, but gives less meaningful predictions of rate changes in response to changes in tone-level. Future work will require models that include inhibitory inputs.

378 Temporal Properties of Fibers in Auditory Nerve and Trapezoid Body to Broadband Noise.

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It has been shown in the cat that, for low-frequency pure tones, fibers in the trapezoid body (TB) show both more precise phase-locking and higher entrainment (spiking on every stimulus cycle) than auditory nerve (AN) fibers. Because a subset of these recordings was derived from spherical bushy cell axons projecting to the medial superior olive (MSO), it was speculated that this enhanced synchronization may be critical for the sensitivity to interaural time differences (ITDs) in MSO. As most natural stimuli are broadband and behavioral ITD-sensitivity is best for broadband noise, we wanted to evaluate the difference in coding of broadband noise in AN fibers and bushy cell TB axons.

We recorded from the TB and AN of pentobarbital-anesthetized cats. We used the poststimulus time (PST) histogram to short tone bursts at the characteristic frequency (CF) to identifiy cell class. Responses to a standard broadband (0.1-30 KHz) noise (duration 1 sec) were obtained at a number of SPLs. For all possible pairs of non-identical spiketrains, we constructed all-order interval histograms. Pairs of identical spiketrains were excluded because their all-order interval histogram overestimates zero intervals and underestimates all intervals with a duration between zero and the refractory period of the fiber. All histograms were added and normalized and we refer to the resulting histogram as a normalized shuffled autocorrelogram (SAC). At low CFs the SAC had the form of a damped oscillation with a peak at delay zero and an oscillation frequency near the CF of the fiber. At high CFs, the SAC had a single central peak. From each SAC we retrieved two parameters: the height of the peak at delay 0 and the width of this peak at half-height. Peak height was significantly higher in TB fibers compared to AN fibers for all frequencies. In low-CF fibers, width at half-height showed a decrease as a function of CF, and was smaller in TB fibers than in AN fibers.

We conclude that enhancement of synchronization of TB fibers relative to their AN inputs is not restricted to pure tones, but extends to broadband stimuli.

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379 Late Developmental Pruning of Collateral Branches Associated with the Main Afferent and Efferent Axons of the Medial Nucleus of the Trapezoid Body

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Much attention has been given to the main afferent and efferent axons associated with the medial nucleus of the trapezoid body (MNTB). Globular bushy cells (GBC) of the cochlear nucleus send large-diameter axons that end in the form of large terminal specializations, Calycies of Held, in the contralateral MNTB. In adult mammals, each GBC axon ends as a single calyx that engulfs the soma of a single MNTB principal cell. Principal cells project their main axons to the nearby lateral superior olive. Intracellular labeling in tissue slices (Kuwabara & Zook,'91) has shown that main MNTB afferent and efferent axons give off small-diameter, collateral axons that contact one or more of each of the identifiable periolivary cell groups surrounding the lateral olive. Collaterals from labeled sets of afferent and efferent axons (the pre- and post-synaptic axons of a single MTNB principal cell) converge upon the same sets of target nuclei and cells (Zook,'92).

In post-natal mice, intracellular labeling in brainstem tissue slices show that, at birth, single collaterals of both MNTB afferent and efferent axons form terminal arborizations that spread widely across all target nuclei. In mice around the onset of hearing, terminal arbors usually form narrow bands across each target nucleus. In older mice, arbors are usually more restricted to small, focal clusters of cells within target nuclei. A similar maturation sequence is seen for short collateral axons that branch and terminate within the boundaries of the MNTB. These developmental sequences are reminiscent of the late-developmental pruning of multiple calycies that takes place within the MNTB (Kuwabara et al.,'91). At birth, single GBC axons with multiple calyces of Held are common. These multiple calyx axons are first spatially restricted within the MNTB around the onset of hearing and then largely disappear, leaving the mature pattern of a single calyx for each GBC axon.

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380 Neural Responses in the Ventral Nucleus of the Lateral Lemniscus to Repetitive Stimulation of the Lateral Lemniscus in a Rat Brain Slice

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Neurons in the ventral nucleus of the lateral lemniscus (VNLL) show several firing patterns in response to depolarizing current injection. Most VNLL neurons respond to electrical stimulation of the lateral lemniscus (LL) with excitatory and/or inhibitory postsynaptic potentials. To better understand how VNLL neurons preserve and convey temporal auditory information, in this study we examined their responses to repetitive stimulation of the LL.

Brain slices of 400 microns were taken in the frontal plane through the VNLL of young rats. The LL just ventral or medial to the VNLL was stimulated by a single pulse and a train of 100 or 200 pulses at 10-300 Hz. Intracellular recordings were made and suprathreshold synaptic responses, i.e. action potentials, were recorded from VNLL neurons. The intensity at which a single pulse could reliably elicit a spike was used for repetitive stimulation. The number of spikes was counted to

calculate the firing probability. The latency of each spike was analyzed to assess temporal precision of the responses.

For most neurons (14/21) the firing probability was greater than 90% at 10 Hz. As the repetition rate was increased, the firing probability decreased. Spike latencies progressively increased in response to a train of pulses. The increase was usually more apparent at higher than at lower repetition rates. For some neurons, the spike latencies showed an increasingly random variation (jitter) in response to a train of pulses. For other neurons, although the progressive increase of spike latency was observed, the extent of jitter in spike latency was essentially constant in response to a train of pulses. The jitter was more pronounced at higher than at lower repetition rates for all of the neurons tested.

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381 Temporal Features of Spectral Interactions in the Inferior Colliculus of Mustached Bat

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Neurons in the inferior colliculus (IC) of mustached bats show facilitated responses to one spectral element in the presence of another at particular delays. Facilitatory interactions appear to be shaped by inhibitory interactions. Here we describe effects of antagonists to receptors for glycine (strychnine or STRY) and GABA (bicuculline or BIC) in creating the inhibitory interactions that shape facilitatory interactions in single IC units. Eighty facilitated units formed 4 groups based on the associated inhibition. In 22%, only facilitatory interactions were observed. In 37%, inhibition was seen at delays shorter than the delays causing facilitation. In 16%, inhibition was seen at delays longer than the delays causing facilitation. In 18%, inhibition was seen at delays shorter and longer than the delays causing facilitation. Consistent with earlier results, facilitatory interactions were eliminated by STRY or STRY/BIC, but not by BIC itself. Inhibition at shorter delays was not eliminated by STRY (100%), STRY/BIC (82%), and BIC (87%). In contrast, inhibition at longer delays was eliminated by STRY (78%) and STRY/BIC (83%) and not by BIC (20%) by itself. These results suggest that: a) inhibition at shorter delays are not created in the IC or may be mediated through different receptors other than glycine and GABAA, and b) inhibition at longer delays are created in the IC and is glycine receptor mediated. These inhbitory interactions may enhance contrast between preferred (i.e., facilitated) and non-preferred delays and may adjust the best facilitated delay of neurons, but they do not appear to sharpen a neuron's tuning to delay.

382 Neural Correlates of Temporal Processing in the Inferior Colliculus of Mice Lacking the Kv1.1 Voltage-Gated Potassium Channel

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The Kv1.1 voltage-gated potassium channel is found throughout the auditory brainstem and in the cochlear nucleus has been linked with units specialized to encode coincident inputs. The channel has also been reported to be moderately expressed in the inferior colliculus. It has a low threshold of activation after depolarization and remains open for a period of time after the initial depolarization event. The characteristics of the Kv1.1 channel implicate it in the formation of fast-spiking excitatory postsynaptic potentials that allow neurons to fire quickly and with high temporal precision, properties found in many IC neurons. We examined the functional contribution of Kv1.1 channels in the IC in coding silent gaps embedded in noise and 100% and 50% sinusoidal amplitude modulated (SAM) noise in a Kv1.1 knockout (KO). Near-field evoked potentials were obtained from 10 +/+, +/- and -/- Kv1.1 mice developed on the C3H strain. Recordings were obtained from bipolar electrodes (25-40 um tips) lowered 500u into the central nucleus. Gap stimuli consisted of two 50 ms noise bursts separated by quiet gaps varying from 0.25 to 96 ms, including a no gap control. SAM noise bursts were 200 msec in duration with modulation frequencies ranging from 10 to 1000 Hz. Each set of stimuli were presented at 40, 60 and 80 dB SPL. We calculated RMS amplitudes (GAPS) and power spectrums (SAM) in 3 time windows in order to compute the respective gap functions and modulation transfer functions. There were no significant differences in minimal gap thresholds (MGTs) or slopes of recovery functions between groups, with MGTS ranging from 1 to 4 msec at the 50% recovery point. The SAM upper cutoff frequency ranged from 80 to 300 Hz, and was lower in KO mice.

These results suggest that the Kv1.1 channel is important for encoding dynamic stimuli which require repetitive firing, but not a one-to-one response to stimulus, as needed in the case of gap detection.

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383 Control of Call Frequency by GABAergic Synaptic Transmission in the Medulla of Echolocating Horseshoe Bats.

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Bats adjust temporal, spectral and intensity parameters of their echolocation calls by precisely monitoring the characteristics of the returning echo signals. However, neuronal substrates and mechanisms for auditory feedback control of vocalizations are still largely unknown in any vertebrate. We used echolocating horseshoe bats to investigate the role of the brainstem for the control of call frequencies in response to changing auditory feedback. These bats accurately control the frequency of their echolocation calls through auditory feedback both when the bat is at rest and when it is flying and compensating for changes in echo frequency caused by flight-induced Doppler-shifts (Doppler-shift compensation, DSC). The relative simplicity of echolocation calls and the robustness of DSC behavior allow one to examine the control of different call parameters independently from one another.

Here we focus on the pre-motor control of vocalizations. Specifically, we injected various GABAergic and Glutamatergic agonists and antagonists into the horseshoe bat medulla and analyzed their effects on the different echolocation call parameters. It is known that in all mammals studied, the anterior portion of NA controls call frequency via the superior laryngeal nerve, whereas the posterior portions control temporal call parameters via the recurrent laryngeal nerve. In addition, vocalization is integrally associated with breathing, both at the behavioral level and at the level of single unit activity within NA and neighboring regions of the lower brainstem.

Using iontophoresis of the GABAA agonist Muscimol and its antagonist Bicuculline methiodide into the anterior NA we were able to affect call frequency independently of other vocalization parameters or of respiration. These preliminary results suggest that GABAergic input to the anterior NA is essential for the (pre)motor control of call frequencies in horseshoe bats both at rest and during DSC behavior.

384 Does Formation of the Vertebrate Ear Modulate Astroglial Connexin 43 Expression in the Hindbrain?

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Connexin (Cx) 43 is a gap junction protein found predominantly in astrocytes of the central nervous system. In the developing vertebrate hindbrain, astrocytes and their precursors define rhombomere boundaries and subsequently guide migration of the neurons as well as their dendritic and axonal outgrowths from the hindbrain. In this preliminary study, we first show that even though a segmentally organized scaffold of fibers exists as early as stage 35/36 of the

developing Xenopus hindbrain, it does not mature into specialized glial boundaries until stage 47. At stage 47, the neural scaffold and the outlying neuropil become abruptly defined by the presence of Cx43. Furthermore, these expression patterns persist during the formation and differentiation of the hindbrain and the ear. We also show that unilateral deletion of the ear at stage 33/34 down-regulates Cx43 expression at rhombomeric boundaries at the outset, and that this effect is cumulative over time. Thus, the emergence and differentiation of the ear not only provides the hindbrain with a general trophic support, but also influences the appearance of coupled glial cells at rhombomeric boundaries that may play a role in the development of hindbrain auditory and vestibular circuitry.

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385 The Effect of Changes in Ambient Oxygen Concentration on the Bioelectric Properties of Middle Ear Mucosa

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The objective of the study was to compare the effect of 24 hours of exposure to 7% O_2 (normal middle ear physiologic conditions) versus 21% O_2 (found in the middle ear after tympanostomy tube placement) on transepithelial Na^+ absorption and Cl^- secretion in cultured gerbil middle ear epithelial cell monolayers.

Mongolian gerbil middle ear epithelial cells previously transformed with SV40 were grown from 4 to 7 days under standard conditions. Then, culture dishes were exposed to 24 hours of 7%, 14%, or 21% O_2 in airtight incubation chambers. Cells were then mounted into an Ussing chamber and transepithelial short circuit current (I_{sc}) was measured.

Among cells exposed to the different O_2 concentrations, no differences in baseline I_{sc} or in transepithelial Na^+ absorption were identified, with similar responses to amiloride blockade of apical Na^+ channels. However, the UTP-induced stimulation of apical Cl^- secretion in the presence of apical Na^+ channel blockade with amiloride was significantly enhanced after exposure to 21% O_2 when compared to 7% O_2 exposure. It is theorized that the increase in Cl^- secretion seen at 21% O_2 (which correlates clinically with the presence of a tympanostomy tube) may lead to a small amount of water secretion into the middle ear, contributing to middle ear humidification and/or enhancing periciliary fluid depth.

386 The Cycle of Entry, Growth and Dissolution of External Canal Epidermis in the Normal Developing Human Middle Ear and Its Relation to Cholesteatoma

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In order accurately to characterize epidermoid formations (EFs) in the middle ear during development we studied the immunohistochemical expression patterns for external canal and middle ear epithelial cytokeratins in paraffin serial sections of several antibodies at different gestational ages of fetuses and young children. Epithelial structures suspected as EFs could then be accurately characterized as epidermoid with age-appropriate antibodies.

A study of the interface between annular external canal epidermis and middle ear epithelium suggested that epidermal cells entered the middle ear epithelium at about 16 weeks from the external canal epidermis.

Each of 22 serially sectioned paraffin-embedded temporal bones from 16 weeks gestation and up to 8 months postpartum, but not before or after, contained one or more, often large numbers of, immunohistochemically confirmed EFs. EF sizes increased significantly with increasing age and EFs *pari passu* underwent increasing epidermoid differentiation. Three large, older EFs showed features of impending dissolution. Mapping of the EFs indicated that they developed anywhere in the annular lateral wall region of middle ear, but the majority were in the anterosuperior region.

Persistent growth in a very few EFs may result in cholesteatoma. The genesis of congenital cholesteatoma from external canal epidermis is thus similar to that of acquired cholesteatoma and, indeed, some cases classified as the latter may be of congenital origin. Factors that relate to regression of EFs must be present, however, in the great majority of developing temporal bones and may, in the future, provide a non-surgical approach to the management of cholesteatoma.

387 Epidermoid Rests in the Developing Mouse Middle Ear

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It is widely believed that congenital cholesteatoma originates from epidermoid cell rests incorporated into the temporal bone during embryonic development. Congenital cholesteatomas of the anterior-superior middle ear may arise from such a rest, known as the epidermoid formation (EF), which is present in many fetal temporal bones up to about 33 weeks of gestation. The EF has been shown to persist into the postnatal period in some cases and may provide the nidus for cholesteatoma formation. To date, no animal model has been established for study of this structure in non-human material.

We have observed nodules of epidermoid cells very similar in light-microscopic appearance to those previously described in human temporal bones in the normal mouse middle ear during the perinatal period. These structures are always located anterior to the tympanic annulus on the lateral wall of the middle ear near the junction of the ciliated eustachian tube epithelium with the middle ear mucosa. In all specimens examined thus far, the mouse EF is a single, rounded structure from 15 to 120 microns wide (mean width 56 microns), which is composed of stratified squamous cells of epidermoid appearance. EFs have been identified as early as embryonic day 17.5 and they persist until at least 7 days after birth. Transmission electron microscopic studies are currently underway to characterize the ultrastructure of the epithelial cells comprising the Efs.

It is hoped that the mouse will prove to be a useful model for studies on the development and fate of epidermoid rests in the middle ear and thereby provide better understanding of factors relating to the etiology of congenital cholesteatoma.

388 Protective Effect of Corticosteroid Against the Ototoxicity of Aminoglycoside Otic Drops.

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Otic drops are commonly used not only for otitis externa but also for otorrhea in the presence of tympanic membrane perforation or tympanostomy tube. Many studies demonstrated the ototoxicity of aminoglycosides. In our previous study, we observed that gentamycin (GM), when activated with liver extract demonstrated significant ototoxicity. The purpose of this study is to assess the protective effect of corticosteroid against the ototoxicity of gentamycin and tobramycin drops using isolated cochlear outer hair cells (OHCs) in vitro with and without liver extract.

OHCs from adult chinchilla cochleae were exposed to standard bathing solution (control), otic drops with and without corticosteroid and liver extract, and liver extract alone. The images of OHCs were recorded using an inverted microscope, and analyzed on the Image Pro-plus 3.0 program. Time to cell death and change of cell length were measured.

Insignificant ototoxicity was demonstrated when isolated cochlear OHCs were exposed to GM or tobramycin otic drops without liver extract. In the presence of liver extract, these aminoglycoside otic drops showed significant ototoxicity in terms of the time to cell death and the percent change in cell length. Addition of dexamethasone

(0.1 %) significantly reduced ototoxicity in terms of the time to cell death and the percent change in cell length even with liver extract activation.

Conclusion: This study demonstrated that GM and tobramycin cytotoxicity of isolated cochlear OHCs requires activation such as liver extract and addition of dexamethasone significantly reduced cytotoxicity.

389 Genomic Plasticity and Shifting Gene Expression Patterns in Biofilm Forming Pseudomonas aeruginosa, a Pathogen of Otorrhea

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Chronic otorrhea is a major cause of acquired hearing loss. One of the most important pathogens associated with otorrhea is Pseudomonas aeruginosa (PA). The paradigm that chronic infections are caused by bacterial biofilms prompted us to study the relationship among pathogenicity, biofilm-formation and genomic plasticity. It is essential to determine the gene sets involved in the above processes and whether they are present in every bacterial cell or distributed in a ?communal gene-pool?. We constructed a highly redundant genomic-DNA library to analyze changing expression patterns of a large number of genes during biofilm development. The genomic DNA of twelve clinical strains was used to build this pooled PA genomic library, comprised of ~250,000 clones. This library has an ~1.4 kb insert size and ~4.5x redundancy.

Sequencing of ~2 million nucleotides revealed that 89 genes are not represented in the genome of the PAO1. Distribution studies of the non-PAO1 genes revealed that only 2 of the unique sequences were found in all the tested 11 strains, 2 were found in 9 strains and 4 in 6 strains, but the 53 remaining genes were found in less than 1/2 of the strains. Overall, the novel genes are well dispersed in the clinical strains of this library.

To test the changing expression pattern of PA genes, the library was printed on nylon membranes. RNAs isolated from planktonic- and biofilm-grown PA were used to probe these membranes. Comparative hybridizations uncovered sets of clones with significantly different levels of expression. Identification, further analysis of these clones and evaluation of their potential involvement in biofilm formation and pathogenicity is in progress.

390 Correlation Between Mucin and NO Metabolite Concentrations in Human and in Experimentally Induced Middle Ear Effusion

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It has been suggested that nitric oxide (NO) is involved in the pathogenesis of mucoid type of middle ear effusion(MEE). The purpose of this study is to determine and correlate concentrations of mucin and nitric oxide (NO) metabolites in humans and in experimentally induced otitis media with effusion (OME).

Samples of human MEE were collected at the time of myringotomy and tympanostomy tube insertions. The type of MEE was classified as serous or mucoid MEE at the time of surgery. Experimental otitis media was induced in chinchillas by injecting NO doner, S-nitroso-N-acetylpenicillamine (SNAP) alone, lipopolysaccharide (LPS) alone, or LPS + SNAP. Samples of middle ear fluid were collected and analyzed for mucin by PAS and NO metabolites using the Griess method. At the end of each experiment temporal bones from animals were harvested for histopathological study.

In human samples, the concentrations of both mucin and NO metabolites (NO2- + NO3-) were significantly higher in mucoid than in serous MEE. In animal models, mucin concentration was the greatest in LPS + SNAP group and least in the SNAP group. Histopathology of animal temporal bones showed the greatest mucosal thickening and inflammation in the LPS + SNAP group. In the chinchilla animal model, exogenous NO in LPS-induced OM increased the mucin concentration in middle ear fluid as well as mucosal thickness and inflammation in middle ear mucosa. A positive correlation between mucin and NO metabolites in humans and in experimentally induced middle ear effusion suggests that NO may contribute to the pathogenesis of mucoid otitis media.

391 Ultrastructural Changes of the Tympanic Membrane in Granular Myringitis

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Granular myringitis is a disease of the tympanic membrane (TM) characterized by focal or diffuse replacement of dermis by granulation tissues. Although clinical features of the disease have been well described, its pathogenesis is still obscure. In order to elucidate the pathogenesis, we investigated on the ultrastructural changes of 6 human TM of granular myringitis patients together with normal TM and middle ear mucosa specimen, which were taken at the surgery

In normal tympanic membrane, there is a outer layer of stratified squamous epithelium and an inner thin mucosal layer of flat cells. In between, there is a lamina propria containing fibrous connective tissue with a few blood vessels. In diseased tympanic membrane, loss of outer squamous epithelium and inflammatory cells infiltration in the lamina propria were the main findings. Electron microscopic findings indicated that cell death with loss of intracellular organelles of outer squamous epithelia were observed at the transitional area from normal to granulation-covered TM. Our results suggest that granular myringitis be a variant form of wound healing disorder.

392 Proinflammatory Cytokine Induction in Response to Respiratory Syncytial Virus Infection in Well-Differentiated Epithelial Cells

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Respiratory syncytial virus (RSV) has been implicated in significant morbidity and mortality among infants, the elderly, and the immunocompromised host. RSV infection typically localizes in the respiratory tract mucosa, thereby resulting in rhinitis, sinusitis, otitis media, tracheobronchitis and pneumonia. Results from both clinical and *in vitro* studies suggest that RSV infection induces proinflammatory cytokine genes in epithelial cells thereby activating the immune response. However, most *in vitro* studies have used cell lines and undifferentiated cells to examine the immune response to RSV

infection. The present study was undertaken to investigate the effects of RSV infection in well-differentiated nasal (WD-NE) and tracheobronchial (WD-TBE) airway epithelial cells at an air-liquid interface. This model more accurately recapitulates the *in vivo* physiology and morphology. A recombinant RSV that expresses green fluorescent protein (GFP) (rgRSV) was used to infect both WD-NE and WD-TBE. In contrast to previous reports, our results demonstrated a relatively minor up-regulation of the proinflammatory cytokines IL-8, IL-6, and RANTES expression in response to infection. TNF-α treatment resulted in significant up-regulation of IL-8, IL-6 and RANTES in both RSV-infected and uninfected cells. Between-group comparisons suggested that RSV infection potentiated the effects of TNF-α. Studies in progress are directed toward understanding the mechanism. These findings suggest that one major effect of RSV infection is its potentiation of a proinflammatory immune response.

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393 Assessment of Otitis Media in 60 Inbred Strains of Mice

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Otitis Media (OM) accounts for 22 million visits to United States physicians every year. Identifying mouse models will allow great insight into the pathogenesis of this childhood disease. With the recent sequencing of the mouse genome, gene mapping and manipulation are easier in the mouse compared to other animal models of OM. In order to identify a potential OM mouse model, a method of assessing middle ear morphology was needed. Tympanometry, a commonly used technique in OM screening in humans, was adapted to detect middle ear abnormalities in mice. A methodology and baseline for tympanometry was established in the mouse. Video otoscopy, auditory brain threshold (ABR) and histological analysis were used to validate any abnormal tympanometry measurements. Thus far, we have tested 60 inbred strains. (At least 10 ears /five mice from each strain were tested with tympanometry.) This large database of tympanograms establishes a reliable reference for normal ear pressure, volume and compliance in the mouse. Some mice from the 129S1/SvImJ, 129X1/SvJ, C57L/J, CBA/CaH-T6/J, LP/J, NZB/B1NJ, and BUB/BnJ strains exhibited abnormal tympanograms. A large percentage of mice from the LP/J strain showed middle ear effusion; this strain therefore provides a good OM mouse model. These results support the feasibility of a large-scale screening program for mice with middle ear abnormalities at The Jackson Laboratory.

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394 Expressions of caspase-3, -8 and NF-κB in human middle ear cholesteatoma

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Middle ear cholesteatoma is characterized by an excessive accumulation of keratin debris within the middle ear together with hyperproliferation of keratinizing epithelium.

Caspase-8 is activated by induction of TNF-α. Caspase-8 is an important mechanism in the activation of caspase-3, which cleaves key regulatory or structural proteins and in particular activates apoptotic nucleases. NF-κB is a transcription factor and is known to inhibit apoptosis induced by TNF-α. In this study, we hypothesized that expressions of caspase-3, -8 and NF-κB are uniquely connected to the proliferation and programmed cell death of keratinocytes during the growth and development of middle ear cholesteatoma. The presence of caspase-3,-8 and NF-κB in cholesteatoma was examined using paraffin-embedded tissue sections, and to correlate the above protein profiles with clinical patient data. Normal skin and ear drum were also stained as control.

With the immunoperoxidase staining method, caspase-3 was detected as a brownish color in the spinous and granular layer of cholesteatoma epithelium. Caspase-8 was densely localized in the granular layer. NF-κB was localized densely in the perinuclear region of granular layer significantly. In the control study using normal mouse IgG and normal goat IgG, there was no staining in cholesteatoma. Normal human skin and ear drum spicimens were not significantly stained in the epithelium. There was a significant correlation between cholesteatoma epithelial cell positivity on anti caspase-3,-8 and NF-κB antibody staining and otorrhea observed just before surgery.

These findings suggest that caspase-3, -8 play important roles in programmed cell death, which results in the accumulation of keratin debris during the growth of cholesteatoma. NF-κB was recognized in cholesteatoma epithelium, but it is suggested that the transcription factor is in an inactivated state in middle ear cholesteatoma.

395 Microarray Analysis of Gene Expression in Rat Middle Ear after Bacterial Pathogen Challenge

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Differential gene expression plays key roles in the pathogenesis of otitis media. To gain insight into the molecular processes of otitis media influenced by Streptococcus pneumoniae, microarray technology was used to examine differential gene expression patterns in the early stages of rat middle ear after bacterial pathogen challenge. Rats were euthanized 6 h, 24h and 72 h after Streptococcus pneumoniae instillation into the middle ear, and gene expression was determined using Atlas rat cDNA expression arrays containing 1176 known genes at each time point. At 6 h in bacteria-challenged ears, the mRNA expression of two macrophage inflammatory proteins (MIP), MIP-1A and MIP-2 were simultaneously upregulated more than 7-fold at 6 h in bacteria-challenged ears, and interluekin-1b mRNA had increased 2.6-fold. More than 97 % genes showed almost no altered gene-expression levels 6 h after stimulation with bacteria. These changes may contribute to the initiation and persistence of middle ear mucosa inflammation and effusion during an episode of bacterial acute otitis media. The cDNA array experimental approach identified functionally significant genes in middle ear mucosa after stimulation with bacteria, providing a powerful technical basis for future analysis of mechanisms of middle ear inflammation.

396 Middle Ear Epithelial Mucin Secretion in Response to IL-6

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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 cytokines play an integral role in the mechanisms of otitis media, investigating the effect of specific cytokines on the regulation of mucin secretion and gene expression is vital to furthering our knowledge of the pathophysiology of otitis media.

We investigated the mucin secretion of cultured middle ear epithelium(MEE) in response to IL-6 stimulation. Primary cultures of chinchilla MEE were established and exposed to 0, 50, 100, and 200 ng/ml concentrations of IL-6 in growth media for 16 hours after exposure to 5 ìCi/ ml tritiated glucosamine. The culture supernatant was then drawn off and loaded on sepharose columns after enzymatic degradation. The radioactivity of 2 ml fractions was measured by liquid

scintillation. Mucin production was determined from the radioactivity of appropriate fractions in comparison to control.

Mucin production from cultured MEE cells increased in a dose response manner in regard to IL-6 exposure. This study demonstrated the ability of IL-6 to up-regulate mucin secretion in cultured middle ear epithelial cells. This investigation and future studies may lead to novel and efficacious treatments for otitis media through cytokine modulation.

397 Is There an OAE Correlate to Behavioral Overshoot?

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The behavioral detection threshold of a tone pip masked by noise improves by 10 dB or more when the pip is delayed 200 ms after noise onset compared to 1 ms delay. An existing model of this overshoot effect posits that the noise onset activates the medial olivocochlear (MOC) efferent system, which reduces the nonlinear component of the basilar membrane response at 200 ms delay in comparison to 1 ms. There also should be observable effects on stimulus frequency otoacoustic emissions (SFOAE) if this model is accurate. In normal-hearing adults, SFOAE thresholds were measured in response to a 4-kHz tone pip at 1 and 200 ms delays after the onset of a broadband frozen noise. The SFOAE was defined as the signal difference at 4 kHz of the pip+noise after subtracting the pip-alone and noise-alone signals, thus eliminating all stimulus response. The SFOAE noise was defined as the SFOAE energy averaged over offband frequencies. SFOAE threshold was the lowest pip level producing a 3 dB signal-to-noise ratio. At spectral noise levels of 40-50 dB SPL, the average SFOAE thresholds were 9-14 dB lower in the 200 ms delay condition. This appears consistent with the overshoot model. However, the average thresholds were unchanged at lower noise levels in apparent contradiction to the level dependence of overshoot. For a notched-noise condition (1 octave notch, spectral passband level of ~30 dB SPL), average SFOAE thresholds were 7 dB lower in the 200 ms delay condition. The potential role of the acoustic reflex on SFOAE thresholds is under study. The different level dependence of the SFOAE thresholds compared to behavioral thresholds in overshoot is problematical, but measurements of SFOAE threshold level differences may have relevance to the interpretation of overshoot models.

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398 The Efferent Control System and Menière's Disease

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The variability of histological and electrocochleographic (ECoG) findings and inconsistent effectiveness of treatments pertinent to the assumed endolymphatic hydrops (EH) suggest that EH may not be essential for Menière's Disease (MD), raising the suspicion of another underlying mechanism.

The efferent system plays a role in vestibulo-cochlear homeostasis. The auditory efferent system, via its distal part, the medial olivocochlear (MOC) system, is involved in setting up the operating point of the basilar membrane through electromotile responses of the outer hair cells (OHCs). Indeed, the ECoG findings following electrical activation of the MOC bundle and contralateral acoustic stimulation are consistent with those in MD and may mimic EH. The vestibular efferent system may also be implicated, considering the finding of selective damage to the vestibular type II hair cells, which morphologically correspond to the OHCs and also receive a direct efferent input.

The accessibility of the efferent system for clinical testing is very low and, at present, only limited information can be obtained from testing the MOC system.

The MOC system in 40 patients with MD was studied by recording of transient evoked otoemissions with and without contralateral acoustic stimulation. The results showed no deficit in MOC suppression. Furthermore, the magnitude of suppression, in comparison to the normal database, tended to be greater: the value over 3dB was found in 40% of patients, while only in 5% of the normal subjects. The relevance of this finding is unclear, as the range of "normal" values of MOC suppression has not yet been defined, although a wider dynamic range of suppression may imply a more sensitive, but unstable olivocochlear system.

Although there is no clear indication of structural abnormality of the MOC pathway, in view of the supporting evidence, the possibility of a dysfunctional efferent vestibulo-cochlear system should be considered in further research on MD.

399 Distinctive Contributions of Olivocochlear Efferent and Middle-Ear Muscle Reflexes to the Alteration of Distortion Product Otoacoustic Emissions by Contralateral Noise

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It is widely accepted that suppression of otoacoustic emissions (OAEs) with contralateral presence of low-level sounds is an auditory reflex mediated by the olivocochlear efferent system, involved in the alteration of cochlear function. The measurement of OAE suppression has potential to be a noninvasive tool for evaluating functional status of the efferents. However, concerns arose regarding the confounding effects of the sound-induced middle-ear muscle reflex on OAE suppression recording. The consequence of this type of auditory reflex is alteration of sound transmission through the middle ear, which undoubtedly influences the recorded OAEs. Although the thresholds of middle-ear muscle reflex in humans have been confirmed at sound levels greater than those routinely used in OAE suppression recordings, we know little about its effect on OAEs. A difficulty in investigating one type of auditory reflex is that the effects of the other reflex cannot be eliminated in almost all human cases. The aim of the present study was to find approaches for separating the contributions of olivocochlear efferent and middle-ear muscle reflexes to the contralaterally sound-induced alteration of OAEs. Distortion product otoacoustic emissions (DPOAEs) were measured as a function of time in normal human ears. Recordings were made at frequencies from 1 to 6 kHz with and without contralateral presence of broadband noise. The noise was present at several levels, equal to and 10 to 20 dB below and above the middle-ear reflex threshold. The preliminary results showed distinctive effects of the two reflexes activated, respectively, with low- and high-level noise on the time course of DPOAEs. The effect is also dependent on the stimulus frequency for DPOAE recording. Characteristic features of the measured DPOAE time functions will be presented in detail.

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400 Comparison of Medial Efferent Rapid Adaptation of Otoacoustic Emission and Cochlear Microphonic Distortion Products

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2F1-F2 rapid adaptation, demonstrated in cats, mice, guinea pigs and humans, has been related to normal medial efferent function. It has not been determined, however, whether this effect is produced by MOC activity at the DP or primary tone frequencies, or some combination of the three. To determine the site of MOC action in rapid adaptation we measured simultaneously the 2F1-F2 DPOAE as well as the cochlear microphonic (CM) at 2F1-F2 (DPCM; the CM to the distortion product) and at the primary frequencies (CMF1 and CMF2; the CM to the test

tone signals). All measures were taken simultaneously in the same ear in lightly anesthetized guinea pigs. In this study, the ratio of F1 to F2 was 1.21; F1 was 70 dB SPL and F2 was 65 dB SPL. Previous investigators have shown that the cochlear microphonic is increased in response to MOC stimulation. Our results, however, show that when the 2F1-F2 DPOAE undergoes rapid adaptation following primary tone onset, the DPCM is likewise decreased, with a similar time course. This result suggests that the MOC is not acting at the 2F1-F2 frequency to produce the observed DPOAE rapid adaptation response. The CMF1 and CMF2 responses are complex, with at least one of the two components showing an increase in amplitude following primary tone onset, suggesting MOC action at the primary frequencies. These findings argue that the MOC acts to suppress the level of one or both primaries and that "rapid adaptation" observed at the 2F1-F2 frequency is a result of the MOC-induced decrease in the primary tone levels. This result is intuitive as previous work has shown that the magnitude of efferent action is proportional to the amplitude of the stimulating tones: In the 2F1-F2 adaptation paradigm, the primary tone levels are typically 40-60 dB above the level of the DPOAE, indicating that there should be considerably more MOC action at the primaries as compared with the 2F1-F2 frequency.

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401 Gentamicin Administration Effects on Onset Adaptation and Contralateral Suppression of DPOAEs in the Rat.

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Recently our lab has shown that onset adaptation and contralateral suppression of DPOAEs in the rat are mediated primarily by the middle ear reflex, whereas in other species it appears to be mediated predominately by the medial olivocochlear (MOC) efferent system. In the rat this was demonstrated by sectioning middle ear muscles (MEMs), after which only small residual effects remained, postulated to be mediated by the MOC system. Smith, et al (1994), showed in guinea pigs that single dose Gentamicin 150mg/kg IM completely abolished contralateral noise suppression of CAPs within 2 hours, and the effect was reversed after 96 hours. The same results were seen in 3 animals in which only stapedius was cut. They explain this effect by Gentamicin-induced blockade of presynaptic calcium channels on MOC efferents. Gentamicin is also known to block neuromuscular junctions at high doses (Pittinger, et al, 1972). Our current study examines whether Gentamicin abolishes the DPOAE effects by blockade of MOC efferents, or by chemical MEM paralysis, in the rat. In our protocol, input/output functions for two DPOAE frequencies were recorded, with and without contralateral noise. Middle ear muscles were then sectioned and the paradigm repeated. Finally, Gentamicin 150mg/kg IM was injected, and after 2 hours, the paradigm repeated. Our data suggests that the small residual DPOAE effect seen after MEM sectioning is abolished, or nearly abolished by Gentamicin 150 mg/kg. In a control group given only Gentamicin with intact MEMs, only a small loss of suppression was seen, comparable to the small residual suppression remaining after MEM sectioning. This data supports the postulate that Gentamicin 150mg/kg inhibits MOC efferent activity, and not the middle ear reflex.

402 Aminoglycoside Effects On Fast Distortion Product Oto-Acoustic Emission (DPOAE) Adaptation in Mice

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Fast adaptation of the DPOAE has been shown to be efferent mediated, and present in several species. Eliminating efferent stimulation of the outer hair cells (OHC) by drug intervention or surgical methods has been shown to reduce or eliminate this effect in several species. Here, we present an exploration of the effects of varying doses of gentamicin and kanamycin on fast adaptation of the DPOAE in mice, compared to guinea pigs. Using Tucker Davis Technologies hardware and Matlab interface, DPOAE adaptation curves were obtained for 72 combinations of primary tones L1 and L2. Adaptation strength was defined as the difference between the onset DPOAE amplitude minus the steady state DPOAE amplitude, and was plotted in a three dimensional curve over the L1/L2 grid. A single dose of gentamicin reduced adaptation strength in guinea pigs without affecting other measures of cochlear sensitivity. However, gentamicin was not well tolerated by mice in combination with anesthetic, and was lethal at doses as small as 100 mg/kg. Wu et al (2001) have demonstrated that kanamycin causes ototoxic effects in mice with cumulative doses, so we tested its effect on adaptation strength. In mice, kanamycin did not have a significant effect on maximum adaptation strength. However, after kanamycin, maximum adaptation occurred at a L1 level higher than the L1 required for maximum adaptation at baseline. In Guinea pigs, kanamycin did not have any effect on adaptation strength, nor the L1 or L2 values required to produce the maximum and minimum adaptation. In conclusion, a single dose of Kanamycin did not reduce amplitude of adaptation in either species, and was not useful in determining whether the effect was efferent-mediated. Gentamicin, a substance known to affect efferent function, was not well enough tolerated in mice to be a useful assessor whether the adaptation seen in mice was efferent-mediated, and different methods need to be employed.

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403 Comparing DPOAE Input/Output Functions, Onset Adaptation and Contralateral Suppression in Rat and Chinchilla using Identical Experimental Paradigms

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Our laboratory has been exploring the properties of the auditory efferent system by measuring DPOAEs. Two ways to study efferent effects on DPOAEs are to (1) measure temporal envelopes of DPOAEs as a function of time and any change during the initial few ms of the DPOAE (called onset adaptation) and (2) measuring the changes in steady-state DPOAEs in the presence of contralateral noise (contralateral suppression). Previous studies in the rat (Relkin et al., 2001 ARO abstracts) and rabbit (Luebke et al., JARO 2002) show significant DPOAE changes due to middle-ear reflex activation, others have indicated no middle ear involvement (Liberman et al., JASA 1996). We have extended the experimental paradigm used to collect data from rats (Relkin et al., 2001 ARO abstracts) to chinchillas. The chinchilla I/O functions appeared to grow almost linearly to 40-50dB SPL in all but one frequency tested. Rat I/O functions grow non-linearly to 20-30dB with a clear notch in all but one frequency. Rats have changes in the DPOAE of 10-15dB in the presence of contralateral noise observed at all I/O levels and frequencies, the chinchilla showed changes of 1dB or less. Onset adaptation in rat is usually 4-8dB at all I/O levels and may "flip" near a notch in the I/O function. Chinchilla's have onset adaptations of 2-4dB that occur only near a notch and also "flip". Cutting middle ear muscles in the rat leaves only 0-3dB residual effects. Attempts to cut the middle ear muscles in the chinchilla were unsuccessful. However, cutting the olivocochlear bundle at the 4th Ventricle in the chinchilla eliminated any measurable onset adaptation or contralateral suppression. Corresponding phase data in both species will be presented.

404 Dynamic Changes of Spontaneous Otoacoustic Emissions Occurring Immediately after the Onset of Contralateral Acoustic Stimulation (CAS)

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Spontaneous otoacoustic emission (SOAE) changes following the onset of a broad-band noise CAS presented at either 65, 55, or 45 dB SPL were measured in human subjects to evaluate the time-course of medial olivocochlear (MOC) efferent effects. Data were sampled for 1.2 s with the onset of the recording window at 0.4 s prior to the abrupt onset of CAS. For each ear, waveform samples were analyzed off-line using the Wigner-Ville distribution (WVD) with frequency zooming and regional smoothing, which provided high time and frequency resolutions for signals with temporal spectral changes. The amplitude and frequency estimators derived from the WVD analysis of each SOAE were averaged over 50 samples. Temporal changes of SOAE amplitude and frequency shifts were approximated by exponential functions to determine time constants and latencies of the CAS effects. The results indicated that: (1) for the vast majority of SOAEs examined, CAS produced an increase of SOAE frequency and a decrease of SOAE level; (2) the magnitude of the CAS effects on the frequency and amplitude shifts of SOAEs diminished with a decrease of the CAS level; (3) temporal characteristics of the SOAE changes following the CAS onset, as well as the relationship between those changes and the CAS level exhibited substantial intersubject variability; (4) CAS altered SOAEs with a latency in the range of tens of milliseconds relative to the CAS onset, and (5) the latency of an SOAE frequency increase was shorter than that of an SOAE amplitude decrease suggesting that the tuning of the SOAE generator is affected by the CAS before a reduction of the cochlear amplifier begins. Clearly visible effects of low-level CAS on SOAEs support the view that contralateral suppression of SOAEs is mediated primarily by MOC activity rather than through the stapedial reflex.

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405 Post-onset DPOAE Adaptation Before Noise Exposure in Animals Shown to be Resistant to Noise Trauma

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The German waltzing guinea pig is a new strain of animal expressing recessive hereditary inner ear degeneration. The homozygotes are deaf at birth and show vestibular disturbance. The heterozygotes, or carriers, have normal hearing and vestibular status. We have in previous studies found that the carriers are less susceptible to noise exposure. Maison and Liberman (The J. Neuroscience, 2000) have shown a correlation between the strength of the Medial Olivocochlear (MOC) reflex and susceptibility to noise-induced hearing loss: the stronger reflex, the lesser hearing loss. Efferent mediated DPOAE adaptation occurs in the first 200-500 ms after the onset of the two primary tones. Typically, the change in DPOAE response amplitude is approx. 3 dB. The variability in both MOC reflex strength and susceptibility to noise-induced hearing loss are great in both humans and animals. The aim of this study was to investigate if the carriers of the German waltzing guinea pigs express a stronger MOC reflex. We used ten carriers of German waltzing guinea pig, and four animals from a normal strain. ABR at 4, 8 and 16 kHz were measured at three time points, before noise exposure, 1 hour post noise (broad-banded noise, 2-20 kHz, for 2 hours at a level of 103 dB (A)) and 2 weeks post noise. Also measurements of the adaptation of the 2f1-f2 DPOAE were performed. The ratio between the two primary tones was f2/f1=1.2. The efferent mediated reflex was measured with the primaries at three different levels with two sets of primary frequencies in order to create the most optimal DPOAE-response. The mean value of the DPOAE amplitude was calculated at discrete time points and plotted as a function of time. As in previous studies the carriers were less affected by the noise as compared to control group. The carrier's reflex strength was also stronger than in the control group. Individual animals of carriers showed extremely strong reflexes with changes in DPOAE amplitude as high as 9 dB.

406 Studies on the Basic Characteristics of DPOAE and its Origin

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OAE originates from cochlear active mechanisms and reflects the sensitivity status of cochlea. We use methods of cochlear efferent activation and high-level sound exposure with DPOAE measurement to study the various aspects of cochlear function and explore the origin of DPOAE. The amplitude and latency of DPOAE were measured with and without contralateral noise before and after high-level sound exposure in guinea pigs and humans. DPOAE latency was measured in 79 normal humans, with and without contralateral noise. In addition, the DPOAE amplitude was measured when a 3rd tone was presented ipsilaterally to obtain suppression tuning curves (STC).

After high-level sound exposure, the DPOAE amplitudes decreased with the biggest decrease occurring when f2 equals the exposing tone frequency. Before exposure, contralateral stimulation significantly decreased the DPOAE amplitude at 1~4kHz while DPOAE latency increased at 2~6kHz. After exposure, only the amplitudes at 2kHz significantly decreased and latency did not change with contralateral stimulation. The DPOAE latency decreased nonlinearly with primary frequency increase and decreased linearly with primary intensity increase. Gender differences were observed; DPOAE latency was longer in males compared to females at low frequencies. Contralateral sound made the latency longer. The STCs were asymmetrical, V-shaped, the tips of which were consistently centered around the f2.

We find that changes in DPOAE are a sensitive measure that reflects the changes of cochlear function induced by sound exposure and contralateral suppression. DPOAE latency is an important index and can reflect various aspects of cochlear function. DPOAE suppression tuning curves support the hypotheses of cochlear tuning and frequency resolution and that the DPOAE originates primarily from the f2 frequency region.

407 Tension Sensitivity of Prestin

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Prestin (Zheng et al., 2000) is the protein that is either the piezoelectric membrane motor or the essential part of the motor localized in lateral outer hair cells. The dependence of the membrane capacitance shows that its voltage sensor has electric charge between 0.9 and 1 electronic charge, similar to that of the membrane motor in outer hair cells. However, the mechanical changes that accompany charge movement has not been analyzed quantitatively. (Santos-Sacchi et al., 2001 and Ludwig et al., 2001).

To examine mechanical changes in prestin that accompany charge transfer, we applied known membrane tension to prestin-transfected cells through the patch pipette. Membrane tension was evaluated by the formula 1/2 rP, where r is the radius of the cell and P is the pressure applied. We found that voltage shifts of the membrane capacitance due to tension were given by $(5 \pm 1)\times10^{-6}$ Vm/N. If the values of tension estimated were indeed applied to prestin, prestin's area changes are (0.7

± 0.2) nm^2, about 1/4 of those in the membrane motor in outer hair cells. This discrepancy can be attributed to absence or presence of components in transfected cells compared with outer hair cells. Since our patch pipettes contained trypsin (1 mg/ml), and since we did not see significant time dependence during recording, we do not think that the cytoskeleton affects the membrane tension applied. Thus we interpret that the motor in outer hair cells is a complex that consists of prestin and helper proteins, enhancing the effectiveness by allosteric interactions between them. This interpretation is also consistent with the observation that the number of 10 nm particles approximately agree with the number the electrical charge of of the motor in outer hair cells. We therefore speculate that an important next step in clarifying the motor mechanism in outer hair cells could be to identify proteins that specifically bind to prestin.

408 Effects of cGMP on prestin-transfected HEK cells

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Outer hair cells (OHCs) in the mammalian organ of Corti express a unique feature called "electromotility", which is thought to provide the local active mechanical amplification of the cochlear response to sound. A widely accepted explanation for the cellular mechanism of electromotility is that a unique "motor protein" in the OHC basolateral membrane changes conformation when electric stimulation is applied, leading to a change in OHC length. We have demonstrated in both in vitro systems that prestin is this motor protein [Zheng et al., Nature 405, 149-155, 2000]. In the past, several compounds, including cGMP have been shown to affect outer hair cell electromotility [Szönyi et al., Hear. Res. 137, 29-42, 1999]. There are at least two positions on the prestin molecule that are potential cGMP–dependent protein kinase phosphorylation sites. Whether these sites are involved in cGMP dependent reaction is as yet unknown.

To address this question, we have established a heterologous system to study prestin function and its potential modification. In this system, prestin cDNA is transiently transfected into a human kidney cell line, TSA 201. The cells expressing prestin are selected to measure nonlinear capacitance, a signature of outer hair cell motility. Different chemicals, including a cGMP analog have been applied to the transfected cells. Our data suggest that application of the cGMP analog can significantly increase nonlinear, voltage-dependent charge displacement in prestin transfected cells. The molecular mechanism of cGMP effect is being further investigated.

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409 Surface Charge Asymmetry Affects the Direction of Voltage Dependent Membrane Movement

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Electrical stimulation of mock transfected (pBK-CMV-GLUT5) Chinese Hamster Ovary (CHO) cells results in membrane movements that are opposite to those of prestin transfected CHO cells (PNAS 2001: 98: 4178-4183). We show that a change in the surface charge asymmetry (i.e. the surface charge density on the internal, σ_i relative to the external, σ_e interfaces) of the membrane can result in a polarity reversal in membrane movements. We do this by calculating the voltage dependent change in surface tension of a membrane, $\Delta\gamma$, assuming it results from changes in membrane polarization. We calculate $\Delta\gamma$ by: (1) estimating the polarization charge, q_p by use of Gouy-Chapman-Grahame theory. (2) substituting q_p into Gibb's adsorption equation derived for a polarizable interface under the conditions that the chemical composition and temperature are constant; and (3) integrating Gibb's adsorption equation for a model membrane polarized from a transmembrane potential, Ψ_o to Ψ. We find $\Delta\gamma$ is a parabolic function of Ψ. We also find that the voltage at maximum tension, which corresponds to the point of zero charge, depends upon the asymmetry of the surface charge density. For $\sigma_i > \sigma_e$, the maximum is at positive potentials and for $\sigma_i < \sigma_e$ the maximum is at negative potentials. The greater the asymmetry the more positive and negative is the voltage at maximum tension. Our analysis suggests that $\sigma_i > \sigma_e$ for prestin transfected CHO membranes, compared to $\sigma_i < \sigma_e$ for mock transfected CHO membranes.

We suggest that one role for prestin in outer hair cells may be to assure the correct electromotile response polarity for the cochlear amplifier.

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410 What Prestin transports

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There is now good evidence that outer hair cell (OHC) electromotility is generated by a 'motor' complex which includes 'Prestin'. Prestin is a member (SLC26A5) of a superfamily of anion-bicarbonate exchangers, transport proteins which are expressed in a variety of tissues. This has led us to explore its role as a bicarbonate-chloride exchanger as well as a transporter of neutral solutes such as sugars known to be taken up by OHCs (Geleoc et al. 1999).

Isolated guinea pig OHCs were perfused in a flow chamber with perilymph buffered with hepes. When external chloride was reduced for 100s by gluconate replacement, cells shortened in length by approximately 6%. The shortening was blocked by 1mM DIDS or by 10mM salicylate included in the external media. However, when the external solution was buffered with 25mM bicarbonate instead of hepes, no length change occurred with this protocol. The data suggest that a chloride-bicarbonate exchanger, implicated in OHC pH regulation (Ikeda et al. 1992) shares many properties of the OHC motor protein and may indeed be SLC26A5. A small chloride conductance (1-2nS) observed in bilaterally symmetric chloride suggests that Prestin also has a low chloride permeability. A simple kinetic model with a unique mechanically compact state can summarize these data.

When expressed in HEK-293 cells, GFP-tagged Prestin (a gift from B. Fakler) shows properties of a neutral solute transporter. Isotonic replacement of external 30mM glucose by fructose led to a reversible 6% increase in the area of cells expressing Prestin. There was no area increase in cells expressing GFP alone or when extracellular solution as control was applied. These data suggest that a distinguishing feature of the SLC26 family is the ability to transport, in addition, some hexose sugars.

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411 Properties of OHC Motors in Prestin-Mutant Mice

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It is generally believed that mechanical amplification by cochlear hair cells is necessary to enhance the sensitivity and frequency selectivity of hearing. In the mammalian ear, the basis of cochlear amplification is believed to be the voltage-dependent electromotility of outer hair cells (OHCs). The motility is thought to arise through voltage-dependent conformational changes in a membrane protein, and prestin has been proposed as this molecular motor (Zheng et al., 2000, Nature). Targeted deletion of prestin in mice results in loss of OHC motility in vitro (Liberman et al., 2002, Nature).

In this study, we attempted to investigate how the absence or reduction of motor proteins is reflected on the membrane surface structures.

Prestin-knockout mice as well as wild types and heterozygotes were used. Freeze fracture replicas of OHCs from three genotypes were obtained by standard procedures. The density and size of membrane particles was determined from selected patches of membrane P-faces using imaging-processing software. Nonlinear capacitance was also measured from OHCs isolated from the three genotypes. The density of motor proteins calculated from nonlinear capacitance measurements was compared to that obtained from freeze fracture experiments. To determine whether the absence of motor protein affects the expression of voltage-gated ion channels, whole-cell currents were recorded from OHCs isolated from wild-type and prestin-knockout mice. Also, the expression level of KCNQ4 from the hair cell tissue from wild-type, heterozygous and prestin-knockout mice was measured using real-time PCR.

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412 Apoptotic Cell Death in the Organ of Corti and Absence of Glut5 in Outer Hair Cells in Prestin Knock-Out Mice

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Characterization of prestin mutant mice demonstrated that prestin is required for eletromotility of outer hair cell and cochlear amplifier (Liberman et al., Nature 419: 300-4, 2002). However, an unexpected phenotype in prestin mutant mice is the scattered loss of both inner and outer hair cells (OHCs) in the basal 25% portion of the cochlea between 7 and 9 weeks while no hair cell loss was observed at 1 week. We further investigated the progression of hair cells loss in prestin mutant mice from 1 to 7 weeks of age. Tunel staining was used for apoptotic cell death on cochleas at different developmental ages. In the basal turn of the prestin null cochleas, apoptotic cell death was observed in the organ of Corti at 3-5 weeks while most prominent at 4 weeks. This result suggests that cochlear physiological measurements of prestin knockout mice could be performed more accurately before the cell death occurs at 3-4 weeks without the complications of hair cell loss at later stages. The hair cell apoptotic death is consistent with the notion that prestin itself may have functions other than motor, such as ion transporter (Dallos & Fakler, Nat Rev Mol Cell Biol. 3(2): 104-11, 2002). Interestingly, we found that Glut5 protein is absent (while its mRNA is still present at P9) in cochleas of the prestin null mice at ages of 2 and 7 weeks. The absence of Glut5 in prestin null mice suggests that Glut5 is part of the outer hair cell electromotor unit, consistent with the previous report (Geleoc et al., Nat. Neurosci. 2(8): 713-9, 1999). Since blocking Glut5 induces apoptosis in the human intestinal epithelial cells (Maresca et al., J Nutr. 132(9): 2723-31, 2002), and Glut5 is likely involved in providing energy in OHCs in the basal cochlea, it is thus possible that the lack of Glut5 contributes, in part, to hair cell loss in prestin mutant mice.

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413 In Vitro Electromotility and in Vivo Cochlear Sensitivity in Mice with Targeted Deletion of Prestin

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Hearing sensitivity in mammals is enhanced by mechanical amplification thought to be generated by outer hair cells (OHCs). In addition to mechanoelectric transduction, OHCs perform electromechanical transduction, whereby transmembrane voltage drives cellular length changes at audio frequencies in vitro. This electromotility is thought to arise via voltage-gated conformational changes in a membrane protein, and prestin has been proposed as this molecular motor (Zheng et al., 2000, Nature; Oliver et al., 2001, Science; Belyantseva et al., 2000, J. Neuroscience).

In this study, targeted deletion of prestin in mice resulted in loss of OHC electromotility in vitro and a 40-60 dB loss of cochlear sensitivity in vivo, as measured via DPOAEs and ABRs. Histopathological analysis revealed loss of OHCs in the basal 1/4 of the cochlea in homozygous mutants and a decrease in OHC height throughout the cochlea compared with place-matched controls. OHC mechanoelectric transduction remained intact, as evidenced by the maintenance of cochlear microphonic potentials. In heterozygous mutants, prestin mRNA levels were half normal, in vitro electromotility was halved, and there was a 6 dB increase in cochlear thresholds in vivo. There was scattered OHC loss in the extreme base, and OHC heights were intermediate between homozygotes and wildtypes.

These results suggest that prestin is indeed the motor protein, and that it is required for both electromotility and cochlear amplification. The results from heterozygotes are difficult to reconcile with existing feedback models of the cochlear amplification process. Thus, either the models need revamping or one must invoke additional active processes to explain cochlear sensitivity in the mammalian ear.

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414 Morphological Changes Caused by C-terminus Mutations of Prestin

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The unique "electromotility" of the outer hair cells in the mammalian cochlea is assumed to play a fundamental role in amplification of sound signals. The key molecule responsible for electromotility is prestin (Zheng et al., Nature 405, 149-155, 2000). The current assumption about prestin's operation is as follows: intracellular chloride anions, functioning as extrinsic voltage sensors, bind to prestin and are translocated across the cell membrane in a voltage-dependent manner. The translocation initiates conformational change of the protein, which then results in cell length changes (Oliver et al., Science, 292, 2340-2342, 2001). Although the chloride-binding site in prestin has not been identified, several amino acids of prestin were found to be critical for normal function. C-terminus of prestin, for example, has been demonstrated to play an important role in the electrophysiology of the protein (Navarrete et al., ARO, 2002). Examining the role of prestin's C-terminus will no doubt aid in understanding the mechanism of prestin's, and thus the OHC's, unique abilities. We have observed that TSA cells, transiently transfected with C-terminus deletion mutants, have different cell morphology than cells transfected with wild type prestin. Cells transfected with deletion mutants Del525-744 and Del590-744 appeared to have a rounded shape. In the case of Del525-744, 70% of transfected cells appeared to be round and less than 10% showed more than 3 protrusions. In contrast, 30% of cells transfected with wild type prestin had more than 3 protrusions. Additional experiments are under way to investigate apoptosis and cytoskeleton changes caused by these mutants.

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415 Protein Kinase C and Voltage-Dependent Capacitance in Prestin-Transfected TSA Cells

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Outer hair cells (OHCs) of mammalian cochlea can change their length in a voltage-dependent manner. This unique "electromotility" is thought to play a crucial role in cochlear amplification. It is well accepted that

nonlinear capacitance can serve as a representation of electromotility. Several protein phosphorylation inhibitors and activators have been suggested to affect nonlinear capacitance and electromotility of OHCs [Frolenkov et al., J. Physiology, 531, 667-676, 2001, Szönyi et al., Hear. Res. 137, 29, 1999]. Prestin is the key molecule response for outer hair cell electromotility. Prestin itself possesses all motor protein properties, including nonlinear capacitance, when expressed in a heterologous system [Zheng et al., Nature 405, 149-155, 2000]. Whether prestin function is modified by phosphorylation is unknown. There are at least six positions on the prestin molecule that are potential PKC phosphorylation sites. Mutation at some potential PKC phosphorylation sites of prestin shifted V1/2 of the nonlinear capacitance curve [Oliver et al., Science, 292, 2340-2342, 2001]. Therefore, potential PKC involvement in prestin's nonlinear capacitance behavior is investigated in this project.

In the past, protein kinase C activator PMA has shown to have no effect on OHCs electromotility [Szönyi et al., Hear. Res. 137, 29, 1999]. We did not observe any change, caused by PMA treatment, on the nonlinear capacitance of prestin-transfected human kidney cells either. However, PKC inhibitors: both RO31-8220 (5 μM, PKC inhibitor) and bisindolylmaleimide III (BIM, 1 μM, PKC alpha-specific inhibitor) shifted the V1/2 in the depolarizing direction. Our preliminary results intimate that voltage-dependent capacitance in prestin-transfected TSA cells might be modulated by a PKC phosphorylation. The molecular mechanism of the PKC effect is being further investigated.

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416 Prestin - an Evolutionary Trace Perspective

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Prestin is a membrane protein found in the outer hair cell (OHC) lateral wall. It plays a central role in OHC electromotility which provides the motor force of the Òcochlear amplifierÓ responsible for the sensitivity and frequency-resolving capacity of the mammalian ear. Its amino acid sequence shows highest homology with members of the SLC26A family of sulfate anion transporters. The mechanism by which these proteins achieve their transport functions and how prestin endows the OHC lateral wall plasma membrane with force generating abilities are unknown. Previous studies have used point mutation of charged residues and a C-terminal deletion to probe prestinÕs functional organization. We are using Evolutionary Trace (ET) analysis to identify functional areas that confer prestinÕs unique properties. ET uses phylogenetic information to rank residues within a protein sequence by evolutionary importance and then maps the ones ranked at the top onto a representative structure. If the residues form structural clusters, they can identify functional surfaces such as those involved in molecular recognition. We hypothesize that the residues most likely to account for prestinÕs unique properties are ÒtraceÓ residues invariant within prestins but not globally. We have compared the distribution of charged residues in prestins and pendrins to determine those that are also ÒtraceÓ residues. N-terminally tagged prestin will then be mutated by site-directed mutagenesis and tested for functionality.

417 Outer Hair Cell Function in AChE Knockout Mice

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Acetylcholine (ACh) is the primary neurotransmitter of the cholinergic system and its activity is regulated through hydrolysis in nerve synapses by acetylcholinesterase (AChE). Cochlear outer hair cells (OHCs) in adult mammals are innervated predominantly by efferent axons that originate in the auditory brain stem. The efferent fibers form chemical synapses at the base of the OHCs with ACh being their principal neurotransmitter. Activation of the efferents can alter micromechanical events withinthe cochlear partition and thereby provide a "gain control" ofthe cochlear amplifier. The efferent action on cochlear mechanics is believed to be mediated by the alpha 9/alpha10 ACh receptors on OHCs. Although the importance of AChE in the function of the nerve system has been known for a long time, its role in development remains enigmatic. So in present study, we attempted to study the influence of AChE on the development of OHC motor function and on the expression of the alpha /alpha10 receptors on OHCs using AChE knockout mice. The first set of experiments was to determine whether OHC motility developed normally. Motility and nonlinear capacitance were measured from OHCs isolated from wild-type and AChE knockout mice using the whole-cell voltage clamp technique and a photodiode-based measurement system. Our results suggest OHC motility develops normally without any delay. In the second set of experiments, the expression of alpha 9 and alpha 10subunits was studied to determine whether excessive ACh in the OHC efferent synapses resulted in down-regulation of the expression of alpha 9/alpha10 ACh receptors. OHCs were collected from wild-type and knockout mice and real-time PCR was used to quantify the expression level of alpha 9 and alpha 10 mRNAs. The results will be discussed.

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418 Intracellular Calcium Stores Modulate the Cholinergic Response of Rat Outer Hair Cells

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Outer hair cells (OHCs) of the mammalian cochlea receive cholinergic innervation from medial efferent neurons of the superior olivary complex. The major efferent transmitter, acetylcholine (ACh), interacts with the calcium-permeable alpha9/alpha10-containing nicotinic receptor on OHCs, leading to the subsequent activation of the small conductance, calcium-dependent potassium channels, SK.

Previous studies (Sridar et al., 1997; Evans et al., 2000) suggested that intracellular calcium stores may also be involved in the ACh-induced response in cochlear hair cells.

We studied the effects of various compounds known to interact with intracellular calcium stores for their ability to modulate the ACh-evoked current in OHCs. Whole-cell patch clamp recordings were performed on OHCs in the apical turn of the organ of Corti excised from the 3-4 week old rat cochlea.

The SERCA antagonist cyclopiazonic acid produced 52 ± 7% (mean ± s.e.m., n = 5 cells) reduction of the ACh-evoked current within 2 min. Ryanodine modified the ACh response in a dose-dependent manner, facilitating it when applied at a low concentration and inhibiting it at a higher concentration. The amplitude of the ACh-evoked current was 60 ± 29% (n = 6 cells) larger in the presence of 0.5 - 1 μM ryanodine, and was reduced by 39 ± 4% (n = 6) in the presence of 100 μM ryanodine. Caffeine (5 mM) caused an initial augmentation of 14 ± 4% (n = 3) followed by a gradual inhibition (65 ± 6% after 8 min in caffeine) of the ACh current.

These data are consistent with the hypothesis that ryanodine-sensitive calcium stores in OHCs modulate cholinergic efferent inhibition. An additional involvement of IP3 receptors, however, has not been ruled out.

419 Implication of Ryanodine Receptors in the Process of Cochlear Amplification and Neurotransmission: an In-Vivo Study

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Ryanodine receptors are known to regulate free cytosolic calcium concentration. This family of intracellular calcium channels plays a significant role in calcium-induced-calcium-release and are implicated in calcium dependent processes that require tight harnessing of time, space and signal amplitude.

Antagonistic concentrations of ryanodine have been shown to enhance the fast and slow effects of cholinergic fibres to outer hair cells in-vivo (Sridhar et al 1997, J Neurosci; 17(1): 428-37). More recently, when isolated mouse inner hair cells were depolarised under voltage clamp, intracellular calcium concentration was increased. Application of ryanodine resulted in signal suppression (Kennedy and Meech 2002, J Physiol; 539(Pt 1): 15-23).

In a series of in-vivo experiments, we perfused both agonistic and antagonistic concentrations of ryanodine into guinea-pig cochleae (n=12).Two simultaneous pure tones (f1 8kHz, f2 9.68kHz, intensities from 30 to 80dB SPL) were presented to the cochleae and compound action potential, cochlear microphonic and distortion products extrapolated.

For the first time, we directly demonstrated that intracochlear perfusion of antagonistic concentrations of ryanodine (50 and 100 ìM) block both evoked potentials of the auditory nerve and distortion products of the cochlear microphonic. Even at high intensities (50 dB SPL and higher), when one would expect the influence of the cochlear amplifier to be shunted, a diminution in the compound action is still observed. Our data strongly suggests a role for ryanodine receptors and CICR both in signal transduction at the inner hair cell level and also at the level of the cochlear amplifier.

420 Changes in Hair-bundle Stiffness Following Disruption of Different Interstereociliary Links

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Up to five link types can be distinguished on chick vestibular hair bundles. Top links connect the kinocilium to the tallest stereocilia, whereas tip links, horizontal top links, shaft links and ankle links connect the stereocilia to each other. Top links, tip links and ankle links are all BAPTA sensitive. Ankle links and shaft connectors are subtilisin-sensitive, whilst tip links, top links and horizontal top links are subtilisin-insensitive.

The effects of subtilisin and BAPTA treatment on the Brownian motion of chick utricular hair bundles were investigated using Nomarski micro-interferometery. The estimated value for the absolute hair-bundle stiffness (0.8 $mN \cdot m^{-1}$) is similar to that reported for hair bundles in preparations from other species. Relative stiffness changes were calculated from the ratio of spectra obtained before and after treatment with BAPTA or subtilisin. Perfusion with low calcium medium containing 5 mM BAPTA caused a sudden (in less than 1.0 min), decrease in hair-bundle stiffness to about 0.56 of the original stiffness. Subtilisin (50 mg/l) also caused a rise in the amplitude of Brownian motion. The stiffness started to decrease after 10 minutes of subtilisin application and declined to a steady value of about 0.45 of the original stiffness after 35 minutes.

Immunofluorescence experiments with three different monoclonal antibodies that recognise top and tip links, ankle links, and shaft links show that the subtilisin-induced decrease in stiffness occurring between 10 and 35 minutes correlates best with the loss of shaft links.

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421 Origins of Negative Stiffness in Hair Cell Stereocilia

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Negative compliance, together with negative damping, could be a mechanism with which the ear can gain sensitivity, a wide dynamic range, and the sharpness of frequency tuning.

Increased compliance due to gating of mechanotransducer channels is known as ``gating compliance'' and provides evidence that these channels are indeed mechanically gated (Howard and Hudspeth 1988, Neuron 1:189--199). An analogous effect on a membrane motor leads to increased compliance in the cell body of outer hair cells. A recent report demonstrated that the compliance of the stereocilia not just increases but can turn into negative as well. This observation is consistent with a prediction from a model proposed by Howard and Hudspeth (1988), which does not assume interactions between channels. However, we show that an alternative model for gating compliance, which does not assume interactions between channels either, does not lead to negative compliance. In an effort to resolve the discrepancy between the two models, we found two possible explanations for the observed negative compliance.

One possibility is an exquisitely fine-tuned organization of the stereocilia bundle, making it possible to impose a one-to-one relationship between the bending of the bundle and the displacement of each transducer channel.

Another possibility is that such a stringent condition for channel displacements cannot be realized by a macroscopic manipulation, and that the observed negative stiffness is a demonstration that gating of the mechanotransducer channel involves cooperativity.

422 FM1-43 Enters Hair Cells from the Onset of Mechano-Electrical Transduction in Both Mouse and Chick Cochlea

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Recent findings suggest that FM1-43 enters mouse cochlear hair cells by acting as a permeant blocker of their mechano-electrical transducer channels (Gale et al. 2001 J Neurosci 21:7013-7025). These findings suggest that FM1-43 could be used as a tool for detecting the onset of transduction in immature hair cells. We validated this approach by monitoring the onset of transduction during embryonic and early postnatal development both electrophysiologically and by means of FM1-43 internalization. Organotypic cultures were prepared at embryonic day 16.5. In the basal coil, transducer currents can be elicited in inner hair cells (IHCs) from the equivalent of the day before birth and in outer hair cells (OHCs) from the day of birth. Consistent with the electrophysiological findings, IHCs and OHCs begin to internalize FM1-43 (3 (M, applied for 10 s) at the same age.

Chick basilar papilla cultures were prepared from embryonic chicks at 6.5 days. Cultures were taken at daily intervals, dipped in FM1-43 and viewed under epifluorescence. They were subsequently fixed and stained for the presence of hair bundles using monoclonal antibodies to the hair-cell antigen. Alternatively they were processed for SEM to examine hair-bundle morphology. Additionally, cultures were grown in the presence of 0.1 or 1.0 mM neomycin. Chick papillary hair cells begin to load with FM1-43 at a stage equivalent to E8.5, suggesting that mechanotransduction begins at the same age. FM1-43 uptake and height-ranked rows of stereocilia developed during neomycin application. This indicates that transduction develops in the presence of channel blockers and that current flow through the channel is unlikely to be required for formation of the stereociliary staircase (Tilney et al. 1988 Dev Biol 116:119-129).

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423 Mechanoelectrical Transduction in the Mammalian Inner Ear investigated by AFM and Patch Clamp

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The mechanosensitive structures – the rod-like stereocilia - located on top of the hair cells transform a mechanical deflection around their pivot point into an electrical signal by so-called mechanoelectrical transduction channels (MET channels). Due to the complex network of cross-links between the stereocilia direct investigation of a single MET channel is difficult. A new experimental approach is reported using Atomic Force Microscopy (AFM) as a nanomanipulator and patch clamp recording to detect the resulting current response of an outer hair cell of postnatal rats in the whole-cell recording mode. In contrast to glass fibers the AFM technique allows to image and displace individual stereocilia and to measure the resulting force. In a micromechanical study a force applied to a single stereocilium resulted in displacement of only the adjacent stereocilia rather than the entire hair bundle even though the stereocilia are connected by a dense network of links. The force measured at the first adjacent stereocilium of the same row declined to 36 % of the maximum force (102 pN) measured at the directly displaced stereocilium. These results were confirmed in a second study using AFM simultaneously with patch clamp. A displacement of a single tallest stereocilium by the AFM tip resulted in transduction currents ranging from 9 to 49 pA supposing an opening of one to five transduction channels. Both, weak force transmission by lateral cross-links and small transduction current amplitudes indicate a weak mechanical interaction between individual stereocilia of the tallest row of stereocilia of outer hair cells from postnatal rat.

424 Cloning, Expression, and Localization of Ion Channel Genes in the Cochlea

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Electrophysiological recordings made from isolated hair cells show that ion channels are acquired in the order of I_K, I_A, and I_{Kca}. We are interested in the regulation of these ion channels during the development of the inner ear. Previously, we reported the cloning of chick Kv1.4 and, more recently, the cloning of a member of the *Shal* subfamily, cKv4.2. Here, we report the localization of cKv4.2 as well as the cloning and expression of other *Shaker* subfamily members, including cKv1.2, cKv1.3, cKv1.5, and a *Shaker*-related cGMP-gated K+ channel during inner ear development. We have localized cKv4.2 to the hair cells and ganglion cells of the cochlea using both immunohistochemistry and *in situ* hybridization. *Shaker* clones were obtained by screening a cDNA library made from the cochlear epithelium of late embryonic chicks. Rat and chick Kv sequences were used to design primers for the purpose of obtaining fragments identical to the cDNA of chick Kv1.2 and 1.3. A mixture of these fragments was used as a probe. Sequence analysis revealed chick homologues of Kv1.2, Kv1.3, Kv1.5, and a cGMP gated K+ channel with a deduced amino acid similarity of approximately 97%, 84%, 75%, and 72%, respectively, to the corresponding mammalian homologues. RT-PCR expression products for cKv1.3 and 1.5 were revealed for embryonic day 3 otocysts, whereas cKv1.2 was expressed in the cochlea on embryonic day 6 followed by the cGMP-gated K+ channel on embryonic day 9. Additionally, these studies revealed RT-PCR product expression of cKv1.2, cKv1.3, cKv1.5, and the cGMP gated K+ channel in the sensory epithelium of posthatched day 17 chickens.

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425 Potassium Channel Localization in Sensory Epithelia of the Rat Inner Ear

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Vestibular hair cells differ in the type of afferent terminals they contact: type I hair cells contact calyx terminals; type II hair cells contact bouton terminals. Presumably, these two hair cells have different roles, which are presently unknown, but these distinct roles are currently the subject of much ongoing research. Different ion channels are involved in the distinct functions of the two hair cell types. Two of the potassium channels that are thought to play a distinct role in the function of type I hair cells are KCNQ4 and erg.

We used antibodies against KCNQ4 (T. Kharkovets and T. Jentsch) and the erg subfamily (Chemicon) to examine the ultrastructural localization of these K channel subunits in the inner ear of the adult Long-Evans rat. These subunits are candidates for negatively activating conductances in hair cells, $g_{K,L}$ and g_{Kn}, (Kharkovets et al, PNAS, 2000; Hurley and Eatock, ARO, 2002). Silver enhancement of immunogold label was used and we counted silver grains per hair cell section in 70 nm sections.

In crista and otolith organs, we found immunoreactivity for both subunits in most hair cells (HCs), but staining was much stronger in the calyx afferent terminals on type I HCs. The calyx terminal label varied with region in a complementary fashion, with higher KCNQ4 staining in the central and striolar zones and higher erg staining in the peripheral and extrastriolar zones. For erg, there were 22 ± 4 (mean ± SEM) grains per type I HC profile (n=23; no difference between peripheral and central zones). Counts were higher in peripheral calyces (235 ± 34; n=11 profiles) than in central calyces (63 ± 8; n=4, $p<0.0001$). For KCNQ4, there were 3.8 ± 1.4 grains per typeI HC profile peripherally (n=18) vs. 9.8 ± 2.8 centrally (n=8, $p<0.025$), and 94 ± 17 grains in peripheral calyx profiles (n=18) vs. 376 ± 118 centrally (n=8, $p<0.025$). Most KCNQ4 grains were on the inner face of the calyx.

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426 Slack Potassium Channels in the Chick Basilar Papilla.

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The Ca-activated K channel Slo-1 is expressed in auditory hair cells, where it appears to play a role in frequency tuning in several species. This channel's properties can be modified by subunits such as beta; other modifying subunits may also be important. A related gene, Slack, was cloned from rat brain and shown to encode channels with properties different from those of either channel alone, when co-expressed with Slo-1 (Joiner et al. Nat. Neurosci. 1, 462; 1998). A Slack ortholog, Slo-2, was also cloned from C. elegans (Yuan et al. Nat. Neurosci. 3, 771; 2000). We find that slack is expressed in chick basilar papilla, and cloned a full-length cDNA from this auditory epithelium. Chick cochlea slack (ccSlack) is highly homologous to rat brain Slack, with differences clustered near the amino and carboxy termini. Isolated sequence differences between ccSlack and chick brain partial Slack cDNAs we also obtained suggest cochlea-specific isoforms and/or alternative splicing. Functional studies of ccSlack in CHO cells reveal properties similar to rat Slack. Currents rectify outwardly in whole-cell patch clamp, and the channel has a unitary conductance of 75-100 pS in symmetrical K. Interestingly, ccSlack-transfected CHO cells exhibit membrane potentials near EK, suggesting ccSlack might act as a leak current and help set membrane potential. Quant. RT-PCRs show a shallow tonotopic gradient in ccSlack expression, with the basal quadrant containing about 50% of the transcript levels of the apical quadrant. Similar studies show a 2X greater expression in the neural, or tall hair cell half of the epithelium as compared to the abneural, or short hair cell half. Our results suggest that Slack channels may help

determine hair cell membrane properties, perhaps in association with Slo-1 channel isoforms.

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427 Cloning And Functional Expression of a Ca++-Activated K+ Channel, SK2, From Mouse Cochlea

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In the cochlea, the outer hair cells receive efferent input via olivocochlear innervation. The synapses at the base of the outer hair cells have been studied and fast inhibitory synaptic currents mediated by the small conductance Ca2+-activated K+ (SK) channels have been described. Consistent with the previous work, the present findings showed the cloning of a complete SK2 cDNA (Gene Bank acc # AY123778) from a mouse cochlea cDNA library (kindly donated by Dr. G. Richardson, University of Sussex, UK). The mouse cochlea SK2 putative protein sequence is identical to the one cloned previously from mouse colon (Seungil et al., Am J Physiol 2001, 281:G964) and differs from the SK2 channel cloned from rat brain (Kohler et al., Science, 273:1709) only at three positions. First, at the amino intracellular domain Gln25 in the cochlea and colon channels replaces His25 in the rat brain channel; second, also at the amino terminal domain of the protein a stretch of Gly is six Gly residues shorter in the cochlear channel; last, in the carboxyl domain of the protein, Ser 546 in the cochlea and colon channels corresponds to Thr in the brain channel. It is not currently known if there is any functional relevance to these sequence differences.

Functional expression in Xenopus oocytes was used to study the properties of the cochlea SK2 channel. Under conditions where only outward K+ currents could be recorded, a Ca2+-sensitive K+ current with single channel conductance of 9.5 ± 0.8 pS (n = 4) was measured, and the current was blocked in the presence of 10 mM dequalinium chloride. Details of the characterization of the cochlea SK2 channel are reported as well as compared to studies of the rat brain SK2.

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428 The SK2 Channel in Efferent Inhibition of Avian Hair Cells

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In the inner ear, acetylcholine released from efferent neurons causes inhibition of mechanosensory hair cells through the activation of apamin-sensitive, calcium-dependent potassium channels that may be encoded by the SK2 gene (Kohler et al., Science. 1996; Dulon et al., Eur J Neurosci. 1998; Yuhas et al., J Comp Physiol [A]. 1999). A chicken orthologue of the rat SK2 gene was cloned previously from a cDNA library and shown to have 93% homology with the mammalian gene (Genbank #AF079372). In this study, the presence of SK2 mRNA in chicken basilar papilla was confirmed by RT-PCR using multiple overlapping primer pairs. Inside-out patch clamp recordings were made from HEK293 cells transfected with cloned chicken (Gallus) SK2 (gSK2) cDNA. The single channel conductance of cloned gSK2 at –100 mV averaged 14 ± 1.4 pS (n=3). Calcium sensitivity curves fitted at -80 mV yielded an average half-maximal calcium concentration of 0.82 ± 0.14 μM and an average Hill coefficient of 2.5 ± 0.8 (n =7). These values are similar to the parameters established for the mammalian SK2 channels (Kohler et al., Science. 1996; Jager et al., FEBS Lett. 2000). The gSK2 channel may subserve cholinergic inhibition in the chicken basilar papilla. In addition to characterizing calcium sensitivity, it may be possible to use these methods to obtain kinetic parameters necessary to simulate SK behavior in a model of efferent inhibition in avian hair cells.

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429 Expression Analysis of Voltage-gated K+ Channel Genes KCNQ1-5 in Rat and Guinea Pig Inner Ear

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Voltage-gated potassium channels are essential for regulation of membrane potential and neuronal excitability. Mutations in these ion channel genes result in human disorders such as cardiac arrhythmias, and congenital deafness, epilepsy. Inner ear function relies mainly on two types of excitable cells, mechanosensory hair cells and primary afferent spiral ganglion neurons type I. Mechanotransduction by cochlear hair cells is further dependent on a K+ gradient across the cochlear partition. These processes involve several types of potassium channels, among them several voltage-gated subtypes. Previously, we have identified time- and voltage dependent sub-threshold non-inactivating K+ current in cochlear outer hair cells. The aim of this study was to identify genetic correlates of these M currents in the cochleas using molecular and electrophysiological approaches. M-like potassium currents were recorded from guinea pig outer hair cells. Here we report on the expression of KCNQ subtype mRNAs as determined by reverse-transcription-polymerase chain reaction (RT-PCR) of total RNA from guinea pig and rat cochleae. PCR fragments were confirmed by sequence analysis. KCNQ2-4 were expressed strongly. Expression of KCNQ2 splice variants was detected. Inner ear expression of KCNQ5 was found (GenBank accession no. AF525937). These results, taken together with ongoing localization of expression, will provide an extended basis for the molecular heterogeneity of M currents in cochlear tissues.

430 Two Classes of Hair Cells with different Potassium Currents in the Hearing Organ of the Zebrafish (*Danio rerio*)

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The zebrafish (*Danio rerio*) is becoming an increasingly important model for the vertebrate genome. Zebrafish have a well developed sense of hearing (up to frequencies of 2.5 kHz) which makes them suited for identifying new genes involved in hearing. To establish electrophysiological data for wild type animals we studied the voltage-activated currents in hair cells from the lagena, the hearing organ of the zebrafish. Whole cell currents were recorded from hair cells at the edges of explants of the lagena since isolating single hair cells would render them leaky. Whole cell recordings (outside solution: 145 mM NaCl, 3 mM KCl, 10 mM CaCl2, 10 mM HEPES, 310 mosm* kg-1, pH 7.2; internal solution: 20 mM KCl, 120 mM K-Gluconat, 0.3 mM GTP, 10 mM Na-Phosphokreatin, 10 mM EGTA, 4 mM Na2-ATP, 2 mM MgCl2, 10 mM HEPES, 290 mosm* kg-1, pH 7.2) revealed voltage-activated outward currents that could be identified as potassium currents. Two types of cells could be distinguished due to differences in the kinetics of current activation and inactivation. One cell type showed a current that activated transiently at negative membrane potentials (about -58 mV) and inactivated rapidly, which was classified as IA. The other type of hair cells, which was lacking the IA current, showed a slowly inactivating current (IK) that was activated at membrane potentials positive to -27 mV. The two types of cells also differed in their peak K+ conductances (IA cells: 8 nS, IK cells: 19.5 nS) and their capacities (IA cells 1.6 pF, IK cells 2.8 pF). Mean current densities were 3.4 nS/pF (IA cells) and 7.3 nS/pF (IK cells). A pharmacological identification of the two groups of K+channels could not be accomplished due to the short lifetime of patch recordings (3 min). We aim at optimization of preparation conditions and solutions to ensure recording time spans of several minutes as in higher vertebrate hair cells.

431 The Potassium Current IK,n Contributes to Maturation of Mouse Cochlear Inner Hair Cells

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The KCNQ4 subunit (Kubisch et al. 1999 Cell 96:437-44) is expressed in outer hair cells (OHCs) and underlies the native OHC conductance IK,n (Marcotti & Kros 1999 J Physiol 520:653-660). Recent in-situ hybrydization and immunofluorescence observations indicate that KCNQ4 is also expressed in inner hair cells (IHCs; Beisel et al. 2000 Mol Brain Res 82:137-149; Kharkovets et al. 2000 PNAS 97:4333-4338). In mouse OHCs, IK,n first appeared when these cells mature functionally at postnatal day 8 (P8). We therefore looked for the developmental appearance of an IK,n-type current in IHCs.

Membrane currents were recorded, using the patch clamp technique, from apical coil IHCs (P8-P30) in acutely dissected organs of Corti from CD-1 mice. From the onset of hearing at P12 onwards, hyperpolarizing voltage steps from a holding potential of –64 mV elicited a current that commenced instantaneously and then deactivated slowly to a steady state. The deactivating currents were similar to the current IK,n previously found in guinea-pig (Housley & Ashmore 1992 J Physiol 448:73-98) and mouse OHCs and could be selectively blocked by submicromolar concentrations of linopirdine (KD=0.6 (M), a known blocker of IK,n in OHCs (Marcotti & Kros 1999). The block of IK,n by linopirdine resulted in a shift of the resting membrane potential of IHCs from about 1 mV at P12 to 11 mV from P19 onwards. The amplitude of IK,n, measured as the deactivating current at –124 mV, gradually increased to about 350 pA, reaching half-maximal size at P16.7. The kinetics and pharmacological properties of this current in IHCs were not significantly different from those of IK,n of OHCs. These results suggest that a small current indistinguishable from IK,n contributes to the functional maturation of IHCs, in addition to the much larger IK,f (Kros et al. 1998 Nature 394:281-284).

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432 Potassium Current Properties in Apical and Basal Inner Hair Cells from Guinea-Pig Cochlea

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Variations in the expression of outer hair cell (OHC) potassium conductances along the tonotopic gradient of the adult mammalian cochlea have been investigated. The basolateral membrane conductance increases from the apex to the base along the length of the cochlea. However, variation in the expression of inner hair cells (IHCs) potassium conductance along the tonotopic axis of the cochlea is unknown. IHCs of guinea-pigs were separately isolated from the apical and basal turn and the potassium currents were measured by the whole-cell voltage-clamp. The potassium current flows through two types of membrane conductance: a fast (Ik,f), TEA-sensitive conductance and a slow (Ik,s), TEA-resistant conductance. Membrane conductance demonstrated no significant differences between apical IHCs and basal IHCs. Reversal potentials were -65 ± 2 mV and -68 ± 5 mV in apical and basal IHCs, respectively. The rate of outward current activation was voltage-dependent and faster in basal IHCs than in apical IHCs. TEA effect was stronger on basal IHCs than on apical IHCs, suggesting that Ik,f is dominant in basal IHCs. We studied the effect of cyclopiazonic acid (CPA), which increased resting cytoplasmic Ca^{2+} by inhibiting sarcoplasmic reticulum Ca^{2+}-ATPase. Three out of four IHCs obtained from the basal part of the cochlea demonstrated augmentation of the current by CPA, whereas three out of four IHCs from the apical part demonstrated inhibition of the currents.

433 Analysis of Cav1.3 (α1D) Ca^{2+} Channel Splice Variants in the Mouse Organ of Corti

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$Ca_v1.3$ L-type Ca2+ channels (LTCCs) are essential for hearing and normal cochlear development. They comprise more than 90% of LTCCs in mouse inner hair cells (IHCs). $Ca_v1.3$ currents in IHCs inactivate much slower (11% after 5-s depolarizations) than heterologously expressed human $Ca_v1.3$ (80%). We therefore investigated if alternative splicing accounts for the different $Ca_v1.3$ channel kinetics.

We analyzed the expression of different $Ca_v1.3$ α1 subunit splice variants using RT-PCR in whole cochlear mRNA and mRNA isolated from single cell IHCs in mice 3 (P3) and 19 days (P19) after birth. Single cell PCR revealed expression of $Ca_v1.3$ α1 in IHCs. Exons 22A (encoding segment IIIS2), 31A (IVS3), 42A (short C-terminus) and 42 (long C-terminus) and 9A (I-II loop) were amplified in cochlear mRNA. Whereas exons 42, 42A and 9A were detected both at P3 and P19, exon 22a was only expressed on P19. To analyze the effect of these splice variants on $Ca_v1.3$ function, these exons were introduced into the human $Ca_v1.3$ α1 subunit, heterologously expressed (+α2-δ + β3) in tsA201 cells, and current properties were investigated using the whole-cell patch clamp technique. As an example, the inactivation properties (including Ca^{2+} - dependent inactivation) of $Ca_v1.3_{42A}$ were indistinguishable from $Ca_v1.3_{42}$, but activation kinetics were significantly faster.

Our preliminary data show that alternative splicing is developmentally regulated and it fine-tunes $Ca_v1.3$ kinetics. Further studies will show if it also accounts for the extremely slow inactivation of $Ca_v1.3$ currents in IHCs.

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434 Impact of Ca^{2+} Channels on the Development of Cochlear Inner Hair Cells

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During postnatal development mouse inner hair cells (IHCs) pass through a period of spontaneous activity. This activity relies on the expression of $Ca_V1.3$ L-type Ca^{2+} channels. We studied IHCs of mice lacking the alpha subunit of $Ca_V1.3$ channels and describe their presynaptic properties. The residual Ca^{2+} current consists of a dihydropyridine sensitive L-type component and a current that is probably mediated by SNX-482 insensitive R-type Ca^{2+} channels. The tiny remaining secretory responses are mainly mediated by L-type Ca^{2+} channels. As expected we did not observe any electrical activity in early postnatal mutant IHCs even when injecting large depolarising currents. Membrane potentials of mutant IHCs, however, displayed spontaneous inhibitory postsynaptic potentials up to at least p25. The voltage dependence of the underlying currents and their biphasic shape at depolarizing potentials characterizes them as potassium currents following Ca^{2+} influx through nicotinic receptors. We failed to observe such spontaneous or evoked inhibitory postsynaptic currents in IHCs from hearing control mice (p25). In line with previous studies (Kros et al., 1998) these cells displayed fast outward currents of large amplitude. On the contrary, mutant IHCs showed only slow outward currents, as if they were lacking the expression of large conductance Ca^{2+} activated K+ (BK) channels. The data suggest a complex developmental failure due to the strong reduction of Ca^{2+} channels.

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435 Calcium Channel Inactivation in Turtle Auditory Hair Cells

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Studies of Ca currents in turtle auditory hair cells identified an L-type channel most likely of the α 1D variety similar to other hair cell preparations. α1D channels show marked calcium-dependent inactivation as compared to initial reports from auditory hair cells. Whole cell recordings were obtained from hair cells at high (0.640±.01) and low (0.34±0.01) frequency positions. Currents inactivated in response to a 20ms depolarizing step to –10mV from a holding potential of –80mV. Inactivation was dependent on interpulse interval (ipi). An exponential relationship was found between peak current and ipi with a time constant of 174±35ms (N=21). The decay in current in response to 1sec.depolarizations to –10mV was best fit with three time constants having values of 6±1ms and 61±ms for t1, 73±5ms and 77±14ms for t2 and 903±132ms and 1179±122ms for t3 for high and low frequency cells respectively, suggesting inactivation was similar between frequency locations. Currents completely inactivated in response to 25sec. depolarizations to –10mV. Inactivation was assessed using a 20ms prepulse protocol that varied the potential between –110mV and 110mV followed by a test step to –10mV. U-shaped inactivation curves suggested inactivation was calcium induced. Half-inactivation voltages of -462±mV and -40±2mV were obtained for low and high frequency cells, corresponding to the more negative activation of calcium currents from low frequency cells. Inactivation was slowed and reduced but not eliminated by replacing external Ca with Ba or by raising the intracellular [BAPTA]. Elevating intracellular calcium led to an irrecoverable loss of current. A hypothesis incorporating known properties of inactivation and channel clustering is proposed to account for the data.

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436 Immunolocalization of the Chloride Channel ClC-K in Inner Ear and Other Histologic Sites

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Although the importance of the transport of cations for inner ear function has been demonstrated in widespread studies, the role of anion transport in the ear has received less attention. The present investigation inquired into the occurrence in inner ear and other tissues of ClC-K, a member of the ClC family of chloride channels. Inner ears from healthy young adult gerbils were fixed briefly with zinc dichromate-formalin, processed into paraffin and immunostained by the avidin-biotin complex procedure employing a 1:500 dilution of ClC-K primary antibody (Chemicon, Tamecula, CA). A broad range of other tissues was examined similarly for comparative assessment of Cl channel function. Selective strong immunostaining for ClC-K was observed in subtypes of inner ear fibrocytes rich in Na,K-ATPase. These included types II, IV and V fibrocytes of the spiral ligament, stellate fibrocytes of the spiral limbus, supralimbal fibrocytes and corresponding fibrocyte types in the vestibule. In addition, intermediate cells of the stria vascularis and an apical focus in the utricular macula showed moderate immunoreactivity. In the survey of gerbil tissues, the apex of superficial columnar cells in the colon, gastric parietal cells, reticular cells in splenic germinal centers, the plexiform layers of the retina and smooth muscle stained for ClC-K. Carbonic anhydrase-positive (type I) fibrocytes in the cochlea and underlying the urothelium in urinary bladder lacked affinity for ClC-K antibody. The observations further document the role of inner ear fibrocytes in ion transport and offer evidence for a conductance that could mediate chloride transport and balance anion and cation fluxes promoted by the Na,K-ATPase and the Na-K-Cl cotransporter present in the fibrocytes.

437 CLC-KB Gene Promoter Expression in the Mouse Cochlea

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CLC-KB chloride channel is a kidney-specific member of chloride channel (CLC) family, and mutations of the CLC-KB gene are known to cause Bartter's syndrome type III, that is associated with congenital deafness and renal failure. This indicates that CLC have important roles in maintaining cochlear ion homeostasis. Although the localization of CLC has been known on the stria vascularis, there were no reports showing the CLC on the fibrocytes. We investigated the CLC-KB chloride channel on the cochlea by using transgenic mice harboring the EGFP gene driven by an 11kbp human CLC-KB gene promoter. CLC-KB activity was seen not only on the stria vascularis but also on type II, type IV and suprastrial (type V) fibrocytes of the spiral ligament, and on limbal fibrocytes. However, the reaction in the type II, IV or V fibrocytes was weak compared with that in the stria vascularis and limbal fibrocytes. These results may indicate that some fibrocytes possessing both CLC and NKCC regulate the cell volume, transport and recycling of Cl- such as seen on the stria vascularis. Moreover, these fibrocytes may recycle chloride ions through CLC that accompany Na+ and K+ into the cell via NKCC, consequently discharged Cl- into the cell was utilized for NKCC again, thus helping to preserve the high K+ concentration in endolymph. These results mean that the cochlear fibrocytes play a contributory role in the maintenance of cochlear ion homeostasis.

438 Analysis of Contributions of Acid Sensitive Ion Channels to the Hearing Of Mice

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Two types of acid sensitive ion channels (ASICs), BNC1 (or ASIC2) and DRASIC (or ASIC3) are known to participate in the detection of touch and acid stimuli in mice. These channels are also detected in the inner ear by RT-PCR amplifications. Immunolabeling localized the ASICs in spiral ganglion (SG) neurons in the mature cochlea, and acid stimuli to cultured SG neurons elicited excitatory responses. To test whether ASICs are involved in mammalian hearing functions, we examined the effect of inactivating ASIC genes on the hearing threshold (HT) and noise sensitivity of the mice.

HTs of wild type and ASIC knockout (KO) mice were measured by auditory brainstem responses (ABRs) elicited by either click or tone bursts. Inactivating ASIC genes showed no significant effects on HTs. They were 27.5ï,±0.8 dB SPL (n=16) and 27.0ï,±2.2 dB (in SPL) (n=10) for ASIC2 wild type and KO mice respectively, and were 33.3ï,±1.6 (n=22) dB and 30.8ï,±3.7 (n=18) dB for ASIC3 wild type and KO mice respectively. However, ASIC2 KO mice were significantly more resistant to noise-induced temporary threshold shift (TTS). Measured one hour after exposing animals to noise (110 dB white-band (WB) for 1 hour), TTS in wild type and ASIC2 KO mice were 6.3ï,±1.8 (n=14) dB and 15.6ï,±1.9 (n=8) dB, respectively. The HTs were recovered to near pre-exposure levels three weeks after noise exposures. In contrast hearing threshold shifts were 20.0ï,±2.4 (n=6) dB and 18.8ï,±1.3 (n=4) dB after exposing animals to 120 dB WB noise for 1 hour.

We are currently testing whether the higher noise resistance is also exhibited by ASIC3 KO mice. In conclusion, our data did not support a direct involvement of ASICs in the transduction apparatus of the mammalian cochlea. The lack of ASICs in SG neurons may reduce acid induced excitatory responses. Therefore, the improved noise resistance may be explained by a decrease in noise-induced excitatotoxicity temporarily damaging hearing sensitivity of mice.

439 Calcium Waves in Rat Cochlea

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Calcium waves were observed in various excitable and nonexcitable cells where they induced physiological responses. It is not clear if there are calcium waves existed in cochlea and its mechanisms. Calcium waves were studied in rat cochlea slices and cultured stria cells. Calcium waves propagated in all directions. The velocities of parallel, antiparallel and orthogonal directions are 5.21 plusmn 3.20 mum/sec (n=20), 5.81plusmn4.56 [mu]m/sec (n=16) and 10.94 plusmn 14.36 mum/sec (n=21). Calcium waves may propagated through two different pathways, intercellular pathways via the gap junction communication, and extracellular pathways related with ATP release and purinergic receptor activation. Calcium imaging studies indicated purinergic receptor antagonist suramin 300 muM preincubation and calcium free bath solution inhibited the calcium waves propagation both in distance and velocity. After 300muM suramin preincubation, the calcium wave propagating velocity reduced to 4.25plusmn1.61 mum/sec (n=14). Immunobiochemistry experiments showed the gap junction component Connecxon C26 existed is cochlea stria area. Thus the calcium waves propagation in cochlea stria is mediated via ATP release and purinoceptor activation. The gap junction involvements need to be studied furthermore.

440 Comparative Analysis of Green Fluorescent Protein-Labeled Chimera in the Wild Type and the Mutation of Connexin 26

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Introduction: Connexin 26 (Cx26) is a subunit composing the gap junction and plays an essential role in the potassium recycling between perilymph and endolymph of the cochlea. Cx26 mutation causes congenital profound sensorineural hearing loss and is considered one of the main mutant genes of the non-syndromic hereditary deafness. In our previous study we have demonstrated the differences in subcellular distribution and function between the wild type and the mutation of Cx26. The purpose of this study is to reveal the differences in the trafficking and assembly of green fluorescent protein (GFP)-labeled chimera between the wild type and the mutation of Cx26 in the living mammalian cells. Materials: Coding sequences of connexin 26 were cloned from the genomic DNA of normal subjects and connexin 26 mutation-related deaf patients and subsequently inserted into GFP-expressing vector to induce GFP-labeled chimeric connexin 26 (Cx26-GFP). The vector construct was transfected into Hela cells using cationic lipids. The expression of Cx26-GFP was traced in the living cells under the fluorescent microscope. Results: Fluorescent microscopy showed that the wild type Cx26-GFP chimeras were expressed, transported and assembled into the cell membrane. In contrast, the mutated Cx26-GFP chimeras were expressed and localized only in the cytosol without assembly. Conclusions: Our preliminary data shows different trafficking and assembling patterns between the wild type and the mutation of Cx26 in the living cells. This experimental system, utilizing GFP, allows us to perform further functional analysis of Cx26 and to prepare basic data for developing novel therapeutic strategies of Cx26 mutation related hearing loss.

441 Towards the Understanding of the Gap Junctions Role in the Inner Ear

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In the cochlea, gap junctions assemble into two independant cellular networks, i.e. epithelial, between the supporting cells of the organ of Corti, and conjonctive between the fibrocytes of the spiral ligament, the spiral limbus, and the basal and intermediate cells of the stria vascularis. To date the expression of 5 different connexins, proteins that compose gap junctions, has been reported in the inner ear. Cx26 and Cx30 are the major connexins of the inner ear and are widely expressed in both cellular networks. The functional importance of gap junctions in hearing is headlined by the fact that mutations in the genes encoding Cx26, Cx30, Cx31, Cx32, and Cx43 cause hearing loss. Although, their role in the cochlea is poorly understood.

Here we present the results of the inactivation of Cx26 in the epithelial gap junction network of the inner ear, and the ubiquitous inactivation of Cx30, which both cause hearing loss.

In mice deleted for Cx26 in the epithelial gap junction network, the organ of Corti, the endocochlear potential, and the concentration of potassium in the endolymph develop normally. However, from the onset of hearing, around P14, the organ of Corti undergoes a degeneration process. It is followed by a decrease of both endocochlear potential and concentration of potassium. The mechanism of cell death and its consequences on the cochlear physiology are now studied. Cx26 epithelial gap junctions could play a role in funneling the potassium secreted by hair cells upon sound stimulation. In absence of Cx26 epithelial gap junctions a local increase of extracellular potassium in the organ of Corti could occur and would lead to cell death.

Ubiquitous inactivation of Cx30 results in the total abolition of endocohlear potential. Degeneration of the organ of Corti is also observed. We therefore propose that Cx30 plays a critical role in both generation the endocochlear potential and for the survival of the organ of Corti.

442 Cellular Distribution and Molecular Assembly of Connexins in the Inner Ear of Mice

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Connexins (Cxs) are protein subunits that assemble to form gap junctions in cell membrane mediating direct intercellular communications. Their importance in the auditory function has been highlighted by the findings that mutations in at least five Cx genes, Cxs26, 30, 31, 32 and 43, cause hearing impairment of cochlear origin. At least 17 different subtypes of Cxs have been cloned in mammals. In this project we investigated subtype specific distribution and molecular assembly of different Cxs found in the cochlea of mice.

Quantitative RT-PCR amplifications detected numerous Cx subtypes in the cochlea of adult mice. Normalized to the Cx26 mRNA expression level (100%), average expression levels of other Cxs in the cochlea were (n=4 for all cases): Cx29 (7.5%), Cx43 (5.7%), Cx45 (4.9%), Cx30 (4.6%), Cx37 (2.8%), Cx36 (2.2%), Cx40 (1.3%), Cx50 (1.0%), Cx57 (0.9%), Cx31 (0.7%), Cx46 (0.7%), Cx47 (0.7%), Cx32 (0.2%). Cxs33 and Panx1 were below the detection limit. Appearances of multiple Cx transcripts suggested that gap junctions in the cochlea may be assembled heteromerically. We found that Cx26 and Cx30 were co-immunoprecipitated, suggesting they form heteromeric connexons. In contrast, Cx26 and Cx32 were not co-immunoprecipitated in the cochlea while positive control showed that they were co-assembled in the liver. These results were consistent with immunolabeling data showing that distribution patterns of Cx26 and Cx30 in the cochlea always overlapped in all developmental stages examined (from E13.5 to adult mice), whereas the expression patterns of Cx26 and Cx32 were segregated. These diverse patterns of Cx molecular assembly in the cochlea are expected to produce gap junctions with complex biophysical characteristics. Subtype specific regional expressions of Cxs suggested that long-range intercellular communications in the inner ear, such as the proposed endolymphatic K+ recycling pathway, depend on concerted actions of multiple types of Cxs.

443 Asymmetric Gap Junctional Permeability and Charge Selectivity in the Cochlear Supporting Cells

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A gap junctional channel connects two adjacent cells and provides an intercellular conduit, which can passage ions and small molecules up to 1-1.5 kDa. Based on size that gap junctional channels can traverse, it has generally been assumed that it would impose little selectivity on the movement of permeants. The cochlea has multiple connexin genes expressed in supporting cells. We previously demonstrated that gap junctional coupling between the cochlear supporting cells possessed a variety of asymmetric gating to voltage polarity and in direction. We have hypothesized that asymmetric gating may consist of gap junctional pathways to transport ions and small molecules directionally in the cochlea. In this experiment, the permeability and selectivity of gap junctional coupling between cochlear supporting cells were investigated. Multiple fluorescent dyes varying in charge and size were combined to inject into cells by patch pipette. Dye transports were measured by time-lapse fluorescence microscopy; voltage gating was also simultaneously recorded under the voltage clamp. Consistent with the revealed asymmetry of voltage gating, dye transjunctional passage exhibited asymmetric movement between the supporting cells. In the intact sensory epithelium (organ of Corti), the injected dyes revealed directional transport, favorable in a basal direction. The transjunctional permeation also revealed highly restricted charge selectivity. Positively charged dyes facilitated to pass through the cochlear gap junctions and had a broad diffusion range extending into several decades of cells; whereas negatively charged dyes were usually restricted in local area and limited in 2-3 adjacent cells. The data demonstrated that multiple connexins formed hybrid channels have asymmetric transjunctional permeability and charge selectivity, which can consist of gap junctional pathways to transport the charged ions and molecules directionally in the cochlear supporting cells.

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444 A SNARE-Like Protein Expressed in Hair Cells and Photoreceptor Cells

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We have isolated a cDNA clone from a chicken basilar papilla cDNA library that putatively encodes a 25kD protein with no obvious homologies to any known protein.

As judged by Northern blotting and in situ hybridization in the chicken, the transcript is robustly expressed by hair cells and photoreceptor cells and weakly expressed in a variety of neuronal tissues. In the mouse, the orthologous transcript is specifically expressed in the retina. An antiserum against the corresponding recombinant murine protein strongly labels the outer segments of photoreceptors.

Tertiary-structural prediction links the novel protein to the tSNARE family. Indeed, the protein displays a low degree of similarity to several members of the tSNARE family in the N-terminal regulatory domain. We found that the novel protein, like many known tSNAREs, can modulate the exocytotic activity of PC12 cells.

The novel protein's weak homology with members of the SNARE family, its predicted tertiary structure, its biochemical properties, and its behavior in the PC12 exocytosis-modulation assay imply that this protein has a SNARE-like function. However, the lack of a characteristic membrane-proximal SNARE domain argues against the protein's direct involvement in membrane fusion events. We therefore hypothesize that the protein plays a role in photoreceptors and hair cells in the targeting of potential interaction partners to relevant membrane compartments or in anchoring them there.

The difference in the gene's expression in hair cells of the chicken and mouse suggests that either expression of this transcript in the internal ear was acquired by birds during evolution or this gene became obsolete with the specialization of the mammalian inner ear.

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445 Store-Operated Calcium Entry in Mammalian Hair Cells, are TRP Channels Responsible?

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The translation of sound stimuli into frequency coded signals in the auditory pathway is carried out by the inner hair cells (IHCs) of the mammalian cochlea. Stimulus evoked depolarisations activate Ca2+ influx, an essential factor in the release of neurotransmitter. Ca2+ influx also activates Ca2+ release from intracellular stores through CICR (Kennedy and Meech, 2002). To maintain these processes the Ca2+ stores must be refilled following release and many cell types possess influx pathways that are triggered when stores are emptied.

We have investigated store-operated Ca2+ influx in mouse inner hair cells (IHCs). Using confocal imaging we have shown that depleting intracellular stores with thapsigargin caused an increase in basal Ca2+i that failed to recover. This was not due to an inability of the cell to regulate Ca2+i (Kennedy, 2002), suggesting that the rise in baseline Ca2+i is due to influx of external Ca2+. To investigate this we applied thapsigargin in a Ca2+-free medium and then briefly applied Mn2+ solution. Under these conditions, increases in fluorescence on addition of Mn2+ indicated the activation of an influx pathway. To identify the influx pathway we used immunohistochemistry to examine the expression of TRP channels in hair cells. We found that TRPC3 was expressed in the plasma membrane of vestibular and IHCs of the cochlea, with little expression seen in the outer hair cells. TRPC1 showed weak diffuse staining in hair cells and TRPC6 appeared to be to be weakly localised to the nuclear region.

Although TRPC3 is thought to be activated in a store-independent way by DAG and IP3 recent evidence suggests that it may also function as a store operated influx pathway (Trebak et al., 2002). This is the first evidence for the expression of TRP channels in mammalian hair cells and we suggest TRPC3 as a possible candidate for the store-operated calcium influx pathway in IHCs.

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446 Conductances in the Post-Synaptic Calyx Ending on the Type I Hair Cell

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Type I hair cells in mammalian vestibular organs synapse onto a large calyx afferent ending that envelops the hair cell. Often following isolation of rat utricular hair cells, we observe calyces still associated with type I cells, sometimes with the afferent stalk attached. Occasionally we can micro-dissect a calyx from its associated hair cell. Here we report data on the ionic conductances of a small number of afferent endings, recorded in whole-cell voltage clamp mode with the ruptured- or perforated-patch configuration.

A common finding is a large Na conductance with half-maximal voltages (V1/2) of activation and inactivation of ~-50 mV and -75 mV. At -25 mV, the current was ~4 nA, the time-to-half-peak was 0.2 ms and the inactivation time constant was ~0.75 ms. The calyces also have negatively activating K conductances that appear heterogeneous in activation range (V1/2's -80 and -50 mV) and kinetics. In one case the conductance was 35% blocked by a KCNQ channel blocker (XE991). The heterogeneity may reflect regional variation in K subunit expression. Lysakowski and Price (this meeting) find that KCNQ4-like immunoreactivity in calyx endings is stronger in the striola than in the extrastriola, and erg-like immunoreactivity has the converse pattern.

Type I hair cells also have a negatively activating current. The pre- and post-synaptic expression of such conductances may underlie novel transmission.

In one case, we recorded from an afferent stalk after pulling it away from the calyx ending. The stalk had a fast, outwardly rectifying K current that activated positive to -60 mV. The steady-state I-V relation was N–shaped initially and showed wash-out, indicating that it includes a Ca-dependent K component activated by Ca influx through L-type channels. These K currents presumably participate with the Na current in generating spikes.

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447 Phase Locking in a Computer Model of Mouse Inner Hair Cells

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Inner hair cells (IHCs) in the mammalian cochlea transform acoustical stimuli into neurotransmitter release. The transduction process comprises several steps. Opening of the transduction channels by the deflection of the stereocilia leads to membrane depolarization which activates voltage gated Ca^{2+}-channels. The entering Ca^{2+} ions trigger exocytosis of neurotransmitter via synaptic vesicles, which then leads to post-synaptic currents in the afferent nerve fibers. Repolarization of the IHCs is mediated by several different types of K^+ currents. The whole process is known to phase-lock to acoustical stimuli up to several kHz (Palmer and Russell, 1986, Hear. Res., 24:1-15).

In a refined version of a computer model for mouse IHCs we included a model for the transduction current based on Géléoc et al. (1997, Proc. Roy. Soc. Lond. B, 264:611-621) to investigate the influence of the composition of the different ionic currents on the ability to phase lock to the stimuli. The findings suggest that the large conductance Ca^{2+} dependent BK-type K^+ currents are crucial in maintaining the phase-locking to frequencies of more than one kHz in mature IHCs. Immature IHCs show only poor phase locking due to the lack of the BK-current, as was suggested by Kros et al. (1998, Nature, 394:281-284).

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448 Heat Shock Response Regulated by HSF1 Protects Sensory Hair Cells Against Acoustic Trauma.

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The purpose of this study was to determine what kinds of stresses induce heat shock proteins in the inner ear and to clarify the role of heat shock response in the inner ear. The inner ears of guinea pigs were exposed to heat stress, sodium arsenite, band noise (130 dB SPL, 3h). Western blot analysis showed that heat shock proteins were up-regulated in the inner ear of guinea pigs after the expose to stresses and immunohistochemical study showed that the heat shock proteins were up-regulated in the organ of Corti of the cochlea.

We established the animal model in which heat shock response was induced only in a unilateral ear to clarify the role of heat shock response. These animals were exposed to noise (130 dB SPL, 1h) and the ABR examination was performed after 2 weeks. The ABR threshold shifts in the ears pretreated with heat stress were smaller than that in control ears. The histochemical study showed that the outer hair cells were not lost in the ears pretreated with heat stress. Furthermore, HSF1 knockout mice were exposed to an intense noise. The outer hair cells of the knockout mice were more lost than that of the normal mice after 2 weeks. These results indicate that the heat shock response regulated by HSF1 plays a critical role in the protection of the sensory hair cells against an intense sound.

449 Heat Shock Factor 1 (Hsf1) Knock-Out Mice Exhibit Decreased Cochlear Recovery Following Noise Overstimulation

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Heat shock proteins (Hsps) can enhance cell survival in response to stress. Heat shock factor 1 (Hsf1) is a transcription factor that regulates the stress-inducible gene expression of the Hsps and certain other stress-responsive proteins. In the unstressed cell Hsf1 exists as an inactive monomer. In response to stress the inactive Hsf1 protein undergoes trimerization and serine hyperphosphorylation, resulting in its activation. We have previously shown Hsf1 in outer and inner hair cells in the organ of Corti, spiral ganglion cells in the modiolus, and in the stria vascularis of the unstressed rat and mouse cochlea. We also demonstrated that the heat shock shown by Yoshida et al. (1999) to precondition the cochlea against noise trauma, results in Hsf1 activation in the rodent cochlea. We now examine the role of Hsf1 in protection from noise overstimulation using an Hsf1 knock-out mouse. Baseline hearing was assessed using auditory brainstem responses (ABRs) and no significant difference in threshold was observed between Hsf1 knock-out mice and their Balb/C background strain, indicating that these mice had normal hearing. Hsf1 knock-out mice and their wild-type littermates were exposed to a 98 dB, 2-20 k, 100% duty cycle noise exposure for 2 hours and ABRs were measured at 4k, 12k, and 20k, at 3 hours, 3 days, and 2 weeks following the cessation of noise. Mice lacking the Hsf1 gene exhibited significantly less recovery of hearing than their wild-type littermates at 3 days and 2 weeks following noise overstimulation, with the most pronounced effects being observed at 20 kHz. These studies suggest a potential role for Hsf1 in cochlear recovery following stress. We are currently assessing the role of Hsf1 elimination on downstream gene expression in the mouse cochlea.

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450 Ebselen Attenuates Temporary Noise-induced Hearing Loss

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Temporary hearing loss can be followed by complete or partial functional recovery. The aim of the present study was to determine whether ebselen could reduce the temporary noise-induced hearing loss. Male pigmented guinea pigs (250-300 g) with normal hearing confirmed by auditory brainstem response (ABR) measurement at 2, 4, 8, and 16 kHz were used in this study. Subjects were randomly divided into control and ebselen treated groups and exposed to 110 dB octave-band noise centered at 4 kHz for 3 hours. One hour before and 18 hours after noise exposure, treatment group was orally given 10 mg/kg ebselen. Hearing was evaluated by ABR measurement immediately, 1, 3, 7, 10, and 14 days following noise exposure. Control group showed approximately 40 dB threshold shift across frequencies immediately after exposure, which returned to normal one week later. Temporary threshold shifts measured immediately after noise were significantly reduced ($p<0.05$) to approximately 4.4 dB in ebselen treated group. This threshold shift returned to normal more rapidly compared to control group. These findings suggest that ebselen can attenuate noise-induced temporary threshold shifts in guinea pigs, probably by

scavenging NO and peroxynitrite excessively produced by intense noise.

451 Ebselen Mediated Protection from Single and Repeated Noise Exposure in Rats.

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In the cochlea, noise evokes the formation of toxic metabolites or reactive oxygen species including the formation of "super-radicals" such as peroxynitrite. Ebselen, a mimic of glutathione peroxidase and an effective scavenger of peroxynitrite, is a neuroprotectant under clinical investigation for acute ischemic stroke. We performed single and repeated noise exposures on 8-week old Fischer-344 female rats (110 dB, 4-16 kHz noise, 4 hours). Rats were dosed with 16mg/kg Ebselen bid, i.p., before and after noise. Controls were dosed similarly with vehicle only containing solutions. Auditory brainstem responses (ABR) were evoked using both click (4-16 kHz) and pure tone (4, 8, 12, 16 kHz) stimuli. Permanent threshold shifts (PTS) were evaluated at 3-4 weeks following noise. Morphologic evaluation of whole mounted cochleae stained with DAPI to detect cell nuclei and FITC-phalloidin to assay F-actin were performed. On average, three times more outer hair cells were lost in control versus Ebselen treated animals. In addition, controls contained regions where all three OHC rows were missing (0.1-0.6 mm lengths of organ of Corti). In Ebselen treated animals, a loss of all three rows of OHCs did not occur. Therefore, Ebselen acts to reduce the total number of hair cells lost after noise exposure and prevents the occurrence of complete OHC loss. Physiologic data from ABR analysis indicated that Ebselen provided significant protection from developing a PTS following both single ($p<0.01$) and repeated noise exposure ($p<0.05$). In addition, these data indicate that the severity of the PTS is highly associated with both the pattern and level of OHC loss.

452 A Clinical Free Radical Scavenger, Edaravone, Protects Cochlear Hair Cells from Acoustic Trauma

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Reactive oxygen species (ROSs) are known as toxic agents derived from acoustic trauma. The purpose of this study is whether edaravone, a free radical scavenger for clinical use, can protect cochlea of guinea pigs from acoustic trauma. To evaluate cochlear function, we assessed auditory brain stem response (ABR) thresholds. And for histological assessment, we observed the sensory epithelium using surface preparation technique. Six Hartley guinea pigs with normal PreyerÕs reflexes and normal tympanic membranes were used in this study. Osmotic pump were implanted in the right ears of all animals, and left ears were kept intact as control. Three days after operation we measured the ABR thresholds of all animals and we confirmed no threshold shift in any of the animals. Immediately after ABR examination, their osmotic pumps were exchanged with other pumps filled with edaravone (1.722×10^{-2} M). Twenty-four hours after the exchange, animals were exposed to intense (130 dB SPL) noise with a center frequency of 4kHz for 3h. Two weeks after sound exposure, ABR threshold were recorded and all animals were killed. Their temporal bones were removed and we observed the organ of Corti of each inner ear incubated with fluorescein isothiocyanate-conjugated phalloidin with surface preparation technique. After noise exposure the 8-kHz threshold shifts in treated ears were significantly less than those of the control ears, and there were fewer defects on outer hair cells of organ of Corti. This result suggests that edaravone protected cochlea from acoustic trauma.

453 Administration of N-acetyl-L-Cysteine Prevents Noise-induced Hearing Loss from Impulse Sound

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We have demonstrated significant success in decreasing noise induced hearing loss (NIHL) due to loud continuous noise exposure by administering various antioxidant compounds either before (Kopke et al., 1999, 2000, 2001) or after the sound exposure (Kopke et al., 2002a, 2002b). Recent investigations have explored the efficacy of one these compounds (N-acetyl-L-cysteine, LNAC) to protect the cochlea from impulse sound. Female chinchillas were injected (I.P.; LNAC at 325mg/kg) twice a day for 48 hrs, 1 hr prior to the sound and then 1 hr and twice a day for 48 hrs post-noise (PN) exposure. The impulse noise consisted of a train of paired impulses (2 impulses /sec) delivered at 155 dB SPL for 75 seconds. A second group of animals were injected with an equal volume of 0.9% saline given over a similar time schedule, exposed to the same noise paradigm and served as controls. The ABR thresholds were determined pre-noise, immediately following noise, 7, and 21 days PN. Noise-exposed, saline injected animals at week three measured ABR thresholds of 27.0, 38.5, 40.5, and 33.5 dB SPL while LNAC-treated animals recovered to 31.2, 8.2, 11.5, and 10.5, dB SPL above average pre-exposure baseline at 2, 4, 6, 8 kHz respectively ($P < 0.01$ at 4, 6, and 8 kHz). This is an encouraging result towards our long-term objective of developing agents for oral administration in clinical populations.

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454 Combination of Antioxidant and Glutamate Receptor Antagonist Can Improve Protective Role Against Noise Trauma in Rats

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Noise exposure causes hearing loss. The mechanism underlying noise-induced hearing loss (NIHL) is still not completely known. However, it is widely accepted that two main mechanisms may be involved in NIHL. One is a direct mechanical process, while the other is indirect via metabolic deficiencies (Borg et al., 1995). The later may be over-release of glutamate from hair cell leading synapse swollen, the formation of reactive oxygen species (ROS), enzyme alteration and ion concentration changes etc. The present study is to investigate the combinative role of antioxidant, N-L-Acetylcysteine (NAC) and glutamate receptor antagonist, Caroverine against impulse noise trauma in rats. The results were shown that the combination of NAC and Caroverine could improve protective role against impulse noise trauma in the rats.

455 Influence of Acrylonitrile on Noise-induced Hearing Loss, Cochlear Glutathione, and Blood Cyanide Levels in Rat

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Acrylonitrile (AN) is one of the 50 most common chemicals with production of billions of pounds per year in industry and estimated exposure to approximately 125,000 workers. The metabolism of AN might lead to ototoxicity via two potential routes. By depleting glutathione (GSH), AN may render the ear more vulnerable to noise-induced injury. In addition, the production of cyanide (CN) from AN

metabolism may inhibit superoxide dismutase (SOD) limiting antioxidant capacity or have direct ototoxic effects (Tawackoli, et al; 2001). This study evaluates the effect of AN on NIHL, cochlear GSH, and blood cyanide (CN) levels in male Long-Evans rats.

Auditory function was measured by recording compound action potentials (CAP) from the round window in animals exposed to noise and AN, noise alone, AN alone, and vehicle only (105 dB octave-band noise for 4 hours centered at 13.6 kHz; 50 mg/kg body weight subcutaneous AN injection). In parallel studies, cochlear GSH and blood CN levels were measured spectrophotometrically following AN injection.

Subjects exposed to AN and noise had marked auditory threshold elevation compared with noise alone. AN alone did not appear to have an impact on thresholds, which were similar to those of subjects receiving vehicle only. In the cochlea, GSH levels were maximally depleted 2 hours following AN injection with depletion observed from 15 minutes and recovery beginning at 8 hours. Blood CN levels rose from baseline to a zenith one hour after AN exposure and then returned to baseline over the next two hours.

As hypothesized, Acrylonitrile potentiates NIHL in rats, but does not produce permanent auditory impairments when given in the absence of noise. Acrylonitrile also depletes GSH in the cochlea and is metabolized to CN soon after it is injected. Proposals for determining the mechanism of AN action will be discussed.

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456 Protective Role of Src-PTK Inhibition on Noise Induced Hearing Loss

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Following a noise exposure the tight cell junctures between the hair cells, as well as the cell-matrix interactions are stressed and disrupted. The stressing of these tight cell junctures initiates apoptosis, termed anoikis. Signals of cell-matrix disruptions are transduced through cell surface receptors to the cell's interior. A key mechanism for transducing the cell-matrix interactions is the activation of Src tyrosine kinases, such as those associated with FAK, or focal adhesion kinases. Phosphorylation by FAK regulates interactions between integrins and actin at the extracellular matrix, which then continue to recruit and activate intracellular signaling adaptor proteins and trigger various regulatory signaling pathways (Wei, et al, 2001, Frisch, et al, 1996).

The current study extends previous work from our lab in examining the role of apoptosis during noise induced hearing loss, and how it can be prevented by using a src protein tyrosine kinase inhibitor (src PTK). A stock solution of CH-65, a src PTK inhibitor developed by David Hanguaer, (Chemistry Dept. SUNY at Buffalo) was first prepared in DMSO (10 mM) and further diluted in saline (30 µM). A 30 µL drop of a 30 µM concentration of CH-65 was placed on the round window of Chinchillas, and a control solution of DMSO and saline was placed on the opposite ear one hour before sound exposure to an octave band noise centered at 4kHz, 105dB SPL for 4hrs. Evoked potential thresholds measured from the inferior colliculus were determined pre-noise, immediately following the noise, 1 day, 3 days, 7 days, and 20 days post noise. The cochleas were harvested at day 20, and standard cochleograms were performed. CH-65 treated ears showed less permanent threshold shift than controls. These results suggest that blockage of apoptotic signaling at the cell matrix can prevent hair cell loss and play a significant role in reducing noise-induced hearing loss.

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457 Prophylactic Effect of Nifedipine on Noise Induced-Hearing Loss

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To determine whether nifedipine could prevent noise-induced hearing loss (NIHL), the whole cochlea perfusion technique was used in this project. Forty health hybrid guinea pigs were divided into 4 groups (10 in each group): (1) nifedipine perfusion of whole cochlea (NIF) for 2h; (2) animals were exposed to white noise (120 dB SPL) (WNE) for 2h; (3) white noise exposure and perfusion with the normal artificial perilymph NAP (WNE+NAP) for 2h; (4) white noise exposure and perfusion with the nifedipine (WNE+NIF) for 2h. The compound action potential (CAP) was evoked by click and cochlea microphonics (CM) were evoked by tone burst (rise and fall 2 ms, plateau 20 ms). In the whole process, CAP and CM were recorded from the round window at 30, 60, 90 and 120 min. There was not significant difference in CAP threshold between WNE+NIF and NIF group ($P>0.05$). There was significant difference in CAP threshold between WNE+NIF group and WNE group or WNE+NAP group ($P<0.01$), CAP thresholds of WNE+NIF group were 15~23 dB SPL less than other groups. The slopes of CM I/O function changed from nonlinearity to linearity in all groups, though CM amplitude in WNE+NIF was 3 mV higher than that of WNE and WNE+NAP group after 2h perfusion. The results suggest that hair cells (HCs) of cochlea were damaged in present noise exposure condition and nifedipine might have partly prophylactic effect on NIHL. Above evidences could be explained as the blocking effect of nifedipine on L-type calcium channel in cochlear hair cells. Noise exposure could induce a Ca++ influx into the hair cell through calcium channel. The motility of OHCs might be partially inhibited by Ca++ overloaded, and this will induce the inner hair cell sensitivity decrease, CAP thresholds enhance and hearing loss.

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458 Novel Inhibition of Noise-Induced Apoptosis in Cochlear Hair Cell Using Inhibitors of pp60$^{c\text{-}src}$ Protein Tyrosine Kinase

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We previously shown that apoptosis is a major cause of outer hair cell (OHC) death following exposure to intense noise. Carney et al., (ARO, 2003) have demonstrated protection against noise-induced hearing loss through inhibition of pp60$^{c\text{-}src}$ protein tyrosine kinase (PTK). This study was designed to explore the role of the pp60$^{c\text{-}src}$ PTK in apoptotic signaling following exposure to noise. Chinchillas were exposed to 75-pairs of impulse noise at 155 dB pSPL. The animals were sacrificed 5 minutes after the noise exposure. The cochleas were examined for activation of the focal adhesion complex using an antibody against focal adhesion kinase (FAK), which is an intrinsic member of the complex. Using the chinchilla animal model, we have demonstrated that focal adhesion complexes are formed in response to extremely high level noise. FAK is activated upon formation of these complexes and is known to initiate several signaling cascades first through a series of autophosphorylation events and subsequently through phosphorylation of downstream peptide substrates. We have demonstrated that apoptotic cells are seen within the lesion surrounded by focal adhesion complexes. Furthermore, addition of the pp60$^{c\text{-}src}$ PTK inhibitor prevents the apoptotic response without preventing the formation of the focal adhesion complex. These data suggest that the downstream signaling through tyrosine phosphorylation by FAK may be an early step in apoptotic signaling of hair cells. Since FAK is activated by shear stress in other organ systems, these observations may represent

the first signaling pathway identified in the ear to be activated by mechanical stress.

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459 The Effects of Direct Infusion of Dexamethasone into the Inner Ear Against Noise-Induced Trauma on Guinea Pigs

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In clinical, steroid hormones are used for treatment of noise-induced hearing loss, however, its mechanism of effect is not clarified. However, previous study reported noise-induced trauma on the mammalian models can be successfully protected by steroids. Current study intended to evaluate the *in vivo* effects of direct infusion of the Dexamethasone(DEX) into the cochlea against the noise-induced trauma both electro-physiologically and morphologically.

Hartley guinea pigs were used in this study, and the DEX administered directly into the cochlea using the osmotic pump.

In the experimental group, DEX was filled in the pump. The control group received artificial perilymph (AP). After maintaining of initial infusion with DEX or AP, on the experimental 4 days, animals were exposed a one octave band noise centered 4 kHz, at intensity of 120dB SPL for 24hr. We investigated the effect of DEX following noise-induced trauma in guinea pigs using Auditory brainstem responses (ABR) and survival rate of hair cells.

ABR were tested just before pump implantation (Day0), noise exposure (Day4), and sacrifice (Day11) with frequencies of 4, 8, 12, 16, and 20kHz.

The cochlea tissues were separated and preceded to fluorescent immunohistochemistry to observe the remaining hair cell with rhodamine phalloidine(Molecular Probes).

The group of 100ng/ml DEX has less threshold shifts than the group of AP at 2,4,8, and 20 kHz. In morphologically, the group of 100ng/ml DEX has more survival rate of outer hair cells than the group of AP.

This experiment indicated the DEX infused into perilymphatic space via scala tympani has protective effects against noise induced-trauma in the cochlea both electro-physiologically and morphologically.

460 Protection of Auditory Function against Noise Trauma with Local Caroverine Administration in Guinea Pigs

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Abstract

Glutamate is the most likely neurotransmitter for the inner hair cell-afferent neuron in the peripheral auditory system. Intense noise exposure may result in excessive glutamate release, binding to the post-synaptic receptors and leading to neuronal degeneration and noise-induced hearing loss. The aim of this study was to investigate the protective effect of caroverine, an N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist, on noise-induced hearing loss. Two different doses of caroverine were applied onto the round window membrane, followed by one-third octave band noise centered at 6.3 kHz (110 dB SPL) for one hour. Auditory brainstem responses were measured at different time intervals. It was found that caroverine offered significant protection against noise-induced hearing loss.

461 Apoptosis-Related Molecular Events in Rat Spiral Ganglion Neurons (SGNs) *in vivo* After the Loss of Hair Cells

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SGNs die after destruction of hair cells. This loss of SGNs is remarkably slow, taking months to years, depending on the species. In rats treated from postnatal day 8 (P8) through P16 with kanamycin to destroy hair cells, the loss of SGNs is not complete until about postnatal week 16, ≈3 months after deafening, although the number of SGNs declines gradually throughout this period. This raises a number of questions, such as, what is the difference between a neuron that dies soon after deafferentation and one that dies much later? Is the time of cell death purely stochastic or dependent on local or cell-intrinsic factors? These questions have implications for therapy to protect SGNs in deaf individuals. To provide a basis for investigation of such questions, we are using immunohistochemistry to determine the status of trophic signaling and the appearance of apoptosis-related events in SGNs *in vivo*. To assess trophic signaling, we are analyzing the level of CREB phosphorylation. To assess the onset of apoptosis we determine the number of SGNs exhibiting cytochrome c release from mitochondria. To assess terminal apoptosis we determine the number of SGNs exhibiting nuclear condensation and DNA fragmentation, using TUNEL. These are quantified at P16, immediately after deafening; at P23, just prior to the onset of detectable SGN loss; at P32, when SGN loss is significant; and at P60, midway through the cell death period. On P32 and P60, >10% of the SGNs appear apoptotic, a remarkably high fraction given that the rate of SGN loss is ≈1%/day. This suggests that SGNs may require several days to commit to and carry out apoptosis after hair cell loss. Concomitant with apoptosis we observe a decrease in CREB phosphorylation in SGNs.

462 Injury-induced Expression of Activating Transcription Factor-3 (ATF-3) in Rat Spiral Ganglion Cells.

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Unilateral labyrinthectomy (UL) induces hearing loss, severe postural and oculomotor asymmetries. This injury model is widely considered and used as a peripheral nervous system (PNS) injury model. After UL, a large number of molecules are upregulated in spiral and vestibular ganglions. Transcription factors are considered to play crucial roles in sensing and responding to nerve injury to execute apoptosis or to survive. Among those, activating transcription factors ATF-3 is recently identified as a peripheral nerve injury associated factor. In this study, we intended to ascribe ATF-3 as a specifically induced transcription factor after inner ear injury using UL model. The adult rat inner ear was chemically destroyed by UL and expression profiles of ATF-3, c-Jun and some related genes were examined by immunohistochemistry, in situ hybridization and RT-PCR. These procedures were made at 1, 3, 7 and 14 day after UL. Our results revealed that in response to UL, substantial expression of ATF-3 was observed in the operated side of cochlea spiral ganglion cells, whereas no expression of ATF-3 was seen in the unlesioned side of spiral ganglion cells. Similarly in vestibular ganglion cells, we found that ATF-3 mRNA was upregurated in the lesioned side. The transcription factor ATF-3 in rat spiral and vestibular ganglion cells is induced by UL. This finding suggests that ATF-3 works as an immediate early gene in those ganglia and would be crucial

to elicite various gene expression responses after the damage of inner ear organs.

463 Cyclic AMP Prosurvival Signaling in Spiral Ganglion Neurons (SGNs) Involves Mitochondrial Activity of cAMP-Dependent Protein Kinase (PKA)

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We have shown that depolarization promotes SGN survival by recruiting CaMK and cAMP prosurvival intracellular signaling pathways. By adding nuclear export or nuclear localization signal squences to a PKA catalytic subunit, we showed that cytoplasmic PKA action is sufficient to promote SGN survival and nuclear activity has no effect. By similarly targeting the normally cytoplasmic PKA inhibitory protein, PKI, to the nucleus, we showed that nuclear PKA activity is dispensable for its prosurvival effect. The Bcl-2 family apoptotic regulatory protein BAD is a PKA target and we showed that cytoplasmic PKA functionally inactivates BAD. BAD promotes apoptosis by translocating to the outer mitochondrial membrane and facilitating the formation of a pore complex, which includes the voltage-dependent anion channel (VDAC) and Bcl-2 family proteins and which allows cytochrome c efflux from mitochondria. The VDAC also is a docking site on the outer mitochondrial surface for proteins, including a PKA anchoring protein, that contain a specific N-terminal targeting sequence. We directed a GFP-tagged PKA catalytic subunit (GPKA) or PKI (GPKI) to the VDAC by adding this outer mitochondrial (omito) sequence. We find that omitoGPKA, but not omitoGFP, expression suffices to maintain SGN survival, while GPKA targeted to the plasma membrane with a myristoylation signal does not promote SGN survival. Moreover, omitoGPKI expression blocks the prosurvival effect of cAMP on SGNs. Similar results were obtained with the PC12 neuronal cell line. These results suggest that cAMP prosurvival signaling is exerted mainly at the outer mitochondrial membrane to prevent formation of the pore, by post-translationally modifying apoptotic regulatory proteins such as BAD.

464 Adenoviral –mediated BDNF and CNTF Enhance Survival of Denervated Spiral Ganglion Cells

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Hair cell loss, the most common cause of deafness, is often associated with degeneration in the auditory nerve. It has previously been demonstrated that direct cochlear infusion of neurotrophic factors such as NT-3, BDNF or GDNF enhances the survival of spiral ganglion cells (SGCs) after inner hair cell loss. In vitro studies indicated that an interaction among the above factors could synergistically improve SGC survival (Hegarty JL, 1997, J Neurosci). BDNF and CNTF were found to be more effective in promoting the survival of neurons in dissociated cell cultures than either factor individually (Hartnick CJ, 1996, J Neurobiol).

The goal of this study was to determine the influence of combined CNTF and BDNF gene therapy on the survival of SGCs after elimination of inner hair cells in mature guinea pig ear. The CNTF and BDNF transgenes were delivered via replication deficient adenoviral vectors. Similar vectors without the gene cassette insert (Ad.empty) served as controls. Seven days after bilateral deafening, 5µl of adenoviral suspension containing a mixture of Ad-CNTF and Ad-BDNF were injected into the left scala tympani through the round window. Animals were sacrificed 28 days after deafening and their inner ear prepared for SGC count. SGC survival was expressed as cell density. The combined CNTF and BDNF transgene expression significantly enhanced SGC survival compared to Ad.empty control animals. The extent of protection afforded by the combination of BDNF and CNTF was greater than that previously seen with GDNF.

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465 In Vivo Infusion of Nucleosides and Nucleotides to Neomycin-treated Guinea Pig Cochleas – Effects on EABR Thresholds and Spiral Ganglion Cell Survival.

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Hearing can be lost to factors such as noise or ototoxic drugs. A cochlear implant (CI) is one "treatment" option, however, success of CIs depends on the presence of surviving auditory nerve cells. Neurotrophins can protect auditory neurons and may also cause neurons to extend neurites.

Extracellular nucleotides acting at P2 receptors can induce cell proliferation and/or differentiation in neurons. Synergy between neurotrophins and neurotransmitters in growth roles has been reviewed (Neary et al, 1996), and protective roles of primarily nucleosides have been described.

A wide variety of P2 receptors exist in the cochlea (Housley, 2000). Using RT-PCR, we have detected expression of P2X2, P2Y1, P2Y2, and P2Y4 receptor mRNA in mouse, rat, and guinea pig cochlea [Järlebark (2001) Proc Mol Biol Hear Deafness].

Our hypothesis is that nucleosides/nucleotides can: (i) preserve auditory function after ototoxic damage; (ii) protect cochlear neurosensory cells; (iii) induce outgrowth of neuronal processes.

Guinea pigs were evaluated with click ABR to ensure normal hearing. All animals were deafened by 10% neomycin (48 h) delivered into scala tympani via osmotic minipumps. They were divided into 5 groups and supplied with (target): Artificial perilymph (control); alpha-,beta-methyleneATP (P2X1, P2X3R); CPA (N6-cyclopentyladenosine; adenosine A1R); UTP (P2Y2, P2Y4R); 2-meS-ATP (P2Y1, P2X2R); (100 nM-1 mM). EABR and click ABR was tested 5 days post-surgery to ensure click ABR threshold shifts of at least 55 dB (animals with less were excluded). EABR was also measured day 13 and pump change was made. Final measurement was day 25, followed by cardiac perfusion (2.5% glutaraldehyde). Cochleas were decalcified (0.1 M EDTA), dehydrated, and embedded in JB4. Mid-modiolar sections were taken at 3 µm, stained and mounted. Spiral ganglion cells in Rosenthal's canal were counted, the area of the canal measured, and the cell density calculated. Results are currently being evaluated.

466 Determining the Fate of Neurotrophins Delivered to the Scala Tympani of Normal and Deafened Guinea Pigs.

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Neurotrophins promote the survival of auditory nerve cells and may benefit people with progressive deafness by preventing degeneration of spiral ganglion cells in the cochlea. Neurotrophins may be locally administered to the cochlea via a mini-osmotic pump. We studied the fate of ^{125}I labeled neurotrophin-3 (NT-3) injected into the scala tympani to examine the safety and efficiency of this technique. Adult guinea pigs were anaesthetized with 40-60 mg/kg ketamine and 4 mg/kg xylazine (i.m.) and deafened with 400 mg/kg kanamycin (s.c.) and 100 mg/kg frusemide (i.v.). 5-7 days later the animal was again anaesthetized and an ABR was performed to confirm absence of hearing. The left cochlea was exposed via a dorsal approach. A cannula was inserted through a cochleostomy near the round window and the cochlea was infused with 20 µl of 750 ng/ml ^{125}I NT-3 over 8 minutes. After varying times between 2 hours and 6 days, the cochleae were fixed, sectioned and processed for autoradiography and immunohistochemistry. Our findings indicate that a very small proportion of NT-3 that is delivered to the cochlea actually reaches its target, the spiral ganglion cells. The greatest proportion of ^{125}I NT-3 accumulates over the basilar membrane, followed by the spiral limbus

and intercellular spaces of the bony regions of the cochlea. In the first 2-24 hours, ^{125}I NT-3 is present in the first 2 turns of the cochlea. After 4-6 days, the neurotrophin has reached all turns of the cochlea. From this information, we can identify the cochlear targets of NT-3, determine whether the neurotrophin has any adverse effects on the cells in the cochlea and compare the efficiency of different administration techniques.

467 Co-culture of Dissociated Spiral Ganglion with Adjacent Non-neuronal Cells Improves Neuronal Survival

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Cultures of dissociated spiral ganglion are valuable for examining basic mechanisms of growth, development and function of the auditory nerve. However, these cultures typically demonstrate poor neuronal survival. Microexplants of the spiral ganglion fare better, but some studies require individual neurons. We grew enzymatically dissociated spiral ganglion (from newborn mice) on different extracellular matrix substrates in the presence of serum and growth factors, and counted the number of surviving TUJ1 positive neurons/cochlea. We found that the substrates made a difference in survival - fibronectin>laminin>>tenascin, but we could not increase the number of surviving neurons/cochlea to more than 430 (approximately 2.5% of the estimated 15,000 spiral ganglion neurons in the mouse cochlea). However, when the spiral ganglion and its attached limbus+spiral lamina (without the epithelium) was dissociated and grown on laminin, 48 hour survival increased to 1200 neurons/cochlea (approximately 8% of estimated total number of neurons). Dissociated cultures of the limbus+spiral lamina alone yielded fewer than 5 neurons. If the limbus+spiral lamina from a newborn cochlea was dissociated and cultured for 24 hours before seeding postnatal day 1 dissociated spiral ganglion into the culture, the resulting culture yielded 1150 neurons/cochlea (approximately 7.7% of the estimated total number of neurons). Conditioned medium from the limbus+spiral lamina culture did not increase the survival of neurons. These observations support the idea that survival of spiral ganglion neurons in culture depends upon cells derived from the limbus+spiral lamina region. We hypothesize that Schwann cells from the spiral lamina are the likely required cells, and suggest that growth atop these cells is responsible for the increased neuronal survival in our culture system.

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468 Neurosteroids Suppress Glutamate and GABA Induced Currents in Spiral Ganglion Neurons

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Neurosteroids, applied locally or directly onto the inner ear, are frequently used in the treatment of sudden hearing loss and tinnitus; however, their effects on cochlear function are largely unknown. In order to explore the effects of neurosteroids on cochlear function, pregnenolone sulfate (PS), a neurosteroid found at high concentrations in brain, was applied directly on to spiral ganglion neurons (SGNs) during whole-cell patch clamp recordings from SGNs in cochlear organotypic cultures of P0-P5 10J mice. SGNs were identified from their location, round, phase-bright somas and their voltage sensitive sodium currents and action potential. Under voltage-clamp (-100 mV), kainic acid (KA), a non-NMDA glutamate receptor agonists, or GABA was perfused on to SGNs with or without PS. Under current clamp, the mean resting potential of SGNs was -53±6mV (n=84). In most SGNs, KA (500μM) and GABA (1mM), induced an inward current under voltage clamp at a holding potential of -100 mV. The KA-induced current was suppressed by CNQX, a non-NMDA receptor antagonist, whereas the GABA induced current was greatly suppressed by bicuculline, a GABA-A receptor antagonist. The neurosteroid, PS, suppressed the current induced by KA (500 μM) by 52% by PS (500 μM) (n=11); this suppression totally recovered after PS wash out. PS (500μM) also suppressed the maximum amplitude of the current induced by GABA (1 mM) by more than 60% (n=6). The suppression of the GABA-induced current recovered slowly after PS washout, consistent with previous reports from granule neurons in cerebella slices. The results indicate that neurosteroids, such PS, can rapidly suppress glutamatergic and GABAergic currents mediated by non-NMDA and GABAa receptors respectively consistent with previous findings from the hippocampus and xenopus oocytes. The effects of other neurosteroids, currently under investigation, will be discussed.

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469 Mechanosensory Epithelial Cells and Ganglion Cells are Clonally Related

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Lineage analysis can provide clues about the timing and types of cell fate decisions made during development. In the ear, we have asked whether neurons and sensory cells share a common progenitor, and examined clonal dispersion across the labyrinth to search for lineage compartment boundaries. We used VSV/RSV pseudotyped retroviruses to infect progenitor cells in the otic cup of the chicken embryo (stages 11-14). Each replication-incompetent vector carries a 24-bp tag and alkaline phosphatase gene as a marker. The surface epithelium of the head was bathed in virus (titer of 2x10^6 i.u./ml) at E2. At E9-13, virus-infected cells were found in the inner ear epithelium or its associated ganglia in 58/77 ears. To date, 113 different oligonucleotide sequences (i.e. distinct clones) have been picked, amplified by PCR and sequenced from 19 ears, revealing relatively large clones. In 3/8 tested cases, sensory cells were found to be related to cells in the auditory (AG) and vestibular (VG) ganglia. In 7/12 cases, cells located in the AG and VG were related. We cannot conclude that these related neurons belong to different sensory systems because vestibular neurons projecting to the lagena macula are located within the AG. With the exception of these ganglion clones, we did not find any examples of clones with members dispersed across anatomical subdivisions of the inner ear. Furthermore, non-sensory cells were not related to sensory cells or neurons (0/8), suggesting that the sensory and neuronal cell fate decisions are made quite early in ear development. There is a hair-cell-bearing sensory organ in middle ear of the bird, and cells in this sensory patch were related to neurons in the middle ear ganglion (2/4), but not to inner ear neurons (0/7). Our data confirm that the neurons and sensory cells of mechanosensory organs can share a common progenitor.

470 Lineage Analysis of the Developing Mouse Inner Ear by Transuterine Microinjection of an Ecotropic Retrovirus

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Cell lineage analysis can provide information about the types and timing of cell fate decisions that underlie cell specification. Our goal is to study cell lineage in a mammalian inner ear. We have adapted a transuterine microinjection technique to introduce a murine ecotropic retrovirus encoding the lineage label human placental alkaline phosphatase (AP) into the embryonic day 11.5 (E11.5) mouse otocyst. The mouse uterus was exposed by a ventral abdominal incision, illuminated with a fiber optic light, and gently palpated to reorient embryos. The replication incompetent retrovirus was injected with a beveled glass capillary pipette driven by an oil-based microinjector. Mouse pups were harvested at postnatal day 6.5-9.5 (P6.5-9.5) and AP-positive (AP+) cells were detected with a chromogenic substrate. Transuterine injection into the E11.5 otocyst was attempted in 55% (191/350) of the embryos present; 83% (292/350) of all the embryos were born; and 89% (261/292) of the neonates grew normally to P6.5-9.5. AP+ temporal bones housing the inner ear were recovered from 105 perfusion-fixed pups, decalcified, and sectioned serially at 25 μm. AP+ cells were detected in 83% (53/64) of the inner ears: 64% (34/53)

were positive in the non-sensory epithelium with the endolymphatic duct most frequently involved (14/53); 11% (6/53) were positive in vestibular (4 saccular maculae) or auditory (2 organs of Corti) sensory epithelia. In addition, AP+ ganglion cells were identified in 17% (9/53) of the ears. We are currently analyzing AP+ cells in the sensory and non-sensory epithelia with a PCR-based sequencing method that will unambiguously define the lineage relationships among our populations of AP+ cells.

471 Lithium chloride changes hair cell fate during the development of the mammalian organ of Corti

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Within the mammalian organ of Corti, the factors that determine whether a progenitor cell develops as an inner hair cell versus an outer hair cell are unknown. In other systems, the canonical wnt/beta-catenin signaling pathway has been shown to play a crucial role in determining cell fate. When this pathway is activated, there is an inhibition of the formation of a glycogen synthase kinase-3beta (GSK-3beta) mediated scaffolding complex that targets beta-catenin for ubiquitin-proteasomal degradation. To examine the role of the wnt/beta-catenin pathway in the determination of cell fate in the organ of Corti, embryonic cochlear explant cultures were treated with lithium chloride, a GSK inhibitor, at embryonic day 13. Treatment with lithium induced a dose dependent increase in inner hair cell number and a decrease in outer hair cells. To verify that this effect represents a change in cell fate and not in cell proliferation, cellular mitosis was assayed by the incorporation of BrdU. Analysis of lithium-treated explants indicated that there was no increase in BrdU uptake within the zone of non-proliferation compared to controls. To confirm that the effects of lithium are mediated through the wnt/beta-catenin pathway, explant cultures were also treated with N-acetyl-Leu-Leu-norleucinal (ALLN), a proteasome-mediated protease inhibitor. Preliminary results showed that this treatment lead to a similar change in hair cell fate. In addition, western blots suggested that there was an increase in beta-catenin and inactivated GSK-3beta in response to lithium treatment. These findings are consistent with an activation of the wnt/beta-catenin pathway via lithium mediated GSK-3beta inhibition and suggest that this pathway plays a key role in regulating hair cell fate.

472 Specification and Patterning of the Organ of Corti

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During development, the specification of the sensory primordium in the cochlea is evidenced by the establishment of a zone of non-proliferating cells (ZNPC) by E14 in mice whose formation coincides temporally and spatially with the onset of cyclin-dependent kinase inhibitors $p27^{Kip1}$ and $p19^{Ink4d}$. Furthermore, the ZNPC is flanked by Connexins26&30 expression domain, indicating the specification of non-sensory domains by this stage as well. However, pathways essential for the domain specification in the cochlea remain elusive. Using candidate gene cloning approaches, we identified a bHLH gene, Math6, in the developing cochlea at early stages. To date, we have generated chimera mice carrying Math6 knockout allele, and are in the process of generating homozygote null mice to investigate its role in the inner ear.

Subsequent to the formation of the ZNPC, a bHLH gene Math1 is upregulated in a subpopulation of cells within the sensory primordium to initiate the gradient of hair cell differentiation. As the organ of Corti along the entire length of the cochlear duct is patterned into precise arrays of hair cells and supporting cells to form a bi-layered structure by E18 from its multi-layered primordium, the sensory organ doubles its length in the absence of significant cell proliferations. These observations collectively suggested the involvement of radial cellular movement in the patterning of hair cell and supporting cell arrays. In the organ of Corti whole mount culture system, we confirmed that the elongation of the organ of Corti is not achieved by cell division. We are developing cochlear slice culture for time-lapse recording to visualize the suggested radial cellular movement in the developing sensory organ.

473 The role of GATA3 in the specification of auditory neurons

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The transcription factor GATA3 is essential for inner ear development but elucidation of its function presents a substantial challenge. Analysis of null mutants is complicated by the need to disentangle direct and indirect effects. We have addressed the issue by knocking down GATA3 expression in 2 different cochlear cell lines. US/VOT33 represents delaminating neuroblasts at embryonic day 10.5 (E10.5) and UB/OC1 represents hair cell precursors at E13.5. The cells are conditionally immortal, proliferating in vitro at 33°C with γ-interferon but differentiating at 39°C without γ-interferon.

Antisense (AS) and Morpholino (MO) oligonucleotides were used to knock down expression of GATA3. Complimentary sense and reverse AS sequences were designed and used as sense controls. Cells were transiently transfected with each reagent for 24-72 hours at 39°C. Expression of GATA3, the bHLH transcription factor NeuroD, βIII-tubulin and α/β-tubulin was assessed by immunolabelling cells or by immunoblotting protein extracts. NeuroD is essential for delamination of cochlear and vestibular neuroblasts but is also expressed in hair cells. βIII-Tubulin is expressed in delaminating neuroblasts but α/β-tubulin is expressed in all cells.

GATA3 and NeuroD were expressed in VOT33 and OC1 at both 33°C and 39°C. When GATA3 was knocked down, NeuroD was down-regulated in VOT33 but not in OC1. βIII-tubulin decreased in VOT33 without GATA3 but no change was observed for α/β-tubulin. In sections of the otocyst at E10.5, GATA3 was expressed in areas of otic epithelium adjacent to the developing cochlear but not vestibular ganglion. Most delaminating neurons expressed NeuroD. Thereafter, cochlear neurons expressed GATA3 without NeuroD but vestibular neurons expressed NeuroD without GATA3. We conclude that NeuroD is a downstream target of GATA3 in auditory neuroblasts and that GATA3 may specify spiral ganglion neurons.

474 Expression of Id Genes in the Embryonic Mouse Cochlea

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The basic helix loop helix (bHLH) protein Math1 has been shown to be both necessary and sufficient for hair cell differentiation in the cochlea (Birmingham et al., 1999 and Zheng and Gao, 2000). However the factors that regulate the temporal and spatial expression of Math1 are unknown. Id proteins function as dominant negative regulators of bHLHs by preventing bHLH proteins from forming functional heterodimers. Moreover, the embryonic expression patterns of Id proteins suggests these factors play a role in regulating the initial onset of bHLH activity. Therefore, Id proteins may play a role in the differentiation of hair cells by negatively regulating Math1. To examine this hypothesis, in situ hybridization was used to determine the patterns of expression for Id1 and Id3 in the cochlea during embryonic development. Id1 and Id3 are expressed broadly in the prosensory region at E12.5 but become restricted to the prospective organ of Corti by E16. In addition, the expression domains of Id1 and Math1 overlap at E14. In contrast, Id1 has been downregulated in cells that express Math1 at E16. In order to evaluate changes in the levels of expression of Id1 and Math1, real time PCR was used to quantify mRNA levels.

Id1 mRNA expression peaks at a point near the onset of Math1 expression. As development continues, expression of Math1 is maintained while expression of Id1 progressively decreases. Based on these results it seems likely that Id1 negatively regulates Math1 and thus participates in the timing of hair cell differentiation.

475 Functional redundancy in Smad3 signaling in the developing mouse inner ear

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Transforming growth factor-beta (TGFß1) is an epithelial-derived signaling molecule that participates in the inductive tissue interactions that guide inner ear development. Members of the TGFß superfamily elicit their biological effects by binding to heterodimeric serine-threonine kinase receptor complexes. TGFß initiates signaling by binding to the type II TGFß receptor, thereby provoking transphosphorylation and activation of the type I receptor (TGFßRI). Once activated, TGFßRI can signal to Smad2 and Smad3. Smad2 and Smad3 are present in the developing mouse inner ear at sites of epithelial-mesenchymal interactions. We used in vivo and in vitro loss of function analysis to discern the role of Smad3 in inner ear development. Development of the inner ear in Smad3 null mutant mice was comparable to the inner ear development of wild-type littermates. Expression patterns of Pax2, a marker of putative sensory epithelium in the developing inner ear, was also not affected by inactivation of Smad3. To determine if the absence of an abnormal inner ear phenotype in the Smad3 null homozygote mice may be due to functional redundancy, Smad2 and Smad3 antisense oligonucleotides were added, either alone or in combination, to a culture model of otic epithelial-periotic mesenchymal interactions to block signaling by these endogenous Smads. When either Smad2 or Smad3 antisense oligonucleotide was added to cultures of periotic mesenchyme containing otic epithelium, differentiation of the periotic mesenchyme was not affected. In contrast, when a combination of Smad2 and Smad3 antisense oligonucleotides was present in culture, there was a marked reduction in the extent of mesenchymal cell differentiation. These observations suggest that functional redundancy between Smad2 and Smad3 may be operant in mechanisms of TGFß signaling in the developing mouse inner ear.

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476 Conditional Knockouts of Type I BMP Receptors Demonstrate Defects in Dorsal/Ventral Patterning of Neural Tube.

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The cell signaling factors, Bone Morphogenetic Proteins (BMPs), play innumerable roles during embryonic development, but unfortunately, classical knockouts of many BMPs and their receptors result in early embryonic lethality. To overcome this problem, we have generated a conditional knockout of the most widely expressed BMP receptor type IA, *Bmpr*, which transduces the signals for several BMP ligands. This conditional knockout has been generated using transcriptional regulatory elements of the *Brn4/Pou3f4* gene that direct expression of Cre recombinase to the neural tube. In the bcre-32 pedigree, Cre expression is also detected in the ventrolateral somatic ectoderm. Conditional knockout of *Bmpr* with the bcre-32 pedigree has demonstrated a role for *Bmpr* signaling in the patterning of the limb (Development 128:4449-61, 2001), gliogenesis, subarachnoid space formation (leading to hydrocephaly in these animals), and formation of hair follicles and external genitalia. Dorsal/ventral patterning of the neural tube, however, appears unaffected. We hypothesized that functional redundancy of type I BMP receptors occurs in the neural tube. Analyses of mice with a "double knockout" of an additional BMP receptor, BMPR-IB, results in dorsal patterning defects, and supports the functional redundancy hypothesis. Defects in the patterning of the neural tube will be discussed. Analyses of hindbrain auditory and vestibular nuclei are being undertaken in the double knockout animals.

477 Retinoic Acid Repression of BMP4 in Inner Ear Development

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Bone morphogenetic protein 4 (BMP4) and all-trans-retinoic acid (RA) are important for normal inner ear development, but whether they are linked mechanistically was not known. We hypothesized that RA down-regulates BMP4 transcription in the developing inner ear. This was tested using a cell line (2B1) derived from otocysts of embryonic day 9.5 Immortomice. 2B1 cells were cultured +/- TTNPB (a specific retinoic acid receptor ligand) followed by ribonuclease protection analysis which showed that TTNPB caused a 3-fold decrease in BMP4 mRNA, with the maximal effect by 6 hours. Nuclear run-on analysis confirmed the decrease in BMP4 expression was transcriptional. Real time RT/PCR analysis of otocyst RNA isolated from 10.5 dpc mouse embryos that had been exposed to RA or vehicle through their mothers demonstrated that BMP4 expression decreased ~2.2-fold in the RA-exposed embryos. The *in vivo* significance of this negative regulation was demonstrated by showing in chick embryos that exposure of otocysts to RA inhibited semicircular canal (SCC) formation, and this effect was overcome by exogenous BMP4. 5' RACE analysis of 2B1 RNA indicated that the major BMP4 transcription start site lies within what is traditionally considered intron 2, suggesting the use of a novel promoter. RT-PCR with intron spanning primers and ribonuclease protection analysis support this conclusion; RT/PCR also demonstrates this novel promoter in mouse embryo otocysts. These data indicate that RA regulates SCC formation by repressing BMP4 expression, and suggest that a novel developmental BMP4 promoter restricts this regulation to specific developing organs, including the inner ear.

478 Early Defects of the Kreisler Inner Ear

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The kreisler mutant strain was generated by X-irradiation in the early 1940's and found to display hindbrain as well as inner ear abnormalities (Hertwig, 1944 and Deol, 1964). Subsequent work by Cordes and Barsh (Cordes and Barsh, 1994) identified the genetic defect as a mutation in a transcription factor expressed predominantly in the developing rhombomeres. Our studies of kreisler show a variable inner ear phenotype manifesting as early as embryonic day 9 in mutant mice. The majority of mutant mice show an absence of the endolymphatic duct primordium and a laterally displaced otocyst. Paint microinjection studies show that the cochlear projection is already abnormal by E12 with dysmorphic semicircular canal plates in most of the specimens. By later embryonic timepoints (E13 and later), rudimentary canal structures are common while the cochlea displays a grossly dilated and non-coiling appearance. These data suggest an early and global ear developmental defect in kreisler that apparently results from the loss of normal kreisler signaling in homozygous mutant mice. Intriguingly, early reports did not describe kreisler expression in the developing inner ear. However, later studies (Eichmann et al. 1997) and our preliminary

data show that kreisler is expressed in the embryonic mouse inner ear. This raises the possibility that altered expression of kreisler in mutants at these time points may be relevant to the final ear phenotype observed in adult kreisler mutants.

479 Blocking Programmed Apoptotic Cell Death Produces a Switch to Non-Apoptotic Cell Death During Ear Development.

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In an effort to determine the role of programmed cell death (PCD) in ear development, we analyzed embryonic chick ears exposed to a chemical caspase inhibitor and embryonic ears from mice with targeted deletions of caspases. Isolated stage 25 chicken otocysts that were incubated in the presence of zVAD.fmk, a pan-caspase inhibitor, demonstrated decreased numbers of apoptotic nuclei. By transmission EM, cells in PCD hot spots lacked apoptotic morphology but had vacuolated, condensed cytoplasm and dense nuclei. We also analyzed developing ears from mice with targeted deletions of caspase 3 (R. Flavell) or caspase 9 (T. Mak). Using hematoxylin and eosin (H&E) staining, apoptotic cells were absent from the otic epithelium and 8th ganglion of caspase 3 (-/-) embryos at 10.5, 11.5, 14.5, and 15.5 days post coitus (dpc). Additional analysis at 14.5 and 15.5 dpc demonstrated that caspase 3 null embryos stained negative for active caspase 3 and had decreased TUNEL staining in locations where cell death occurs in normal littermates. H&E staining also revealed at these sites abnormal shrunken cells with denser nuclei containing granulated chromatin, but lacking the highly condensed chromatin or fragmentation typical of apoptotic cells. Based on sectioned material, there does not appear to be an overt ear phenotype in caspase 3 null specimens. Results were similar for one caspase 9 (-/-) ear at 17 dpc. Our results indicate that cells in the developing ear that are programmed to die by apoptosis are switching to a non-apoptotic mode of cell death when caspase activity is blocked. A similar switch was reported for some populations of developing CNS neurons (Oppenheim, et al., 2001, J. Neurosci., 21:4752), while other neuronal cells were profoundly affected by caspase deletion (Kuida, et al., 1996, Nature 384:368). The mechanism for this switch in ear is not yet known.

480 A Mutation in the Transcription factor AP-2α Leads to Middle Ear and Ocular Defects in ENU-generated Doarad Mice

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The middle ear is a developmentally complex system with the skeletal elements, the middle ear cavity and the tympanic membrane arising from different origins. Mutations causing middle ear anomalies usually coincide with other abnormalities and have expanded our knowledge of middle ear development. Transcription factor AP-2α (*Tcfap2a*) null and chimeric mice show various developmental defects, among them missing middle ear bones and tympanic ring. AP-2α is a member of a family of transcription factors that function in a wide range of biological roles, including developmental processes, apoptosis and the cell cycle and it is overexpressed in various tumors. Here we report a new dominant *Tcfap2a* mutation named Doarad (*Dor*) that has an N-ethyl-N-nitrosourea (ENU)-induced missense mutation in the PY motif of its transactivation domain. *Dor/+* heterozygous mice have a misshapen malleus, incus and stapes, leading to hearing impairment, without any other apparent phenotype. *Dor/Dor* homozygous mice die perinataly, exhibiting prominent facial abnormalities and a severe ocular phenotype, ranging from lens and retinal defects to anophthalmia. *In vitro* assays suggest that this mutation causes a 'gain of function' in terms of the transcriptional activation abilities of AP-2α. These mice enable us to address more specifically the developmental role of *Tcfap2a* in the eye and middle ear and are the first report of a mutation in a gene specifically causing middle ear abnormalities, leading to conductive hearing loss.

481 Hearing Dysfunction in *Mitf$^{Mi\text{-}wh}$/+ Mice*, a Model for Human Waardenburg and Tietz Syndromes

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Humans heterozygous for mutations in the *MITF* gene can have either Waardenburg syndrome type II or Tietz syndrome, congenital deafness-pigmentation syndromes with features shared by mice of the *microphthalmia* series of coat color alleles caused by mutations in the murine homolog *Mitf*. *MITF/Mitf* encodes a transcription factor that is essential for pigment cell development and survival. Previous examination of a heterozygous mutant, *Microphthalmia-white (Mitf$^{Mi\text{-}wh}$/+)*, revealed loss of pigmentation of the stria vascularis and other inner ear abnormalities consistent with a proposed functional hearing deficit.

We used embryological, physiological, and morphological analysis to characterize the hearing deficit in *Mitf$^{Mi\text{-}wh}$/+* mice and wild-type (+/+) littermates. *Mitf$^{Mi\text{-}wh}$/+* mice exhibit threshold elevations at P18 and P28 of 63 and 69.5 dB at 12 kHz compared to wild-type littermates. Preliminary results at P45 suggest that the hearing loss is progressive. *Mitf$^{Mi\text{-}wh}$/+* embryos have substantially fewer melanoblasts in the region of the developing inner ear after melanoblast migration from the neural crest than +/+ littermates. While the apical cochlear area is relatively normal in *Mitf$^{Mi\text{-}wh}$/+* mice, hair cell loss appears to progress towards the base at P24 and P46.

These results demonstrate a high frequency hearing deficit in *Mitf$^{Mi\text{-}wh}$/+* mice during early post-natal life. Further studies of the EP and the morphology of the basal cochlea will help determine the site of the initial pathology and the impact of impaired melanoblast migration in the developing and mature inner ear.

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482 Development of *Xenopus* Larvae under Chronic Acceleration (Hypergravity)

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We are interested in understanding the role of gravity in the development of the inner ear. Results presented here are part of a three year study conducted at the NASA Ames Gravitational Biology Facility. The goal of this research is to evaluate effects of gravity on development of the vestibular system when *Xenopus laevis* are exposed to a chronic acceleration (hypergravity) stimulus. *Xenopus* (Stages 28-50) used in these experiments represent periods of larval life during inner ear vestibular organogenesis. Modules for aquatic habitats were constructed to meet engineering requirements of the large radius 24 ft and 20g centrifuges. Habitats were developed for the modules that permitted free-swimming behavior and continuous video observation during centrifugation. *Xenopus* larvae were exposed to 2g and 3g for up to 10 days with the 24 ft centrifuge and to 4.2g for up to 15 days with the 20g centrifuge. On center and 1g controls were included in the experimental protocol. The centrifuge was stopped every 1-3 days for 15-120 minutes for sample analysis, collection and aquarium maintenance. Results indicate that developmental progression, survival

rates and length were comparable between hypergravity and control groups. Swimming behavior at 2g and 3g was similar in hypergravity and control larvae. However, some S28 and S50 larvae (10-30%) showed altered swimming behavior after 5-10 days of centrifugation at 4.2g. Inner ear samples collected for histology (SEM, light and confocal microscopy) and molecular biology (cDNA library construction, DNA microarray) are currently under analysis. These results suggest that *Xenopus* can adapt to prolonged hypergravity conditions during larval development.

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483 Development of Hair-Bundle Specializations in Outer Hair Cells of the Postnatal Mouse Cochlea

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Three types of linkages are found on mature mouse cochlear OHCs; the tip link, the horizontal top link which connects the upper end of adjacent stereocilia, and the tectorial membrane-attachment crown at the tip of the tallest stereocilia. However in early postnatal mice, the surface of the hair bundle looks very different; although tip links are present, there are no distinct horizontal top links and attachment crowns are seen on all three rows of stereocilia. In addition, ankle links are seen at the base of the stereocilia and a thick cell coat is present over the bundle.

In this study we tracked postnatal changes in the distribution of these hair-bundle features in apical-coil OHCs in acutely dissected organs of Corti from P2 to P22 (day of birth is P0), and assessed their sensitivity to BAPTA and subtilisin. Ankle links are seen from P2 to P9 but disappear by P12. At P2, tip links are already visible and transducer currents can be recorded. Horizontal top links are present by P12 and become well defined at P14. Attachment crowns are restricted to the tallest row of stereocilia by P14. The thick cell coat is extensive at P5, but is reduced by P9 and disappears by P14. Tip links are BAPTA-sensitive but resistant to subtilisin. Attachment crowns are resistant to BAPTA, but subtilisin-sensitive. Ankle links are sensitive to BAPTA and subtilisin, whereas horizontal top links resist both treatments. This study suggests that each of these four specializations are biochemically distinct, that ankle links are a transient feature and that the OHC bundle surface has a mature appearance at P14.

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484 Short Hair Cells Start Out Tall

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Like mammals, birds show a differentiation of auditory hair cells into two extreme forms: tall hair cells (THC) and short hair cells (SHC). It is believed that THC and SHC are functionally analogous to inner and outer hair cells of mammals, respectively. This is most strikingly evident in their innervation. However, SHC are cytologically very different to mammalian outer hair cells and probably show a different kind of motility. As their name implies, SHC have short and wide cell bodies. In their most extreme form, in the basal, high-frequency regions of the basilar papilla, SHC somata contain little cytoplasm and are almost completely filled by the cuticular plate and nucleus. Here we show that the THC and SHC morphologies differentiate slowly from a uniform tall-looking hair cell type.

The development of THC and SHC was followed in barn owls (Tyto alba), aged from embryonic day 16 (50% of total incubation time) to 1 month posthatching. Serial light-microscopical sections were used to evaluate hair-cell heights and widths at regular intervals along the basilar papilla. Young embryos (E16-22) showed uniformly tall hair-cell shapes at all positions along and across the papilla. Around hatching, shortening of abneural cells had begun along the entire papilla, but all hair cells were still taller than wide. Within the first week posthatching, shortening of basal-abneural hair cells continued and approached the adult pattern. However, even at one month of age, cells still had not quite reached adult dimensions.

In the extreme case of basal SHC, the cells contracted to about 20% of their nascent height during maturation. This represents a major cellular reorganization over a prolonged period of time and highlights the specialized nature of SHC.

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485 Post-Natal Elaboration of the Actin-Spectrin Cytoskeleton in Gerbil Outer Hair Cells

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The cortical cytoskeletal lattice of the outer hair cell (OHC) maintains cell shape by opposing active changes in cell length (Holley & Ashmore, 1990). The development of the OHC cortical cytoskeleton was examined in post-natal gerbil organ of Corti using immunofluorescence. Specifically, an antibody to fodrin, a brain spectrin, was visualized with indirect immunofluorescence while actin was detected by direct immunofluorescence with labeled phalloiden. Label appeared in the lateral membrane at different rates for each structural protein. At P0 spectrin and actin label was present only in the cuticular plate. Between P3 and P6, the spectrin label extended down the lateral cell membrane towards the cell nucleus. By P9 the spectrin label was present throughout the cell membrane while actin remained confined to the cuticular plate. By P12, however, label for both actin and spectrin was present throughout the cell cortex. Such descriptions of the development of the actin-spectrin cell cortex may lend further insight into the role of the cortical cytoskeletal lattice in OHC motility and auditory signal amplification.

486 Headbanger: An ENU Induced Mouse Mutant With Stereocilia Bundle Defects.

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A phenotypic approach has been adopted in the mouse to identify molecules involved in ear development and function. Mutant mice were obtained using *N*-ethyl-*N*-nitrosourea mutagenesis and were screened for dominant mutations that affect hearing and/or balance. One of these mutants, *Headbanger*, is presented.

Headbanger mutants display behaviour indicative of vestibular dysfunction, including hyperactivity and head shaking. *Headbanger* mice have a strong Preyer reflex, so are not deaf. However, analysis of the organ of Corti and utricle by scanning electron microscopy has revealed defects in the stereocilia bundles of the sensory hair cells. In the apex of the cochlea, mutant outer hair cell bundles form an O-shape rather than the usual V-shape. At postnatal day 20, in the apex inner hair cell bundles begin to fuse forming giant stereocilia. Utricular hair cells show long, thin and wispy stereocilia when compared to littermate controls. The mutation has been mapped to chromosome 7 in the region of the unconventional myosin gene, *Myo7a*. Sequence analysis of *Myo7a* revealed a mutation in the motor head domain of the gene causing an isoleucine to phenylalanine substitution of a highly conserved amino acid. Additional functional studies are required to determine if this mutation is responsible for the *Headbanger* phenotype.

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487 Delineation of the Phenotypic Expression in a Large Japanese Family with A1555G Mitochondrial Mutation

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The homoplasmic A1555G mutation in the mitochondrial 12S ribosomal RNA gene is known to be associated with maternally inherited susceptiblily to aminoglycoside ototoxicity as well as non-syndromic sensorineural hearing loss even without using aminoglycosides. Clinical phenotype associated with the A1555G mutation ranges from profound congenital SNHL, through moderate progressive SNHL of later onset, to completely normal hearing. The cause for the variation in the phenotype has been undetermined, but involvement of genetic and environmental factors other than aminoglycosides has been postulated. We have identified a large Japanese family with the homoplasmic A1555G mutation that included 124 maternally related family members, with no exposure to aminoglycosides. Phenotypic expression patterns of hearing loss, tinnitus, and vestibular symptoms, incidents associated with the occurrence of phenotypes, and genetic backgrounds were examined in this family. This study suggested the involvement of recessive nuclear modifier genes for the phenotypic expression, existence of environmental factors that induce phenotypic expression, and the significance of tinnitus as an important clinical sign for future hearing loss in Japanese individuals with the A1555G mutation.

488 Molecular analysis of the PDS gene in 16 families with Pendred syndrome

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Pendred syndrome is an autosomal recessive disorder characterized by congenital sensorineural hearing loss combined with goitre. It may account for as many as 10% of the cases of hereditary deafness. Mutations in PDS gene have been observed in patients with Pendred syndrome or with non syndromic deafness linked to 7q31 (DFNB4). We have studied the PDS gene in 16 unrelated families with Pendred syndrome. All the patients were prelingually deaf, with moderate or profound deafness. Cochlear malformations or a widened vestibular aqueduct was documented in 13/16. Goitre and or an abnormal perchlorate discharge test was present in all cases. We have developped a DGGE/sequencing method analysis of the entire coding part of the PDS gene.

A large spectrum of mutations have been observed, but three mutations (V138F, T416P, Y530H) have been observed in respectively 12.5, 9.3 and 6.2% of all the mutated alleles. Three new mutations have been identified : S133X, IVS14+1G/A and S137P. The phenotype associated with these mutations will be described.

489 Temporal Bone Histopathology in Alport Syndrome

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Alport syndrome (hereditary nephritis and sensorineural hearing loss [SNHL]) is caused by mutations in genes that code for novel chains of type IV collagen. Novel type IV collagen is localized to the organ of Corti basement membrane in both mouse and man. Previous temporal bone studies have failed to identify histopathologic correlates for the SNHL. We examined bones from 9 individuals with Alport syndrome, including an individual (CA) with a 1564 cysteine to serine mutation in the COL4A5 gene on Xq22. By light microscopy, 7 of the 9 cases (including CA) demonstrated two unique pathologic changes: 1) A "zone of separation" between the basilar membrane and overlying cells of the organ of Corti. 2) Presence of cells filling the tunnel of Corti and extracellular spaces of Nuel. Electron microscopy (bone CA) demonstrated: 1) Abnormalities of the basement membrane subjacent to cells of the organ of Corti, including irregular thinning and the presence of electron dense particles. 2) The zone of separation occurred between the basement membrane and the basilar membrane. 3) The cells within the tunnel of Corti and spaces of Nuel were morphologically similar to supporting cells. 4) The basement membrane of blood vessels within the spiral ligament and stria vascularis appeared normal. In our cases, the cytologic losses of hair cells, stria vascularis and spiral ganglion cells were insufficient to account for the observed SNHL. Our findings suggest that the histopathologic correlates of SNHL in Alport syndrome are abnormalities of the basement membrane of cells of the organ of Corti and cellular infilling of extracellular spaces of the organ of Corti. We hypothesize that these abnormalities may result in SNHL by altering cochlear micromechanics.

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490 Quantifying Cochlear Basement Membrane Width in Alport Mice Reveals Site-Specific Changes

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Gross accumulation of extracellular matrix proteins has been noted surrounding the strial capillaries of 129Sv-Col4a3 mice. These mice lack the α3(IV) gene and are models of Alport syndrome, a basement membrane disorder whose phenotype includes kidney, auditory and visual defects. Previous studies have noted that the strial capillary basement membranes are markedly thickened and contain increased amounts of type IV collagen, laminin-1, entactin and heparan sulfate proteoglycans when compared to normal littermates. The present study sought to quantify the thickness of the cochlear basement membranes in the "Alport" mouse, particularly the strial capillary basement membranes.

Temporal bones of nine-week old 129Sv-Col4a3 mice and their normal littermates were perfused, decalcified, epoxy-embedded and stained in preparation for transmission electron microscopic evaluation of cochlear basement membranes. Measures of basement membrane thickness obtained at a constant magnification were analyzed with ANOVA and a posthoc Student-Newman-Keuls. The strial capillary basement membrane of the Alport mice was significantly thicker than that of normal littermates. No effect of basal/apical cochlear location was noted. In addition, the strial capillary basement membrane was often multilaminated while the capillary lumen was constricted by the misshapen endothelial cell. In contrast, all other basement membranes were significantly thinner in the Alport mice than in the normal controls. Our quantitative data confirm previous impressions of aberrant basement membrane thickness in the Alport mice. The extensive increase in strial capillary basement membranes suggests that strial dysfunction is the basis for the hearing loss associated with Alport syndrome.

491 Characterizing and Treating the Progressive Hearing Loss Associated with Lysosomal Storage Diseases

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Most lysosomal storage diseases (LSD) result from inherited deficiencies in the activity of particular lysosomal enzymes. Undegraded substrate accumulates in lysosomes, leading to impairment of many organ systems. Progressive hearing loss has been linked to subsets of LSD, particularly those affecting degradation of proteoglycan carbohydrates (mucopolysaccharidoses, MPS) or cell surface glycolipids (sphingolipidoses). The MPS VII mouse, which lacks beta-glucuronidase activity and consequently accumulates chondroitin, dermatan, and heparan sulfates, has been used for investigating the hearing loss in MPS (Ohlemiller et al., Hearing Res 169: 69, 2002). Enzyme replacement or bone marrow transplant (BMT) therapy initiated at birth normalized ABR thresholds during the first few months of life (Vogler et al., Pediatr Dev Pathol 4: 421, 2001). However, BMT-treated animals showed slowly increasing thresholds later, as has been reported for human MPS patients after BMT (Papsin et al., Otolaryngol Head Neck Surg 118: 30, 1998). Systemic adeno-associated virus-mediated gene therapy initiated at birth did not completely normalize hearing, but arrested progression through 18 months of age (Daly et al., Gene Ther 8: 1291, 2001). To determine the mechanisms underlying the peripheral hearing loss seen in some but not all LSD, hearing is being examined in knockout (KO) mouse models of MPS and sphingolipidoses. Preliminary results indicate that both MPS I and MPS IIIB mice have progressive threshold shifts, indicating that lysosome-mediated turnover of heparan sulfate, which accumulates in all three of these MPS, may be involved in maintaining hearing function.

492 Allelic Interaction and Phenotypic Expression of Two Novel *CDH23* Mutations at the *DFNB12* and *USH1D* Loci

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Nonsyndromic recessive hearing loss at the DFNB12 locus is associated with missense mutations of *CDH23* (encoding cadherin 23), whereas Usher syndrome type 1D (USH1D) is associated with frameshift, nonsense, splice site, and other missense alleles of this gene. Here we report novel missense (F1888S) and frameshift (8882-8883insT) mutations of *CDH23* segregating with nonsyndromic hearing loss in a large American family of Swiss-German descent. Homozygosity for F1888S can result in a distinctive phenotype with profound deafness at high frequencies and normal hearing at lower frequencies. F1888S homozygotes have normal vestibular responses to caloric stimulation and normal retinae by funduscopy. Two F1888S homozygotes had normal electroretinograms (ERGs), whereas one had a subnormal ERG. A compound heterozygote for F1888S and 8882-8883insT has prelingual, severe-to-profound deafness affecting all frequencies, normal caloric responses, normal retinae, and a normal ERG. This suggests a model in which Usher syndrome alleles are dominant over DFNB12 alleles of *CDH23* in the cochlea, whereas DFNB12 alleles are dominant over Usher syndrome alleles in the retina and vestibular neurosensory organs. This may reflect differences in minimal levels of cadherin 23 required for proper functioning of the three neurosensory organs. These results have important implications for genetic counseling and communication rehabilitation.

493 A Mouse Model For the Dominant, Y1870C, Deafness Mutation in the Zona Pellucida Domain of TECTA

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Alpha-tectorin is a major, non-collagenous protein of the tectorial membrane. Dominant (DFNA8/12) and recessive (DFNB21) mutations in the human alpha-tectorin gene, *TECTA*, cause deafness. Thus far five families have been identified with dominant missense mutations in *TECTA*, and the clinical phenotypes vary from being mild to severe, and can be either stable or progressive. An Austrian family with a missense, Y1870C mutation in the zona pellucida domain of TECTA has a stable, 80 dB hearing loss thought to be pre-lingual in origin. 'Hit and run' methodology was used to introduce the Y1870C mutation at the equivalent tyrosine residue of mouse Tecta (also Y1870C). In mice heterozygous for the Y1870C mutation, the tectorial membrane lies over the organ of Corti and is attached to the spiral limbus. However, the limbal attachment zone is strikingly reduced in thickness. Also, in the medial zone of the tectorial membrane, striated sheet matrix is missing and the collagen fibrils are aberrantly organised. Heterozygous mice respond with a Preyer reflex to a 20 kHz, 100 dB SPL click. DPOAEs are an indication of the contribution of outer hair cells to cochlear sensitivity. Between 2-48 kHz, DPOAEs of heterozygotes are ~20 dB less sensitive than those of wild type mice, and also more compressive, especially below 20 kHz. The round window CM is similar in wild type and heterozygous mice but the CAP of heterozygotes is ~70 dB less sensitive between 2 and 20 kHz, and ~40 dB less sensitive between 20 and 48 kHz. These results indicate the tectorial membrane is essential for transmitting the mechanical responses of the cochlear partition to the inner hair cells.

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494 Phenotypic Characterization of Head Tossing/Circular Mouse

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Mouse genetics has made significant contributions to several complex areas of mammalian biology and helped to understand the systematic and comprehensive function of mammalian gene. Genotype-based approaches along are insufficient due to the complexity and redundancy of gene functions. On the other hand, mutagenesis is one of the phenotype-driven approaches that identify new genes for specific defects. We have used N-ethyl-N-nitrosourea (ENU), an alkylating agent, to induce the mutagenesis and screen the mice with phenotypes of impaired hearing and balance. One of the inbred strain (BALB/c) exhibited head tossing/circular behavior with autosomal recessive inheritance. Preliminary analysis of phenotypic characterization was performed. Young adult mice showed about 60 – 70 dB hearing level on a click sound ABR test. Gross anomaly of temporal bone viewed by CT (computed tomogram) demonstrated that one of the semicircular canal was missing. The phalloidin staining of hair cells in each sensory organ showed no specific abnormalities. However, the otolith was irregular in size and sparsely distributed. Background strain mice were served as a control group. Linkage analysis and genetic mapping are now currently undergone for cloning of disease related gene using positional cloning.

495 Estrogen Receptors and Hearing in a Receptor Beta Knock-Out Mouse

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There have been suggestions that estrogens may have a protective role on the hearing just as there have been discussions that they are protective against osteoporosis and cardio-vascular disease and in the brain. There are also well documented ABR differences between women and men, with shorter latencies for the females. In the normal population elderly males have a 10-25 decibel worse hearing loss in the high frequencies than the females in the same age. These differences cannot only be explained by occupational noise or anatomical variations. Another example is Turner Syndrome (45,X), where a loss of estrogens is one of the major characteristics and where both an early presbyacusis and longer ABR latencies are seen.

In women estrogens are mainly produced by the ovaries. However, there is a minor production in several non-endocrine tissues, such as the brain and adipose tissues, by the conversion of testosterone to estrogens. This is also is the case in men.

The action of estrogens is believed to be mediated through estrogen receptor a (ERa) and the more recently discovered estrogen receptor b (ERb), which are intracellular receptors. In the brain estrogens reduce cell death, increases axonal sprouting, increase regeneration, and effect synaptic transmission. In a recent study by Wang and coworkers it was shown that in the brain of the estrogen receptor b knockout mouse, a global degeneration of cortical neurons was found that increased with age.

We have earlier shown that estrogen receptors are present in the inner ear of mouse, rat and in human.

In the present study we wanted to look at the development of the inner ear, the content of the a-receptor and the development of hearing in a strain of mice where the b receptor has been knocked out. Results indicate that inner ear and hearing in these mice develop normally up to the age of 8 months.

496 Degenerative Pathways of the Outer Hair Cells and Deiter's Cells Significantly Differ in Mpv17-/- Mice.

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Loss of function of the peroxisomal Mpv17-protein leads to kidney failure and to early sensorineural deafness (Weiher et al., 1990; Meyer zum Gottesberge et al., 1996; 2001; Müller et al., 1997). The organ of Corti subsequently undergoes degeneration, leading to nearly complete loss of the cochlear neuroepithelia. The upset of the degeneration appeared restricted to the outer hair cells that displayed severe degenerative changes. A focal disruption of Hensen's body and endoplasmic reticulum followed by vacuolization of the cytoplasm and lysis of the cells occurred. The floppy and wrinkly appearance of the OHC lateral membrane (after P18) arises the question whether the motor protein of the OHC- prestin might be affected. Anti-prestin immunostaining indicated no significant difference in the distribution of prestin between wild type and Mpv17-/- mice. The immunostaining was present as long as OHCs could be identified (also in case when loss of the auditory function was apparent). The supporting cells (Deiter's cells) were also affected; however, the degeneration pattern differed clearly from that of outer hair cells. Gap junctional connections between Deiter's cells were affected or completely lost during the degeneration. Based on ultrastructural observation and TUNEL staining the degeneration of Deiter's cells seemed to be of necrotic origin. In contrast, the structural degeneration pattern of the OHC appears to be similar to the recently described paraptosis, an alternative form of programmed cell death, discussed to cause distinct forms of neurodegeneration (Sperandino et al. PNAS USA 97:14376:2000; Oppenheim et al. J Neurosci 21:4752:2001).

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497 Cochlear Spiral Ganglion Cell Degeneration of the Mpv17-negative Mice and its Wild Type.

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Although the Mpv17 has been originally described as a glomerulosclerosis gene, recent results indicate that this protein is essential for the function of the inner ear and may play an important role in sensorineural degenerative processes. Age related loss of spiral ganglion cells (SGCs) was described in wild type and transgenic Mpv17-negative mice, however, the onset of the loss of auditory function and the pattern of SGC degeneration clearly differs indicating diverse cellular mechanism of the degeneration process (Weiher et al., 1990; Meyer zum Gottesberge et al., 1996; 2001; Müller et al., 1997). In order to study the differences in the degeneration pattern between Mpv17-negative and wild type mice ultrastructural evaluation and morphometric analysis of the remaining neurons were performed. The ganglion cell diameters in young wild type animals (less than 2 months) reveal an unimodal distribution except for the basal region, where a bimodal distribution was indicated. In animals over 5 months of age in both strains the tendency of a bimodal distribution of the ganglion cell diameters in all three regions (basal, medial and apical) was observed which could be due to a more selective degeneration of myelinated type I ganglion cells. Vacuolization of the cytoplasm, cytolysis of the organelles of the neurons, loosely arranged lamellae of the myelin sheath, invagination of the myelin sheath into Schwann cells, formation of myelin-like bodies and debris found in Schwann cells indicated an ongoing demyelinization. Almost all remaining neurons were arranged in clusters. Severe patchy-like degeneration of the SGCs appeared to be a result of a missing function of the Mpv17-gene coding for the Mpv17 peroxisomal protein involved in redox homeostasis of the cells.

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498 MT/ret Transgenic Mouse as a Novel Model for Research of Melanin in the Cochlea

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Melanin in the cochlea has been supposed to protect the cochlea from traumas, including noise and ototoxic aminoglycosides. But there are conflicting opinions about protective effects of melanin against noise-induced sensorineural hearing loss. These conflicting opinions might have come from results of studies, which compared albino and pigmented guinea pigs in pigment research, although these strains had large amount of genetic variability in factors other than pigmentation. Another problems in previous investigations were lack of quantitative analysis of both melanin contents in the cochlea. Melanin is mainly classified into eumelanin and pheomelanin. Then we investigated a novel transgenic mouse (MT/ret transgenic mouse of line 242) model what had severe systemic melanosis and almost same genetic background as C57BL/6 mouse. No statistically significant difference was observed about the mean ABR thresould between transgenic mice and C57BL/6 mice. A electron microscopic study revealed that the transgenic mouse had abundant melanin in the intermediate cells of the stria vascularis. High performance liquid chromatography analysis indicated that this transgenic mouse contained about three times as

much eumelanin as C57BL/6 mouse had, whereas the content of pheomelanin was about the same between these strains. These results suggest that this transgenic mouse can be a suitable model for investigation about cochlear eumelanin's function.

499 Prestin Expression and Electromotility in Genetically-Induced Hypothyroidism

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Recent reports suggest that peripheral auditory deficits associated with hereditary congenital hypothyroidism resulting from a mutation in the thyrotropin receptor (Tshr), include an enduring transduction defect that diminishes the gain of the cochlear amplifier (Walsh & McGee 2001). Of the many possible factors that might produce such a defect, the absence of, or diminished expression of the molecular motor of outer hair cells (OHCs), prestin (Zheng et al. 2000), must be considered a primary candidate. As anticipated on the basis of previous reports focused on the consequences of chemically inducing hypothyroidism (Weber et al. 2002), RT-PCR results indicate that prestin mRNA is present in the cochleae of Tshr mutant mice. Furthermore, the prestin protein is localized in the OHCs of Tshr mutants and its distribution pattern resembles that described for chemically-induced hypothyroid rats; i.e., prestin is distributed throughout the basolateral membrane, a finding clearly differentiating hypothyroid mice from their euthyroid counterparts in whom the basal pole of OHCs is prestin-free. Given these findings, it is not surprising that OHCs of Tshr mutants are motile, as observed using a whole cell patch clamp approach. Although preliminary indications suggest that the magnitude of motility may be reduced relative to that observed in OHCs from normal animals, length changes in response to voltage steps were apparent and current-voltage curves from OHCs harvested from hypothyroid mice were essentially normal. Based on the corollary finding that OHCs from Tshr mutants appeared anatomically abnormal in vitro, we will consider the possibility that diminished electromotility represents one of multiple OHC defects associated with congenital hypothyroidism. In that context, it is interesting that both synaptophysin immunolabeling beneath OHCs and KCNQ4 protein expression are distinctly diminished in Tshr mutants.

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500 Functional Consequences of Natriuretic Peptide C-receptor Mutation

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The specific role of natriuretic peptides and their receptors in hearing is unclear. This study determined the functional consequences of a spontaneous mutation of one natriuretic peptide receptor, NPR-C, by comparing auditory brainstem response (ABR) and endocochlear potential (EP) data in NPR-C deficient (NPR-C-/-) and control mice.

NPR-C/- mice had functional cochleae but exhibited a progressive high frequency hearing loss that differed significantly from deficits previously described for DBA/2J mice. At postnatal day 25 (P25), thresholds of NPR-C-/-mice were 5-15 dB and 20-40 dB higher than DBA/2J and CBA/J controls, respectively, for frequencies >4 kHz. Moreover, considerable variability existed in the thresholds of NPR-C-/- mice (e.g., from 10-65 dB SPL at 8 kHz). At P100, thresholds of both the DBA/2J and NPR-C-/- mice exceeded 80 dB SPL at all frequencies >4 kHz, while high frequency thresholds of CBA/J mice improved between P25 and P100.

At P25, EPs measured in the apex of the cochleae of both DBA/2J and NPR-C-/- mice were higher than previously reported values for CBA/J mice. In contrast, EPs in the base were lower than in the apex in NPR-C mice but not in DBA/2J controls. Endolymph potassium ion concentrations ([K+]) exceeded 150 mM and were lower in the cochlear base compared to apex in NPR-C-/- mice but not DBA/2J mice. Perilymph [K+] was higher in NPR-C-/- mice than in DBA/2J controls.

These data suggest that the NPR-C mutation produces a defect in cochlear homeostasis that results in early onset, high frequency hearing loss.

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501 Role of Class D L-type Ca2+ Channels for Morphology of Vestibular Sensory Epithelium

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By controlling the majority of depolarization-induced Ca2+ entry, voltage gated Ca2+ channels formed by subunits tightly control neurotransmitter release from vestibular hair cells.

Using á1D-deficient mice, we have recently shown that the absence of these channels can cause deafness and degeneration of outer and inner hair cells (Platzer et al., 2000). The aim of the present study was to investigate the time – dependent patterns of the degeneration during postnatal development in the á1D-mouse vestibular endorgans using light and electron microscopy. At age P90, electron microscopy revealed no morphological aberrations in sensory cells as well as afferent and efferent nerve endings. At P244 however, a beginning degeneration of the nerve endings could be demonstrated by electron microscopy. A loss of vestibular hair cells was evident.

502 Cloning of CTL2, the Target Antigen of Antibody-Induced Hearing Loss, from Guinea Pig and Human Inner Ear

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Autoimmune hearing damage is suspected to be a cause of rapidly progressive hearing loss. We developed a murine monoclonal antibody KHRI-3 that binds to supporting cells in the guinea pig (GP) inner ear and causes hearing loss when infused into the cochlea. Antibodies from patients with rapidly progressive hearing loss, who are suspected to have autoimmune hearing loss, often have antibodies that bind to GP supporting cells with the same pattern as KHRI-3. KHRI-3 immunoprecipitates from GP inner ear a protein doublet of 68 and 72 kDa, which we subjected to MS/MS sequencing. Ten peptide fragments identical to human Choline Transporter-Like 2 (hCTL2) were identified. To determine the GP mRNA sequence of CTL2, we back translated the peptide sequences and synthesized four primer sets. Two of the four primer sets successfully amplified GP sscDNA. To complete the sequence we used the walking primer approach and performed a 3' and 5' RACE. We found that the GP CTL2 cDNA sequence is 86.5% identical to the hCTL2 cDNA and the GP CTL2 protein sequence is 90.5% identical to the hCTL2 protein. Human inner ear vestibular tissue also expresses abundant CTL2 mRNA since we were able to amplify the expected human sequence from RNA isolated from vestibular tissue using all of the primer sets. We can now clone

the full-length GP and human CTL2 cDNAs and use these to produce proteins to use for screening sera from patients suspected to have autoimmune hearing loss. Using cloned GP CTL2 protein we can also determine the function of this protein, allowing us to understand the mechanism of antibody induced hearing loss.

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503 Comparision of Western Blot Results in Patients With Progressive Sensorineural Hearing Loss.

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Progressive bilateral sensorineural hearing loss in a subset of patients is suspected to be of autoimmune origin. We have collected sera of patients with and without progressive bilateral hearing loss after they have signed the proper consent forms. The sera were tested using western blotting on guinea pig inner ear tissue. The western blotting was performed and analyzed by a person who was blinded to the patients' history. Sera were selected and prepared by a person with access to the case histories to allow a mixed panel of samples from patients with and without hearing loss. We noted that each serum produces distinct reactivity patterns that are fully reproducible from one experiment to another; in fact it is possible to recognize a previously tested serum from its pattern of bands on western blots. To analyze these complex patterns, we assessed all of the prominent bands produced by each serum. The bands common to patients without progressive hearing loss were identified these bands were excluded from the analysis of the sera from progressive hearing loss patients. The bands unique to progressive hearing loss patients are considered to be of importance for follow through with further investigation. We scored the bands using a regression plot and the results are as follows: 30-35 (kDa) 11%, 36-41 (kDa) 33%, 65-70 (kDa) 39%, 113-118 (kDa) 22%, 155-160 (kDa) 22%, and 197-202 (kDa) 22%.

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504 CTL2, the Target Antigen of Antibody Induced Hearing Loss is Expressed in the Human Vestibular Epithelium

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We recently isolated, sequenced, and cloned the inner ear supporting cell antigen that is the target of monoclonal antibody-induced hearing loss in guinea pigs. The glycoprotein identified by the KHRI-3 antibody is the guinea pig homologue of human CTL2, a choline transporter-like protein. This protein has 10 predicted transmembrane spanning regions, is expressed in a punctuate pattern at the cell surface in supporting cells of the guinea pig organ of Corti, saccule, utricle and ampulla. We previously reported that patients with suspected autoimmune sensorineural hearing loss have antibodies that stain the guinea pig organ of Corti with the same pattern as KHRI-3, suggesting that they might have antibodies against CTL2. To examine the expression of CTL2 in the human inner ear we raised antibodies against CTL2. peptide, purified and tested against guinea pig inner ear. The purified antibodies stain the supporting cells with the same pattern as the KHRI-3 antibody. We tested this antibody on human vestibular epithelium taken from consenting patients undergoing translabyrinthine surgical procedures. The CTL2 antibody produced a strong punctate staining of supporting cells in human saccule, utricle and ampulla with a honeycomb pattern like that observed in guinea pig vestibular epithelium. The large size of the stained spots and the strong intensity of staining are consistent with a multi-molecular complex in the membrane of the supporting cells. These findings demonstrate that the CTL2 protein is highly expressed in human inner ear tissue. Therefore, in some individuals with autoimmune disorders, antibodies against the CTL2 protein could cause hearing loss like that induced by the KHRI-3 antibody in guinea pigs.

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505 Gene Expression Analysis of *Shaker-2* Cochlear Transcriptome Using cDNA Microarrays

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Shaker-2 is a recessive mouse deafness mutant due to a mutation in *Myo15*, which encodes for an unconventional myosin. Affected mice exhibit auditory and vestibular defects by 3 weeks of age, including lack of ABR response (at all frequencies up to 90 dB) and circling behavior. Closer examination of the auditory sensory epithelium revealed very short stereocillia in both outer and inner hair cells. In addition, there is a long actin-containing protrusion (cytocaud) initiating beneath the cuticular plate and extending toward the basal end of inner hair cells. These observations suggest that Myo15 is important for actin organization. In this study, we have taken a molecular approach and used cDNA arrays to delineate the underlying cellular pathways involved. To evaluate cochlear gene expression profiles, we have chosen two microarray methods: GeneFilters on nylon membranes and Affymetrix Genechip. The GeneFilters arrays contain more than 5,000 genes, including known genes and many uncharacterized cDNAs. In order to identify changes in gene expression that might result from compensation for the absence of Myo15, but not those involved in cell dealth, we determined gene expression at early times and prior to large degree of cell dealth, e.g. 3 weeks and 3 months. Results obtained from the cochlear RNA of the *shaker-2* homozygotes were compared to those from the heterozygote littermates. No reproducible differences were observed between homozygotes and heterozygotes at 3 weeks or 3 months old. Preliminary studies indicated a global shift in gene expression pattern between these two age groups. We are currently performing further analysis using the Affymetrix Genechip. Understanding changes in gene expression levels affected by the *Myo15* mutation may provide insight into the molecular pathways leading to actin disorganization.

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506 The Usher Syndrome Proteins Cadherin 23 and Harmonin Form a Complex by Means of PDZ-Domain Interactions

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Usher syndrome type 1 (USH1) patients suffer from sensorineuronal deafness, vestibular dysfunction, and visual impairment. Several genetic loci have been linked to USH1, and four of the relevant genes have been identified. They encode the unconventional myosin VIIa, the PDZ-domain protein harmonin, and the putative adhesion receptors cadherin 23 (CDH23) and protocadherin 15 (PCDH15). We show here that CDH23 and harmonin form a protein complex. Two PDZ domains in harmonin interact with two complementary binding surfaces in the CDH23 cytoplasmic domain. One of the binding surfaces is disrupted by sequences encoded by an alternatively spliced CDH23 exon that is expressed in the ear, but not the retina. In the ear, CDH23 and

harmonin are expressed in the stereocilia of hair cells, and in the retina within the photoreceptor cell layer. Since CDH23-deficient mice have splayed stereocilia, our data suggest that CDH23 and harmonin are part of a transmembrane complex that connects stereocilia into a bundle. Defects in the formation of this complex are predicted to disrupt stereocilia bundles and cause deafness in USH1 patients.

507 Identification of Interacting Proteins to the Human USH1C Protein (Harmonin) Involved in Genetic Deafness.

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The Usher syndrome type1c gene (USH1C) was recently identified. The gene encodes a protein (harmonin) containing PDZ domains with at least one coiled-coil domain. These proteins are thought to participate in the organization of macromolecular complexes associated with specific domains of the plasma membrane. More recently, different mutations in USH1C have shown to underlie a form of non-syndromic recessive deafness (DFNB18) without RP. In the present study, we have used the yeast two hybrid strategy to identify the protein(s) interacting with USHIC. The bait strain (pBD-CCD) was obtained by inserting the first coiled coil domain (CC1D, 93F-154Q) fragment generated by PCR from a human brain library into the GAL 4 DNA domain binding of the Yeast expression vector pBridge (Clontech). The positive clones were selected from 3x106 transformants obtained from the co-transformation of the yeast strain AH109 with pBD-CC1D and a human brain cDNA prey library using medium stringency media. The positive clones are then sequenced and Genebank Blast analysed. Our data analysis identified 18 potential candidate CC1D-interacting genes as well as known genes including myosin light polypeptide, calmodulin, microtubule-associated protein 7 that may interact with CC1D. Unigene clustering analysis showed that the isolated novel clones, are expressed in the inner ear and/or the retina. Characterization of the potential novel proteins and confirmation of the interactions using co-immunoprecipitation are in process.

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508 The Wolfram Syndrome 1 Gene (WFS1): Heterogeneity of the Mutations Responsible for Low Frequency Nonsyndromic Hearing Loss

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Non-syndromic low frequency sensorineural hearing loss (LFSNHL) affecting only 2000 Hz and below is an unusual type of hearing loss. The loci of autosomal dominant LFSNHL were reported including DFNA6, DFNA14 and DFNA38. And further study revealed DFNA6, DFNA14 and DFNA38 are allelic. LFSNHL appears to be genetically nearly homogeneous, as only one LFSNHL family is known to map to a different chromosome (DFNA1). WFS1, the gene responsible for Wolfram syndrome, an autosomal recessive disorder characterized by diabetes mellitus and optic atrophy, and often, deafness, was considerated to be responsible for LFSNHL too. Herein we report two different heterozygous missense mutations (2016 G→T,2766 G→A) in the WFS1 gene found in two LFSNHL families. Mutations in WFS1 were identified in all LFSNHL families tested. None of the mutations was found in at least 280 control chromosomes. An increased risk of sensorineural hearing loss has been reported in such carriers. Therefore, we conclude that mutations in WFS1 are a common cause of LFSNHL.

509 Reversal of Vasospasms of the Spiral Modiolar Artery (SMA): A Potential new Approach for the Treatment of Sudden Hearing Loss (SHL)

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Vasospasms of the SMA may cause an ischemic stroke of the inner ear that manifests itself by SHL. Vasospasms are induced by hypercontraction of vascular smooth muscle cells (VSMC); the intracellular mechanisms of hypercontractions are still unknown, and the elucidation of these mechanisms remains an important clinical issue. Endothelin-1 (ET1) induces vasospasms of the SMA via activation of ETA-receptors. Here we tested the hypotheses that these vasospasms are a) reversible by ETA-receptor antagonists, b) mediated by a Ca^{2+}-sensitization of the contractile apparatus via a Rho-kinase induced inhibition of myosin light chain phosphatase (MLCP), and c) prevented by the second messenger cAMP and the vasodilator CGRP. The VSMC Ca^{2+}-concentration ($[Ca^{2+}]i$) and the vascular diameter were determined simultaneously by fluo4-microfluorometry and videomicroscopy, respectively. The Ca^{2+}-sensitivity of the contractile apparatus was evaluated by a correlation between the $[Ca^{2+}]i$ and the vascular diameter. ET1 induced vasospasms were prevented but not reversed by the ETA-receptor antagonists BQ-123 and BMS-182874. The Ca^{2+}-sensitivity was increased by ET1 and by inhibition of MLCP with the selective inhibitor calyculin A. ET1 induced Ca^{2+}-sensitization and vasospasms were prevented and reversed by the selective Rho-kinase antagonist Y-27632, CGRP, and dbcAMP. We conclude that ET-1 induces vasospasms of the SMA via an ETA-receptor mediated activation of Rho-kinase. These vasospasm were reversed by CGRP via a cAMP-mediated desensitization of the contractile apparatus. The observation that vasospasms were reversed by Y-27632 and CGRP but not by ETA-receptor antagonists suggests that Rho-kinase and CGRP-receptors, rather than ETA-receptors, are the most promising pharmacological target for the treatment of vasospasms and SHL.

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510 PMCA2 Expression is Driven by Two Promoters in Mouse Cochlea

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Deafwaddler (*dfw*) mice are deaf and exhibit a wobbly gait. The *dfw* mutation was localized to *Atp2b2*, encoding a plasma membrane Ca^{2+}-ATPase, PMCA2 (Street et al, 1998). Heterozygous mutants of *Atp2b2*, while not deaf, have significant high-frequency hearing loss, implying that expression is tightly regulated. PMCA2 is highly expressed in stereocilia of outer hair cells, suggesting that it is important for hair cell Ca^{2+} homeostasis. It is also abundant in cerebellar Purkinje neurons and lactating mammary gland. To understand the pathways that control or restrict PMCA2 expression, we have examined transcriptional regulation of the *Atp2b2* gene. Using a primer at the start of the ORF, 5'-RACE was performed using RNA from adult CBA mice. Sequencing of clones identified two alternative first exons, implying regulation through distinct promoters. One transcription start (1c) predominated in cerebellum. A second (1m) was the main form in lactating mammary gland. In cochlea, clones containing either initial exon were observed. Real-time RT-PCR confirmed the highly preferred usage of exon 1c in cerebellum (1c:1m>1000), and exon 1m in lactating mammary gland (1m:1c>100). In cochlea, 1c was only ~15x more abundant than 1m, suggesting that both may play an important role, possibly in different cell types such as hair cells and spiral ganglion neurons. Genomic sequence upstream of exons 1c and 1m was cloned via database and

PCR, and *in silico* analysis of putative regulatory elements was performed. Neither promoter contained TATA or CCAAT-boxes, but both contain multiple sp1 and E-box sites, which are common in TATA-less genes. Both also contain consensus sites for NFκB, CRE, and SRE that may be important for stress response and Ca^{2+}-dependent transcription. This preliminary promoter characterization has identified multiple pathways by which *Atp2b2* may be regulated. Further analysis will determine cell-specificity of promoter use in the cochlea.

511 The Role of *FOXI1* in Pendred Syndrome

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Pendred Syndrome (PS) is an autosomal recessive disorder characterized by severe-to-profound sensorineural hearing loss (SNHL), thyroid goiter and temporal bone malformations (dilated vestibular aqueduct (DVA) and Mondini malformation). Mutation screening of the causative gene, SLC26A4, has facilitated diagnosis, however in many individuals with this phenotype no SLC26A4 mutations are found. In other persons, only a single SLC26A4 mutation is detected. These findings suggest genetic heterogeneity and/or epistatic interactions with other genes. Recently, cochlear abnormalitites and SNHL were demonstrated in mouse mutants with targeted deletions of a winged helix transcription factor, Foxi1. In situ hybridization studies of Foxi1 showed that this gene is expressed in the endolymphatic duct and sac in a pattern similar to that seen with Slc26a4. Further studies have shown that Slc26a4 expression is absent in Foxi1 -/- mutants. Based on these observations we hypothesized that mutations in FOXI1 cause PS in humans.

We screened a cohort of 133 families with a PS phenotype who were negative for SLC26A4 mutation screening. We identified eight single nucleotide polymorphisms (SNPs) in FOXI1. Four SNPs (276: GàA; 969:GàC; 1011:GàA; 1041:CàT) are wobble bases, causing no change of amino acids. Four nucleotide changes (674:CàT; 724:CàA; 769:GàC; 797:GàA) identified in three affected persons result in missense mutations (T199I, P216T, R231G, R240Q) in FOXI1. In mutations screening of 153 random controls, P216T was detected in two individuals and T199I was detected in one individual. While only two changes are conserved across species (T199I and R240Q), all cause significant alteration in size and polarity of the amino acids. Based on these data, we calculate that muations in FOXI1 are responsible for 10% of the PS genetic load and are one-fifth as common as mutations in SLC26A4.

512 Autoregulation of the Gene Encoding the Hair Cell Differentiation Factor Brn-3.1

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The POU-domain transcription factor Brn-3.1 (Brn-3c; POU4F3) is expressed by hair cells from the time of their commitment to the lineage and throughout life. Deletion of the murine gene encoding Brn-3.1 leads to failure of hair cell differentiation, and eventual death of the cells. Lifelong expression of the gene suggests the possibility of positive feedback. To determine whether Brn-3.1 autoregulates expression of its own gene, we crossed mice null for the Brn-3.1 gene with transgenic mice in which GFP is driven by Brn-3.1 regulatory sequences. Backcrosses resulted in Brn-3.1 null animals in which cells that normally express the gene expressed GFP. This allowed us to track these cells in vivo and in vitro, and to explore signals controlling gene expression.

GFP expression was observed in large numbers of Brn-3.1 null, undifferentiated auditory and vestibular hair cells immediately after birth. However, the level of GFP expression was substantially lower than that seen in hair cells of Brn-3.1 wild-type or heterozygous mice. This suggests that while maintenance of some Brn-3.1 gene expression does not require Brn-3.1 protein, there is substantial positive auto-regulation of the gene.

In the organ of Corti, GFP-expressing cells were visible for a few days, while some vestibular GFP+ cells were visible for weeks. The disappearance of GFP expression over time may be related to hair cell death, or to complete failure of GFP expression. To explore this, organ of Corti from newborn Brn-3.1 null mice expressing GFP was placed either in standard culture medium, or in medium containing the pan-caspase inhibitor zVAD-FMK. Untreated, the undifferentiated hair cells disappeared in a few days, as was observed in vivo. However, GFP+ cell disappearance was strongly inhibited by zVAD-FMK. This suggests that loss of GFP+ cells was indeed due to hair cell death, and that the hair cells die by an apoptotic process.

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513 Transcriptional Control of the Cochlear Motor Protein Prestin

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Electrical stimulation during the hearing process induces rapid length changes of cochlear outer hair cells. The molecular motor (prestin) has recently been identified (Zheng, J. et al. 2000 Nature 405, 149-155). In our effort to find transcriptional regulators of the prestin gene we have identified a functional Thyroid Hormone Response Element (PresTRE) at position -416 in relation to the ATG codon of rat prestin (Weber, T. et al. 2002 PNAS 99, 2901-2906). We determined the start site of transcription in the rat using RACE-PCR. After alignment of the RACE sequence data with the homologous human genomic sequence we found that PresTRE is located downstream from the startpoint of transcription, probably in the second intron. We therefore started to analyse up to 1 kb upstream of the transcriptional start point of the human prestin gene using computer programmes. We were able to note a number of putative regulatory elements including another TRE. Fragments differing in their length from within these first 1 kb have been cloned. First studies analysing specifity and function of distinct binding sites in this region will be presented and discussed in the context of their function for normal prestin activation.

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514 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 defects (Mitchem et al., Hum. Mol. Genet. 11:1887-1898, 2002; Naz et al., Amer. J. Hum. Genet. 71:632-636, 2002). Mouse Tmie encodes a novel protein of 153 amino acids; the human TMIE protein is 156 amino acids in size. The protein is predicted to contain a signal peptide and at least one transmembrane domain. The C-terminus of the protein is rich in charged amino acids (36/74), and includes two clusters of lysine residues. The identification of Tmie homologs in the fish Danio rerio and Fugu rubripes indicate that this gene is conserved across many vertebrates, with the highest sequence similarity in the central region of the protein, including the transmembrane domain and a portion of the predicted cytoplasmic tail. Northern analysis with a probe derived from the 3’ UTR of mouse Tmie demonstrated expression of the gene in brain, kidney, liver, and lung.

Three alternative transcripts were identified, at least one of which appears to be due to the use of an alternative brain-specific promoter and first exon. Localization with an anti-peptide antibody indicated membrane association of TMIE that was ectopically expressed in cultured fibroblasts, consistent with the predicted transmembrane domain in the protein. TMIE in the fibroblasts was concentrated in filopodia-like projections of the cortical actin cytoskeleton, suggesting the protein may have some role in anchoring cytoskeletal elements to the membrane.

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515 Processing and Secretion of Normal and Mutated Cochlin, the Affected Protein in the Sensorineural Deafness and Vestibular Disorder, DFNA9

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Missense mutations in the FCH/LCCL domain of cochlin result in the autosomal dominant hearing loss and vestibular dysfunction disorder, DFNA9. Recombinant FCH/LCCL has a novel fold disrupted by 3 of 4 previously analyzed DFNA9 mutations. Characteristic eosinophilic deposits in DFNA9-affected inner ear structures could be the result of aberrant folding, secretion, or solubility of mutated cochlins, as misfolded proteins accumulate and aggregate, causing toxicity in certain other pathologic states. To study the biological consequences of cochlin misfolding, we expressed normal and mutated cochlins in cultured mammalian cells. Three missense mutations associated with DFNA9 were introduced separately into full-sized cochlin cDNAs, which were transfected into 293T-HEK, COS-7, and 3T3 fibroblast cell lines. Immunocytochemistry revealed localization of normal and mutated cochlins in perinuclear structures consistent with the Golgi apparatus/endoplasmic reticulum.

Western blot analysis of lysates prepared from transfected cells and conditioned media showed equal amounts of normal and mutated cochlins. Normal and mutated cochlins were proteolytically processed and glycosylated equivalently, and non-reducing SDS-PAGE failed to detect any evidence of abnormal cross-linking of the mutated polypeptides. These findings suggest that the pathology associated with cochlin mutations is unlikely to result from abnormalities in secretion, processing, or cross-linking. Alternatively, DFNA9 mutations may disrupt protein-protein interactions involving the FCH/LCCL domain and other components of the extracellular matrix of the inner ear.

516 Calcium-Activated Potassium Channel Genes in *Xenopus laevis* and *Xenopus tropicalis*

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The calcium-activated potassium channel (*slowpoke*, KCNMA1, KCa1.1) plays a key role in establishing the acoustic frequency selectivity of auditory hair cells (Fettiplace and Fuchs, 1999). We are interested in the regulation of *slo* expression during development of the *Xenopus* inner ear and present here preliminary data comparing *xslo* genes between two *Xenopus* species, *X. laevis* and *X. tropicalis*. The diploid species, *X. tropicalis*, has a shorter generation time than the tetraploid *X. laevis*, and has been selected by the DOE for shotgun sequencing of the genome. We identified expressed *xslo* inner ear sequence by synthesizing and probing a *Xenopus* inner ear SMART (Clontech) cDNA library (Serrano *et al.*, 2001) with a *xslo* cds partial clone. Isolated clones shared 98% nucleotide identity with a full length *xslo* coding sequence (AF274053). We have begun to use PCR-based techniques (Genomewalker, Clontech) to clone the *X. laevis* and *X. tropicalis slo* promoters. Recent studies in *Drosophila* demonstrate that the *slo* transcriptional control region is large and complex (Bohm *et al.*, 2000). To date, we have cloned over 1 kb of *xslo* genomic sequence upstream of the translational start site, and this region shares 96% nucleotide identity between the two species. We determined *xslo* gene copy number using *Xenopus* muscle to prepare genomic DNA for Southern blots. The genomic DNA was restricted with *Bam*HI, *Eco*RI, *Hin*dIII, and *Pst*I, and probed with a *xslo* cds partial clone. In both species, genomic Southern analysis provided strong support for the presence of a single copy of the gene. Taken together, the data provide insight regarding the conservation of *xslo* genes between divergent *Xenopus* species.

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517 Expression of a Dominant-Negative Connexin26 in Mice Causes Disorganization of Organ of Corti and Non-syndromic Deafness

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Hereditary deafness affects about 1 in 2,000 children and mutations in the GJB2 gene, which encodes gap junction protein connexin26, are the major cause in various ethnic groups. However, the pathogenesis of deafness due to GJB2 mutations remains obscure. Mice with targeted disruption of the gene were embryonic lethal in a previous study. To elucidate the pathological role of connexin26 in the inner ear, we produced transgenic mice carrying a R75W mutation in the GJB2 gene, which was identified in a hereditary deafness pedigree and showed a deleterious dominant-negative effect.

The R75W+mice showed severe hearing loss from an early stage of development. Histological analysis of the mutants revealed hyperplasia of supporting cells, failure in the formation of the tunnel of Corti, and degeneration of sensory hair cells. Despite robust expression of the transgene, no obvious structural change was observed in the stria vascularis and spiral ligament that are rich in connexin26 and generate the endolymph. The high resting potential in cochlear endolymph essential for hair cell excitation was normally sustained.

These results indicate that the GJB2 mutation associated with sensorineural deafness affects the differentiation of supporting cells resulting in disorganization of the organ of Corti, rather than affecting endolymph homeostasis, in mice and probably in human.

518 A Proposed Mechanism of the Gene *Ahl* for Increased Susceptibility to Noise-Induced Hearing Loss.

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Animals and humans show differing susceptibility to noise damage even under very carefully controlled exposure conditions. This difference in susceptibility may be related to an uncontrolled genetic component. Common experimental animals (rats, guinea pigs, chinchillas, cats) are outbred?their genomes contain an admixture of many genes.

About 10 years ago Erway et al (1993) demonstrated a recessive gene associated with early presbycusis in inbred mice: *Ahl*. A series of studies have shown that mice homozygous for *Ahl* are more sensitive to the damaging effects of noise.

Recent work has shown that mice homozygous for *Ahl* are not only more sensitive to noise, but also are probably damaged in a different manner by noise than mice containing the wild-type gene.

Recent work in Noben-Trauth?s lab (Di Palma et al., 2001) has shown that the wild-type *Ahl* gene codes for an outer-hair cell specific cadherin. Cadherins are calcium dependent proteins which hold cells together at adherins junctions to form tissues and organs. The cadherin of interest is localized to outer hair and has been termed otocadherin or cdh-23. Reduction in, or missing otocadherin may allow stereocilia to be more easily physically damaged by loud sounds and by aging.

519 Towards Functional Analysis Of Protocadherin 15, The Gene Associated With Ames Waltzer (av) Mouse Mutation And Usher Syndrome 1F

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The av mutation causes deafness and circling behavior in mice. The gene that harbors the av mutation codes for a protocadherin, Pcdh15. Mutation in the human homologue of the mouse Pcdh15 causes Usher syndrome type 1F, establishing the av mouse as a model for deafness in USH1F. Analysis of the predicted amino acid sequence of Pcdh15 shows 11 cadherin repeats, a transmembrane domain and a unique intracellular domain with 2 proline-rich regions, which could serve as binding sites for domains such as SH3 (Src Homology 3). SH3 domains regulate protein localization and often participate in the assembly of multi-component signaling complexes. Using the yeast 2-hybrid system interacting proteins are being screened and candidates genes, such as myosin VIIa, are being tested for interaction in this system. To investigate gene regulation, we analyzed the 'TATA-less' promoter region of Pcdh15 for critical regulatory elements and characterized alternatively spliced products derived from Pcdh15. Results from these experiments will be presented. In the cochlea, electron microscopy of hair cells from the null allele av-3J show severe disorganization of stereocilia bundles as early as E17.5. Hair cell stereocilia from alleles carrying less deleterious mutations (ex. av-J& av-2J) appear fairly normal at P0. However, some cuticular plates of hair cells from av-J and av-2J alleles appear rotated on the apical cell surface by P2, compared to age matched controls. By P5, this rotation is more conspicuous. Although abnormal function of the vestibular system is apparent electrophysiologically (see abstract by Jones SM et al) and behaviourally (waltzing), stereocilia of all vestibular receptors in all alleles studied appear normal into adulthood. Based on current observations it appears that protocadherin 15 is required for hair cell development and function and that Pcdh15 may mediate its function through interacting proteins.

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520 Identification Of The Rodent USH2A And USH3 Genes And The Cellular Source Of The USH2A And USH3 Transcripts

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The autosomal recessive disorder, Usher syndrome is defined by the association of sensorineural deafness and visual impairment due to retinitis pigmentosa (RP). Variation between families corresponds to three clinical types distinguishable by difference in the severity of hearing loss and vestibular dysfunction; all three forms have RP. Each subtype is heterogeneous and twelve genetic loci have been identified. Five genes have been already cloned and characterized: USH1B, USH1C, USH1D, USH2A and USH3. We have recently identified and characterized the human USH2A and USH3 genes. Murine and rat orthologues of these genes were also identified. To identify the cellular origin of the USH2A and USH3 transcripts we visualized the distribution of the transcripts by in situ hybridization. The transcripts for USH2A are found in the rod and the cone cells of the outer nuclear layer. The USH3 transcripts were detected in the outer and inner nuclear layers. Laser capture microdissection (LCM) coupled with reverse transcription-polymerase chain reaction (RT-PCR) data confirmed the in situ results. Both USH2A and USH3 proteins are highly conserved in human, mouse and rat. Immunohistochemistry suggests that the USH2A protein is excreted into the interphotoreceptor cell matrix (IPM) of the retina. Antibodies against the USH2A protein specifically bound only to the apical surface of the retinal pigment epithelial cells. The USH3 protein was immunodetected in the cells of the outer nuclear layer of the retina.

521 Overexpression of Fibroblast Growth Factor (FGF2) in the Adult Mouse Protects Against Synaptic Degeneration in the Cochlear Nucleus Following Acoustic Overstimulation.

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FGF2 has been implicated in the early development of the cochlear nucleus in the fetal mouse, but there is relatively little of it expressed there postnatally. Previous experiments in the adult (C57/BxCBA/J) mouse cochlear nucleus demonstrated a loss of synaptophysin-stained synaptic endings within 7-14 days after noise damage to the cochlea, while FGF2 expression increased in astrocytes. On this basis we hypothesize that FGF2 upregulation represents a compensatory reaction on the part of cochlear nucleus cells to the degenerative changes. If so, one might prevent synaptic degeneration by providing FGF2 to the cochlear nucleus challenged by overstimulation. To test this hypothesis, we noise-exposed transgenic mice, in which the full-length cDNA for FGF2 is overexpressed and higher levels of the FGF2 protein might protect synaptic endings against damage. The wild type from the same strain and the FGF2 overexpressor heterozygous mice, aged 3-4 months, were exposed to 115 dB SPL for 6 hours. After 7 and 14 days' survival, the brains were fixed and the cochlear nucleus sections were immunostained for SV2 (a synaptic vesicle protein, used as a measure of the number of synaptic endings). The cochleograms of both the wild type and FGF2 overexpressor showed complete loss of outer hair cells and loss of inner hair cells restricted to the region of noise exposure. In the wild type mouse there was a loss of synaptic endings in the parts of

the ventral and dorsal cochlear nucleus corresponding to inner hair cell loss. In contrast, in the FGF2 overexpressor, there was minimal loss of SV2 staining. These findings suggest that FGF2 can protect the cochlear nucleus against the loss of synaptic endings in response to acoustic overstimulation.

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522 The Influence of One Copy of the Prestin Gene on Cochlear Anatomy and Physiology in 129S1 Mice

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Outer hair cells (OHC) undergo length changes resulting from voltage-dependent conformational changes in prestin, the recently discovered motor protein (Zheng et al., Nature 405, 149, 2000). This electromotility has been proposed as the mechanism of cochlear amplification in mammals. In order to test this proposal, targeted deletion of the prestin gene was attempted in 129S1 mice in collaboration with Xenogen Biosciences. Because homozygous mice died in utero, heterozygotes with only one copy of the prestin gene were compared with wildtype controls to determine if they displayed a similar phenotype. If auditory function is compromised in heterozygotes, then animals with only one copy of prestin would exhibit haploinsufficiency. In this project, compound action potential (CAP) threshold curves and CAP tuning curves at 12 kHz were acquired at the round window to determine if cochlear sensitivity and frequency selectivity were different in heterozygotes and wildtype mice. Following euthanasia, the animals were evaluated in three additional experiments. First, whole-cell, voltage clamp recordings were used to estimate the density of motor proteins from the maximum charge movement and to evaluate electromotility in dissociated OHCs. Second, prestin mRNA was assessed using RT-PCR; prestin protein, using immunocytochemistry. Finally, some animals were cardiac perfused to preserve the cochleae. Gross anatomy of the organ of Corti, including the measurement of OHC length, was determined using the hemicochlea technique to learn whether morphological changes occur in heterozygotes. Results from all experiments were collated to determine the influence of one versus two copies of the prestin gene on cochlear anatomy and physiology.

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523 Expression of a Dominant-Negative Connexin26 in Mice Causes Disorganization of Organ of Corti and Non-syndromic Deafness Please Replace With Your Title.

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Hereditary deafness affects about 1 in 2,000 children and mutations in the GJB2 gene, which encodes gap junction protein connexin26, are the major cause in various ethnic groups. However, the pathogenesis of deafness due to GJB2 mutations remains obscure. Mice with targeted disruption of the gene were embryonic lethal in a previous study. To elucidate the pathological role of connexin26 in the inner ear, we produced transgenic mice carrying a R75W mutation in the GJB2 gene, which was identified in a hereditary deafness pedigree and showed a deleterious dominant-negative effect.

The R75W+mice showed severe hearing loss from an early stage of development. Histological analysis of the mutants revealed hyperplasia of supporting cells, failure in the formation of the tunnel of Corti, and degeneration of sensory hair cells. Despite robust expression of the transgene, no obvious structural change was observed in the stria vascularis and spiral ligament that are rich in connexin26 and generate the endolymph. The high resting potential in cochlear endolymph essential for hair cell excitation was normally sustained.

These results indicate that the GJB2 mutation associated with sensorineural deafness affects the differentiation of supporting cells resulting in disorganization of the organ of Corti, rather than affecting endolymph homeostasis, in mice and probably in human.

524 Vestibular System Problems: Righting the Balance

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ARO members are developing new genetic and cellular therapeutic mechanisms for diagnosing and rehabilitating hearing loss. For decades they have had standard diagnostic tools to determine the degree of hearing loss, its cause and its epidemiology and extent in society. Yet this is manifestly not the case regarding vestibular disorders. Vestibular disorders are typically mis-diagnosed, and are often treated with clinical indifference or misunderstanding, leading to poor data acquisition that is required for epidemiological studies. The following speakers, with national reputations in their respective fields, will present and discuss with the whole ARO membership:

(i) The impact of vestibular disorders on an individual's life

(ii) The epidemiology of vestibular disorders

(iii) The problems facing vestibular researchers

(iv) The use of animal models in vestibular research

(v) The application of vestibular research in clinical practice.

This workshop also introduces the areas where new and innovative research for rehabilitating vestibular disorders is urgently required.

525 Living with Vestibular Disorders.

Susan Hickey vestibular patient, Legacy Clinical Research & Technology Center, Portland, OR

I first experienced balance problems in March 1996. It took almost two years -- and 10 medical specialties -- to receive a diagnosis. During this time, I struggled to function as the Chief Operating Officer of a large electric utility before accepting a disability retirement. For the last four years I have focused my efforts on treatment and recovery, studying vestibular problems through first hand experience.

In this session, I discuss the impacts of multiple balance problems including fistulas, hydrops and BPPN on my career and my daily life. This description includes symptoms, events or occurrences that cause symptoms and the resulting limits on my activities. Through these remarks I hope to interest researchers in developing improved diagnostic and rehabilitation tools so that future patients with vestibular disorders have a greater chance of full recovery.

526 The Epidemiology of Balance and Vestibular Disorders

Howard J. Hoffman, Daniel A. Sklare Epidemiology & Balance/Vestibular Sciences Program, Scientific Programs Branch, Division of Extramural Research, NIDCD, Bethesda, MD

A literature search reveals few epidemiologic investigations of disorders of balance/vestibular function. Over the past decade, NIDCD has collaborated with the National Center for Health Statistics to obtain prevalence estimates. Questions incorporated into the 1994/1995 Disability Supplement (DS) to the National Health Interview Survey (NHIS) have yielded prevalence estimates of chronic (3+ months) dizziness and imbalance by age, gender and race/ethnicity, as well as estimates of co-morbid conditions and risk factors. Currently underway, a second investigation involves interviewing and balance testing (modified Romberg) of adults aged >40 years, as part of the

National Health and Nutrition Examination Survey (NHANES), 1999-2004. We will present findings from the DS/NHIS and from the first two years of the NHANES questionnaire.

527 Problems Facing Vestibular Researchers (Emphasis on Clinical Application).

F. Owen Black Neurotology Research, Legacy Clinical Research and Technology Center, Portland, OR

The list of impediments to the growth and development of vestibular scientists capable of addressing clinical problems is formidable:

1) no educational "critical path" or generally accepted "pipeline" model,

2) multi-disciplinary skills required from both basic science and clinical disciplines,

3) no certification standards or requirements,

4) no coherent mechanism to support capable and motivated students,

5) small, geographically dispersed community ("critical mass") of peers and

6) funding sources disparate and difficult to access.

Vestibular researchers are also hampered because of the expensive equipment and technical support required to conduct key research projects.

528 The use of animal models in vestibular research.

Galen David Kaufman Otolaryngology, University of Texas Medical Branch, Galveston, TX

The phylogenetic primacy of a vestibular system means that even far-flung animal models can teach us useful things about how an organism detects self position and motion. Especially at the level of transduction and neuronal plasticity, many details of vestibular organization likely remain similar between species. These fundamentals then become shaped to serve the wide behavioral diversity seen between species (e.g. herbivore vs predator). Using the relatively adjacent comparison of rodent with human, Dr. Kaufman will discuss a few examples of how findings in one species pointed towards questions or protocols in another.

529 Advances in Application of Vestibular Research in Clinical Practice

Llyod B. Minor Depts. of Otolaryngology--Head & Neck Surgery, Biomedical Engineering, and Neurosci, The Johns Hopkins University, Baltimore, MD

The anatomy and physiology of the vestibular receptors in the inner ear are remarkably similar across vertebrate species. Information from these receptors is used to control reflexes that enable us to maintain our balance and to keep our gaze steady during head movements. The organization and control of these reflexes have been subjects of extensive basic research. The principles gleaned from these studies are providing a basis for diagnosing and treating many vestibular disorders.

The identification of superior canal dehiscence syndrome and development of an effective surgical treatment provide examples of the application of basic research to clinical problems. Patients with this disorder develop vertigo in response to loud noises and/or to changes in middle ear or intracranial pressure. Analysis of the eye movements evoked by these stimuli provided the basis for localizing the abnormality. Other areas where advances in basic research are having an impact on clinical problems include studies of vestibular adaptation and compensation following injury to the inner ear, the effects of ototoxic drugs on the vestibular system, and the responses of vestibular nerve fibers to electrical stimulation.

530 Functional Subdivisions within Human Auditory Cortex : An Overview

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As in other mammals, anatomical studies suggest that the human auditory cortex consists of a primary 'core' surrounded by non-primary regions, each with multiple subdivisions. A goal of human neuroimaging studies has been to determine the function of these various cortical regions. Although, neurophysiological studies in non-human primates have concentrated on determining the response properties of the core, those in non-primary areas suggest that posterior fields show a greater sensitivity to sound location and anterior fields show a greater sensitivity to con-specific calls. Human neuroimaging studies probing the functional organisation of the auditory cortex have used sounds that vary in frequency, sound level, pitch, bandwidth, spectro-temporal modulation and position. Irrespective of the type of sound presented, sound-induced activation often occupies a common region on the supratemporal plane. Nevertheless, broad subdivisions can be determined from the spatial segregation of response sensitivity. The primary auditory cortex (located on the medial part of Heschl's gyrus) appears to be more sensitive to variations in frequency and sound level than do the non-primary areas. Both primary and non-primary areas appear to be tonotopically organised, but the number and orientations of non-primary tonotopic fields is uncertain. While modulations in amplitude or frequency particularly engage posterior non-primary fields on the planum temporale, with a lateral focus, sound position or movement also engage the planum temporale, but with a more medial focus, and may favour the right hemisphere. Clearly, a gross outline of the functional organisation of the human auditory cortex is emerging, but we still have a poor understanding of any fine-grained subdivisions. Greater progress in describing the functional architecture might be achieved by distinguishing the response patterns to a range of acoustic cues within individuals, rather than across groups, and by measuring temporal dynamics of the response.

531 Structural and Functional Parcellation of the Auditory Cortex – a Historical Review.

Edward Jones The Center for Neuroscience, University of California, Davis, Davis, CA

The extent of neocortex devoted to auditory function, particularly in primates, is second only to that associated with the visual system. Current research based on functional imaging, single and multiunit recording, connection tracing and chemical neuroanatomy is beginning to reveal many new details that permit a finer functional parcellation of this large expanse of cortex.

This review traces the history of the auditory cortex, commencing with the ablation studies of Munk and Ferrier, and follows the progressive refinement of knowledge that came about as the result of application of techniques of progressively higher resolution.

Cyto- and myeloarchitectonic studies of Campbell, Brodmann, Flechsig, and the Vogts first located the auditory koniocortex in primates and Cajal presented details of its cellular structure that have still not been superseded. Connections with the medial geniculate body were initially defined by Le Gros Clark and Walker using retrograde degeneration techniques and by Poliak and others using the Marchi technique.

Modern parcellations commenced with the correlative evoked potential and anatomical studies of Rose and Woolsey, which revealed the presence of multiple, tonotopically organized cortical areas having differential connections with the nuclei of the medial geniculate complex. These studies set the stage for all subsequent analyses. Recent studies have tended to follow this tradition, although achieving higher resolution as the result of application of single and multiunit techniques and more sensitive anatomical tracing techniques, which extended the study of connections to cortico-cortical and corticofugal pathways.

Most recently, histochemical and immunocytochemical staining patterns have permitted parcellations of the auditory regions that extend earlier schemes of subdivision. These newer parcellations are meaningful in revealing different cortical termini of parallel auditory pathways running through the medial geniculate complex and provide an increasingly valuable basis for the interpretation of functional imaging studies on auditory perception.

532 Organization of Primate Auditory Cortex: Refinement of the Model

Troy Alan Hackett Hearing & Speech Sciences, Vanderbilt University, Nashville, TN

Current models of auditory cortical organization in monkeys are anchored by a central *core* region with structural and functional characteristics of primary auditory cortex. Flanking the core on its caudal, lateral, and medial borders is a ring of fields known as the *belt*. These fields have topographic connections with the core, and also with the *parabelt* region bordering the lateral belt fields on the superior temporal gyrus. Rostral and caudal divisions of the lateral belt and parabelt target functionally-distinct domains in temporal, frontal, and parietal cortex, consistent with models of stream segregation in auditory cortex. Architectonic studies indicate that homologues of the core region exist in other primates, including chimpanzees and humans. The belt and parabelt regions may also have homologous counterparts in other primates, but the available data vary greatly with respect to this issue. Compared with the *lateral belt* region, the organization of the *medial belt* region is not well defined. The results of ongoing studies of fields in the medial belt are consistent with their inclusion in auditory cortex, but suggest that they subserve different roles than areas in the lateral belt. Thus, four distinct regions comprise auditory cortex in primates. This is consistent with classical architectonic and modern studies of structure and function in the superior temporal region of nonhuman primates and humans.

533 Temporal Processing in the Primate Auditory Cortex

Xiaoqin Wang Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD

Temporally modulated sounds are fundamental components of human speech and animal communication sounds. It is known that auditory cortical neurons have much more limited stimulus-synchronized responses compared to neurons of the auditory-nerve and subcortical nuclei. How the auditory cortex represents rapidly occurring, but still perceptible, stimuli has long puzzled neurophysiologists. Recent findings from our laboratory show that there are two largely distinct populations of neurons in the auditory cortex of awake primates: one with stimulus-synchronized discharges that represents slowly occurring sound sequences explicitly with a temporal code, and the other with non-stimulus-synchronized discharges that represents rapidly occurring acoustic events implicitly with a rate code. Both populations of cortical neurons transform acoustic transients within a temporal integration window into a discharge rate-based representation. Our results suggest that the combination of temporal and rate codes in the auditory cortex provides a possible neural basis for the wide perceptual range of temporally modulated sounds. The significant reduction in the maximum stimulus-synchronization rate of auditory cortical neurons compared to subcortical neurons and the accompanying temporal-to-rate transformations in the auditory cortex have an important functional implication. They suggest that cortical processing of sound streams operates on a "segment-by-segment" basis, rather than on a "moment-by-moment" basis as found in the auditory periphery. This is necessary for more complex stimulus processing and representation, which requires a longer integration window around the point of interest. The slow-down of the temporal response rate in the auditory cortex would allow rapidly occurring auditory information to be integrated in the cerebral cortex with information from other sensory modalities that have intrinsically slower sensory receptors.

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534 Emergent Cortical Representations of Spatiotemporally Complex

Michael M. Merzenich Keck Center for Integrative Neurosciences, University of California, San Francisco, CA

Studies of developmental and post-critical period plasticity conducted in the auditory cortex of rodents and primates (including humans) have provided us with a new level of understanding of the origins of complex signal processing and processing specialization in the mammalian forebrain. Cortical processing is initially shaped by exposure-dependent plasticity limited to an early "critical period" of development. As in the visual system, progressive exposure-dependent plasticity leads to the maturation of that modulatory control systems that "gate" plasticity and limit it to a behavioral context in older brains. In such a behavioral (alert, attending, rewarding, punishing) context – but no longer with passive exposure to environmental stimuli - adult plasticity is powerfully expressed separately or inseparably in the cortex, in spectral, temporal, and intensive parametric domains. These plasticity studies demonstrate that the processing machinery of the mammalian forebrain establishes its initial representational machinery to reflect a primary specialization for the sounds to which the infant animal is exposed. Auditory cortical systems subsequently refine cortical processing machinery, as a function of the spectral, temporal, intensive, and abstracted spatial parameters of behaviorally important stimuli. Some of the theoretical and practical implications of these studies will be discussed.

535 Tonotopic Maps in the Human Auditory Cortex

Elia Formisano Dept. of Cognitive Neuroscience, Universiteit Maastricht, Maastricht, Netherlands

The decomposition of a complex sound into its frequency components is the first processing stage of hearing. It is believed that cortical neurons that respond selectively with respect to the spectral content of a sound form one or more map in which neighboring patches on the cortical surface respond to similar frequencies (tonotopic maps).

In a number of species, the existence of such tonotopic maps in the primary auditory cortex (AC) has been demonstrated using various invasive methods.

In humans, tonotopy in AC has been investigated non-invasively using electro- and magneto-encephalography (EEG, MEG), positron emission tomography (PET) and functional magnetic resonance imaging (fMRI). These studies have provided evidence for the presence of one or multiple systematic representations of frequency in temporal regions, but only coarse and inconclusive information on their cortical topography. Even fMRI studies at relatively high-spatial resolution have failed to obtain detailed topographical representations of frequency-selective responses. In particular, fMRI studies have been less successful in the auditory than in other sensory domains. In the visual domain, for example, fMRI studies have succeeded in defining the retinotopic layout and the borders of early human visual areas.

In the present talk, I will review functional neuroimaging investigations of tonotopy in human AC. I will then illustrate a recent fMRI study at ultra-high magnetic field (7 Tesla), in which novel imaging techniques in combination with a silent, event-related protocol and a cortical surface-based analysis of functional data were used to delineate the detailed topography of frequency-selective responses in human primary AC. Results demonstrate the existence of two tonotopic maps in two adjacent subdivisions of PAC. These two maps are mirror-symmetric and clearly resemble those of presumably homologue fields in the macaque monkey (AI and R).

536 A Functional Parcellation of the Auditory Cortex in Humans Using fMRI

*André Brechmann Non-Invasive Imaging, Leibniz-Institute for Neurobiology, Magdeburg, Germany

At present the only potential correlates of different auditory cortex fields in fMRI studies are multiple foci of activation. But in view of the large gross-anatomical variability of the human temporal lobe and its variable topographic relation to the rest of the brain it is a major problem to establish identity of such activation foci across individuals. Nevertheless, there are some anatomical landmarks which are comparable across induviduals, namely the first transverse sulcus and Heschl's sulcus along which we find stripe-like clusters of fMRI activation. By using mainly these landmarks we established a framework of territories which resembles the stripe-like organization of core, belt and parabelt areas of the non-human primate auditory cortex.

This topographic framework of territories was used to study the representation of linearly frequency modulated tones (FM) with a low-noise FLASH-sequence. In several experiments FM were varied in frequency range, duration, level or ear of presentation and tested during different tasks (passive listening, categorization of FM-direction (up vs. down) and of FM-duration (short vs. long).

Results show that the territories can be distinguished in terms of their level-dependent activation and their response to different types of tasks using the same set of FM. Furthermore, the results of all experiments clearly point to a specialization of the right auditory cortex for processing of the direction of frequency modulated tones.

In conclusion, analyzing fMRI activation in each individual brain is a feasible approach towards a functional parcellation of the human auditory cortex which has to be refined in future studies.

537 Functional Organization of Human Cerebral Cortex Involved in Hearing and Speech

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Fields on human temporal, frontal and parietal cortex are involved in processing complex sound, including speech. To better understand the locations, organizations and interconnections of these cortical fields we carry out experiments involving direct cortical recording and electrical stimulation in patients undergoing surgical treatment for medically intractable epilepsy. Multicontact subdural recording arrays are placed over the surface of the left or right cerebral hemisphere; multicontact modified depth electrodes are inserted into Heschl's gyrus (HG). Intraoperative photographs, MRI and x-ray aid electrode localization. Electrode placements are based on the clinical considerations. (protocols were approved by the Univ. Iowa Human Subjects Review Board). Sounds are delivered via insert earphones. A tonotopically organized primary field (AI) is localized to mesial HG. Functional properties of AI distinguish it from fields adjacent fields and to a field on the posterolateral surface of the superior temporal gyrus (field PLST). PLST, which overlaps the classic Wernicke area, responds robustly to a wide range of simple and complex sound, including speech sounds. Functional connections between acoustically mapped fields are studied by electrically stimulating selected sites (single, imperceptible, bipolar, charge balanced, 0.2 ms pulses) while recording from other electrode contacts. AI and PLST exhibit reciprocal and complex functional connections. Stimulation of PLST activates cortex of the inferior frontal gyrus, a region presumably included in the classic Broca area. Results provide evidence for cortical fields and their functional interconnections underlying speech that were postulated more than a century ago.

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538 Inhibition of Inner Ear Morphogenesis by Sprouty Overexpression

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Coupling of positive regulatory signals with negative feedback modulators is a mechanism of regulation of embryonic development. Sprouties are cell membrane-anchored proteins that act as feedback inhibitors of FGF/FGFR signaling, seemingly by inhibiting the Ras/MAPK pathway. Sprouties were first identified in Drosophila and later their homologues were characterized in chicks and mammals. To date, four mammalian Sprouty genes have been identified. Overexpression of Sprouties has been shown to cause organ phenotypes that resemble those of loss or reduction of function of FGFs or FGFRs. We have previously shown that FGF10/FGFR2(IIIb) signaling regulates early inner ear morphogenesis, specifically stimulating budding morphogenesis at the otocyst stage (Pirvola et al., J. Neurosci, 2000). Motivated by the observations that Sprouties are expressed in the embryonic inner ear, we have now investigated whether they can regulate morphogenesis of this organ. As a model system we have used transgenic mice in which human Sprouty 2(hSpry2) is misexpressed under Pax2 upstream region. Pax2 is prominently expressed in the early-developing otic epithelium. Ectopic hSpry2 expression caused a severely malformed inner ear phenotype. The otic vesicle was formed, but further outgrowth of the semicircular canals and cochlear duct was impaired. Compartmentalization at the level of saccule and utricle was also perturbed. Some of the hSpry2 mutants survive until adulthood. These mutants showed a behavioural phenotype that was consistent with the structural alterations seen in the mutant inner ears. Because of similarities in the inner ear phenotype of hSpry2 overexpression mice and Fgfr2(IIIb) and Fgf10 null mutant mice, and because endogenous Sprouties are expressed in the otocyst, our data suggest that Sprouties modulate budding morphogenesis of the inner ear. We are further investigating the mechanisms by which these genes cause the defects in inner ear development.

539 A Role for FGF Signaling During Commitment and Differentiation in the Organ of Corti

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The Fibroblast Growth Factor (FGF) signaling pathway is involved in the regulation of cell growth, commitment, differentiation, and patterning throughout embryonic development. Here we characterize a specific role for FGF signaling during inner ear organogenesis. Deletion of FGF Receptor 3 (FGFR3) from the sensory epithelium of the mammalian cochlea has been shown to disrupt the development of pillar cells (PCs). To further examine the effects of FGFR3, the receptor was inhibited in embryonic cochlear explant cultures using SU5402, an FGFR antagonist, or a soluble form of FGFR3 that acts by binding to endogenous ligand(s). Receptor inhibition resulted in a reversible suspension of PC development that could be resumed upon removal of the antagonist. In contrast, when FGFR3 was activated beyond physiological levels by exogenously applied FGFs (FGF2 or FGF17), a dramatic increase in the number of cells that assumed a PC fate was observed, as evidenced by an increased expression of the PC marker p75-lntr. The additional pillar cells appeared to be derived from a large pool of FGFR3-positive precursor cells located adjacent to the developing inner hair cells within the sensory epithelium. During normal development, subsets of these cells presumably develop as PCs, outer hair cells, Deiter's cells and Hensen's cells. In contrast, in FGF17-treated explants markers for Deiter's cells and outer hair cells were absent but inner hair cell and pillar cell markers remained. Therefore, it seems likely that a significant percentage of the precursor population may initially be competent to develop as PCs, but the availability of

FGFs within the epithelium ultimately limits the number of FGFR3-positive cells that will assume a pillar cell fate.

540 The FGF Antagonist Sef is Expressed in the Auditory System and May be Required for Hearing.

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Using gene trap mutagenesis, we have generated a battery of mice with mutations in genes that encode secreted and transmembrane proteins. Integration of a DNA vector mutates the gene and inserts a marker, β-galactosidase, which labels the cells that express the trapped gene. In the *KST223* line of mice, β-gal activity was detected in the E9.5 otic vesicle, with expression restricted to the ventral half by E11.5, mostly in the non-sensory epithelium of the developing cochlea. The inferior colliculus (E11.5, P6) and cochlear nuclei were also labeled (P6).

KST223 homozygotes show diminished Preyer reflexes by 8 weeks. We are recording Auditory Brainstem Responses (ABR) to better understand the nature of the deficit. Our preliminary analysis of 12-14 week old animals indicates that homozygotes (n=3) do not show significantly different thresholds or latencies from heterozygotes (n=5) or wild type controls (n=5). However, especially at 60-80 dB, the amplitudes of the first and second ABR peaks in mutants are strikingly lower than those of wild type animals, with heterozygotes exhibiting an intermediate effect. We are extending analysis of ABRs at this and other ages in order to better understand and quantify this difference.

We cloned the *KST223* gene trap insertion site by 5' RACE, and found that it encodes mouse Sef (for Similar Expression to FGFs), a novel protein that is hypothesized by others to act as an FGF pathway antagonist. We are now using standard histological techniques to look for defects in regions of Sef expression: the ear, cochlear nuclei and inferior colliculus. Based on its proposed role in FGF signaling, it is possible that the *KST223/Sef* mutant phenotype is due to changes in the proliferation or survival of cells in any of these regions.

541 Fgf3 and Fgf8 Dependent and Independent Transcription Factors Are Required for Otic Placode Specification

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Previous studies suggested that competent ectodermal cells respond to signals from adjacent tissues to form the otic placode and inner ear in vertebrates. Fibroblast growth factor (Fgf) family and Distal-less (Dlx) family members have been implicated in this signal-response pathway. To study the roles of these factors in the otic development, we used mutations and morpholino anti-sense oligonucleotides to compromise their functions in zebrafish embryos. We show that compromising Fgf3 and Fgf8 signaling blocks ear development; only a few scattered otic cells form. Removal of *dlx3b*, *dlx4b* and *sox9a* genes together also blocks ear development, although a few residual cells form an otic epithelium. Combined loss of Fgf signaling and the three transcription factors completely eliminate all indications of otic cells. Expression of *sox9a*, but not *dlx3b* or *dlx4b*, depends on Fgf3 and Fgf8. Our results provide evidence for Fgf3-Fgf8 dependent and independent genetic pathways for otic specification and suggest that Fgf3-Fgf8 function induces both the otic placode and the epithelium.

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542 Fibroblast Growth Factors Promote Canal Development in the Chicken Inner Ear by Inducing Bmp2 Expression.

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The vestibular apparatus that is responsible for sensing angular acceleration consists of three semicircular canals and their sensory organs, the cristae. In the chicken inner ear, the anterior and posterior canals are derived from an epithelial outpouch in the dorsal region of the otocyst starting at embryonic day 3 (E3). Over time, two regions in the center of the canal outpouch undergo resorption, leaving behind two tube-like structures in the margin of the canal pouch, the anterior and posterior canals that are joined together by the common crus. The molecular mechanisms required for the specification of the canals and the common crus are not clear. Using a recombinant avian retrovirus encoding Fgf3 (RCAS-Fgf3), or beads soaked with FGF2, we show that FGFs promoted canal development and disrupted the formation of the common crus in the chicken inner ear. FGFs mediated these effects by inducing Bmp2 expression, which has been postulated to be important for the canal pouch outgrowth. The upregulation of Bmp2 and 7 in the dorsal region of the canal pouch, in particular, disrupted the formation of the common crus. In support of this hypothesis, treatments with Noggin, an antagonist of BMPs, rescued the loss of common crus induced by FGF2. Furthermore, depending on the stage of development, the loss of FGF functions in response to the application of the FGFR1 inhibitor, SU5402, resulted in disruption of canal formation or induction of an ectopic common crus-like structure. Together, these results suggest that FGFs by inducing BMPs, play important roles in specifying and regulating normal canal development.

543 Expression of Mouse Fibroblast Growth Factors and Fibroblast Growth Factor Receptors During Early Inner Ear Development

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The inner ear, which contains the sensory organs specialised for audition and balance, develops from ectoderm adjacent to the developing hindbrain. This tissue is first apparent morphologically in the mouse when it thickens at the 7-8 somite stage in the vicinity of rhombomeres 5 and 6 to form the otic placode. The placode subsequently invaginates during the 13-20 somite stage and forms a closed vesicle by 21-29 somites (Kiernan et al., 2002). The otic epithelium then initiates cellular differentiation and morphogenesis, which ultimately results in the exquisitely complex inner ear. Transplantation studies have established that the region of surface ectoderm that can form an otic vesicle is initially quite large. As development proceeds, the region of otic competency becomes progressively restricted and the placodal tissue adjacent to the hindbrain becomes specified for an otic fate. Tissue grafting and recombination experiments suggest that placodal development is directed by signals arising from the underlying mesoderm and adjacent neurectoderm. Several intercellular signalling molecules, including the fibroblast growth factors (FGFs) are involved in these processes.

We have previously analysed the roles of Fgf-3, Fgf-8 and Fgf-10 in inner ear development. To determine the potential roles of other Fgf family members, we have examined the expression patterns of the remaining 19 members as well as members of the Fgf receptor family from embryonic day 8 – 9.5. To date, we have identified four members of the Fgf family that are expressed in tissues relevant to inner ear development and whose role in these processes has not been determined. In addition, we have examined inner ear development in mice that lack *Fgf-15*, the putative homologue to chick *Fgf-19*. We will present evidence that *Fgf-15* and *Fgf-19* play different roles in otic

development as well as data showing that additional members of the Fgf family may play a role in mouse inner ear development.

544 A Network of Fibroblast Growth Factors in the Induction of the Inner Ear.

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The inner ear is derived from the otic placode, a thickened ectodermally derived disc induced from the non-neural ectoderm, between stages 7 to 9. Recently we have shown in the chick embryo that the intersection of localized fibroblast growth factor signaling, provided by fgf-19, with wnt signals, provided by wnt-8c, constitutes the molecular nature of this induction. We now compared the expression of *fgf-3* and *fgf-8* in the chick embryo, with that of *fgf-19*, as both are expressed early enough to play a role in inner ear induction, and both have been shown to have some function in the development of the inner ear. We wished to order these molecules into a hierarchy of inductive interactions leading to the formation of the chick otic placode. We show that the endoderm fated to underlie the caudal cephalic paraxial mesoderm plays a role in otic induction and we hypothesize (and provide evidence of its sufficiency) that this role is fulfilled by *fgf-8*. Fgf-8 in the endoderm may act as a trigger, initiating both *fgf-19* and *fgf-3* expression in the paraxial mesoderm, allowing the otogenesis to occur.

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545 A Transcription Factor Gene Expression Profile of Regenerating Chick Cochlea and Utricle Sensory Epithelia.

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The ears of many nonmammalian vertebrates have the ability to regenerate sensory hair cells. In order to characterize the signaling pathways that regulate this process, we have analyzed temporal changes in gene expression in chick cochleae and utricles during the early stages of regeneration in vitro. We employed a unique, custom built oligonucleotide microarray that interrogates the expression of >1600 human transcription factor (TF) genes. By analyzing multiple samples and hybridizations we have obtained statistically robust profiles of the patterns of TF genes expressed after hair cell injury and during the onset of regeneration. Hair cells in explanted sensory organs were damaged by incubation in neomycin. Cultured epithelia from the utricle were injured by laser microbeam. Samples were isolated and TF profiled at various timepoints after injury (30 mins-48 hours). Comparisons across the two treatment timecourses for utricle epithelia revealed a set of 113 TFs that significantly changed as hair cells recovered. Among the genes showing the most dramatic changes were TCFL1, PAF65A, BCL11B, DEAF1, SHH and CEBPG. A set of 36 TFs showed similar patterns of expression in the regenerating cochlea and utricle. These include LAF4, HSF1, HMX2, MSC, EEF1A1 and BAT1. This TF profile should lead to development of new molecular markers for the study of hair cell development and regeneration, as well as identification of the transcriptional pathways that are important for these processes.

546 Viral-Mediated Transgene Expression In Non-Sensory Cochlear Epithelial Cells

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The need for clinical intervention in the cochlea usually arises from hearing impairment due to hair cell (HC) loss. The future ability to regenerate HCs depends on genetic manipulations of cells that survive in the sensory epithelium after HCs are lost, namely, the non-sensory cells. To manipulate gene expression in the non-sensory cells in vivo, it is necessary to introduce genetic material into these cells. The purpose of this study was to examine the feasibility of virus-mediated gene transfer into the organ of Corti (OOC) via the scala media. Guinea pigs were anesthetized and the scala media of their left ears (3rd cochlear turn) inoculated with 5 mL of an adenovirus vector with a reporter gene insert (Ad.LacZ, 1011 viral particles/mL). Control animals were inoculated with artificial endolymph. Animals were sacrificed 5 days after surgery, and the temporal bones were removed, decalcified, cryo-sectioned at the mid-modiolar plane and processed for transgene detection using antibodies to b-galactosidase. The mechanical trauma associated with violating the scala media resulted in severe HC loss, especially near the site of inoculation. Transgene expression in experimental animals was detected in all supporting cells in the OOC as well as non-sensory epithelial cells in the inner sulcus and interdental cell areas. No transgene expression was found in control animals. In conclusion, we demonstrate that inoculation of Ad.LacZ into the scala media of the guinea pig inner ear leads to transduction of several types of non-sensory cells in and around the OOC. The ability to successfully deliver transgenes into these cells, combined with a better understanding of the genetic control of HC differentiation, will allow us to attempt a phenotypic conversion of supporting cells for replacing lost HCs.

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547 Development of a Knock-In Model for Mammalian Hair Cell Regeneration

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We propose to test the hypothesis that mammalian hair cell (HC) regeneration is possible if mature HCs or supporting cells (SC) are induced to reexpress a critically important growth factor, bone morphogenetic protein 4 (BMP4). BMP4 is hypothesized to initiate an expression cascade critical for HC development and regeneration. Since the characterization of SC-specific promoters is not sufficiently far along to allow their use, we have chosen the pTetOn system (Clontech) to express genes downstream of the HC- specific promoter Brn3.1 (Erkman et al, 1996). The Brn3.1 promoter-gene cassette was inserted into the pTRE2 vector that contains the tetracycline response element (TRE), which is activated by the reverse Tet repressor (rTetR) encoded in the pTetOn vector. When a cell line derived from the Eday9 (p.c.) Immortomouse inner ear (2D2) containing pTetOn is exposed to the Tet analog doxycycline (dox), rTetR is expressed, which then activates TRE allowing expression of genes downstream. 2D2 cells express HC markers Brn3.1, alpha-10 AChR, myosin 7A, the receptors BRK-1 and BRK-3 & chordin, noggin, jagged-1 & -2 and notch-1. 2D2 cells express green fluorescent protein under control of the Brn3.1 promoter. 2D2 cells stably transformed with pTetOn and transiently transfected with lucifersase/pTRE show a 6.8 fold induction when stimulated with dox (228-fold when the plasmid encoding tet silencer (pTS) is included). We have recently cloned myc-tagged BMP4 into the pTRE2 vector and transiently transfected 2D2/pTetOn cells. Myc-tagged BMP4 is being analyzed by Western blot for induction by dox. We are also analyzing Brn3.1 myc BMP4 constructs in the pTRE2 vector, prior to producing transgenic mice, which will be tested for their ability to regenerate a sensory epithelium.

548 The Transcription Factor GATA3 Identifies the Position of the Striola During Sensory Regeneration the Avian Utricle

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Members of the GATA family of transcription factors have diverse roles in the development of blood, heart, smooth muscle, and ectoderm. Previous studies have suggested a role for GATA3 in inner ear development (Rivolta and Holley, 1998; Karis et al., 2001; Lawoko-Kerali et al., 2002; Hawkins et al., this meeting), and haplo-insufficiency for GATA3 leads to syndromic hearing loss in humans (Van Esch et al., 2000). We have used conventional immunocytochemistry to characterize GATA3 expression in the otolithic maculae of chicks. In the utricle and lagena, GATA3 expression is confined to a 6-8 cell-wide region that is centered in the reversal zone at the center of the striola. This expression pattern appeared unchanged between post-hatch days 5-60. Treatment of cultured utricles for 24 hours in 2 mM streptomycin (which kills nearly all hair cells within the sensory epithelium) had no observable effect on the size or location of the GATA3 region.

In order to determine the possible influence of the extracellular matrix (ECM) on GATA3 expression, we used thermolysin to remove entire sensory epithelia from utricles and allowed them to attach to uniform fibronectin substrates. The sensory epithelia were maintained in culture for five days, and were then fixed and processed for GATA3 immunoreactivity. The size and configuration of the GATA3 region appeared unaffected, suggesting that contact with the native ECM is not required to maintain proper GATA3 patterning. In other experiments, we treated epithelial cultures for five days with either retinoic acid, TGF-β1 , or Sonic Hedgehog. None of these factors lead to any detectable change in the pattern of GATA3 expression.

These data suggest that GATA3 expression is regulated by factors intrinsic to the sensory epithelium. In addition, GATA3 expression may serve as a 'marker' for the position of the striolar region during hair cell regeneration.

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549 $P27_{kip1}$ and Ath-1 Immunoreactivity During Recovery of the Newt Inner Ear Sensory Epithelium

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Hair cells of the mammalian auditory system once lost, through ototoxic agents, ageing, or noise-induced damage, are not replaced. The mammalian vestibular organs exhibit a limited capacity to regenerate. Comprehensive investigations into recovery have centred on using the bird as an animal model. As an alternative we have developed a culture system using explants from newt, a urodele amphibian. Urodeles exhibit a remarkable capacity to regenerate a variety of tissues. Studies of other amphibia have suggested both non-mitotic production of hair cells from supporting cells and repair of sub-lethally damaged hair cells.

Exposure of cultured explants to gentamicin leads to total ablation of all hair cells within the saccule. Hair cells die by apoptosis and the dying cell bodies are taken up by supporting cells. Following hair cell ablation new hair bundles are apparent by 12 days post-treatment. Incubation with BrdU, a synthetic thymidine analogue, reveals that a proliferative response amongst supporting cells is generated specifically in areas of hair cell loss. This suggests cell death promotes the response and that the signal initiating proliferation appears to be highly localised. The pattern of expression of proteins that control cell cycle re-entry in the inner ear are also being assessed. $P27_{kip1}$, an inhibitor of cyclin dependent kinases is up-regulated when progenitor cells withdraw from the cell cycle. Immunohistochemistry has been used to investigate the expression of $p27_{kip1}$ during the recovery period following hair cell ablation. $P27_{kip1}$ does not appear to be down-regulated. In addition we have looked at Ath-1(atonal homologue 1) expression, a hair cell fate determinant, expressed following terminal mitosis during development. Ath-1was found to be present in hair cells prior to damage but not apparent in the hair cells of recovered sensory epithelia.

Supported by Defeating Deafness

550 Spontaneous Formation and Retraction of Microvilli in Living Cells Observed by Scanning Ion Conductance Microscopy

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Scanning ion conductance microscopy (SICM) allows visualization of the surface of living cells with a resolution about one order of magnitude better than optical microscopy (~50 nm). We used SICM to make time-lapse observations of the surfaces of various living cultured cells, both epithelial and non-epithelial, as well as the apical surfaces of the supporting cells (inner and outer phalangeal cells, Hensen's cells) in cultured organs of Corti of three-day-old mice. We found that short (<500 nm) microvilli covering the surfaces of these cells are dynamic structures, spontaneously forming, retracting, and re-arranging. The majority of them undergo life cycle of around 12 min: fast formation/assembly (5 nm/s), steady state, and relatively slow retraction/disassembly (1.2 nm/s). We hypothesized that these short, presumably non-specialized, microvilli are generally not static but rather dynamic cellular organelles. The observed dynamics implies continuous assembly, disassembly or utilization, and lateral movement of the characteristic central core of microvilli. This core presumably consists of parallel actin filaments densely packed by cross-linking actin-binding proteins. Bundle-based actin structures have been previously considered as relatively stable, in contrast to actin network structures like filopodia, pseudopodia or lamellipodia. It has also been presumed that the extensive dynamic changes of microvilli in development are initiated by specific signals. In contrast, our results suggest that the cell may use the intrinsic dynamic properties of microvilli to build complex cytoskeletal structures. Several features of stereocilia genesis, i.e. the spontaneous formation of microvilli on the surface of hair cells, lateral re-arrangement of microvilli, and selective re-absorption of microvilli (Tilney LG, Cotanche DA & Tilney MS, Development, 116:213-226, 1992), could be related to the microvillar dynamics observed in our experiments.

551 Gap Junctional Communication in the Avian Inner Ear: A Potential Role During Hair Cell Regeneration

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Gap junctional intercellular communication (GJIC), known to play an important role in maintaining homeostasis and regulating cell growth, differentiation, and wound healing, has been characterised by fluorescence recovery after photobleaching (FRAP). Photobleaching experiments were carried out on organotypic cultures of the basilar papilla and utricle of chicken hatchlings (E21). The epithelia, comprising live hair cells and supporting cells, were loaded with calcein AM. The 488-nm line of a 100mW argon ion laser of a confocal laser-scanning microscope was used for bleaching and excitation. An area of 50 μm^2 was bleached through the entire depth of the tissue with maximum laser power. The redistribution of calcein-molecules was subsequently monitored over 5 min by an attenuated laser beam. During this period, the fluorescence recovered only in supporting cells but not in hair cells. Treatment of the tissue with carbenoxolone (CBX), a pharmacological blocker of GJIC, inhibited fluorescence recovery in supporting cells. A unidirectional flow of calcein from the abneural to the neural edge of the basilar papilla was observed, which indicates a rectifying gap junction population in supporting cells.

To determine whether gap junctions play a role during hair cell regeneration, we examined the effects of a disrupted GJIC on the proliferation of supporting cells in response to hair cell death. Cultures of the basilar papilla and utricle were exposed to gentamicin to induce hair cell loss, and subsequently incubated for 3, 5 and 7 days in media containing bromodeoxyuridine (BrdU) and either CBX, or its inactive analogue glycyrrhizic acid (GZA). CBX-treated tissue showed a marked reduction of BrdU labelled nuclei at all time points, compared to their controls. These results point to a role for GJIC in the regulation of supporting cell proliferation.

Supported by Defeating Deafness

552 Laser Lesioning of Chick Vestibular Epithelial Cultures Triggers Localised MAPK Phosphorylation and Proliferative Activity

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Damage to avian hair cells triggers the proliferation of nearby supporting cells. In order to study the signalling events that stimulate proliferation in hair cell epithelia we have adapted a culture preparation using thermolysin-treated utricular epithelia (Warchol, *J. Neurosci.*, 22, 2607, 2002). Epithelia were used within 4 days of dissection to maintain organotypic structure. A laser ablation system attached to the microscope allowed control of the timing and magnitude of damage. An antibody was used to label the dually phosphorylated form of extracellular signal-related kinases 1 and 2 ($MAPK^{erk1/2}$). BrdU was used to assess the number of cells in s-phase. Ten minutes after laser lesioning $MAPK^{erk1/2}$ phosphorylation was observed in cells close to the lesion. By 30 minutes cells more distal to the lesion were labelled. This pattern suggests that $MAPK^{erk1/2}$ phosphorylation spreads out from the lesion site in a time-dependent manner. S-phase activity was analysed over the 6 to 26 hr time period after laser ablation using 2 hr BrdU pulses prior to fixation. At least 4 epithelia were used for each time point. BrdU-positive nuclei were counted within a 120 μm radius circle centred over the lesion site. The temporal data suggests that laser ablation triggers two waves of s-phase entry occurring 12 and 24 hours after damage, with increases of 270 and 380% over control levels respectively. Further analysis using antibodies to phospho-histone 3 is in progress to confirm this data. Additional spatial analysis shows that s-phase entry is delayed in cells proximal to the lesion site compared to more distal cells. We suggest that the initial and primary function of cells close to the lesion is repair of the wound, thus delaying proliferation in those cells.

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553 Perceptual Learning of Speech Patterns

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Native language acquisition is a prime example of experience-dependent learning. Despite the fact that this learning involves the enhancement of some and attenuation of other perceptual sensitivities, depending on the system of contrasting categories of the ambient language, the ability to learn novel speech patterns is apparently retained well into adulthood. For example, adult monolingual listeners are able to learn to discriminate and identify spoken words that include novel non-native language speech sounds and to perceptually adapt to the "deviant" speech patterns of a foreign-accented talker. However, the success and speed with which adults learn to perceive non-native and foreign-accented speech patterns depend on several factors, including the similarities and differences between the native language and target language systems of contrasting categories, the training conditions, and psychoacoustic and contextual factors that influence the discrimination and identification of speech sounds. This talk will review current data and theories of perceptual learning of speech patterns, and will identify the procedures that are most effective for speech perception training and that may have broad applications for clinical populations with known speech perception deficits.

Work supported by NIH-NIDCD grant DC 03762

554 Human Discrimination Learning on Basic Auditory Tasks

Beverly A. Wright

Human listeners can learn to discriminate between sounds that are initially indistinguishable. To better understand the nature of this learning, we have been using behavioral techniques to examine training-induced improvements on basic auditory discrimination tasks. In this talk, I will describe how multiple-hour training differentially affects the discrimination of sound frequency, intensity, location, and duration, how learning on a given discrimination condition generalizes, or fails to generalize, to untrained discrimination conditions, and how different training regimes can either enhance or degrade learning and generalization. I will discuss how these data supply useful baseline information for comparison with learning on tasks employing more complex sounds such as speech, contribute to our understanding of the mechanisms underlying performance on particular trained tasks, inform the development of therapeutic training schemes, and provide insights into the neurobiology of learning and memory.

Supported by NIH.

555 Auditory Learning and Conductive Hearing Loss

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Conductive hearing loss, occurring clinically or experimentally, has been found to change neural connections and to reduce neural activity in auditory brainstem nuclei, and to influence auditory perception beyond the time for which the conductive loss is present. These findings suggest that conductive loss produces a form of auditory learning in which experience with impoverished input leads to changes in the brain. However, the changes are not permanent. For example, children with a history of chronic otitis media with effusion (OME) have poorer than normal binaural unmasking for some months to years after the disease has resolved, but they eventually recover. In this talk, I will focus on the effects of OME in children and of ear plugging in ferrets on measures of central auditory function. In children, a 'threshold' level of OME is required to produce impaired binaural hearing. In ferrets, binaural unmasking, auditory temporal resolution and sound localization are all impaired after prolonged ear plugging. The recovery of sound localization accuracy during plugging depends on experience (active learning), rather than time. Bilateral ablation of the auditory cortex impairs recovery, suggesting a role for the cortex in this form of auditory learning.

Supported by the MRC, The Wellcome Trust and Defeating Deafness

556 Perceptual Learning and Cortical Self-Organization

*Michael P. Kilgard Cognition and Neuroscience, University of Texas at Dallas, Richardson, Texas

Sensory cortex is continually reorganized to meet changing behavioral needs. However, the general principles that allow behaviorally useful reorganization in large populations of neurons remain unclear. In this talk, I will describe a series of experimental manipulations that offer new insight into the neural basis of perceptual learning. These experiments provide strong evidence that modulatory neurotransmitters inform cortical neurons which particular sensory events to learn. Once relevant stimuli have been identified, network-level plasticity rules control how these inputs alter cortical connectivity and dynamics. For example, the same modulatory stimulation, when associated with different forms of auditory input, can generate dramatic changes in A1 frequency map organization, temporal processing, or sequence selectivity. Understanding how sensory experience guides neural plasticity will be critical for the development of new therapies for neurological rehabilitation.

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557 Dendritic Remodelling as a Mechanism for Cortical Plasticity

Mike Brian Calford School of Biomedical Sciences, The University of Newcastle, Newcastle, Australia

Traditionally, neurons have been classified by the shape of their dendrites. That shape was thought to be sculpted in early development, but then to become fixed in the adult brain. It is now well recognized that dendritic proteins and structures have a half-life of a few days and that mechanisms for recapitulating or regulating this shape must exist. In this talk I will consider two possible mechanisms regulating dendritic form in the mature brain, internal cellular processes and experience-dependent input activity. We have used the 'barrel field', the representation of the whiskers (vibrissae) in the cortex of rodents, to address this question. Functionally, a single barrel (aggregation of cells) processes the movements of a single vibrissa and neurons within a barrel show a preferential orientation of dendritic arbors toward the barrel center. We examined the geometry of barrel neurons in adult rats 8 weeks after input activity was reduced by removing the corresponding vibrissa. Deprivation destroyed the dendritic orientation bias without affecting the length or areas of the dendrites, indicating dendritic growth away from the barrel. Specific patterns of afferent activity were therefore necessary to maintain the dendritic form established in infancy. When activity patterns changed, dendritic elements responded by growing in the direction of the more active inputs. These findings should be applicable to all areas of cerebral cortex and define a new mechanism for plasticity.

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558 Norma Slepecky and Her Scientific Career

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Norma Slepecky is no longer with us, and we miss her. We miss her as a friend, a colleague, a coworker, an adviser, a teacher, we miss her cheerful personality. Norma was one of the most popular participants at the ARO meetings and one of the most productive too. Norma was only 57 when she died on May 2, 2001 almost two years ago. Our memories of her are still vivid.

When she died, she was a professor in the Department of Bioengineering and Neuroscience and a Member of the Institute for Sensory Research at Syracuse University. She started her scientific career as an undergraduate biology student at the same university and received her master?s degree in Microbiology. Subsequently, she was awarded the Ph. D. degree from the Department of Anatomy and Cell Biology at the State University of New York Health Science Center in Syracuse. The subject of her dissertation, ?Anatomy of the Stereocilia and Sensory Hair Cells of the Inner Ear: Ultrastructure, Protein Composition, and Functional Implications.? was the starting point of the main line of her future research. But her research branched out to other structures of the organ of Corti and, more broadly, of the cochlea, and to other sensory organs, including those of the sense of touch. Norma cooperated in her research with many colleagues locally, nationally and internationally and never refused to lend her expertise to others ? she seemed to be completely unselfish and not concerned about her own career. One of her colleagues called her his heroin and characterized her research contributions as ?the wind beneath many of our wings.? Yes, Norma contributed much more than she received credit for.

I had the great pleasure of cooperating with Norma on two occasions. One produced somewhat enigmatic results and, although they were already published, their description may be worth repeating on this occasion. They concern the question of ionic coupling between the OHCs and the supporting cells of the organ of Corti. The accepted view is that there is none but our results suggested the opposite. I want to outline them briefly for your comments.

559 Contributions of Specialized Fibrocytes to Inner Ear K^+ Homeostasis

Bradley A. Schulte Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, SC

Maintaining ionic and water balance between the unique fluid compartments in the inner ear is essential to auditory and vestibular function. Potassium homeostasis is of particular significance, because K^+ is the major cation carrying transduction and silent currents in the inner ear. Data form physiological, morphological, histochemical and more recently molecular and genetic studies have provided a conceptual framework for the thesis that K^+ effluxed from hair cells is actively recycled back to endolymph. Current models of this process postulate the existence of distinct lateral and medial recycling pathways from cochlear outer and inner hair cells, respectively. Functionally similar routes are proposed for recirculation of K^+ in the vestibular end organs. All of these putative recycling pathways invoke the cooperative activity of a variety of cell types. Central to each is the participation of several highly unique subpopulations of fibrocytes. Strategically located in discrete regions of the spiral ligament and limbus and in the connective tissue underlying vestibular neurosensory epithelia, inner ear fibrocytes have been classified into at least five subtypes based on morphologic and histochemical specializations peculiar to cells actively involved in fluid and ion transport. However, they can generally be grouped into two major phenotypes; cells that actively resorb K^+ from perilymph and cells that transport the resorbed K^+ down its concentration gradient. Presumably both of these activities are critical to normal inner ear function and thus serve as potential targets in hereditary and noise-, ototoxin- and disease-induced hearing and balance disorders. This presentation will summarize the evidence in support of K^+ recycling in the inner ear and highlight the role of specialized fibrocytes in this process.

560 The Membranous Organization of Outer Hair Cells.

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The outer hair cells of mammals are well organized with respect to their membranous specialization. Although the morphological descriptions of these membranous structures are well made, the functional characterizations of the various membranous structures are not yet clearly established. The distinct membranous structure of the outer hair cell consists of subsurface cisternae that surround the lateral walls of the cell body and is attached to the plasma membrane by elaborate cytoskeletal structures. The subsurface cisternae are characterized by the attachment of numerous mitochondria. The basal portion of the subsurface cisternae extends to form subsynaptic cisternae which are apposed by the efferent nerve endings. Thus, the subsurface cisternae are under medial efferent control. Hensen's body is the membranous structure found in the upper part of the outer hair cell near the cuticular plate and is often connected to the subsurface cisternae. At the very apical portion of the subsurface cisternae, there is a specialized membrane structure associated with the apical plasma membrane. Structures resembling the Golgi complex are often found below the cuticular plate. In addition, there are numerous vesicles found throughout the hair cells associated with fine cytoskeletal structures. Some of these vesicles near the basal pole of the cell are probably neurotransmitter vesicles. A cluster of mitochondria occurs below the nucleus and it is known as the Retzius body. Here we have attempted to characterize the membranous structures using specific markers and advanced imaging techniques in order to illuminate their possible specific cellular functions.

561 Imaging Cochlear Glycoconjugates

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The purpose of this presentation is to provide an overview of the characterization and distribution of glycoconjugate (GC) macromolecules within the cochlea. GCs consist of carbohydrates attached to either a lipid or protein core. GCs have been broadly classified into three groups: glycolipids, glycoproteins, and proteoglycans. The structure of these macromolecules ranges from the relatively simple glycolipids to the highly complex proteoglycans. GCs are major components of the extracellular matrix, and occur as fibrous (collagens) and adhesive (fibronectins) macromolecules. The tectorial and basilar membrane and the spiral ligament contain abundant GCs. GCs also occur within cell membranes as glycolipids (GM1), glycoproteins (integrins), and proteoglycans (syndecan). Some GCs are composed primarily of proteins (collagens), and they are readily preserved and visualized using standard chemical fixation and staining methods. However, other GCs are composed primarily of carbohydrates (proteoglycans), and are difficult to preserve and visualize. The cochlea contains a vast and diverse array of GCs whose functions are important for normal hearing. Congenital defects in the synthesis and catabolism of GCs have also been implicated in different forms of hearing loss.

I will attempt to review the structure, composition, distribution, and anatomical methods used to explore these fascinating macromolecules that occur within the cochlea.

Supported by the NIDCD.

562 Recent Advances in the Cell Biology of Acoustic Trauma

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Sound and drugs have unique effects on different cell types in the auditory system. Past and current research into the causes of acoustic trauma and its prevention suggest that different cell types respond to their unique stresses in distinct ways. The biology of these different cells and the nature of their role in the hearing process help predict the nature of their vulnerability to insult, and the future path of repair or regeneration. Some cells are poisoned in their own metabolic waste, while others are most susceptible to mechanical damage. Norma Slepecky's curiosity extended to every individual cell in the auditory system. Current evidence suggests that in normal function and under the stress of acoustic trauma, we should consider these cells as she did -- individually.

563 Essential cytoskeletal components in sensory stereocilia development, function, and renewal.

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Studies on the localization and role of cytoskeletal and muscle-like contractile proteins in inner ear sensory hair cells were pioneered by the seminal work of Slepecky and colleagues in the 1980's. We have been extending this work by studying the dynamic behavior and ultrastructural localization of these proteins. We have focused on the role of these proteins in the stereocilia. Each stereocilium contains a core of densely packed bundle of actin filaments that emerges from and progressively grows above the apical surface of the cells during development to reach different, yet predictable lengths and widths. Besides actin, several other cytoskeletal molecules have been identified as having a vital role in stereocilia formation and function because they are specifically expressed in or around the stereocilia and mutations in their genes lead to deafness. Three are unconventional myosins: myosins VI, VIIA and XV. In addition, espin, a novel protein that is depleted in a deaf mouse mutant cross-links stereocilia actin filaments. We have been using a combination of rapid freezing, immunogold labeling, and electron tomography to examine the precise localization of these proteins within the structure of the stereocilia. The dynamic localization of these proteins is followed using confocal microscopy of primary cultures of inner ear sensory tissues after transfection with mRNAs constructs encoding GFP-tagged proteins. We exploited the preferential localization of these proteins in the stereocilia to show that the entire stereocilia are continuously being remodeled and renewed based on an actin molecular treadmill mechanism. This combination of molecular and cell biological techniques is being used to gain insight on how the stereocilia develop to precisely specified structures and how they renew and adapt to keep the resting tension on the transduction channels constant thus maintaining optimum mechanosensitivity.

564 An Insight into the Works of the Organ of Corti

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Techniques such as laser heterodyne interferometry have provided detailed information about the micromechanical properties of the organ of Corti. Although measuring the cochlear vibratory responses with great temporal and spatial resolution these 'one point' techniques give only limited information about the overall response pattern. Using confocal microscopy a more 'pattern-assessing' approach can be applied. The technique offers high-resolution visualization of structures deep inside the organ of Corti and provides a means for monitoring stimulus-induced cellular changes. For example, the micromechanical events in response sound-like stimuli have been investigated using an isolated temporal bone preparation. By applying a variable pressure gradient across the cochlear partition, it is possible to gradually shift the position of the basilar membrane and the hearing organ from scala tympani towards scala vestibuli. Animations and detailed analysis of the resulting alterations illustrate the complex nature of the motion pattern. The results may assist in providing a more integrated view of the cellular interactions and a new insight into the works of the organ of Corti.

565 Molecular Genetic Analysis of Connexin 26 Heterozygotes for Variation Within the GJB2-GJB6 Region

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At least 10% of congenital deafness is attributed to GJB2 (connexin 26) mutations, and molecular analysis of this gene is an important diagnostic tool. However, screening of individuals with nonsyndromic hearing loss reveals that as many as 40-50% of subjects with GJB2 mutations are heterozygotes. Interpretation of an individual case is difficult; hearing loss could be due to an undetected second GJB2 mutation, an interaction with another gene mutation, or due to an unrelated cause. Recent studies described GJB2 heterozygotes that also carry a 342 kb deletion that disrupts the coding exon and removes the entire 5' upstream region of the GJB6 (connexin 30) gene located 30 kb distal to the GJB2 gene. To ascertain the incidence of the mutation and to identify variation within the GJB2:GJB6 region (35 kb) we developed a series of highly polymorphic markers to screen for deletions that appear as apparent homozygosity in the region, as well as

fluorescent in situ hybridization probes to visualize deletions in interphase nuclei and metaphase chromosomes. We screened 100 patients with congenital nonsyndromic deafness for the del(GJB6:D13S1830) mutation, identifying it in 2 of 22 GJB2 heterozygotes, but not in 24 subjects with 2 GJB2 mutations or in 54 subjects with no mutations. Analysis of polymorphic markers in the deletion region showed an incidence of homozygosity in controls of 11.5% compared to 7.4% of subjects with no GJB2 mutations, 50% of subjects with 1 mutation and 54% of subjects with 2 mutations. These studies suggest that greater than 90% of Cx26 heterozygotes have mutations other than the del(GJB6:D13S1830) that remain to be identified We hypothesize the deletion removes regulatory sequences that coordinate the expression of connexin 30 and /or connexin 26. In support of this hypothesis, comparative sequence analysis of GB2-GJB6 regions in mammals reveals several clusters of highly conserved noncoding regions (CNSs), which may regulate their expression.

566 Use of a Multiplex PCR/sequencing Strategy to Detect both the Connexin 30 (*GJB6*) 342 kb Deletion and Connexin 26 (*GJB2*) Mutations in Cases of Childhood Deafness

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Mutations in Connexin 26 (Cx26) account for a high proportion of deafness, but 30-55% of deaf patients with Cx26 mutations have only one mutant allele. Recently a large deletion in another DFNB1 gene, Connexin 30 (Cx30) was identified and suggested as a common mutation causing deafness in homozygotes and double heterozygotes with Cx26 mutations.

We describe a multiplex polymerase chain reaction (PCR) procedure followed by direct sequencing for simultaneous detection of Cx26 mutations and Cx30 deletions. Three sets of anonymous DNA samples made from residual clinical material included a deaf subject set (108), carrier subject set (28), and control subject set (64). PCR amplified DNA fragments simultaneously with three sets of primers [10-20pmol: Cx30d-F/Cx30d-R; Cx30c-F/Cx30c-R; Cx26-F/Cx26-R] in a multiplex state with products verified by gel electrophoresis. If a Cx30 342 kb deletion was not found, bi-directional Cx26 sequencing was performed.

2/108 deaf samples had double heterozygote mutations (E47X/342 kb deletion and 167delT/342 kb deletion) in Cx26 and Cx30. 69/108 had only Cx26 mutations; 40 had 1 mutation, and 29 biallelic mutations. Two novel Cx26 mutations 511-12insAACG and 358-360delAG were found. No deaf subject was found to have only a Cx30 342 kb deletion. 37/108 deaf samples had no detectable mutation in Cx26 or Cx30. All 28 carrier samples had a Cx26 mutation but none had a Cx30 342 kb deletion. No controls had a Cx30 342 kb deletion or a Cx26 mutation.

We present a method for simultaneously detecting Cx30 deletions and Cx26 mutations in deaf patients. It can detect large deletions of Cx30 extending to Cx26, or a large Cx26 deletion that would go undetected using mutation-detection assays. Although the approach distinguishes homozygous from heterozygous Cx30 342 kb deletion, it cannot determine if both a Cx30 deletion and Cx26 mutation are in *cis* or *trans*.

567 Mutational Analysis of the Six Cloned Usher Genes Using a Sequential Screening Strategy

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Usher syndrome (USH) is an autosomal recessive disorder characterized by congenital hearing impairment and reptinits pigmentosa. Three clinical types are known and there is an extensive genetic heterogeneity, with at least 12 genes implicated. The causative genes have been identified for USH1B, USH1C, USH1D, USH1F, USH2A, and USH3. We analyzed exons of these six genes using a sequential screening strategy in DNA samples of 230 probands with USH We have identified a variety of novel mutations and polymorphisms in MYO7A, USH1C, CDH23, PCDH15, USH2A, and USH3 in these patients. We found that 30% of probands with USH1 carried MYO7A mutations and 40% of probands with USH2 had USH2A mutations. Two of the 15 MYO7A mutations account for the greatest percentage of observed mutant alleles (25%). The most common USH2A mutation, 2299delG, accounts for 62% of mutant alleles. Three mutations were found in USH1C in our patients' panels, accounting for 1.65% of non-Acadian USH1. 29% of the cases with USH1 were found to carry CDH23 mutations, supporting the view that Cadherin 23 is the second most common type of Usher type I. A mutation, 193delC, accounts for 27.8% of CDH23 mutant alleles, suggesting that it may be a common mutation in CDH23. In PCDH15, Seven novel mutations including two missense and five truncating changes were detected. No one of USH3 mutations was detected in our patients with USH2 and atypical USH. Interestingly, we have identified two USH probands with a heterozygous change in a USH gene and a heterozygous mutation in another USH gene, indicating that the possibility of digenic inheritance among USH genes. Identification of novel mutations may provide insight into the phenotypic variation. Moreover, identification of patients/families that do not have mutations in the cloned USH genes would be essential for reducing the impact of heterogeneity on the search for new USH genes.

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568 A 4pb-Insertion in the Eya-Homologous Region of EYA4 Causes Hearing Impairment in a Family Linked to DFNA10

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Hereditary hearing impairment (HHI) is a heterogeneous class of disorders showing various patterns of inheritance and involving a multitude of different genes. Mutations in the EYA4 gene are responsible for postlingual, progressive, autosomal dominant hearing loss at the DFNA10 locus. EYA4 is homologous to the Drosophila gene eya („eyes absent"), a key regulator of eye formation. EYA4 plays an important role in several developmental processes. Here we report a DFNA10 family displaying sensorineural, progressive hearing impairment and linkage to 6q23. By mutation analysis of EYA4 an insertion of 4 bp (1558insTTTG) was detected. This insertion creates a frameshift and results in a stop codon at position 379. Hence, in the EYA4 protein nearly the complete „eya homologous region" (eyaHR),

which is essentiell for the protein function, is deleted. Our family is the third one linked to DFNA10 and revealing a mutation in the EYA4 gene. In all three families the mutations are localized in different regions of the eyaHR suggesting that this protein contains several functional subregions with different tissue specific importance.

569 Nonsyndromic Hearing Loss in a Japanese Family with a 7472insC Mutation in the Mitochondrial tRNA$^{Ser(UCN)}$ Gene

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We report a Japanese family with nonsyndromic hearing loss associated with a 7472insC mutation. The transmission of hearing loss was compatible with a maternal inheritance pattern. No family members had any neurological symptoms. The 7472insC mutation was heteroplasmic in white blood cells taken from two affected patients; the percentages of the mutant mitochondrial DNA were 94 % and 98 %. Audiometric examination in the two patients showed sensorineural hearing loss with U-shaped and sloping audiograms. They showed good speech discrimination scores. Testing of auditory brainstem responses (ABR) in one patient demonstrated wave V with normal latencies.

To date, four mitochondrial mutations in the tRNA$^{Ser(UCN)}$ gene (A7445G, 7472insC, T7510C, and T7511C) have been reported to be associated with nonsyndromic hearing loss. Recently we reported the second family with a T7511C mutation (Ishikawa et al, 2002). The 7472insC mutation was first reported in a Sicilian family (Tiranti et al, 1995). In contrast to our family, some members with hearing loss in the Sicilian family had ataxia, dysarthria and focal myoclonus. This mutation also was found in a large Dutch family with nonsyndromic hearing loss in all family members, except for a single person suffered from ataxia and myoclonus (Verhoeven et al, 1999). To our knowledge, the present family is the first one to have the 7472insC mutation in Asia. Our results add to previous evidence for the 7472insC mutation as a pathogenic mutation associated with nonsyndromic hearing loss. In addition, mutations in the tRNA$^{Ser(UCN)}$ gene should be analyzed in families with maternally inherited nonsyndromic hearing loss, even if they are Asian or not.

570 Molecular Diagnostic Determination of Usher Syndrome type IIa.

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Usherin deficit is responsible for Usher IIA syndrome characterized by moderate to severe down sloping hearing loss and adolescent onset of retinitis pigmentosa. Usherin is expressed in many tissues including the basement membranes of the retina and the inner ear and salivary gland tissue. We present photomicrographs showing absence of Usherin in the basement membranes of the minor salivary glands in subjects homozygous for the 2299 mutation which is considered to be a null mutation. Robust immunostaining for usherin is observed in salivary glands from normal individuals. Placental tissue is also immunopositive for usherin, allowing a non-invasive diagnostic screen for newborns thought to be at risk for Usher IIA. Reverse-transcription polymerase chain reaction (RT-PCR) from these same tissues shows an absence of RNA in subjects homozygous for the 2299 mutation. Further, RT-PCR was able to reliably determine carriers for this mutation, since only half the normal complement of usherin mRNA was present in these samples. Thus**,** this work demonstrates that diagnosis of Usher IIA caused by null mutation is feasible using these methods, and that carrier status can also be established. Accurate and inexpensive diagnostic techniques are critical for efficient identification of candidates for treatment of this disease, should therapies for Usher IIA be developed. In addition, early diagnosis will provide a longer window for treatment protocols designed to improve the visual and hearing aspects of Usher IIA syndrome.

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571 A Defect in Prestin, a Cochlear Motor Protein, Underlies Non-Syndromic Recessive Deafness

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Prestin, a membrane protein that is predominantly expressed in the outer hair cells (OHCs) of the cochlea, is a member of a newly recognized class of molecular motors. The discrete localization of prestin expression in the OHCs makes it a strong candidate gene for a human deafness. Here we report the cloning and characterization of multiple variants of human prestin gene, SLC26A5. SLC26A5 has at least four isoforms that differ at the last exon, as well as at some internal exons. The prestin gene spans a genomic region of over 70 kb on chromosome 7. Histochemical studies of mouse cochlear preparations showed immunostaining for prestin only in the OHCs. The expression profile of prestin was also studied through RT-PCR and northern blot. Preliminary results suggest that the prestin isoforms are associated with developmental stages. Moreover, we have identified a 5'-UTR splice acceptor mutation (IVS2-2A>G) in this gene which underlies recessive non-syndromic deafness in two unrelated families, thereby assigning an essential function to prestin in the hearing process.

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572 Assessment of the Relative Contribution of Autosomal Recessive Genes to Childhood Non-syndromic Sensorineural Hearing Impairment- Implications for Genetic Testing and Counselling

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About 1 in 2000 children are born with a genetic hearing impairment, mostly inherited as a non-syndromic autosomal recessive trait, for which more than 30 different genes have already been mapped.

Although previous studies have shown that connexin 26 (*CX26*) can account for up to 30-60% of such deafness, the relative contribution of the many other genes is not known, making it difficult to establish a routine diagnostic genetic testing service.

One hundred and forty one sib pairs with early onset non-syndromic hearing impairment were recruited. Those in whom deafness could not be attributed to *CX26* mutations were investigated further by analysis of microsatellite markers flanking each mapped recessive gene, followed by mutation analysis of those genes which have been identified.

The genetic basis of 51 cases (36.2%) was established, the majority (31.2%) being due to mutations in the *CX26* gene. Analysis showed that none of the remaining loci identified (DFNB2-29) make a significant contribution, although mutations in the *PDS* gene did account for at least 2.8% of all cases and as such are the next most common cause of childhood deafness. We also describe novel disease causing mutations in the *TMPRSS3, OTOF* and *TECTA* genes.

Based on our findings, *CX26* and *PDS* should form the basis of any genetic testing programme and we also highlight a number of important issues for genetic testing and counselling.

573 The Cytosine Insertion at Position 961 Coexisting with A1555G Mutation in Mitochondrial 12S rRNA Gene in a Large Chinese Pedigree with Maternally Inherited Hearing Loss

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Mutations in mitochondrial DNA have been shown to be one of the important causes of sensorineural hearing loss. Here, we reported the characterization of a large Chinese family (507 members in seven generations) with maternally inherited non-syndromic hearing loss. Members of this family showed variable severity and age at onset of hearing impairment. In addition, the average age at onset of hearing loss in this family exaggerated from 49 year (generation III) to 3.3 years (generation VI). Sequence analysis of the complete mitochondrial genome in this pedigree revealed the presence of a homoplasmic A1555G mutation in the 12S rRNA gene and other nucleotide changes. Of these changes, the C insertion at position 961 in the 12S rRNA gene was of special importance, as mutations at this position have been associated with aminoglycoside induced deafness in genetically unrelated families. This suggests that mitochondrial haplotypes, specifically with the 961 mutation, acting as the secondary factor(s), may play a synergic role in the development of deafness phenotype associated with A1555G mutation.

574 Genotypic and Phenotypic Characterization of a DFNA6 Hungarian Family Showing Low-frequency Sensorineural Hearing Impairment

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Hereditary hearing impairment is a heterogeneous sensory defect with more than 70 identified loci. Only two of these loci (DFNA1 and DFNA6/14/38) are associated with low-frequency sensorineural nonsyndromic hearing impairment. DFNA6 and DFNA14 loci was mapped to non-overlapping adjacent regions on chromosome 4p16. This critical region includes WFS1 gene. Recessive mutations in the WFS1 gene are responsible for Wolfram syndrome; missense mutations inherited in an autosomal dominant way result in low-frequency sensorineural hearing impairment (LFSNHI).

In this study we analyzed a five-generational hungarian family with LFSNHI and linkage to DFNA6. The family contains 14 affected persons. We have performed mutation screening of all WFS1 coding exons in family. We have detected missense mutations changing amino acids and one of them (T699M) segregated completely with the affected haplotype. The second missense substitution (R818C) did not segregate with the hearing impairment, it most likely represents a polymorphism. In general, these patients showed a postlingual, sensorineural, bilateral, symmetric, nonsyndromic low frequency hearing impairment with a slow progression. The hearing impairment is accompanied by normal vision, vestibular responses and no malformations of the inner ear detectable by computer tomography or magnetic resonance imagine.

575 Auditory Neuropathy in a Family with Mohr-Tranebjaerg Syndrome

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Mohr-Tranebjaerg Syndrome (MTS) is characterized by early-onset post-lingual deafness, late-onset dystonia or ataxia and progressive visual deterioration. Other symptoms can include paranoid psychotic features and mental deterioration. Mutations in TIMM8a, located on Xq22, have been shown to cause MTS. A family with three affected males in two generations have been found to have a nonsense mutation, 135 C>T (Q34X), in exon 1 of the TIMM8a gene. These males have many of the classic features associated with MTS, but in addition, the hearing loss is an auditory neuropathy. Auditory neuropathy is characterized by absent or abnormal auditory brainstem response with normal outer hair cell function. It was predicted that an auditory neuropathy phenotype would be associated with MTS by Merchant et al. (Otol Neurotol. 2001 Jul;22:506-11) who found near complete loss of spiral ganglion cells and preserved organ of Corti in the temporal bone of an MTS patient with the 151delT mutation in TIMM8a. The family presented here is the first case where outer hair cell function was determined clinically in an MTS patient. This patient had an unsuccessful trial with hearing aids and has since stopped wearing them. While cochlear implants have been shown to be beneficial in some cases of auditory neuropathy, the family has been informed of the studies by Merchant, et al. and been advised that a cochlear implant might not work due to the progressive loss of the spiral ganglion. This family verifies the prediction that MTS is associated with an auditory neuropathy and demonstrates the importance of genetic diagnosis in therapeutic decisions for hearing disorders.

576 EphA4 Regulates Segregation of Nucleus Magnocellularis Inputs to Nucleus Laminaris

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Nucleus laminaris (NL) in the chick auditory brainstem is a sheet of neurons with symmetric bitufted dendrites. Inputs from ipsilateral nucleus magnocellularis (NM) contact dorsal NL dendrites and somata while inputs from contralateral NM are largely restricted to the ventral NL dendrites and somata. The segregation of NM inputs to distinct regions of NL cells facilitates computation of interaural time differences, which are used in sound localization. We are interested in identifying the molecular mechanisms underlying the development of this innervation pattern. We previously showed that EphA4 receptor tyrosine kinase localizes to dorsal but not ventral NL dendrites at the stage when NM-NL synapses begin to form. In the present study, the role of EphA4 in establishing precise binaural segregation was assessed. The region of the chick hindbrain that gives rise to the auditory nuclei was targeted using in ovo electroporation to introduce plasmids

encoding GFP (controls), full-length EphA4/GFP or a kinase inactive form of EphA4 (kiEphA4)/GFP. Embryos were transfected at E2 and were allowed to develop to E10, when NM and NL are identifiable and connections are present. Gene expression persisted through E10. At E10 NM-NL axons were labeled in vitro with rhodamine dextran amine. The number of axons that grew across the line of NL cell bodies was assessed in control and experimental embryos. Expression of kiEphA4 resulted in a significant increase in the number of axons that extended aberrantly across the line of NL cell bodies compared to control transfections. Overexpression of full-length EphA4 resulted in a more modest increase. In addition, transfection with kiEphA4, but not GFP alone, was associated with abnormal NL morphology in some cases. These results suggest that EphA4 has a role in the maturation of NL and in the formation of binaural segregation in the projection from NM to NL.

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577 EphA4 Repulsion of Spiral Ganglion Neurites is Blocked by Anti-Ephrin B2/B3 Antibodies

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The ephrins and Eph receptors make up two large families of bi-directional signaling molecules that are known to play a role in the development of the nervous system. Recently, van Heumen et al. (2000) observed expression of EphA4 in the developing cochlea, with strong expression in the osseous spiral lamina through which afferent dendrites must pass to reach the organ of Corti. They also noted ephrin B2 and B3 expression by spiral ganglion (SG) neurons. We have found using a stripe assay that the neurites of neonatal rat SG neurons are repulsed by EphA4 in vitro (Brors et al., 2001). The ephrins that mediate this response may be B2 and B3. However, because there are 9 at least ephrins, other isoforms may be involved. To assess the roles of ephrins B2 and B3 in EphA4 signaling, we cultured SG explants near EphA4 stripes or on uniform EphA4 surfaces. To stimulate neurite outgrowth, all explants were cultured with NT-3 in the media. The behavior of neurites from control explants was compared to that of neurites from explants treated with anti-ephrin B2 and/or B3 blocking antibodies.

Treatment with either anti-B2 or B3 antibodies significantly reduced the repulsion response observed at the border of an EphA4 stripe in control cultures. Moreover, when both antibodies were used together, neurites crossed onto EphA4 stripes with no evidence of repulsion. Abnormal fasciculation and turning of neurites observed on uniform EphA4 surfaces were also eliminated by treatment with anti-ephrin B2 and B3 antibodies. In other systems, the anti-ephrin antibodies used have been reported to be specific for B2 and B3, respectively, at the concentrations we employed. However, the possibility of cross-reactivity with other ephrins must still be considered.

The results suggest that ephrin B2 and B3 together mediate the response of SG neurites to EphA4. These ephrins presumably also play a role in the guidance of SG neurites toward their targets during development.

578 Adhesion Molecule Expression During Migration of the Inner Ear Cell Line, VOT-33, Mimics That Observed in Delaminating Neuroblasts *In Vivo*

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Cells destined to become the neurones of the cochlear-vestibular ganglion (CVG) originate within the otic epithelium, migrate out and reaggregate to form the CVG early in development. This involves modulation of cell-cell and cell-substratum interactions that are mediated by adhesion molecules, including the integrins. We have shown that, at E10.5, α3 and α6 integrin subunits are expressed at high levels within the murine otic epithelium but that expression is decreased in the developing CVG, suggesting that integrin regulation is associated with neuroblast delamination. To study the role of adhesion molecules in CVG development further we used two conditionally immortal cell lines, VOT-33 and VOT-36, which were derived from the ventral otocyst of the Immortomouse at E10. Expression of selected markers suggested neural and epithelial phenotypes for VOT-33 and VOT-36 respectively. Consistent with these phenotypes, VOT-36 expressed β4 integrin and its F-actin was arranged into sub-cortical bundles. VOT-33 did not express β4 and exhibited a stress fibre-rich actin morphology. In keeping with expression patterns observed within the murine otic epithelium, both cell lines expressed α3, α6 and β1 integrins as well as cadherins. A monolayer wound assay was employed to model the migratory processes involved in neuroblast delamination. VOT-33 exhibited faster wound closure than VOT-36 over 24hrs, covering 80% and 50% of the wound area respectively. Moreover, expression of α6 integrins in VOT-33 was lower in cells adjacent to the wound whilst cadherin expression was maintained. This is consistent with expression patterns observed in delaminating neuroblasts *in vivo*. We are now investigating the role of integrins in VOT-33 migration using blocking antibodies in this assay.

579 Normal Postnatal Development of Banded Afferent Projections to the Inferior Colliculus of the Ferret from the Superior Olivary Complex

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The inferior colliculus (IC) is one of the major sites of auditory convergence and one that integrates descending cortical and ascending brainstem auditory information. Inputs to the IC from the cochlear nucleus, medial and lateral superior olives and the dorsal nucleus of the lateral lemniscus are highly ordered and organized into series of afferent bands or patches. While the development of the structural organization of some aspects of the ascending afferent projections to the IC has been well characterized in the rat, little information is available on this organization in the developing ferret that begins hearing on postnatal day 32 (P32) versus P15 in the rat. To determine whether a banded pattern of afferent input develops in the IC before the onset of hearing, ferret kits were reared to either P7 or P14. At these time points, the animals were given an overdose of ketamine and xylazine and perfused through the heart with 4% paraformaldehyde fixative. The brains were removed and glass pins coated with crystals of the lipophilic dye 1,1'-dioctodecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) were placed in the fixed tissue in either the superior olivary complex of the whole brain or in transverse sections of the brain beginning at the caudal superior olive. Our results indicate that during normal development, bands of axons from the lateral superior olive are in the IC by P7. The bands are denser and more mature in appearance by P14, 18 days prior to the onset of hearing and the time somatic synaptogenesis in the superior olive is just beginning.

580 Postnatal Formation of Synaptic Connections at the Calyx of Held

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We used a combination of serial section electron microscopy, whole-cell recording and intracellular labeling to study the innervation of MNTB cells by calyces of Held in mice. We identified synaptic and punctum adherens contacts between axon terminals and postsynaptic structures and investigated the presence of mitochondria-associated adherens complexes (MACs) at early stages of innervation. At

postnatal days 0 and 2 (P0, P2), synaptic and punctum adherens contacts occurred primarily with identified MNTB cell dendrites or non-somatic structures in the adjacent neuropil. The proportion of non-somatic contacts (synapses 92%; puncta 77%) decreased by P2 (synapses 86%; puncta 57%). At P2 we occasionally observed axons in contact with long stretches of the MNTB cell body. At P0 and P2, MNTB cell bodies appeared multipolar with several primary dendrites. Intracellular labeling confirmed these observations and revealed that dendrites could extend for long distances with little branching. Midline stimulation at these ages evoked synaptic currents that varied from cell to cell in their sensitivity to antagonists of excitatory and inhibitory neurotransmission. By P4 immature, cup-shaped calyces frequently enveloped the MNTB cell body, and MAC structures had formed. Our results indicate rapid maturation of the calyx between P2 and P4, along with a shift from dendritic to somatic sites of innervation. Based on the timing of their appearance, MACs may play a role in synapse stabilization, but probably not in the early stages of synapse formation.

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581 Synapse Development in Endbulbs of Held

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The endbulb of Held is one of the largest synaptic endings in the brain. It arises from myelinated auditory nerve fibers and makes axosomatic contact with spherical bushy cells in the anteroventral cochlear nucleus. Its size, multiple synaptic release sites, and physiological properties have implicated it in the conveyance of precise timing for acoustic events. A light microscopic study in cats demonstrated that endbulbs develop from a flattened, club-shaped ending into an elaborate, highly branched arborization (Ryugo and Fekete, 1982). Using the electron microscope, we have been studying ultrastructural changes of the endbulbs, including membrane and synaptic specializations, that accompany the branching transformation. In neonates, the endbulb surface forms an irregular and highly invaginated interface with the postsynaptic bushy cell. This surface specialization serves to increase the membrane apposition between these two elements. Over the next 60 postnatal days, the surface becomes less corrugated, greatly reducing cell-cell contact. The number of postsynaptic densities (PSDs) decreases, whereas the length of each increases. The net result is a decrease in the total amount of PSDs present in the membrane. The reduction in PSDs may reflect increased efficiency in synaptic function. Mitochondrial volume fraction increases two fold, possibly reflecting a greater metabolic need for increased activity in auditory nerve fibers. Synaptic vesicle density remains constant at approximately 60/μm^2. We will extend our analysis of endbulbs to include postnatal 120- and 180-day cats. There are clearly dynamic interactions occurring at this synaptic junction whose significance may be revealed through further study.

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582 Analysis of Molecular Guidance Cues Directing Formation of the Calyx of Held

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In contrast to other sensory systems, little is known about the cues guiding the development of auditory circuits. We are interested in determining the molecular factors that establish tonotopic projections from the CN to the contralateral MNTB that terminate in calyces of Held. We assessed the developmental progression of this projection by placing DiI in the CN in intact mouse brains from E14.5 to P3. The brains were immersed in fixative following DiI placement. Despite intense labeling of trapezoid body fibers extending as far as the contralateral IC by E16.5, only sparse projections into the territory of the SOC were apparent prior to P0. In an effort to determine the factors guiding the calyceal projection, we analyzed the expression patterns of the midline-attractant growth factor, netrin-1, and its receptor, DCC. Analysis by in situ hybridization at E14.5 and E16.5 shows netrin-1 expression at the midline of the ventral brainstem. In addition, fibers in the trapezoid body express DCC protein at E15.5 during CN fiber extension. These results are consistent with the utilization of netrin-1/DCC signaling in attracting CN fibers to the midline during calyceal development, as has been shown in other decussating projections of the CNS. We are currently attempting to characterize the functional role of the netrin/DCC signaling system in auditory development.

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583 Development of GABAB Receptor Immunoreactivity in the Avian Auditory Nuclei

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Nucleus magnocellularis (NM), nucleus angularis (NA), and nucleus laminaris (NL), second and third order auditory nuclei in the avian brainstem, receive GABAergic input primarily from the superior olivary nucleus (SON) and from a small number of multipolar neurons residing in the neuropil surrounding NM and NL. Previous physiological studies have suggested that both GABAA and GABAB receptors are involved in the inhibitory action of GABA on the firing properties of NM neurons. We sought to characterize the distribution of GABAB receptor expression in these nuclei in the mature system, during embryonic development, and following removal of eighth nerve input. We used a polyclonal antibody raised against GABAB receptors (Clark et al. 2001). Serial sections through NM and NL were examined at 4 stages of embryonic (E) development E10, E14, E21, and at posthatch day (P) 20. Preliminary results show that at P20 and E21, when the structure and function of the auditory nuclei is know to be mature, GABAB immunoreactivity is characterized by discrete puncta covering the somas of NM, NL, and a subset of NA neurons. At E14, when the GABAergic synapses are forming, similar labeling is observed. However, at E10, when GABAergic fibers are entering the auditory nuclei, immunoreactivity is present in somas as diffuse staining with few puncta. Unilateral cochlear removals were performed in P5 animals. After a 15-day survival period we did not observe any qualitatively observable changes in the distribution of in GABAB receptor immunoreactivity in NM, NL or NA. The characterization of GABAB receptor distribution through development and under conditions of afferent deprivation enriches our understanding of the full complement of inhibitory influences on auditory processing by these nuclei.

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584 Activity Deprivation Eliminates Depolarization and AMPA Receptor Mediated Calcium Influx

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During development of the chick cochlear nucleus, nucleus magnocellularis (NM), afferent activity from the eighth cranial nerve plays a critical role in the maturation of NM neurons. Early elimination of this input causes increased cell loss and profound changes in the properites of surviving neurons. Previous studies have shown that several calcium homeostasis mechanisms (including metabotropic glutamate receptors, plasma membrane calcium pumps, and sodium/calcium exchangers) depend upon this afferent input during development for normal expression levels and patterns. The goal of the present study was to determine the effect of activity deprivation during development on the cellular calcium homeostasis of NM neurons.

Seven chick embryos underwent unilateral otocyst ablation, and 6 embryos underwent bilateral otocyst ablation, at embryonic day (E)2.5.

Removal of the otocyst prevents formation of the middle ear and eighth nerve thus removing all excitatory input to the ipsilateral NM. NM neurons in normal E15-18 chicks show robust calcium increases in response to 50 micromolar kainate (KA) or 80 mM KCl due to influx through calcium-permeable AMPA receptors and voltage gated calcium channels. NM neurons in E15-18 animals that had undergone bilateral otocyst ablation (6/6) and ipsilateral to unilateral ablation (6/7) showed no calcium responses to KA or KCl. In the seventh unilateral animal, both KA and KCl elicited calcium responses that were attenuated 63% and 52%, respectively. NM neurons contralateral to unilateral ablation responded normally to KA and KCl.

These results suggest that voltage gated calcium channels and calcium permeable AMPA receptors in NM neurons depend upon afferent input from the eighth nerve to develop normal abilities to flux calcium in the presence of a ligand (AMPA receptors) or depolarization (KCl).

585 Early Sensorineural Interactions within the Prenatal Mouse Cochlea

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During prenatal development, auditory nerve fibers are guided into radial and longitudinal pathways and towards their appropriate target hair cells by means that are largely unknown. To begin to clarify these mechanisms, we examined the formation of cochlear neuron arbors in relation to hair cell development within the prenatal mouse cochlea. From embryonic day (E) 11.5 through birth (P0), cochlear nerve fibers were labeled using antibodies for five neuronal intermediate filament proteins (alpha-internexin, NF-L, NF-M, NF-H, and peripherin) and newly differentiated hair cells were labeled using antibodies that detect myosin VI or VIIa. Both alpha-internexin and NF-M staining revealed nerve fibers entering the prosensory zone of the cochlea and engaging newly differentiated hair cells (identified with myosin VI antibodies) at timepoints up to one day earlier (E13.5-14) than previously reported. Furthermore, analyses of the timing and fine architecture of the fiber ingrowth into the developing cochlea indicates that cochlear neuron growth cones may be able to discern discrete cellular microdomains that both define the extent and delimit the boundaries of inner and outer hair cell zones. These findings should eventually provide a foundation for more detailed studies of the molecular determinants involved in the guidance and sculpting of all of the classes of cochlear innervation.

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586 Expression of the High-affinity Choline Transporter (ChT1) is Consistent with Medial Olivocochlear Innervation during Development

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Acetylcholine serves as the primary neurotransmitter for synaptic transmission between efferent olivocochlear (OC) axons and cochlear hair cells in the mammalian inner ear. Recently, the cDNA for the high affinity choline transporter (ChT1) has been characterized in C. elegans, mouse and rat. Choline is an essential precursor for the synthesis of several membrane lipids and choline transport is thought to be the rate-limiting step for acetylcholine synthesis in presynaptic axon terminals. Using a rabbit polyclonal antiserum for ChT1, we investigated the possibility that the high affinity choline transporter (ChT1) is expressed in both medial and lateral OC axons and that the expression of ChT1 may be developmentally regulated in the mammalian cochlea.

In both the embryonic and postnatal cochlea, the pattern of ChT1 immunoreactivity was consistent with the developmental pattern of medial OC axons. In basal regions of the cochlea, ChT1 immunoreactivity was found below inner hair cells (IHCs) as early as embryonic day 18 (E18) in mouse and rat organs of Corti. By postnatal day 4 (P4), ChT1 was localized beneath IHCs and the first row of outer hair cells (OHCs) in both animals. Between P6 and P15, ChT1 was visible within the inner spiral bundle, tunnel-crossing fibers and below OHCs. By P20, the majority of ChT1 was localized below OHCs. In all of the ages examined, ChT1 expression was a subset of either GAP43 immunoreactivity, a marker of growing efferent axons or synapsin/snap25 immunoreactivity, a marker for efferent terminals in the inner ear. Our observations suggest that high-affinity choline transport is involved in the synthesis of acetylcholine in the mammalian inner ear and may play a role in the formation of mature efferent synapses between OC axons and outer hair cells. Further, ChT1 may be expressed preferentially by medial OC axons.

587 Developmental Expression of Voltage-Gated Potassium Channels in the Cochlear Nucleus

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In order to develop electrical properties allowing them to process and convey acoustic signals, auditory neurons must express specific sets of ion channel proteins according to precisely regulated spatial and temporal expression programs. Here we show the major characteristics of the developmental program leading to the normal adult distribution in neurons of the cochlear nucleus (CN) of Kv1.1 and Kv1.2, two proteins involved in the assembly of voltage-dependent potassium channels with rapid activation kinetics.

We have used a combination of in situ hybridization, with oligoprobes for specific sequences of Kv1.1 or Kv1.2, and immunocytochemistry with polyclonal antibodies binding to specific regions of the C-teminal portions of Kv1.1 or Kv1.2. The oligoprobes and the antibodies were used to localize the corresponding mRNAs or polypetides in cells from histological sections of the CN from postnatal rats at ages ranging from postnatal day 1(P1) to postnatal day 60 (P60).

Kv1.1 and Kv1.2 transcripts were detected in many CN cell populations from P1 to adulthood. Kv1.1 and Kv1.2 protein products detected by immunocytochemistry were also seen in cell bodies as early as P1 and up to adulthood (P60). However, by the end of the third postnatal week (P21) Kv1.1 and Kv1.2 immunolabeling shifts from cell bodies to neuronal processes, likely axons. These findings provide evidence that the expression of voltage-dependent potassium channels is developmentally regulated in auditory neurons. They also indicate that axonal targeting of these ion channels lags well behind hearing onset and synaptogenesis in the rat, suggesting that at least some mature electrical properties are acquired relatively late in development.

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588 Dynamic Changes of GABAergic Neurons in the Auditory Midbrain of Young Rats following Brief Acoustic Stimulation

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Mild sounds, when presented early in life, can shape central auditory sensitivity. One of the key neurotransmitters in the central auditory system is γ-aminobutyric acid (GABA). There has been some evidence that GABAergic system may change after loud sound exposure or cochlear lesion. Whether similar effects may occur following brief stimulation of sounds presented at a physiological level remained unclear. In this study, we delivered a mild tone to young animals and

studied changes of their GABAergic system in the auditory midbrain using the techniques of GABA immuno-histochemistry, and immuno-blotting of glutamate decarboxylase (GAD) and $GABA_A$ receptor.

Young rats (Sprague Dawley, 2-week old) were first conditioned inside a sound room for 8 hrs. A monotone (4 kHz, 65 dB SPL) was then presented for 30 minutes and animals sacrificed 1 or 2 hrs later. Control animals were put in the same environment without sound. Immuno-histological results showed that the GABA positive neurons in the auditory midbrain increased drastically in number 1 hr after sound exposure. Similarly the protein level of GAD and the $GABA_A$ receptor also increased. Such changes returned to around pre-stimulus level 2 hrs later. In animals of an older age (4-week old), such GABAergic responses were less prominent.

Results showed that at postnatal week 2, the GABAergic system in the auditory midbrain is relatively plastic. Brief acoustic stimulation is sufficient to induce the synthesis of GABA. Such dynamic expression of GABA to sounds should be taken into consideration when interpreting the results on GABA-related immuno-histochemistry or immuno-blotting. Questions remain whether the GABAergic neurons are active at this time of observation and whether they exert inhibitory effects on other neurons. We speculate that if such effects are cumulative, prolonged stimulation of sound in the early postnatal period could lead to long-term changes in GABAergic system. Such long-term changes may be related to the occurrence of tinnitus in animals whose GABAergic system may also be altered in a similar way.

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589 Clustering of Nicotinic Acetylcholine Receptors in Hair Cells.

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Nicotinic acetylcholine receptors (nAChRs) mediate synaptic transmission between hair cells and olivocochlear (OC) axons in the mammalian cochlea. During development, it is not clear which synaptic proteins are required for the clustering and stabilization of nAChRs in hair cells. At the neuromuscular junction (NMJ), agrin, rapsyn, muscle-specific kinase (MuSK), and other synaptic molecules form a multimeric scaffold that is essential for the clustering and stabilization of nAChRs. We have isolated partial transcripts of rapsyn and MuSK from rat cochlear RNA using gene-specific primers and RT-PCR. Our data suggest that rapsyn is expressed at a relatively constant level the first two weeks of postnatal development. In contrast, MuSK expression peaks after the first week of postnatal development and then persists at slightly lower levels during the second postnatal week.

We have further investigated the clustering of nAChRs by labeling nicotinic receptors with α-bungarotoxin (αBTX) using either freshly dissected cochlear spirals or dissociated hair cell preparations. At early postnatal ages, inner hair cells (IHCs) exhibit a range of αBTX labeling from diffuse to punctate. Diffuse labeling patterns consistently show αBTX along the basolateral surface of the IHCs. Punctate labeling patterns show anywhere from 1 to 3 discreet plaques typically along the basolateral surface. In postnatal day (P) 9 and older cochleae, there is a decrease in the number of IHCs with identifiable αBTX plaques and diffuse labeling patterns are absent. These data provide evidence that nAChRs may cluster in hair cells during efferent synaptogenesis. These data are also consistent with the idea that there is a transient innervation of IHCs by medial OC axons during development. Our results raise the possibility that the mechanism of clustering and stabilizing nAChRs proposed for the NMJ may be similar for nAChRs in hair cells.

590 Formation of Ion Channel Clusters and Synaptic Connections in the Developing Bullfrog Saccule

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During development, electrically resonant hair cells (HCs) in the bullfrog saccule, a sensor of linear acceleration, form ion channel clusters and develop synaptic connections with vestibular neurons. To examine the temporal relationship between these events, we used fluorescent derivatives of dihydropyridine (Fl-DHP) and charybdotoxin (Fl-CbTx), verifying with patch-clamp recordings that these toxin derivatives retained their specificity for L-VGCC and BK channels. We then labeled saccules for 15 mins with Fl-DHP and/or Fl-CbTx, immunolabeled saccules with myosin VI and neurofilament antisera, and used confocal and electron microscopy to study the distribution and composition of ion channel clusters and the relationship between these clusters and synaptic endings.

Mature HCs displayed varying numbers of puncta on their basolateral surfaces, indicating that they possessed both L-VGCC and BK channels. These puncta, which ranged from <0.25-1.0 um in diameter, were restricted to the subnuclear regions of mature HCs and co-localized with each other. They also were associated with synaptic endings, partially or completely surrounding the synaptic active zone, providing a mechanism by which HCs could increase the number of these channels while maintaining their stoichometric ratio. L-VGCC channels in immature HCs, by contrast, were confined to the supranuclear region, were not necessarily co-localized with BK channels, and were not associated with synaptic endings, implying that HCs were capable of initiating synaptogenesis. Extramacular HCs had L-VGCC but not BK channels, implying that they did not exhibit electrical tuning. They also were innervated by neurons regardless of their state of differentiation, implying that synaptogenesis could also be initiated by neuronal innervation.

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591 Apoptosis and Regeneration of Hair Cells in Neuromasts Following Brief Exposure to Calcium Free Water

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Hair cells of superficial neuromasts are reported to be crucial in helping fish exhibit rheotaxis (i.e., to properly orient in currents) (Baker, C.F. and J.C. Montgomery, 1999; J. Comp. Physiol. A, 184:519-527). Previously, we found that a single 15 s immersion in calcium free water significantly decreased rheotaxis. A spontaneous recovery to control levels of rheotaxis required 9 days, sufficiently long to suggest that calcium free water caused serious trauma to the hair cells. We now find that a single 15 s immersion in calcium free water results in a significant decrease in mean number of hair cells per neuromast 48 h after immersion. Furthermore, the traumatized hair cells die by apoptosis as indicated by an activation of caspase. Apoptotic cell death is accompanied by an up regulation of mitosis as indicated by BRDU incorporation into cells entering S-phase. The mean number of hair cells per neuromast recovers to control levels by 72 h. Clearly, brief exposure to calcium free buffers severely traumatizes neuromast hair cells, leading to cell death and regeneration.

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592 Cell Proliferation in the Mammalian Inner Ear After Aminoglycoside Application

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The discovery of hair cell regeneration in the avian inner ear raises the possibility that hair cell regeneration might occur in the mammalian cochlea as well. Mitotic activity in the cochlea has been studied in 3-week-old gerbils exposed to acoustic trauma (Roberson and Rubel, 1994) and in 4- to 5-weeek old mice treated with dihydrostreptomycin (Yamashita et al., 1999). These studies showed no evidence of hair cell regeneration or of any cell division within the normal sensory epithelial structures, but suggested that cell proliferation can be induced in other areas of the cochlea by acoustic trauma and ototoxic agents. In the current study, we examined the mitotic activity in the cochlea of rat and guinea pigs following application of aminoglycoside. The young albino guinea pigs (250-300 g) and rats (250-300 g) were deafened by kanamycin (KM, 400 mg/kg, s.c.) and ethacrynic acid (EA, 50 mg/kg, i.v.). The other guinea pigs (250-300 g) received a 4 mg dose of gentamicin (GM) injected into the middle ear. Bromodeoxyuridine (BrdU,150 mg/kg, i.p.) was injected once a day for 5-9 days following injection of EA and KM or GM. Then the temporal bones were fixed with 10 % formalin, decalcified, and dehydrated. The cochlea, utricle, and ampulla were embedded in paraffin, and the embedded tissues were sectioned, mounted, and stained with a BrdU Staining Kit. In the damaged cochlea, no evidence of hair cell regeneration or of any cell division within the normal sensory epithelial structures was seen. Frequent cell division was seen in other regions of the damaged cochlea. In GM-treated guinea pigs, a small number of BrdU labeled nuclei were seen in the sensory epithelium of the utricle and ampulla. These findings suggest that regeneration does not occur in the cochlear sensory epithelium in the guinea pigs or rats, as in gerbils or mice, but that aminoglycosides can induce cell division in non-sensory regions of the cochlea as does acoustic trauma.

593 Increased Hair Cell Regeneration Accompanies Increased Supporting Cell Proliferation in p27 Knockout Mice Cochleae Following Aminoglycoside Ototoxicity.

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We have previously demonstrated that the genetic deletion of p27Kip1 allows for continued cell division in neonatal and adult mouse organ of Corti, well after terminal mitosis has normally occurred (Lowenheim et al., 1999, PNAS, 96, 4084-4088). In addition, supporting cell proliferation is induced after aminoglycoside treatment in p27-/- and p27-/+ mice. In both genotypes, evidence of inner and outer hair cell regeneration was observed (Kil et al., 2000, ARO abstracts, 23, 273).

Here we quantitatively analyze the different organ of Corti cell types that participate in both proliferation and regeneration following aminoglycoside induced hair cell injury and loss in vivo. Hair cells were killed using repeated systemic injections of amikacin sulfate (P7-P12) followed by bromodeoxyuridine (P10-P12), a nucleoside analog which is incorporated into the DNA of proliferating cells during S-phase. Mice were sacrificed two days after the final BrdU injection on P14. Cochlea were dissected and immunoreacted as whole mounts. Serial section analysis was performed on 4 micron thick plastic cross sections. Some loss of the organ of Corti occured during sectioning, although the majority of the organ of Corti was analyzed. Cell type determination was based on immunocytochemistry and morphology.

After amikacin treatment, an increase in cell proliferation was observed in inner phalangeal, Deiter's, and Hensen's cells. In addition, the number of BrdU positive IHCs and OHCs increased following amikacin treatment. These data indicate that many of the supporting cell types in the organ of Corti of p27-/- mice exhibit increases in proliferation in response to amikacin treatment and that the level of hair cell regeneration also increases in response to aminoglycoside ototoxicity.

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594 Activation of Early Stage Apoptotic Pathways in Short-Term Cultures of the Chick Basilar Papilla

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We have shown through in vivo studies that early steps of the apoptotic pathway are activated in hair cells of the chick basilar papilla 6-12h after a single systemic injection of gentamicin (300 mg/kg). The cells that exhibit these early steps proceed to express later, terminal stages of apoptosis when gentamicin-damaged hair cells are being ejected from the sensory epithelium. Here, we have used short-term in vitro cultures of the chick cochlea to examine the initial steps of apoptosis activation in the hair cells.

Chick cochleae were dissected and immediately bathed in cochlear culture media (MEM, 30% glucose, NaHCO3, 1M Hepes, FBS, L-glutamine). Varying concentrations of gentamicin were added to the culture media used to incubate the cochleae. Cultures were then incubated at 37?C for either 1 hour, 3 hours, or 6 hours. After incubation, the cochleae were immediately fixed in 4% paraformaldhyde. Immunocytochemistry and fluorescence microscopy were used to view the whole mount cochleae in order to assess the extent of TIAR translocation out of the hair cell nucleus and into the cytoplasm at each time point and gentamicin concentration.

Our results show that at all time points, control chick cochleae exhibit localization of TIAR only in the hair cell nuclei. However, cochleae treated with gentamicin (0.5 mM) translocate TIAR after 1 hour of incubation and involve a majority of the hair cells in the cochlea by 3 hours. Lower doses of gentamicin (0.3mM) induce TIAR translocation by 6h of incubation. These results indicate that chick cochleae in culture initiate the apoptotic program within an hour of treatment with appropriate doses of gentamicin. This suggests that in our in vivo studies it takes between 6 and 12 hours for gentamicin to reach high enough levels in the cochlea to initiate apoptosis in the most proximal (basal) hair cells.

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595 Direct Transdifferentiation Gives Rise to the First New Hair Cells in Regenerating Avian Auditory Epithelium

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In hair cell regeneration, most new hair cells arise via mitosis; a minority arise by direct transdifferentiation (DT). The goal of this study was quantify the role of DT and characterize its time course. A single dose of 300 mg/kg of gentamicin caused total hair cell loss in the basal end of the chick auditory papilla. BrdU was continuously infused via implanted pump-cannula system. Continuous presence of BrdU allows the identification of new hair cells that have arisen by DT, since they are unlabeled. We used an anti-myosin VIIA antibody to label new hair cells. We examined regenerating epithelia at 2, 3, 4 5, 6 and 10 days after gentamicin with confocal microscopy and cell counts.

BrdU labeled precursor cells appear at 3 days post gentamicin; BrdU labeled, myosin labeled cells (new hair cells) were first seen at day 5. Myosin labeled cells which were BrdU negative (DT hair cells) were first seen at 2-3 days, and were the majority of hair cells through day 6. From 6-10 days, the number of myosin DT hair cells stabilized and the number of mitotic hair cells increased. At 10 days, 2/3 of the new hair cells were BrdU labeled (mitotic) and 1/3 unlabeled (DT). Regenerating

hair cells with a "transitional" morphology were common; the majority were BrdU labeled but some were BrdU negative.

These results demonstrate that DT gives rise to the earliest new hair cells in regenerating epithelium, and is the major source of new hair cells for the first 6 days after a single gentamicin dose. Thereafter, DT ceases and mitotis is increasingly important, ultimately giving rise to about 2/3 of all new hair cells. The "transitional" morphology has been shown to be a normal stage of mitotic regeneration (Stone and Rubel, 2000); these data confirm that finding and demonstrate that it is also a normal stage in DT.

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596 Trophic Factor Instillation Causes Both Functional and Morphological Recovery from Gentamicin-Induced Vestibular Toxicity in the Guinea Pig

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This study will determine the effects of brain-derived neurotrophic factor (BDNF), retinoic acid (RA), insulin-like growth factor one (IGF-1), and transforming growth factor-alpha (TGF-α) on the functional and morpholigic recovery after gentamicin instillation. Semicircular canal function was measured by sinusoidal Earth-vertical axis rotation (HVOR). HVOR gain was the primary measure of HVOR function. Otolith function was measured by constant rotation about an axis that is 30 degree from Earth-vertical (OVAR). For OVAR rotation, we used modulation sensitivity and bias velocity (offset of slow phase eye velocity from zero velocity). For animals instilled with gentamicin, and only TGF-α, RA, and IGF-1, the functional results showed an increase from the gentamicin-only group that did not reach normal levels. In general, animals instilled with gentamicin and any combination of growth factors that included BDNF showed a large degree of recovery of HVOR gain, OVAR modulation sensitivity and OVAR bias. From the morphologic results, it was obvious that gentamicin reduced the number of ampullar type I cells, and BDNF was needed for the return of type I hair cells. Type II hair cells were not changed significantly when treated with gentamicin, or any trophic factor. Thus, all four trophic factors are necessary to achieve the result of full functional recovery, and that full recovery is correlated with the return of type I hair cells. The return of type I hair cells is associated with the presence of BDNF. The presence of the other trophic factors is associated with limited functional and morphologic recovery of the vestibular periphery. Hence, it appears that the morphologic recovery that is produced by exogenous BDNF is paralleled by recovery of semicircular canal and otolith function.

597 Spontaneous Renewal of Vestibular Hair Cells following Gentamicin Exposure in Postnatal Rat Utricular Explants

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To investigate vestibular hair cell renewal in the mammalian inner ear we established an *in vitro* model of long-time culture (i.e. up to 28 days) of 4 day-old rat utricular maculae. In one series of experiments the utricles were exposed to 1 mM of gentamicin for 48 h and then allowed to recover in either unsupplemented medium or medium supplemented with the anti-mitotic drug, aphidicolin. In a parallel series, control explants were not exposed to gentamicin. Utricles were harvested from the 2nd to the 28th day *in vitro*. Whole-mount utricles were immunostained with phalloidin-FITC and stereociliary bundles were then systematically counted. In a 2nd series of parallel experiments BrdU was added to the medium. At harvest, these explants were immunostained with an anti-BrdU antibody tagged with either FITC or biotin and labeled cells counted.

Loss of hair cell stereociliary bundles was nearly complete 3 days after gentamicin exposure with the stereociliary bundle density only 3-4% of their density prior to exposure. Renewal of hair-cell bundles was robust in explants recovering in medium without aphidicolin, where a peak (i.e. a 15 fold increase) of stereociliary bundle renewal was reached on day 21 *in vitro.* However, sparse renewal of stereociliary bundles did occur in the presence of aphidicolin. The anti-mitotic efficacy of aphidicolin on the utricular epithelium was verified in the second series of experiments.

Our results suggest that spontaneous renewal of vestibular hair cell stereociliary bundles after gentamicin damage does include mitotic events within the epithelium of the utricular macula explants. Using our *in vitro* model in similar experimental series we are currently analyzing LM and SEM preparations as well as preparations that are doublestained for BrdU and myosin VIIa.

598 The Inferior Colliculus: A Model for the Search of Molecules Implicated in the Inhibition of Axonal Regeneration

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It is well known that axons fail to regenerate following sectioning in the adult mammalian central nervous system. We have previously shown that the inferior colliculus (IC) represents an interesting model to study axonal regeneration in vitro (Hafidi et al. 1995, J Neurosci, 15:1298-1307). Sectioning the IC commissure in gerbil organotypic cultures at early postnatal development stages (P6) is followed by extensive axonal regeneration. The regenerated axons form synaptic contacts in the controlateral IC lobe. However, the commissural axons sectioned later during development (P12) stop sprouting when in contact with the healing zone (Hafidi et al. 1999, J. Neurobiol, 41: 267-80). In the present study, we used the suppressive subtractive hybridization technique on mice commissural tissues dissected at P6 and P12 stages to identify molecules involved in the inhibition of axonal regeneration at P12. False positive clones were eliminated by dot-blot analysis of forward and reverse subtractions. After sequencing more than 300 clones, we selected 30 potentially interesting molecules of which some are of poorly known (e. g. Rho N, TMS II) and/or of unknown function (e. g. RIKEN/IMAGE clones). We are currently studying the expression and distribution of these candidate molecules in the mice brain by quantitative PCR and in situ hybridization.

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599 Survival, Migration and Neurite Outgrowth of Dorsal Root Ganglion Neurons following Xenograft Implantation into the Adult Rat Inner Ear

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The poor regeneration capability of the sensory epithelium and neural components of the mammalian inner ear has initiated different approaches to enhance the functionality of these structures after injury. An interesting alternative is to use a biological implant with the potential to establish synaptic contacts with the cochlear spiral ganglion neurons and with the perspective to develop into a functional auditory unit. This hypothesis was tested by xenograft implantation of embryonic dorsal root ganglion (DRG) neurons into the adult inner ear. The DRG

neurons were taken from transgenic animals expressing green fluorescence protein (GFP) or LacZ at embryonic days 13-14. The implants were transplanted into the scala tympani of adult rat inner ears. To enhance cell survival and possible neurite outgrowth, nerve growth factor (NGF) was perfused into the inner ear using a miniosmotic pump. The results show that transplanted DRG neurons survived for a postoperative survival time ranging from three to ten weeks, verified by GFP fluorescence, histochemical detection of LacZ, mouse neural marker neurofilament and Thy1.2 antibodies. The surviving DRG neurons in the scala tympani were frequently attached to the osseous spiral lamina, close to the organ of Corti. They were also observed within the Rosenthal's canal, among the spiral ganglion neurons and their peripheral processes to the organ of Corti. NGF application stimulated extensive neurite outgrowth of DRG neurons. These results illustrate not only the survival, migration and neurite outgrowth of xenografted DRG neurons in the adult inner ear but also the feasibility of neural transplantation into the adult auditory system, thereby creating possibilities to replace injured spiral ganglion neurons in the cochlea.

600 The Effects of Gentamicin Damage on Hair Cell Death and Regeneration in the Oscar Inner Ear

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Fishes, like humans and other vertebrates, detect sound using inner ear sensory hair cells. In humans and other mammals, sensory cells that are killed by intense sounds, drugs, or aging, are lost permanently. In contrast, fishes not only continue to proliferate large numbers of sensory hair cells throughout much of their lives, it is also known that hair cells damaged by aminoglycoside antibiotics are repaired or replaced within 20 days of treatment (Lombarte et al. 1993, Hear. Res., 66:166-174). However, it is not known if aminogloycoside damage leads to hair cell death and replacement by newly proliferated cells, or whether the hair cells are damaged by the drug and then repaired. The purpose of the present study is to differentiate between these two competing hypotheses of cell death and replacement vs. damage and repair. Oscars (*Astronotus oscellatus*) were administered a single high dose intramuscular injection of gentamicin and sacrificed according to a specific time course to follow hair cell death/damage and regeneration/repair. Phalloidin staining was used to evaluate hair bundle damage at each time point. Cell death was detected by morphological characteristics such as the presence of pyknotic nuclei or cell extrusion. Apoptotic cells were also identified using a fluorescent substrate that binds active caspase. The results of this investigation will lead to a better understanding of cell fate following ototoxic damage in fish.

601 Production and Utilization of a Mouse "Inner Ear/Organ of Corti Pertinent In Silico Microarray

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Functional genomic analyses using cDNA microarrays have demonstrated a great utility in drug discovery, disease diagnosis, identification of candidate and new genes, gene profiling, and pathway assembly of complex biological systems and gene networks. Use of this technology in the expressional analyses of the inner ear is limited. Two technical dilemmas that prevented its widespread use in the inner ear are: 1) the lack of extensive inner ear mouse cDNA arrayed sets and 2) the concentrations of total RNA needed to generate reliable data. We have utilized two mouse cDNA libraries: The RIKEN inner ear subtracted cDNA library (http://www.ncbi.nlm.nih.gov/UniGene/lib.cgiORG=Mm&LID=572) and the NIDCD organ of Corti cDNA library (http://neibank.nei.nih.gov/Ear/ NbEar.shtml) for production of an inner ear "pertinent" chip representing ~6,000 non-redundant cDNAs. About 2/3 of these cDNAs represent known genes or defined Unigene EST clusters. The remaining cDNAs are ESTs not found in GenBank NR or EST databases. They are localized to specific chromosomal locations using NCBI/NIH and Ensembl mouse genomic BLAST searches.

Presently, direct and indirect labeling (e.g., indirect amino-allyl labeling, and the dendrimer labeling method) of nucleotide probes requires 20 µg and 2.5 µg respectively of total RNA. We are now using our microquantity-cDNA protocol to test the feasibility of generating probes for expression analyses using pg amounts (i.e., a few cells worth) that represent unique cell types in the inner ear (e.g., inner and outer hair cells). Initial comparisons among subdissected fragments [spiral ganglion, organ of Corti, and the cochlear lateral wall (stria vascularis)] are being done to classify each cDNA according to its cochlear expression pattern. These inner ear-specific cDNA clones could be used as the first step in determining the functional/structural roles of these genes and as potential candidates for non-syndromic hearing disorders.

602 Real-time Quantitative Polymerase Chain Reaction for Low-Abundance Transcripts and Dilute Samples from the Inner Ear

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Real-time quantitative RT-PCR (qRT-PCR) is a relatively new, highly sensitive technology that allows high throughput quantification of gene expression levels. This technique should be, in principle, of great aid to study gene expression in the inner ear, where the small tissue size prevents the use of techniques such as Northern blot. However, working with RNA from inner ear has some specific difficulties: 1) the ear is a small organ encapsulated in bone, which complicates reliable surgical extraction of anatomically distinct parts, 2) RNA yield from individual specimens is low, beyond the limit of detection by conventional spectrophotometers, 3) RNA purity cannot be routinely determined from RNA gels since they require 1 µg of sample - the entire RNA yield from two cochleae, 3) cDNA synthesis from samples with these low RNA concentration is inefficient, 4) cDNA samples are often dilute, making detection of low-abundance transcripts and small changes in these transcripts difficult.

To allow reliable quantification of small changes in low-abundance transcripts in the inner ear we tested many parameters and reagents to optimize these procedures. We found the following steps to result in optimal qRT-PCR reactions. 1) Extraction of total RNA by RNeasy spin-column system (Qiagen), 2) on-column and off-column DNase treatment of RNA, 3) analysis of RNA purity, concentration and integrity by Agilent 2100 bioanalyzer, 4) cDNA synthesis using Omniscript kit (Qiagen) and random hexamers. Other important parameters to be worked out are the identification of an appropriate normalizing gene, design of primers that behave linearly and do not produce primer-dimers. Using these steps and SYBER Green fluorescence dye as a detection method we have successfully quantified the levels of mRNA for several trophic factors in the inner ear of wild type and transgenic mice, measuring differences as little as 2 fold in gene expression.

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603 Inner Ear Specific Gene Disruption in Neonate Mice in vitro

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Although targeted gene disruption is a good way to analyze the function of the gene in vivo, phenotypes including physiological functions in

adult stages cannot be studied in the genes which have important roles in development because of embryonic lethality. It is possible to avoid this problem using conditional knockout system where the gene disruption is limited in specific organs or at specific time. In Cre/loxP system, for example, Cre protein which is expressed under the control of organ specific or drug dependent promoter excises out the sequences flanked by two loxP sequences at 5' and 3' ends respectively. As for inner ears there are few Cre transgenic mice reported which specifically express Cre protein in the inner ear and some of them survive only until perinatal period.

To solve these problems we tried inner ear specific gene disruption using explant culture of organ of Corti from neonatal Cre reporter mice which express lacZ protein only after Cre protein excises out stop codon flanked by loxP sequences. This time we used adenovirus vector to express Cre in the explant culture of organ of Corti. Three days after infection almost all cells in outer hair cell region express lacZ protein although only few cells express lacZ in inner hair cell region.

The high gene disruption rate in outer hair cell region means that this system is very useful in studying the influence of gene disruption on electrophysiological activity, homeostatic maintenance and chemical impairment of outer hair cells in the inner ear.

604 The Role of Promoters and Adenoviral Construct on Persistence of Gene Expression in Organ of Corti and Macular Cultures

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The utility of adenoviral vectors for studying gene transfer in the cochlea has been demonstrated in numerous studies. Most studies have used a first generation E1/E3 deleted vector with the transgene driven by a CMV promoter. Most studies have suggested that gene expression using this system lasts only a few days. Using a luciferase expression system a variety of promoters and adenoviral constructs were tested on postnatal rat organ of Corti cultures and adult mouse utricular cultures. Intensity of luciferase expression was used to determine the promoter and vector combination that maximized gene expression in terms of degree and duration. Using a green fluorescent protein expression system the distribution of vector within organ of Corti and macular cultures was determined. The data suggest that robust gene expression can be maintained in auditory neuroepithelium using a variety of promoters. The potential to deliver genes to hair cells as well as damaged neuroepithelium was demonstrated by treating cultures that had been pretreated with aminoglycosides. By varying the promoter used, low to high level expression can be achieved for varying time periods. This combined with the observation that adenovirus can be concentrated into a small volume makes this vector ideal for auditory and vestibular applications.

605 A Cre Recombinase Expressing Mouse Line for Hair Cell-Specific Gene Targeting

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Hair cell specific gene targeting is necessary for the determination of the gene function in hair cells of the inner ear. The Cre/LoxP system has been used successfully for cell-type specific gene targeting in other tissues including the nervous system. In this study, we hope to create mouse lines in which Cre is specifically expressed in hair cells of the inner ear. We cloned a 9-kb genomic fragment from the mouse Prestin gene that contains the putative TRE, exon III (the first coding exon) and part of intron III. Following the 9-kb fragment, we inserted an internal ribosome-entry site (IRES), a 1.7-kb Cre coding region with nuclear localization signal (NLS) and the SV40 large T antigen polyadenylation signal. A total of 11.3-kb fragment of this construct was prepared for micro-injected into the pronuclei of fertilized one-cell eggs from FVB/NJ. Three founders were identified by PCR and Southern blot analyses. The founders were mated with the reporter mouse line, ROSA26R. Mice positive for both Cre and LacZ were analyzed using X-gal staining for Cre activity. One of the three founders was found to have Cre activity specifically in hair cells of the organ of Corti and vestibular end organs. In addition, Cre activity is detected in spiral and vestibular ganglia and in other tissues such as testis, kidney and brain, but not in liver. In the organ of Corti, Cre activity was first detected at postnatal day 14 (P14) in inner hair cells (IHCs), and subsequently at P75 in both IHCs and outer hair cells (OHCs). This Cre transgenic mouse line can readily be used by the research community for crossing with floxed (LoxP) mice for studying gene functions in hair cells of the organ of Corti and vestibular end organs.

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606 Non Viral Gene Delivery in the Auditory System: The Role of Dendrimers and Non Lipid Polymers.

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Liposomal gene delivery using agents such as lipofectamine have been demonstrated in the inner ear both in vitro and in vivo. Overall traditional liposomal gene therapy has been found to be very inefficient. Newer non viral delivery techniques are available that use novel polymers to condense DNA into small particles. Using a variety of these polymers, plasmids carrying a CMV promoter and an enhanced green fluorescent protein were introduced into cultures of neonatal organ of Corti and adult macular neuroepithelium. The distribution of gene expression was assessed using microscopy and semi-quantitative RT-PCR. A dendrimer preparation was found to function well in vitro and was used to test gene expression in an in vivo preparation. The expression plasmid and an AAV plasmid carrying the GFP gene were introduced into the inner ear via a cochleostomy and by applying the vector-dendrimer mix directly to the round window. Distribution of GFP, duration of expression and effect on hearing was examined for different vector and delivery strategies was examined.

607 Sendai Virus Vector Mediated Transgene Expression in the Cochlea and Middle Ear In Vivo

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Inner and middle ear gene transfer is an attractive new approach for hearing diseases. Sendai virus (SeV) is an enveloped virus with non-segmented negative sense genome RNA and the recent recombinant SeV technology have shown that the vectors designned from the virus very efficiently mediate gene transfer and expression. The transgenes introduced by the SeV vectors are present as RNAs in cytoplasm thereby suggesting that this gene transfer system are substantially free from genotoxicity. There are no reports had appeared reporting its severe pathogenicity to human. SeV vectors carrying the green fluorescent protein gene (SeV-GFP) and lacZ (SeV-lacZ) were injected via guinea pigs middle ear, cochleostomy (via scala tympani and scala vestibuli). We have investigated which cell types transduce in pigmented guinea pig middle ear, cochlea and mouse cochlea in vivo. Immunohistochemical analysis demonstrated that this vector can be transfected with very different types of cells of middle ear mucosa middle ear, scala tympani of cochlea, fibrocytes of stria vascularis and the organ of Corti in cochlea including supporting cells. The Preyer's

reflex remains intact in all animals, suggesting that SeV vectors did not affect hearing function. It might stimuli hair cell regeneration will be a powerful tool for repairing the organ of Corti and SeV vectors might be useful to compensate the functions of these mutated genes.

These data suggest that the SeV vector- mediated transgene expression is a useful modality for the remedy of disease affecting the mammalian middle ear mucosa and cochlea.

608 Evolution of a Dual-function Gene

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The human *CTBP2* gene encodes two known proteins that have apparently unrelated functions. One product, known as C-terminal binding protein 2 (CtBP2) is a transcriptional co-repressor that binds zinc-finger proteins. The other product (RIBEYE) is a cytoplasmic protein that appears to be a structural component of "ribbon-class" synapses in hair cells and photoreceptors, where it has been hypothesized to serve as a molecular motor. In mammals, CtBP2 is encoded by nine exons, with a large intron between exon1c and exon2. Exon1R of Ribeye is contained within this large intron. The two isoforms thus have different N-termini, each of which is encoded by single 5' exons (A domains), that are spliced onto a common C-terminus (B-domain) encoded by the same eight exons. To understand how novel developmental and physiological functions can evolve from a single gene, we have been investigating the genomic structure and embryological function of *ctbp2* in pufferfish, zebrafish, humans, and cows. Our preliminary data suggests that the last common ancestor of fish and mammals already had a single copy of the *ctbp2* gene, and that a later duplication in the fish lineage generated a second copy of this and several flanking genes in pufferfish and zebrafish. Developmental analysis is revealing changes in gene function after the teleost gene duplication.

609 AAV5 and AdV5 Transduce Different Cell Type in the Mouse Cochlea: *in vitro* and *in vivo* Analysis.

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We investigated AdV5(E3-)CMV-GFP (from the University of Iowa Gene Therapy Vector Core) and AAV5-CMV-GFP (from Targeted Genetics) both *in vitro* and *in vivo* for their relative ability to transduce cells in the mammalian cochlea. *In vitro*, cochleae of neonatal (P4) Swiss Webster mice were cultured in insert wells for 24 hrs. Cultures were then transfected for 48 hrs and recovered for another 48 or 96 hrs. In Corti's organ, Deiter's cells were the predominant cell type transduced by AdV5. In contrast, Hensen's, Cladius, and outer and inner hair cells (IHC) were transduced by AAV5.

In vivo, adult mouse cochlea (2-3 months old) were injected with a 2uL solution of AdV5 ($1.3x10^7$ pfu/uL) or AAV5 ($2.1x10^{13}$ DNaseI Resistant particles/uL) through a surgically created cochleostomy. Mice were allowed to recover for 7 to 14 days. Following cochleostomy in the basal turn, a gradient of transduction was observed for both AdV5 and AAV5, with more cells being GFP positive near the site of cochleostomy. AdV5 transduced Deiter's cells in Corti's organ. It also transduced the saccular supporting cells, cells within the basilar membrane, Reissner's membrane, and stria vascularis. On the other hand, AAV5 transduced mainly IHCs, with Claudius and Hensen's cells to a lesser extent. These data demonstrate the utility of AdV5 and AAV5 in differentially transducing organ of Corti cell types both *in vitro* and *in vivo*.

610 Expression of Musashi1, a Neural RNA-Binding Protein, in the Mouse Inner Ear

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The Musashi1 (Msi1) is an RNA binding protein highly expressed in neural stem cells. One of the targets of Msi11 is *m-Numb*, and its product m-Numb is a Notch antagonist. Msi1 activates Notch signal by translational repression of m-Numb. Notch signaling is involved in maintaining the undifferentiated state and the renewal ability of mammalian neural stem cells in central nervous system. In mammalian inner ear, recent studies have shown the role of Notch signaling in hair cell fate determination. Math1 induces hair cell differentiation, but when Notch signal is activated, Math1 is inhibited and hair cell differentiation is prevented. In this case, the cells are thought to become supporting cells and may continue to hold a kind of renewal ability. We hypothesized Msi1 is expressed in the cells, which has potential to differentiate into hair cells. To this end, we investigated the Msi1 expression in the mouse inner ear by immunohistochemistry using monoclonal antibody against Msi1. In the vestibular periphery, the immunoreactivity of Msi1 was observed in supporting cells , a few Type… hair cells in the cristae ampullaris. It was also found in a small number of supporting cells in utricular and saccular macula. This result inspired us a kind of relation between Msi1 positive cells and predicted hair cell progenitors. In a cochlea, strong immunostaining of Msi1was found in Deiters cells and root cells, but not in hair cells. Moderate staining was seen in some other supporting cells. We suggest that this mosaic pattern of the Msi1- immunoreactivity may reflect Notch signaling-mediated lateral inhibition during the inner ear development.

611 Altered Neurotrophin Expression in the Cochleae of Transgenic Mice Expressing a Dominant-Negative erbB4 Receptor Under the Control of the GFAP Promoter

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Degeneration of cochlear sensory neurons is an important component of sensorineural hearing loss, and long-term survival of these neurons is critical for successful functioning of cochlear implants. The mechanisms involved in the survival of adult cochlear sensory neurons are not clearly defined. We generated transgenic mice that implicate neuregulin (NRG)/erbB receptor signaling in the survival of cochlear sensory neurons (Rio et al, ARO'99 abstract). NRG-erbB signaling in the inner ear of these mice is disrupted due to postnatal expression of a dominant-negative erbB receptor in cells expressing GFAP. Mutant mice show severe hearing loss and 80% postnatal loss of spiral ganglion neurons, without concomitant loss of sensory cells. DNerbB4 expression in the cochlea is localized to supporting cells abutting cochlear sensory cells. Based on these results we hypothesized that erbB receptor signaling in supporting cells plays a critical role in the maintenance of neuronal survival, and that this is mediated by the neurotrophins.

To test this hypothesis we first studied the pattern of expression of NRG and erbB receptors in the postnatal cochlea by immunostaining. We found that NRG is expressed by spiral ganglion neurons while erbB2 and erbB3 are expressed by supporting cells of the organ of Corti. Then, by quantitative RT-PCR we compared the levels of neurotrophin expression in the cochlea of wild-type and mutant mice. As predicted, we found a significant reduction in the levels of expression of both neurotrophins in the mutant ear (2.5x for NT3, 2x for

BDNF). Interestingly, in the cochleae of DNerbB4 transgenic mice, there is a 2.8x increase in GDNF mRNA levels compared to wild type, indicating that the reductions in NT3 and BDNF are specific. These results suggest that survival of postnatal cochlear sensory neurons depends on reciprocal interactions between neurons and supporting cells, these being mediated by NRG and neurotrophins.

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612 The Role of the Brn-3c Transcription Factor in Regulating Neurotrophin Gene Expression in Hair Cells.

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The Brn-3c transcription factor (also known as Brn-3.1 and POU4F3) is necessary for maturation and survival of hair cells in the inner ear. Hence, mutations in Brn-3c are associated with inherited non-syndromic hearing loss in humans and deafness in mice due to inner ear hair cell loss. Brn-3c must mediate its effect through altering expression of specific target genes in hair cells. These genes remain largely unidentified. In this study we investigated the possibility that Brn-3c could regulate neurotrophin gene expression in two hair cell lines. Co-transfection experiments were performed with various neurotrophin promoter reporter gene constructs and Brn-3c expression vectors. These identified specific neurotrophin promoters that were up-regulated in both hair cell lines in the presence of exogenous Brn-3c. DNA footprinting and deletion analysis was used to map the Brn-3c binding sites within the neurotrophin promoters. Bandshift assays and supershift experiments with extracts from hair cell lines confirmed the presence of Brn-3c binding to these putative response elements. Furthermore naturally occurring mutations in Brn-3c which are known to cause hearing loss abolish the ability of Brn-3c to activate the neurotrophin promoters. This *in vitro* data although needing to be confirmed *in vivo* do suggest a role for Brn-3c in regulating neurotrophin gene expression in hair cells with implications for understanding the mechanisms involved in inner ear innervation and ganglia cell survival.

613 Cochlear Function in Mice Lacking α-calcitonin Gene-related Peptide (αCGRP)

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α-Calcitonin Gene-Related Peptide (αCGRP) has been implicated in neurotransmission between olivocochlear (OC) efferent terminals and their cochlear targets. Throughout the mammalian cochlea, OC terminals in the IHC area are immunopositive for CGRP; in mouse and rat, OHC terminals are CGRP-positive, as well [Maison et al., 2002, J Comp Neurol, in press]. In the lateral line, bath application of CGRP increases spontaneous discharge rates in afferent fibers [Bailey & Sewell 2000, J Neurosci 20:5163-9].

To clarify the cochlear role of this neuropeptide, we characterized the auditory phenotype of αCGRP-null mice [Lu et al., 1999, Mol Cel Neurosci 14:99-120] by measuring 1) ABRs to tone pips at 7 log-spaced frequencies from 5.6 to 45.2 kHz, 2) DPOAEs evoked by primaries with f2 at the same 7 frequency values, and 3) the magnitude of classic OC suppressive effects on DPOAE amplitude evoked by electric stimulation of the OC bundle. Groups of mice homozygous for the αCGRP deletion were compared with wildtype littermate controls. Lack of cochlear CGRP was confirmed by immunohistochemistry.

Cochlear threshold sensitivity was unchanged by αCGRP deletion, as measured by 1) visual inspection of ABR waveforms obtained at 5 dB level increments and 2) DPOAE iso-response contours interpolated from amplitude-vs-level functions. Similarly, the magnitude of classic OC suppressive effects was unaffected in homozygous mutants. Significant differences were seen, however, in supra-threshold neural responses. Suprathreshold ABRs in αCGRP-null mice showed a frequency-independent amplitude reduction of roughly 20% of the response; whereas, DPOAE suprathreshold amplitudes were indistinguishable from normal. These data are consistent with an excitatory effect of CGRP release by the lateral OC system on auditory nerve response, either by pre- or post-synaptic effects in the IHC area.

614 Postnatal Expression of Cytosolic Phospholipase A2 in the Gerbil Inner Ear

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The expression of cytosolic phospholipase A2 which regulates the production of eicosanoids was investigated immunohistochemically in the gerbil inner ear during postnatal development. Cochlear inner hair cells, vestibular type I hair cells and ganglion neurons showed immunoreactivity for cytosolic phospholipase A2 as early as 1 day after birth and continued to stain in the mature inner ear. On the other hand, staining for the enzyme in cochlear outer hair cells and type II vestibular hair cells was transient. Outer hair cells demonstrated immunoreactivity for cytosolic phospholipase A2 between 4 and 16 days after birth. Immunostaining of type II vestibular hair cells was present at birth but also disappeared in the adult. Although the significance of these changes in expression of cytosolic phospholipase A2 with development remains unknown, we speculate that this enzyme may be involved in regulating the expression of calretinin and thus influence calcium flux during postnatal development. Such an association is indicated by the coincidental expression patterns of these mediators in the postnatal developing ear.

615 Purinergic Regulation of Resting Membrane Potential in Deiters' Cells

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Recent research has suggested that cochlear supporting cells, including Deiters' cells, play an important role in auditory signal transduction. These cells help to maintain the structural integrity , but they may also actively participate in regulating sound transduction. Change of the micromechanics in Deiters' cells can influence the whole organ of Corti. In perilymphatic fluid around the Deiters' cells, the ATP concentration is increased by stresses such as ischemia or noise exposure, implying that the neurotransmitters acting on the Deiters' cells influence cochlear mechanics. The resting membrane potential can be maintained by the ionic currents along the cell membrane. In this study, we investigated the control of resting membrane potential by ATP and acetylcholine in Deiters' cells.

Deiters' cells were isolated from guinea pig organ of Corti using collagenase and pipette. Whole cell patch clamps were performed under an inverted microscope and the current was measured with pClamp software.

The resting membrane potential of Deiters' cells was -21.1 ± 3.5 mV (n=7) in normal Tyrode solution (2 mM of calcium). ATP (100 μM),

applied by continuous flow system, depolarized the potential to -3.1 ± 1.1 mV, while each of acetylcholine and carbachol (100 μM) had no effect on the membrane potential.

ATP-induced depolarization was reproduced repeatatively. In calcium-free solution with EGTA, membrane potential was maintained and the also depolarized by ATP.

In conclusion, ATP-gated purinoceptors may regulate the transduction processes by controlling the membrane potential in Deiters' cells. The component of the ionic currents affected by purinoceptor will be discussed.

616 Immunohistochemical Detection of Caveolin-1 in the Cochlea of Mice

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Introduction: Caveolin is an intrinsic membrane protein with a molecular weight of approximately 25 kDa. Three types of the protein, caveolin 1, caveolin 2 and caveolin 3 have been identified and caveolin 1 is abundantly expressed in many tissues. Caveolin is involved in signal transduction mechanisms, including protein phosphorylation by protein kinases and G-protein-mediated signal transduction. The purpose of the present study is to investigate the expression of caveolin 1 in the cochlea of mice.

Materials/methods: Seven 7-week-old C57BL/6 mice were used as experimental animals. Auditory brain stem responses (ABR) were measured to evaluate auditory function. The cochleae were fixed in 4% paraformaldehyde overnight after cardiac perfusion, decalcified in 10% EDTA and embedded in paraffin. Mid-modiolar sections were incubated with a primary antibody to caveolin-1 (mouse monoclonal, Transduction Laboratories). A biotinylated secondary antibody was used for accentuation. Processing was ultimately performed by an HRP-streptavidin complex and nickel-enhanced DAB, with subsequent observation under a light microscope. Results/conclusions: ABR recordings showed that the 7-week-old mice showed normal hearing. All of them showed normal Preyer' s reflex. Immunohistochemical investigations revealed that caveolin 1 was expressed mainly in the supporting cells of the organ of Corti, spiral ganglion cells and cells in the spiral ligament. These results suggest that caveolin 1 may be associated with signal transduction mechanisms in the cochleae of C57BL/6 mice.

617 Expression of Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) and its Receptor, PAC1-R, in the Inner Ear

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a member of the glucagon superfamily of peptides that has close amino acid sequence similarity to VIP and GHRH, and can couple to adenylyl cyclase activation with 100 times the potency of VIP. With specific primers and RT-PCR, we have obtained evidence of cDNA expression for PACAP precursor protein in organ of Corti, lateral wall and spiral ganglion microdissected subfractions of the rat cochlea. Primers, targeting conserved cDNA sequence for PACAP in teleosts, directed amplification of products of predicted size for cDNA of a model hair cell preparation from the trout saccule, with nucleotide sequences corresponding to those of respective PACAP transcripts in trout brain. The action of PACAP in these inner ear tissues is predicted to occur through activation of PACAP receptors. Specific primers encompassing the splice variant region in the third intracellular loop of the PACAP receptor (PAC1-R), with five known splice variants, directed amplification of a 819 bp PCR product corresponding to GenBank no. Z23279 in all three microdissected cochlear subfractions, with nucleotide sequence confirmation. An additional PCR product, 926 bp in length, possibly corresponding to one of the splice variants, was detected in the lateral wall subfraction. Immunohistochemical analysis of peptide expression in the rat inner ear suggests PACAP is present in afferent nerve fibers of the cochlea. Light immunostaining was observed in the stria vascularis, consistent with sites of expression on basolateral extensions of marginal cells. PACAP immunoreactivity appeared to be associated with neural elements in the sensory epithelia of mammalian vestibular end organs and with afferent nerve and hair cells in the trout saccule. PAC1-R receptor immunoreactivity has been localized in the rat cochlea to the auditory nerve, the nerve fiber region at the base of outer hair cells, with light staining in the stria vascularis. For the rat vestibular end organs, saccule, utricle and crista ampullaris, PAC1-R immunoreactivity was primarily associated with the nerve calyces surrounding the type I vestibular hair cells. A role for PACAP and PAC1-R in modulation of afferent neural activity in the inner ear is hypothesized, possibly through interaction with dopaminergic efferents.

618 The Presence of Substance P in Afferent Pathways of the Human Labryinth

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In peripheral nerve fiber systems of the human labyrinth, both cochlear and vestibular sensory hair cell populations are innervated by afferent neurons. Substance P,released from capasaicin-sensitive afferent nerve fibers, is a neuroactive substance of somatic sensory systems and a member of the tachykinin family: tachykinins are peptides believed to reduce potassium conductance for depolarization of excitable membranes and elevate intracellular calcium concentrations. Though the presence of this protein has been investigated in ultrastructure components of rodent and human labyrinth, Substance P has not yet been documented in the human organ of Corti. This study presents the immunohistochemical presence and distribution sites of Substance P within nerve fiber terminals of the normal human adult, inner ear. Utilizing light and electron microscopy, positive immunoreactivity could be confirmed in the vestibula and the cochlea of both human and rat. Findings revealed the presence of SP like-immunoreactivity within three afferent nerve fiber pathways of the cochlea: the inner radial fibers of the inner hair cell region, the tunnel crossing fibers and the outer spiral bundles of the outer hair cell region. Concordant with the cochlea, afferent components of all five vestibular endorgans expressed a positive SP reactivity. Immunoreactive sites were consistently concentrated along distal portions of Type I and Type II hair cell regions within the neurosensory epithelium. Moreover, in both the cochlea and vestibula, sensory and supporting cell populations were cytochemically absent of Substance P like-reactivity. Results and functional implications are presented and discussed.

619 Dopamine Receptor Expression in the Inner Ear

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Dopamine has been shown to have a neuroprotective role in the inner ear, in particular, in the cochlea, presumably mediated by dopamine receptors. Previously, we demonstrated that of the dopamine receptor subtype transcripts, specifically those for D1B and D2L were expressed in an organ of Corti microdissected cochlear subfraction in the rat (Kewson et al., *ARO Abstr.* 22: 123, 1999). In the present investigation, immunoreactivity to dopamine D2L receptor protein has been localized beneath the inner hair cell in the apical turn, consistent with the site of lateral efferent axon/type I afferent dendrite interaction. Immunostaining was also observed associated with the base of the outer hair cell with light microscopy, apparently concentrated in neural endings of small caliber (presumably efferent). Immunoreactivity was also observed associated with supporting cells, including both Hensen's cells and Claudius cells. Dopamine D1B immunostaining, visible primarily in the apical turn, appeared associated with nerve fibers in cross section overlapping the apical and basal regions of outer hair cells. Staining, possibly neural, was also associated with Hensen's cells and Claudius cells. The presence of a dopaminergic pathway in vestibular end organs has been suggested by electrochemical detection of dopamine-related metabolites in rat vestibule (Gil-Loyzaga et al., *Brain Res*. 746: 265-8, 1997) and in teleost saccular macula and vestibular nerve fractions (Drescher et al., *ARO Abstr.* 21: 113, 1998). We obtained evidence that the teleost hair cell preparation contained mRNA for the dopamine D2 receptor (Oh et al., *ARO Abstr*. 21: 111, 1998). We now show with RT-PCR and degenerate primers targeting dopamine D1 sequence conserved across vertebrates, mRNA expression of dopamine D1A3 receptor in the teleost model hair cell preparation. Immunoreactivity directed to the dopamine D2L receptor was detected in the sensory epithelia of the saccule and crista ampullaris and the D1B receptor in the saccule of the rat. The staining appeared to be associated primarily with neural elements at the base of the sensory epithelia. The specific localization of dopamine receptors in both cochlear and vestibular sensory epithelia supports the hypothesis of dopaminergic neuromodulation of afferent signal.

620 Expression Analysis of Histamine Receptor Subtypes in The Rat Cochlea.

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It is reported that histamine may have a function as a neurotransmitter or a neuromodulators within the vestibule. On the other hand, it remains unclear even if histamine has a function within the cochlea. Previously, we reported that low concentrations of histamine (10 mM, 50 mM) increased the CAP (Compound Action Potential) amplitude without a change of CM (Cochlea Microphonic Potential) amplitude by perilymphatic cochlear perfusion in guinea pigs. We also reported the expression of histamine H1/H2/H3 receptors mRNAs in the rat cochlea at last this meeting. This time, we performed immunohistochemical stains using specific antibodies for histamine receptor subtypes to make sure the existence and detailed distribution of the receptors¡¦proteins.

Temporal bones were obtained from 4~8weeks old male Wistar rats. 8 *f*Ým-thick cryostat sections were cut in the midmodiolar plane. Immunohistochemical examinations were made using the avidin-biotin complex immunoperoxidase technique with vectastain ABC-PO (rabbit IgG) kit (Vector Laboratories Inc.). The location of the proteins was visualized by incubating the sections with diaminobenzidine.

H1, H2, and H3 receptors were detected immunohistochemically in the organ of Corti, spiral ligament, stria vascularis, spiral ganglion and auditory nerve.

These findings suggest that histamine has a function as a neuromodulator on the auditory system via histamine receptors.

621 Involvement of Vanilloid Receptors in Hearing: Physiological and Immunohistological Evidences

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Vanilloid receptors (VRs), which were primarily found in sensory neurons for noxious perception, have been found recently to exist in non-neural cells and tissues. Accumulating data suggest a possible presence of the VRs in the cochlea that may play a role in cochlear physiology. In this study, capsaicin or the more potent VR agonist, resiniferatoxin (RTX), were infused into the guinea pig cochlea and their effects on cochlear sensitivity were investigated. Capsaicin (20 micro M) elevated the threshold of auditory nerve compound action potential (CAP) and reduced the magnitude of cochlear microphonic (CM) and electrically evoked otoacoustic emissions (EEOAEs). These effects were reversible and could be blocked by a competitive antagonist, capsazepine. Application of 2 micro M RTX resulted in cochlear sensitivity alterations similar to that by capsaicin, which could also be blocked by capsazepine. A desensitization phenomenon was observed in the case of prolonged perfusion with capsaicin or RTX. Transient increase of cochlear blood flow by capsaicin was confirmed, and the endocochlear potential was not decreased. Basilar membrane (BM) velocity growth functions near the best frequency and BM tuning were altered by capsaicin. Immunohistochemistry study revealed the presence of TRPV1 receptors (the first cloned VR receptors) in the hair cells and supporting cells of the organ of Corti. The results indicate an action of capsaicin mainly on the outer hair cells and suggest that VRs in the cochlea may play a role in regulating the cochlear homeostasis and hence the cochlear sensitivity.

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622 Immunolocalization of Mu and Delta Opioid Receptor in the Mouse Cochlea.

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Previous studies in the mammalian cochlea have demonstrated enkephalin-like immunoreactivity in the lateral olivocochlear efferent fibers that primarily form axodendritic synapses on the afferent fibers under the inner hair cells. The circumnuclear, but not basal, efferent terminals on the outer hair cell are also enkephalin-like immunoreactive (Altschuler and Eybalin et al. review). The enkephalins have high binding potency to mu and delta opioid receptors. Therefore, we undertook the immunolocalization of the mu (MOR) and delta (DOR) opioid receptors in the mouse cochlea. Young adult mice (1-3 months old) were deeply anesthetized, perfused transcardially. The whole cochlea was microdissected to obtain surface preparations, then incubated at room temperature for 30 minutes with a blocking solution. The specimen was incubated with either of the primary antibodies delta or mu polyclonal antibody at room temperature for 16 hrs. The secondary antibody labeled with Texas red (1:800 in PBS containing 1% NGS) was applied. The nuclear dye DAPI was used. For light microscopy the ABC rabbit Vectastain kit together with DAB was used to visualize the antigen-antibody reaction. . MOR and DOR immunoreactivity were found in the tunnel crossing fibers, and the

putative large circumnuclear efferent terminals opposing the outer hair cells. Immunoreactivity was also found in thin fibers and their small (~1 micron diameter) terminal endings underneath the inner hair cells. The terminals directly synapsing onto the inner hair cells were not immunoreactive. The outer hair cells were moderately immunoreactive, and the inner hair cells were non immunoreactive. The spiral ganglion neurons were moderately immunoreactive. The immunolocalization pattern is similar to that of enkephalin like-immunoreactivity, suggesting that the endogenous opioid peptide, met-enkephalin, may act on mu and delta autoreceptors modulating the efferent neurotransmission.

623 Caffeine and Ryanodine Demonstrate a Role for the Ryanodine Receptor in the Organ of Corti.

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The hypothesis that the release of Ca2+ from ryanodine receptor activated Ca2+ stores in vivo can affect the function of the cochlea was tested by examining the effects of caffeine (1-10 mM) and ryanodine (1 - 333μM), two drugs that release Ca2+ from these intracellular stores. The drugs were infused into the perilymph compartment of the guinea pig cochlea while sound (10 kHz) evoked cochlear potentials and distortion product otoacoustic emissions (DPOAEs; 2f1-f2 = 8 kHz, f2=12 kHz) were monitored. Caffeine significantly suppressed the compound action potential of the auditory nerve (CAP) at low intensity (56 dB SPL; 3.3 and 10 mM) and high intensity (92 dB SPL; 10 mM), increased N1 latency at high and low intensity (3 and 10 mM) and suppressed low intensity summating potential (SP;10 mM) without an effect on high intensity SP. Ryanodine significantly suppressed the CAP at low intensity (100 and 333 μM) and at high intensity (333 μM), increased N1 latency at low intensity (33,100 and 333 μM) and at high intensity (333 μM) and suppressed low intensity summating potential (100 and 333 μM) and increased high intensity SP (333 μM). The cochlear microphonic (CM) evoked by 10 kHz tone bursts was not affected by caffeine at high or low intensity, and ryanodine had no effect on it at low intensity but decreased it at high intensity (10, 33,100 and 333 μM). In contrast, caffeine (10 mM) and ryanodine (33 and 100 μM) significantly increased CM evoked by 1 kHz tone bursts and recorded from the round window. Caffeine (10 mM) and ryanodine (100 μM) reversibly suppressed the cubic DPOAEs evoked by low intensity primaries. Overall, low intensity evoked responses were more sensitive and were suppressed to a greater extent by both drugs. This is consistent with the hypothesis that release of Ca2+ from ryanodine receptor Ca2+ stores, possibly in OHCs and supporting cells, affects the function of the cochlear amplifier.

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624 Link Between the Connexins and the OCPs

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OCP1 and OCP2, the two most abundant proteins of the organ of Corti (OC) have been shown to correspond to subunits of an E3-SCF ubiquitin ligase system, where OCP2 is a close homolog of Skp1, and OCP1 is an inner ear-specific F-box protein (Henzl et al. 2001). The proteins are expressed exclusively in the nonsensory cells of the OC, coinciding with the boundaries of the epithelial gap junction system, suggesting that connexins (Cx) are targets for ubiquitination by SCF^{OCP1} complex. Testing this hypothesis we determined that recombinant OCP1 and OCP2 form a high affinity, heterodimeric complex similar to that between Skp1 and 2 in the protypical SCF^{Skp2} of *S. cerevisiae*. Next we determined that OCP1 forms a specific noncovalent complex with recombinant Cx26. These data support the hypothesis that OCPs are involved in the regulation of gap junction activity. Because of energetic constraints of the OC it is conceivable that one of the evolving, nontraditional ubiquitination mechanisms is operational, wherein gap junctions are not degraded, but internalized temporarily and subsequently retargeted to the plasma membrane. Moreover, since cullin-1, another SCF subunit, is present at far lower levels than the OCPs, the OCP1-OCP2 complex may function autonomously, independent of the SCF paradigm, serving as temporary storage reservoir for the binding of F-box target proteins. These data should be viewed in the context of the importance of Cx26 mutations in the pathogenesis of hereditary hearing loss.

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625 A Chicken Transmembrane Cochlear Expressed Gene (ggTmc2) is Specifically Expressed in the Inner Ear

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The human non-syndromic dominant and recessive deafness disorders DFNA36 and DFNB7/11 and the murine dominant and recessive inner ear defects Beethoven (*Bth*) and Deafness (*dn*) are caused by mutations in the transmembrane cochlear-expressed gene 1 (*TMC1*). TMC1 is expressed by cochlear hair cells and mutations in TMC1 ultimately lead to hair cell degeneration. Changes in hair cell and auditory functions before the onset of degeneration ranged from normal in *Bth*/+ hair cells to no electrophysiological responses in *dn*/*dn* mice.

We have isolated a Tmc homologue from chicken cochlea using degenerate RT-PCR and cDNA library screening. We found several independent cDNA clones all of which encode identical putative protein with eight predicted membrane-spanning regions. The protein's 864 amino acid residues are 73% identical with mouse Tmc2 and 61% identical with mouse Tmc1. Therefore this gene is named chicken Tmc2 (ggTmc2).

Our Northern blot analysis revealed a 4.4 kilobase ggTmc2 transcript in the cochlea. We did not detect ggTmc2 mRNA in any other organ analyzed, including forebrain, cerebellum, lung, liver, heart and skeletal muscle.

Extensive database searches revealed multiple Tmc-related genes in human and mouse. This analysis also exposed Tmc homologues in puffer fish (*Fugu rubripes*), nematode (*Caenorhabditis elegans*), fruit fly (*Drosophila melanogaster*) and mosquito (*Anopheles gambiae*). Tmc proteins therefore form a family of presumptively integral membrane proteins that display high conservation within their central parts. The proteins' highly hydrophilic, presumably cytoplasmic, amino- and carboxyl-termini differ significantly among Tmc family members. The restricted expression of Tmc2 in the chicken and mouse inner ear implicates this protein in general hair cell function.

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626 Cochlin Is Expressed in the Supporting Cells of the Organ of Corti and Co-Precipitates with CTL2

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Antibody-induced damage to the inner ear is suspected to be a cause of rapidly progressive bilateral sensorineural hearing loss. We developed an animal model of antibody induced hearing loss in the guinea pig using the KHRI-3 monoclonal antibody. Infusion of highly purified KHRI-3 into the guinea pig inner ear results in binding to supporting cells, subsequent loss of hair cells and hearing loss. To identify the supporting cell antigen we used immunoaffinity purification of the

target protein from guinea pig inner ear extracts with the KHRI-3 antibody. The precipitated proteins were additionally purified with SDS-PAGE gel electrophoresis. Three distinct bands were cut out and sent for tandem MS sequencing. The 68-72 kDa bands contained ten peptide sequences identical to human CTL-2, a member of choline transporter family. The prominent 60 kDa band that co-precipitated with CTL-2 was found to be cochlin, a protein product of the COCH-5B2 gene. This gene is the target gene of DFNA9, a congenital hearing loss locus on human chromosome 14. Individuals with mutations of COCH-5B2 develop progressive hearing loss. Antiserum raised against an antigenic peptide within the cochlin sequence was tested on surface preparations and western blots of guinea pig organ of Corti. On western blots the cochlin antibody identifies 3 distinct bands of 61, 49, 40 kDa. Using immunofluorescence we observed co-localization of CTL2 and cochlin in the supporting cells of the organ of corti. This is the first demonstration of Cochlin expression in the organ of Corti. We propose a working hypothesis that CTL2 and cochlin are part of a macromolecular complex with a possible transporter function in the inner ear that is sometimes the target of autoimmune attack and that autoantibodies against these proteins may cause hearing loss.

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627 Increasing Deafness of KCC4 Knockout Mice Following the Onset of Hearing

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Sensitivity and selectivity of normal hearing depends on the active amplification by outer hair cells (OHC). The energy source of the so called “active process” is the driving force across the apical membrane of sensory hair cells. During sound exposure K+ enters OHCs through mechanosensitive channels and exits probably through KCNQ4 K^+ channels in the basal membrane.

Mice lacking the K^+/Cl^- cotransporter KCC4 showed no obvious abnormalities, while the acoustic Preyer reflex was absent in adult mice. ABR to clicks were recorded (400-2000) in anaesthetized mice (120mg/kg Ketamin, 16mg/kg Xylazinhydrochoride) from postnatal day 14 (P14) onwards. Hearing thresholds of KCC4 -/- mice were normal at P14, but deteriorate quickly during the following weeks. While wild type (WT) mice had no significant hearing loss at week 10, KO were nearly deaf, with a hearing loss of 70-80dB compared to WT.

Histological analysis revealed that the inner ear development was normal until P14. However at P21 basal OHCs were almost totally lost, whereas IHCs were still present. In adult mice the organ of Corti was completely absent, but some residual hair cells were found in the apical turn. The degeneration of hair cells was found to precede the loss of Deiters's cells.

Staining with antibodies against KCC4 revealed that KCC4 was confined to a subset of supporting cells. Deiter's cells were labeled along their lateral membrane as well as supporting cells of IHC's.

Our data support the hypothesis that KCC4 is important for the removal of K+ that is secreted into the extracellular space of OHC's during sound stimulation. Loss of KCC4 leads to a reduced salt uptake and interruption of the K+ recycling pathway via the gap junction system to the stria vascularis. As a consequence the ionic environment of OHC's is altered after they become sensitive to sound and leading to the death of these cells.

628 A Domain-Specific Usherin/Collagen Interaction is Essential in Stabilizing Usherin in the Basement Membrane Superstructure

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Usherin is a basement membrane protein encoded by the gene associated with Usher syndrome type IIa, the most common deaf/blind disorder. Genetic and biochemical approaches were used to explore the role of type IV collagen binding in usherin function. We demonstrate an interaction between the LE domain of usherin and the 7S domain of type IV collagen. A purified fusion peptide comprising the first four LE modules was shown to compete with native recombinant usherin for type IV collagen binding. However, synonymous fusion peptides with single amino acid substitutions resulting from missense mutations known to cause Usher syndrome type IIa in humans failed to compete. Only mutations in loop b of the LE domain abolished binding activity. Co-immunoprecipitation and Western blot analysis of testicular basement membranes from the Alport mouse model show that a 70% reduction in type IV collagen is associated with a similar reduction in usherin, suggesting the usherin/collagen (IV) interaction stabilizes usherin in the basement membrane. Thus, the domain-specific interaction between usherin and type IV collagen is essential to usherin function in vivo, and loss of this interaction results in Usher pathology.

629 Domain-Specific Integrin Binding may be Essential to Usherin Function in Humans.

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Usher syndrome type IIa (USH2A) results from defects in the gene encoding usherin, a new class of basement membrane protein. Usherin is found in virtually all of the basement membranes of both the inner ear and the retina, but how its absence or dysfunction results in pathology is unknown. To gain insight into the mechanism USH2A pathology, we attempted to identify receptors on both endothelial cells and epithelial cells that bind to usherin. We devised a cell adhesion assay where plates were coated with recombinant usherin. Domain-specific fusion peptides were used as competitive inhibitors for usherin binding to retinal pigment epithelial (RPE) cells. Three of the four functional domains of the protein would compete, suggesting multiple usherin receptors were present on RPE cells. Since many of the canonical domains shared by usherin and related proteins have integrin binding activity, we used neutralizing antibodies for integrin subunits as competitive inhibitors in our cell adhesion assay. Neutralizing antibodies against integrin $\alpha1$, $\alpha3$, $\alpha5$, and $\beta1$ blocked cell dhesion. To confirm their presence, we prepared plasma membranes from RPE cells, added recombinant usherin, immunoprecipitated complexes with anti-usherin antibodies, and analyzed the precipitated material by western blot using subunit-specific anti-integrin antibodies. Again, only $\alpha1$, $\alpha3$, $\alpha5$, and $\beta1$ subunits were detected. A fusion peptide comprising the thrombospondin domain of usherin in this same assay would precipitate only integrin $\alpha1\beta1$. Introduction of a missense mutation into this domain that is found in USH2A patients abolished the capacity to co-immunoprecipitate integrin $\alpha1\beta1$. Combined, these data suggest that usherin binding to integrin receptors (and specifically Ts domain binding to integrin $\alpha1\beta1$) may be essential to usherin function in humans.

630 Localization of Ocsyn, a Syntaxin-Interacting Protein, in Mammalian Vestibular Hair Cells

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Ocsyn, a protein recently isolated and cloned from a two-hybrid library of the guinea pig organ of Corti probed with syntaxin 1, was identified in organelles situated at the subapical region of inner hair cells, associated with tubulovesicular elements (Safieddine et al, Mol Cell Neurosci, 20:343-353, 2002). In this region, an intense vesicular traffic is present that may require specific SNARE proteins. Similarities in mechanoelectrical transduction properties between inner hair cells and vestibular hair cells lets expect closely related membrane recycling events in both cell types. In the present study, we analyzed the distribution of ocsyn in rat vestibular hair cells by immunocytochemistry and confocal microscopy.

Ocsyn labeling was characterized by intense immunolabeled spots distributed throughout type I and II vestibular hair cells. In the subcuticular regions, spots were densely packed under the cuticular plate. In the medial and basal regions, spots were scattered with a denser occurrence close to the cytoplasmic membranes.

In inner hair cells, Safieddine et al (2002) suggested that ocsyn might be mainly involved in protein trafficking occurring in the subapical regions and might be associated with recycling endosomes. In rat vestibular hair cells, the presence of ocsyn in structures both of the subapical and mediobasal regions leads to the hypothesis that this protein could be involved in vesicle trafficking in the apical cytosol and at neurotransmitter release sites.

631 An Investigation of the Cochleotopic Distributions of GLAST, vGLUT1 and GluR4 in the Guinea Pig Organ of Corti using Immunocytochemistry

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Glutamate is thought to be the neurotransmitter used by inner hair cells (IHCs) at synapses with afferent nerve terminals. Several proteins associated with glutamate neurotransmission have previously been reported in the cochlea including GLAST, a glutamate transporter in supporting cells, vGLUT1, a vesicular glutamate-specific transporter detected in hair cells using rtPCR, and AMPA-type glutamate receptors such as GluR4 which are present on the afferent terminal. Because afferent innervation density varies along the cochlea, we have investigated the cochleotopic distributions of these proteins in guinea pig using immunofluorescence for confocal microscopy and immunogold for transmission electron microscopy. Immunofluorescent intensity for GLAST was greatest in inner phalangeal cells in the middle region of the cochlea (approx 8 - 10 mm from the apex) and immunogold labelling density was usually greater in the 10-mm than the 1-mm point. Immunofluorescent labelling for the AMPA receptor GluR4 appeared as puncta around the IHC base, and immunogold labelling was confined to the afferent synapse. The number of puncta tended to be larger in the middle region of the cochlea than elsewhere. vGLUT1 immunoreactivity was detected in the synaptic region of the IHC and in the inner spiral bundle. Levels of vGLUT1 immunoreactivity were often greater in the middle region than elsewhere, although in some cochleae it was equally strong apically. We also attempted to label for vGLUT2 but no significant immunofluorescence was detected. To control for possible variation in penetration of antibodies in immunofluorescent preparations, anti tubulin-FITC was used for double labelling in some cochleae. There was no equivalent peak of tubulin immunofluorescence intensity around the 10-mm point. These data suggest that the expression of proteins putatively involved in glutamate neurotransmission by IHCs tends to be correlated and to be greatest in middle regions of the cochlea.

632 Immunohistochemical Localization of Alpha 9 and Alpha 10 Nicotinic Acetylcholine Receptor Subunits in the Rodent Inner Ear

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The α9 and α10 subunits of the nicotinic acetylcholine receptor (nAChR) are believed to be the efferent receptor subunits mediating cholinergic neurotransmission in the cochlear and vestibular efferent systems. *In situ* hybridization studies have localized α9 and α10 subunit mRNA to the pituitary, tonsil, cochlea, and vestibular periphery [Elgoyen et al., 1994, *Cell* 79:705-715, 1994; Elgoyhen et al., 2001, *Proc Natl Acad Sci U S A*.;98(6):3501-6;; Lustig et al., 2001, *Genomics*. 73(3):272-83; Sgard et al., 2002, *Mol Pharmacol*. 61(1):150-9].

Various tissues of the brain (pituitary) and inner ear (crista, otolith organ, cochlea) were examined for the presence of immunoreactivity to nicotinic acetylcholine receptor subunits α9 and α10. Interestingly, while both α9 and α10 subunit mRNA are found in the pituitary, the α9 subunit is localized to the posterior and intermediate pituitary, and the α10 subunit is localized to the anterior and intermediate pituitary.

Immunohistochemical staining was observed in all endorgans of the inner ear. In the guinea pig cochlea, α9 subunit protein was present in inner and outer hair cells, whereas the α10 subunit protein was present predominately in outer hair cells. Coimmunoprecipitation experiments show that α9 and α10 receptor subunits are complexed to form a nicotinic acetylcholine receptor.

In the vestibular periphery, calyx and dimorphic afferents stained positive for α9 whereas the peripherin-postiive bouton afferents were not positive for the α9 subunit. Similar analyses are underway to determine the α10 subunit afferent class staining patterns.

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633 Linopirdine is a Competitive Antagonist of α9α10 Containing Nicotinic ACh Receptors

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Studies of the electrophysiological response to ACh in mammalian outer hair cells (OHCs) are hindered by the presence of a large potassium current ($I_{K,n}$). Since $I_{K,n}$ can be blocked by linopirdine, cholinergic effects might be better revealed in the presence of this compound. However, initial attempts to use linopirdine suggested it may also affect the hair cell's cholinergic response. In inner hair cells (IHCs) (P7-12) recorded in excised apical turns of the rat cochlea, responses to 100 μM ACh were reduced ~40% by 100 μM linopirdine, both at –90 and –40mV. In OHCs (P28-30) ACh responses at –30 mV were reduced by ~50%. Linopirdine could be acting on either the ACh receptor (α9α10) and/or the SK potassium channels underlying the hair cell's ACh response. We tested the effects of linopirdine on recombinant α9α10 ACh receptors expressed in *Xenopus laevis* oocytes. Currents evoked by 100 μM ACh (V_{hold}: -70 mV) were reduced by linopirdine in a concentration-dependent manner (IC_{50} 5.3 ± 0.9 μM). This reduction was reversible and voltage-independent. Using

increasing concentrations of ACh in the presence of 10 μM linopirdine, we found a parallel rightward shift with an increment in the EC_{50} value (EC_{50} = 13.8 ± 1.7 μM for ACh and 382.5 ± 37.9 μM for ACh + 10 μM linopirdine). No change in the maximal response was observed, suggesting a competitive mechanism of block. Thus, linopirdine interacts with α9α10 receptors, and must be employed with caution when studying the hair cell's response to ACh.

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634 Quantitative RT-PCR of nAChR alpha9 and alpha10 Levels from the Mammalian Inner Ear

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Functional expression studies of the heteromeric nicotinic aceteylcholine receptor (nAChR) composed of alpha9 and alpha10 subunits have demonstrated a physiologic response very similar to that of the cholinergic receptor that mediates the inhibitory efferent effect on cochlear outer hair cells. This has led to the hypothesis that the nAChR alpha9/10 is the functional heteromeric receptor that underlies efferent synaptic transmission in the outer hair cell of the inner ear. To date, alpha9 and alpha10 have been localized to the vestibular and auditory end-organs by RT-PCR, in-situ hybridization, and immunohistochemistry. However, none of these techniques are highly quantitative. Determining the relative levels of alpha9 and alpha10 subunits within the various end-organs of the inner ear may provide valuable clues to the function of the receptor within these tissues.

To this end, we have performed quantitative RT-PCR of alpha9 and alpha10 mRNA within the rat cochlea, ampullae, saccule, and utricle using the Roche Light-Cycler®. These studies demonstrated that, when normalized for beta-actin, alpha9 expression was greatest in the saccule, followed by the ampullae, cochlea, and utricle, respectively. In contrast, alpha10 expression was highest in the saccule, followed by the ampullae and utricle, and was an order of magnitude lower in a whole cochlea preparation as compared to alpha9. The relative expression of alpha9/10 also differed between tissues. In the saccule, the alpha9/10 ratio = 6.9, cochlea = 3.7, ampullae=1.1, and utricle=0.49.

Surgically harvested human vestibular tissue expressed alpha9 and alpha10 at levels comparable to rat vestibular tissue. It will be of interest to learn whether these patterns of expression correspond to differences in cholinergic physiology among these end-organs.

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635 The Cholinergic Response of Inner Hair Cells is Correlated with Expression of the α10 Nicotinic Subunit.

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In the developing mammalian cochlea, inner hair cells (IHCs) are transiently innervated by efferent fibers. This synapse is inhibitory and mediated by a nicotinic cholinergic receptor (nAChR) probably formed by the α9 and α10 subunits. Expression of α9 mRNA persists in adult rat IHCs, while that of α10 does not. We have characterized cholinergic input to IHCs during this developmental transition. We used whole cell recordings of IHCs in acutely excised apical turns of the rat organ of Corti spanning the onset of hearing (P12). At a holding potential of –90 mV, ACh elicited inward currents (-449 ± 74 pA) in 8 of 8 IHCs at P7-9 and 5 of 5 cells exhibited efferent synaptic currents upon K^+ depolarization. At P10-12, ACh elicited currents of –264 ± 43 pA (16 of 16) and 14 of 15 IHCs had efferent synaptic currents. At P13-14, ACh elicited currents of smaller amplitude (-29 ± 5 pA) in 3 of 5 IHCs and efferent synaptic currents appeared in 2 of 4 cells. By P18, only 1 of 6 IHCs responded to ACh and 0 of 10 cells tested had efferent synaptic currents. In situ hybridization was used to show α10 expression in IHCs at P3. Expression of α10 in these cells decreased at P13, and by P21 was undetectable. The correlation of cholinergic sensitivity with subunit expression strengthens the hypothesis that the functional IHCs cholinergic receptor is composed of both the α9 and α10 subunits.

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636 The Role of Tyrosine Hydroxylase and the Cochlear Lateral Efferent System in Sound Conditioning

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Protection against noise trauma is demonstrated by preconditioning guinea pigs to a low level, non-damaging acoustic stimulus (1.0 kHz tone at 81 dB SPL for 24 hours) before exposure to a noise exposure that causes a temporary threshold shift (TTS) (2.7 kHz, 103 dB SPL, 30 minutes). Sound conditioning results in a 17-28 dB protection of the auditory brainstem response (ABR) threshold compared to the group exposed to the TTS noise exposure. A significant increase in TH immunoreactivity was found in the lateral efferent system after animals were either sound conditioned, or treated with the combined exposure of sound conditioning and noise trauma, while in contrast, noise trauma alone resulted in a significant decrease. Pre-treatment with 6-hydroxydopamine (6-OHDA) blocked the protective effect of sound conditioning. Likewise, when animals were pretreated with either a D1 antagonist or a D2 antagonist and then sound conditioned, protection against noise trauma was not found. In contrast, pretreatment with a D1 agonist before a TTS noise exposure, resulted in near complete protection.

These findings demonstrated an up-regulation of dopamine during sound conditioning and suggests that dopamine release from the lateral efferent system protects against excitotoxicity at the afferent dendrites beneath the inner hair cells.

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637 Temporal and Genetic Influences on the Efficacy of Hypoxic Conditioning against Noise Injury

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The possibility of protecting the cochlea from injury by engaging endogenous mechanisms has received increasing attention (e.g., Yoshida et al., 1999; Wang and Liberman, 2002). We showed that hypoxic conditioning can protect the cochlea of CBA/J and /CaJ mice from noise injury (Ohlemiller et al., ARO 2002). Exposing young mice (4 mos±2 wks) to 8% oxygen for 4 hrs reduces hearing loss at 20-40 kHz caused by noise exposure 24 hrs later by ~20 dB. However, Chen (ARO 2002) reported detrimental effects when hypoxia is simultaneous with noise exposure. The temporal parameters of hypoxia are clearly critical to the balance between harm and benefit. To explore this issue, we varied the delay between hypoxia and noise, and the duration of hypoxia. To uncover possible genetic influences, we also examined conditioning in C57BL/6J mice.

CBA/J mice (8/group) were hypoxically-conditioned or 'air-conditioned' for 4 hrs, then exposed to broadband noise (4-45 kHz, 110 dB SPL, 1.5

hrs) at delays ranging from 0-48 hrs. While significant protection (as determined by ABR) was found only at 24 hrs, some protection was also found at 48 hrs. Delays shorter than 12 hrs appeared detrimental. Immediate noise exposure increased threshold shifts by up to 45 dB. Mice conditioned for 8 hrs, followed by noise 24 hrs later also showed increased threshold shifts. Conditioned C57BL/6J mice exposed to either 15 or 30 min of noise at 24 hrs did not differ from controls.

Potential benefits of hypoxia against noise injury accrue only under narrow timing conditions, and depend on genetic background. The transition from detrimental to beneficial effects at ~12 hrs suggests an interplay between two or more factors with different temporal characteristics. We are examining the expression profiles of HSP72, HIF1α, and reactive oxygen species to see whether these can account for our results.

638 Increased Resistance to Free Radical Damage Induced by Low-level Sound Conditioning

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Cochlear conditioning, where the ear's resistance to noise is increased after prophylactic noise exposure, is a well-established phenomenon in a number of mammals (Canlon, et al, 1988, Campo, et al, 1991). Theories have been proposed to explain the changes that occur during conditioning, including, increased effectiveness of the efferent system (Kujawa & Liberman, 1997), generation of protective heat shock proteins (Yoshida, et al., 1999), and increased endogenous antioxidant activity (Jacono, et al., 1998).

The current study extends previous work from our lab, and focuses on the role of ROS in cochlear conditioning. It is well established that exposure to traumatic noise can increase levels of ROS (Yamane, et al, 1995, Ohlemiller, et al, 1999, Ohinata, et al., 2000), and ROS are implicated as damaging agents in noise induced hearing loss. Given that prophylactic cochlear conditioning enhances the antioxidant protection available in the cochlea, the current studies were designed to evaluate the generalized protection afforded by the increase in antioxidant activity by testing it against paraquat. Paraquat reacts with O_2 and produces $O_2\cdot$. It has been previously reported that paraquat place on the round window causes hair cell loss (Nicotera, ARO abstract). Chinchillas were exposed to a conditioning noise, 500Hz OBN at 95dB for 6hr/ day for 10 days, followed 5 days later with paraquat application to the round window. Controls underwent the paraquat application surgery, without prior conditioning. Evoked potential thresholds measured from the inferior colliculus were determined pre-conditioning, at day 1, 5, and 10 during conditioning, 5 days post-conditioning, and at day 1, 3, 7 and 20 following paraquat application. The conditioned animals showed less permanent threshold shift and reduced hair cell loss than controls. These results suggest a predominant role for antioxidants as mediators of the conditioning effect.

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639 Can Adenovirus-Mediated gene be Delivered to the Cochlea via the Round Window Membrane in Guinea Pig?

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We demonstrated changes in cochlear gene delivery on the round window membrane treated with various drugs. Under general anesthesia, the left round window membrane of twenty white guinea pigs was treated with either physical saline (control), gentamicin, mannitol or phenol solution for 15 minutes. After removal of those solutions, an adenovirus lacZ vector solution or an adenovirus lacZ vector-soaked gelatin sponge (Sponzel®) was placed on the round window membrane for 4 days. After removal of the left bony labyrinths, all cochleae were fixed with 2% glutaraldehyde and 4% paraformaldehyde for 3 hours, then embedded in paraffin. Paraffin sections of all cochleae were stained using immunohistochemical technique (ABC method), and observed under a light microscope. The sections occasionally showed the formation of fibrotic tissue and inflammatory matrix inside the round window membrane in animals treated with phenol, gentamicin or mannitol but not in control animals. The hair cells were morphologically preserved in control and experimental animals. In animals treated with phenol, marked expression of lacZ was observed in the organ of Corti, stria vascularis and mesothelial cells lining the perilymphatic space in the cochlea. There was no lacZ expression identified in the either neuroepithelium or mesothelial cells in the vestibular labyrinth. These data suggest that efficient gene transfer can be facilitated by injury of the round window membrane in the guinea pig.

640 Efficacy of Gene Transfer Through Round Window Membrane: an In Vitro Model

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Cochlear gene transfer studies in animal models have utilized mainly three delivery methods: direct injection through the round window membrane (RWM), intra-cochlear infusion through cochleostomy and a less invasive method, a vector–transgene soaked gelatin-sponge, for transgene delivery through an intact RWM. The gelatin sponge method has been shown to be successful in mediating transgene expression across an intact RWM in mouse *in vivo* but the method has not been tested *in vitro*. We have created an *in vitro* model to test the feasibility of gene delivery through an intact RWM.

The round window with the bony niche from 30 CD1 mice was removed under the microscope and fixed with glue on a petri dish containing a hole. As testing the method, 5 ul of toluidine blue was injected to the niche containing Merogel. The niche was closed with a fascia. A plastic tube was fixed on the opposite side and 200 ul of PBS was injected into the chamber from which the samples were collected at different time points. The concentration of toluidine blue was evaluated spectrophotometrically.

5 ul of adenoviral vector containing EGFP marker gene was injected to the gelatin sponge in the niche and the lateral side of the sample was sealed as described above. Samples were collected from the opposite side at different time points. The presence of the EGFP marker was studied by PCR.

The outer surface of the RWM was treated with detergents, histamine, or AgNO3 to change the permeability of the membrane. The passage of Toluidine blue was not increased after these treatments. In contrast, the passage of adenovectors was increased after AgNO3 treatment.

Adenoviral vectors pass directly through the RWM. The permeability of the RWM can be modulated by different detergents and AgNO3. Compared with more invasive gene delivery methods, this technique represents a safer and clinically more viable route of cochlear gene delivery.

641 Hair Cell Targeted Adenoviral Gene Therapy

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Adenovirus-mediated gene transfer has shown promise in preclinical studies as a potential novel molecular treatment for cochlear pathology.

Although the adenovirus is a "highly efficient vector", it is still limited by the extent of effective in particular inner ear cell type. One of the limitations for adenovirus-mediated gene transfer and gene therapy is the widespread cellular expression of adenoviral receptor, which can lead to inappropriate vector targeting to other sites. In addition, variable expression of adenoviral receptor on different type cells in the inner ear can significantly alter the efficacy of adenovirus-mediated gene therapy. It has been demonstrated that hair cells in the organ of Corti express high-affinity fibroblast growth factor receptors (FGFR). The receptor can thus be used for the targeted delivery of molecules conjugated to FGF into the cytoplasm of the hair cells. The Fab-FGF conjugate was constructed. Modified recombinant FGF was linked with the Fab fragment from a blocking monoclonal antibody generated against the adenovirus serotype 5 knob fiber protein. Recombinant adenoviral ß-gal construct (Ad-ß-gal) was used as a reporter for transgene expression. Approximately 1 x 1010 pfu/ml Ad- ß-gal was transfected into the cochlear explants with or without Fab-FGF. The ß-gal expression was widely detected in the cells of different inner ear cell types when treated with Ad-ß-gal alone. Interestingly, the explants treated with FGF-targeted Ad-ß-gal demonstrated a targeted ß-gal expression in the hair cells. The present study suggests that retargeting the adenovirus via the FGFR pathway allow extremely efficient transduction of targeted hair cells.

642 Experiences with Liposome Mediated Gene Transfer in the Rat Cochlea

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Sensorineural hearing loss refractory to conventional therapy may be one issue for gene therapy in the future. Gene transfer to the cochlea has been approched mainly using various viral vectors (e.g. adenovirus, herpes simplex virus). Liposomes have been tested to find an alternative transfer mode instead of recombinant viral vectors. However, liposome vectors have been reported in comparison to various viral vectors to be least efficient in terms of gene transfer (Staecker et al, 2001). Others reported successful gene transfer after placement of liposome-soaked gelatine sponge at the round window membrane (Jero et al., 2001). We tested liposomes carrying a plasmid with the green fluorescent protein and a CMV promotor. The liposomes were placed at the round window membrane or injected into the cochlea after utriculostomy (Praetorius et al., 2002). The results using the polycationic transfecting agent Metafectene varied according to the application method. In cases of the utricular approach, a thorough transfection of the modiolar neuronal structures were observed. However, the extend of the expression of the reporter gene GFP was variable. Until the viral vector systems can be substituted by a competitive transfective agent the search has to go on.

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643 Neurotrophin Application into the Inner Ear by Transplantation of Neural Stem Cells of Mice

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(Aim) Previous studies have indicated that direct application of neurotrophins in to the inner ear has the efficacy to promote cell viability of spiral ganglions and sensory hair cells. However, the strategy for application is still a matter of debate. In this study, we evaluated the potential of cell therapy for application of neurotrophins in inner ear. (Materials and Methods) The neuroepihtelium of the dorsal telencephalon of GFP-transgenic mice at age of E11.5 were transferred into the neurosphere culture medium. Secondary spheres were then collected for transplantation. C57BL/6 mice at age of 6 weeks (n = 5) were prepared as recipient animals. Under general anesthesia, the medium containing neural stem (NS) cells suspended at a density of 1 x 105 cells/µl was injected from the lateral semicircular canals. The temporal bones were collected on day 28 after transplantation, and made into cryostat sections. To determine cell fate and the ability to produce neurotrophins of transplant-derived cells, immunohistochemistry for GFAP, MAP2, GDNF and BDNF was performed. (Results) Transplant-derived cells were found in each turn of the cochlea, and predominantly localized in the perilymphatic space. Immunohistochemical analysis revealed that 90% of transplant-derived cells differentiated into glial cells and 10% into neural cells, and that expression of GDNF was found in 90% of transplant-derived cells and that of BDNF was in 10%. (Conclusion) NS cells can survive in the inner ear and differentiate into glial or neural cells. In addition, NS cell-derived cells may have the potential for production of neurotrophins in the inner ear. Therefore, cell therapy can be a strategy for application of neurotrophins into the inner ear.

644 Trans-Utero Gene Transfer into the Mouse Otocyst

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Gene therapy is a promising method for treating sensory neural hearing loss (SNHL). Experimental works using inner ear gene therapy have been limited acquired SNHL models such as noise or ototoxic trauma. Congenital (hereditary or environmental) SNHL is common, and its treatment is an attractive application for gene therapy.

As a first step to treating congenital SNHL, it is necessary to design interventions that will allow inoculation of the therapeutic agent into the developing cochlea. We tested viral-mediated gene transfer into the otocyst of mouse embryos. Timed pregnant mice (CD-1) were anesthetized at E11.5 (vaginal plug detection = E 0.5). The uterus was exposed by low midline laparotomy. The embryo was placed on a transparent surgical stage and illuminated from beneath with a fiber optic beam. Approximately 100 nl of Adenovirus (ad) encoding the reporter gene *β-galactosidase* was injected into the otocyst through the uterus wall with 0.1% fast green solution via heat-pulled glass micropipettes. Four days after the injection the embryos were harvested and fixed in 4 % paraformaldehyde. Embryos were embedded in OCT compound and frozen sections were cut with a cryostat. Immunostaining was performed with rabbit polyclonal anti-β-galactosidase antibody to determine transgene expression. Results show reporter gene expression in the inner ear. Transgene positive cells were observed in sensory epithelial areas and in non-sensory regions within the inner ear of the manipulated embryos. It is therefore possible to use this method of in-utero adenovirus-mediated gene transfer for experiments on the treatment of hereditary and environmental congenital SNHL.

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645 Something Old and Something New in NIHL

*Joseph E. Hawkins, Jochen Schacht

With the wide-ranging response and exquisite sensitivity of our hearing organ comes an unfortunate sensitivity to loud sound. This may not have been an evolutionary oversight since aside from an occasional thunderclap, nature does not inundate us with high levels of noise. Noise-induced hearing loss seems to be a modern affliction in the wake of gunpowder and the Industrial Revolution. Today 12-15% of all employed people in developed countries are exposed to potentially damaging noise levels of 85 dB or more, expanding on a tradition that in the past had been limited primarily to blacksmiths, millers and artillerymen.

Sir Francis Bacon (1561-1626) was one of the first to associate excessive sound with deafness but systematic investigations of noise-induced hearing loss did not begin in earnest until early in the 20th century. Today we understand well the audiological, pathophysiological and micro-anatomical effects of noise trauma, and we are beginning to explore the underlying cellular mechanisms. Furthermore, recent studies in experimental animals give hope that therapeutic prevention of noise-induced hearing loss is within reach.

646 The Relationship Between Noise-Induced Hearing Loss and Hair Cell Loss in Rats

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The relationship between noise-induced hearing loss (NIHL) and hair cell loss is not consistent. Interestingly, hair cells may still survive after a complete loss of their auditory function. The present report compares hair cell loss at different cochlear locations with CAP (compound action potential) threshold elevation at related frequencies in rats. CAP threshold elevation and hair cell loss were determined 4 weeks after noise exposure. No hair cell loss was observed in the low-frequency region (<8 kHz) even when CAP threshold elevation exceeded 60 dB. In the middle turn, significant hair cell loss was not observed until NIHL exceeded about 40-50 dB. In the basal turn, while inner hair cell (IHC) loss was not observed until NIHL exceeded about 50 dB, outer hair cell (OHC) loss was observed in almost all of the noise-exposed rats, even in some cases without detectable NIHL. OHC-loss increased gradually with NIHL and in the region of the highest frequencies tested in this study (30-40 kHz), a linear NIHL/OHC-loss relationship was observed. The data indicate that the NIHL/hair cell loss relationship is cochlear location dependent and many hair cells survive under a severe NIHL.

647 Noise-Induced Hair-Cell Loss Versus Total Energy: Analysis of a Large Data Set

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The relation between total noise-exposure energy, recovery time, or rest & % hair-cell (HC) loss was examined in 378 chinchillas. The continuous exposures were either a 4-kHz octave band of noise (OBN) at 47-108 dB SPL (N=169) for 0.5 h to 36 d, or a 0.5-kHz OBN at 65-128 dB SPL for 3.5 h to 432 d (N=131). Recovery times varied from 0-365 d. With both OBNs, other animals were exposed on interrupted schedules [6 h/d, /2d or /wk for 9-365 d (N=78)]. HC loss as a function of age in 117 non-exposed animals was used to correct for loss due to aging. For the 4- & 0.5-kHz OBN, the noise-exposed cochleas (N=607) were separated into 3 groups: 1) Acute (≤ 9 d exposure, 0 d recovery; N=90 & 56) to characterize the primary effects of the noise; 2) Chronic (> 9 d exposure, 0-730 d recovery; N=184 & 144) to determine secondary effects & 3) Interrupted (N=46 & 87) to show the effect of rest during the exposure. Cluster & regression analyses were performed in the basal & apical halves of the cochlea to determine the specific rates (relative to doubling of total energy, recovery & rest) at which primary & secondary effects produced HC loss & rest prevented loss. The effect of recovery time was isolated by subtracting the primary effect determined in the Acute group from the loss in the Chronic group. The effect of rest was isolated by subtracting the primary effect from the Acute group & the secondary effect determined from the Chronic group from the loss in the Interrupted group. It was found that: 1) When the OBN was above a critical level, there was no relation between total energy or recovery time & HC loss; 2) Below a critical level, there were highly significant log-linear relations, but at a low rate; 3) Except for the highest exposure levels, the majority of HC loss from the 4-kHz OBN occurred after the exposure had terminated, while that from the 0.5-kHz OBN occurred during the exposure & 4) Rest periods during either OBN exposure significantly reduced HC loss.

648 Can Infrasound Protect the Cochlea from a Damaging Level of Noise?

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Infrasounds (ie, < 20 Hz for humans; < 100 Hz for chinchillas) are not audible, but they produce large movements of cochlear fluids (Salt & DeMott, 1999). It was speculated that infrasound might bias the basilar membrane & perhaps minimize noise-induced hearing loss (NIHL). Chinchillas were simultaneously exposed to a 30 Hz tone at 100 dB SPL & a 4-kHz OBN at either 108 dB SPL for 1.75 h or 86 dB SPL for 24 h. One tympanic membrane (TM) was perforated prior to exposure to attenuate infrasound transmission to that cochlea. Controls were exposed to infrasound or the 4-kHz OBN only. ABR threshold shifts (TS) & DPOAE level shifts (LS) were determined post-TM-puncture & immediately post-exposure, just before cochlear fixation. The cochleae were dehydrated, embedded in plastic, dissected as flat preparations & evaluated for hair-cell (HC) losses. For each animal, the magnitude & pattern of functional & HC losses were compared between their right & left cochleae. The infrasound exposure alone resulted in a 10-20 dB TS below 1 kHz, no LS & no HC damage/loss. Exposure to the 4-kHz OBN alone at 108 dB produced a 50-60 dB TS for 1-16 kHz, a 40-50 dB LS for 2-12 kHz & severe OHC loss in the middle of the first turn. When infrasound was added, the functional and HC losses extended much farther apically & basally than for the 4-kHz OBN alone. Exposure to the 4-kHz OBN alone at 86 dB produced a 40 dB TS for 3-12 kHz & 30 dB LS for 3-8 kHz, but no HC loss in the middle of the first turn. When infrasound was added, no differences in the functional and HC losses were found compared to the 4-kHz OBN alone. We hypothesize that exposure to infrasound & an intense 4-kHz OBN increases cochlear damage because the large fluid movements from infrasound cause more intermixing of cochlear fluids through the damaged reticular lamina. Simultaneous infrasound & a moderate 4-kHz OBN did not increase cochlear damage because the reticular lamina rarely breaks down during this exposure.

649 The Influence of Varying Degrees of Permanent Hearing Loss on a Polynomial Model of Mechano-Electric Transduction

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Excessive noise exposure damages cochlear structures and alters mechano-electric transduction (MET). Assessment of the pathophysiology associated with the disruption of cochlear MET can provide a better understanding of sensory hearing loss, and may invite new aural rehabilitative options. In our previous work, we characterized MET with a third-order polynomial equation and showed that physiologic indices derived from the equation are sensitive to different cochlear pathologies. Here we explore the influence of various degrees of permanent noise-induced hearing loss on the polynomial model of MET.

Mongolian gerbils (N=43) were exposed to an 8 kHz narrow band noise at 117 dB SPL. Exposure duration ranged from 1 to 128 hours.

Auditory brainstem response thresholds at 16 kHz were monitored weekly until thresholds stabilized. Subsequently, compound action potential thresholds and cochlear microphonics (CM) were recorded from a round window electrode. MET was characterized by a third order polynomial equation obtained from applying a nonlinear system identification technique to the CM evoked by Gaussian noise.

The results showed differences between MET functions for low and high frequencies. Low frequency MET was characterized by an increase in the quadratic term as well as long group delays. This suggests that after damage apical hair cells dominate the low frequency CM and their MET is asymmetric. In contrast, high frequency MET showed a decrease in the contribution of the model's nonlinear terms, indicating that MET is more linear after damage. Furthermore, the linear term decreased proportionally with extent of hearing loss, indicating that the sensitivity of MET is inversely related to the degree of hearing loss.

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650 Relationship Between Acoustical Stress and Hearing Sensitivity in Fishes

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Fishes are often exposed to environmental sounds associated with sources such as seismic experiments, sonars, aquaculture pump systems, and/or boat traffic. While efforts have documented the negative effects of anthropogenic sounds on marine mammals, effects of such sounds on fishes remains poorly understood. We examined the effects of increased ambient sound on the hearing and stress of two fish species differing in hearing capabilities: goldfish (*Carassius auratus*) and tilapia (*Oreochromis nilotica*). We reared each species in 600-L aquaria under quiet (110 dB re: 1 µPa) or noisy (white noise, 170 dB re: 1 µPa) conditions and individuals were removed after different durations of noise exposure. We then measured alterations in noise exposed fish relative to hearing capabilities (using auditory brainstem responses), pathology of the inner ear (using SEM), and stress (using plasma cortisol and glucose levels). We found that while tilapia exposed to white noise for 28 days showed minimal hearing threshold shifts, goldfish exhibited considerable threshold shifts of up to 25 dB after only 7 days of exposure. There was a positive linear relationship between noise-induced threshold shifts and the sound pressure level difference between the noise and the fish's baseline hearing thresholds. These observations will be correlated with plasma cortisol and glucose levels and SEM analysis of the inner ear hair cells of these fish. This study provides insights into differential susceptibility of a hearing specialist (goldfish) and a hearing generalist (tilapia) to noise-induced hearing loss and stress associated with acoustic exposure.

651 Quantitative Analysis of Apoptotic and Necrotic Outer Hair Cells after Exposure to Different Levels of Continuous Noise

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Two modes of outer hair cell (OHC) death, apoptosis and necrosis, have been identified in the cochlea exposed to an intense noise. However, the quantitative data regarding the prevalence of apoptosis and necrosis is not known. This study was designed to quantitatively analyze the occurrence of apoptotic, necrotic and missing OHCs and to evaluate the changes of these parameters with time after noise exposure. Chinchillas were exposed to a narrow band noise at either 104 or 108 dB SPL for 1 hour. The animals were sacrificed at either 1, 4 and 30 days after the noise exposure and the cochleas were collected for detection of OHC death modes. The apoptotic and necrotic OHCs were distinguished by examining the OHC nuclear morphology and confirmed by staining for caspase-3 activity or TUNEL assay. ABR thresholds for click stimuli were used to monitor changes in auditory function. Several trends were evident after the noise exposure. In the subjects exposed to 104 dB noise, damage to the organ of Corti was limited (120-130 OHCs). Most dying OHCs (apoptotic and necrotic) were scattered in the basal part of the cochlea and a small subset of dying cells formed a focal lesion. The sum of necrotic, apoptotic and missing cells at 1-day was equal to the number of missing cells at 30-day; therefore there was no growth of the lesion during this period. In contrast, the subjects exposed to 108-dB noise exhibited a severe cochlear injury. The extent of the cochlear lesions increased with time after the noise exposure. At days 1 and 4, there were more than twice as many apoptotic OHCs than necrotic OHCs. The number of missing cell increased progressively from day 1 to day 30. The increase of missing cells at day 30 could not be accounted for by summing apoptotic, necrotic and missing cells at day 4, indicating that the lesion continued to grow between 4 and 30 days.

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652 Mitochondrial Functions in Apoptotic and Necrotic OHCs Following Exposure to Impulse Noise

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Our previous studies have shown that the outer hair cell (OHC) can die through either apoptosis or necrosis following exposure to intense noise. However, the cellular mechanisms by which the dying OHCs are driven to a specific death pathway are not known. This study was designed to explore the role of the mitochondrial energetic function in regulating the OHC propensity toward apoptosis or necrosis following exposure to an impulse noise. Chinchillas were exposed to 75 pairs of impulse noise at 155 dB pSPL. The animals were sacrificed 5 or 30 minutes after the noise exposure. The cochleas were examined for two indicators of the mitochondrial energetic function: the activity of succinate dehydrogenase (SDH) and mitochondrial membrane potential. The specimens were also stained with propidium iodide (PI) and TUNEL assay to assess the spatial correlation between dying OHCs and their mitochondrial functions. Five minutes after the noise exposure, the OHCs with condensed nuclei and TUNEL positive staining could be noted in the first cochlear turn. These apoptotic OHCs maintained a normal level of SDH staining. Surrounding these cells, cells appeared viable exhibited a decreased or lack of SDH staining. Thirty minutes after the noise exposure, OHCs with swollen nuclei appeared in the original area of SDH depress. Some of these necrotic OHCs exhibited a normal level of SDH staining, whereas others showed a decreased or lack of SDH staining. Surprisingly, virtually all the apoptotic OHCs maintained the level of SDH staining. In addition, mitochondrial membrane potential monitored by the staining of rhodamine 123 maintained in the OHCs with condensed nuclei immediately after the noise exposure and collapsed in the OHCs with swollen nuclei 30 min after the noise exposure. These observations suggest that the status of the mitochondrial energetic function may play an important role in determining the OHC death pathway.

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653 Mitochondrial Degeneration in Chinchilla Hair Cells After Acoustic Overexposure

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Glutamate excitotoxicity is characterized by an increasing damage to cell components and primarily targets the mitochondria (Schinder 1996). In acoustic overexposure, reactive oxygen species are elevated,

which causes further mitochondrial damage. The purpose of this study was to observe the acute and long-term ultrastructural changes of hair cell mitochondria after acoustic overexposure. Female chinchillas were exposed to 105 dB SPL centered at 4kHz octave band noise for six hours. Animals were humanely euthanized at different time periods post-noise exposure. Their cochleae were dissected, processed and observed using transmission electron microscopy. Two hours after noise exposure, mitochondria were swollen with increasing spaces between the crista of the matrix. Localized destruction of the crista was observed in the center of the mitochondria. Three weeks post-noise exposure, crista exhibited shortening, blurring and dissolution. Some mitochondria demonstrated disrupted crista and internal membranes but maintained intact external membranes. Other mitochondria showed damaged external membranes, which separated from internal membranes with well-formed crista. Seven months after noise exposure, mitochondria showed extreme swelling and vacuolization. The external and internal membranes were destroyed, and numerous mitochondria were ruptured. Osmiophilic substance deposits and myelin figures were observed in mitochondria. The findings of this study indicated that the mitochondrial damage occurred at the early stage of sound over stimulation and increased with time. Early mitochondrial damage plays a key role in the noise-induced cochlear damage and initiation of cell death processes.

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654 Noise Exposure Alters Cochlear Oxidized and Reduced Glutathione Levels as a Function of Exposure Duration in the Chinchilla

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The purpose of this study was to determine the time course of changes in reduced glutathione (GSH) and oxidized glutathione (GSSG) levels in response to noise exposures of different durations. Five groups of 6 adult female chinchillas were exposed to 105 dB SPL octave band noise centered at 4 kHz for the following time periods: group 1: no noise (controls), group 2: 30 minutes, group 3: 2 hours, group 4: 4 hours, group 5: 6 hours. Animals were immediately sacrificed after their exposure times and the cochleae preserved in liquid nitrogen. Cochlear tissues were then pooled by group and homogenized in 0.6N PCA to ensure adequate homogenate for analysis. GSH and GSSG levels, as well as the GSH/GSSG ratio, as a measure of oxidative stress, were determined by HPLC separation and coulometric detection, testing 4 samples per homogenate. GSH and GSSG levels were quantified in nmoles/mg protein for each group. As compared to controls without noise exposure, GSH levels significantly increased ($p \leq .001$) to 189% of control for group 2 exposed to 30 minutes and to 181% of control for group 3 exposed to 2 hours of noise. However for group 4 exposed to 4 hours of noise, the GSH level decreased to 24% of control ($p \leq .001$). For group 6 exposed to 6 hours of noise, GSH levels were not significantly different from controls. GSSG levels decreased ($p \leq .001$) to 67% of control values at 30 minutes, and 71% of control at 2 hours, but then increased ($p \leq .001$) to 147% of control at 4 hours, and 156% at 6 hours. Therefore while GSH levels had recovered after 6 hours of exposure, GSSG levels remained high. The GSH/GSSG ratio, a measure of oxidative stress, initially increased at the 30 and 2 hour time-points as compared to control, but was markedly below control value for the 4 and to a lesser degree for the 6-hour time-points. These data suggest that short-term noise exposure markedly alters cochlear GSH and GSSG levels.

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655 Late Mergence of Free Radicals After Noise Exposure

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Reactive oxygen species (ROS) and reactive nitrogen species (RNS) play an important role in noise-induced hearing loss (NIHL). ROS rapidly but transiently appear in the cochlea after noise exposure (Ohinata et al., Brain Res. 878:163-173, 2000) while loss of hair cells progresses for 3 weeks or more. Although the delayed loss may be due to slowly progressing apoptotic pathways, an alternate hypothesis is that a continued formation of free radicals contributes to cell death. To evaluate this hypothesis, we measured auditory brain stem responses (ABRs), hair cell loss and free radicals in the guinea pig following noise trauma (5 hr, 120dB SPL, 1 OCB). Nitrotyrosine (NT) and 4-hydroxy-2-nonenal (4-HNE) were used as histochemical markers of RNS and ROS, respectively. Measurements were made immediately, 3, 7, 10 and 14 days after exposure with a final hair cell count at 21 days. Immunoreactivity to NT and 4-HNE was low initially and reached a maximum at 7 to10 days. During this time the staining shifted from Hensen, Claudius and inner sulcus cells to include Deiters and hair cells. ABR thresholds showed a maximal increase immediately after exposure with a partial recovery stabilizing at 7 to 10 days. Correlating with the delayed formation and changing localization of ROS/RNS there was a progressive hair cell loss, stabilizing at approx. 3 weeks. Based on these findings, we suggest that initial hair cell damage after noise may primarily reflect mechanical events plus transient intense ROS formation while continued formation of ROS/RNS contributes to the long-term cell loss. The delayed formation of free radicals may provide a window of opportunity for pharmacological rescue during this period. The formation of both RNS and ROS suggests that scavengers of these radicals, possibly in combination, may be effective in attenuating NIHL.

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656 Involvement of Nuclear Factor-kappa B in Acoustic Trauma

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Nuclear factor-kappa B (NF-kappa B) is a ubiquitous transcription factor regulating a battery of inflammatory genes. In the present study, we investigated the localization of NF-kappa B in the cochlea. We also examined whether Caffeic Acid Phenethyl Ester (CAPE) and Sulfasalazine, inhibitors of NF-kappa B, protect the cochlea against acoustic trauma.

Albino guinea pigs weighting 300~400g were used. Immunofluorescence staining was performed on the cochleae of 5 untreated animals to determine the localization of NF-kappa B. Thirty-five animals were exposed to 2 kHz pure tone of 120 dBSPL through the hallow ear bar to induce acoustic trauma. CAPE, Salfasalazine or physiological saline solution was intraperitoneally administrated to the animals just before the acoustic exposure.

Staining of NF-kappa B was observed in outer and inner hair cells, supporting cells, interdental cells, and fibrocytes in the spiral ligament. Although CAPE and Sulfasalazine did not ameliorate cochlear dysfunction induced by acoustic trauma just after the acoustic exposure, statistically significant decrease in the CAP threshold shifts was observed in the animals treated with salfasalazine and CAPE 1week after the acoustic exposure.

The present findings strongly suggest that NF-kappa B plays an important role in the generation mechanisms of acoustic injury.

657 Expression of Ref-1 (Redox factor-1) in the Guinea Pig Cochlea

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Exposure to intense noise can lead to permanent damage of the sensorineural epitherium of the cochlea, based primarily on the loss of the sensory cells, the inner and outer hair cells. There are many evidences that noise-induced hearing loss (NIHL) is a result of free radical formation in the cochlea. A causal relationship between reactive oxygen species(ROS) and hearing loss is suggested by observations that depletion of the endogenous antioxidant glutathione leads to increased damage while, conversely, administration of free radical scavengers can attenuate NIHL. Furthermore, it has been known that intense noise might reduces the cochlear blood flow. It has been observed that noise exposure increase hydroxyl radicals in cochlear fluids as well as superoxide anion radicals and level of the radical scavenger glutathione in the stria vascularis.

We recently demonstrated that transcription factor, Activator protein-1(AP-1), is dramatically activated in response to the intense noise exposure in the guinea pig lateral wall tissue in the cochlea.

It has been suggested that the cells respond to such adverse conditions by altering their intracellular reduction/ oxidation (redox) state and making their ultimate decision between survival and apoptosis. Ref-1 was cloned as Redox factor, also known as apyrimidinic endonuclease (APE). Ref-1 is important for the activation of transcription factors, such as activator protein-1 (AP-1), nuclear factor kappa B (NF kappa B), p53, and hypoxia inducible factor-1 alpha (HIF-1 alpha). It has been reported that Ref-1 could be antiapoptic factor in endothelial cells. We observed the expression of Ref-1, a controller of transcription factors, in the guinea pig cochlea. The Ref-1 expression was relatively heavy in the cochlear lateral wall tissue. The finding that Ref-1 express in the cochlear endotherial cells may have potential clinical relevance in the acute sensorineural hearing loss.

658 Tip Link Loss and Recovery on Short Hair Cells of Chick Basilar Papilla

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Hair cell hair bundle tip links interconnect stereocilia at the apical ends of the hairs. Tip links, important to transduction, are vulnerable to low Ca++ and overstimulation. Chick tall hair cells (THCs) suffer a ~30% tip link loss after a 48-h intense sound exposure, recovering to near control levels by 3-4 d post-exposure. This experiment examines tip link loss on overstimulated short hair cells (SHCs).

One-day-old chicks were exposed to a 120 dB SPL, 0.9 kHz pure tone for 4, 24, or 48 h. Those exposed for 4 or 24 h were not allowed to recover. The 48-h exposed animals recovered for 0, 24, 48, 96, or 288 h. Basilar papillae were harvested, prepared, and viewed by scanning electron microscopy. The percentage of hair cell tip links was measured on surviving SHCs in the "patch" lesion, and on adjacent THCs. Control data were obtained from the same areas of unexposed papillae. Control SHCs and THCs had 76% and 73% of tip links intact, respectively. The only statistical difference in tip link loss occurred at 48 h exposure, where losses, relative to control levels, for SHCs and THCs were 37% and 29%, respectively ($p = 0.001$). Within 3-4 d post-exposure SHC tip links recovered to near control levels. Complete recovery was seen by 12 days post-exposure. At 12 days post-exposure, the newly regenerated hair cells had >80% of their tip links intact.

One explanation for the increased tip link loss in SHCs is that they are more violently stimulated because of their location over the basilar membrane. The fact that regenerated cells had more tip links than controls suggests that there may be a chronic level of tip link breakage and repair which has not yet reached equilibrium in the regenerated hair cells at 12 d post-exposure.

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659 Dependence of Loud Sound Induced Cochlear Stria Vascularis Injury on Poly (ADP-ribose) Polymerase

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Using immunohistochemistry, we tested the hypothesis that poly (ADP-ribose) polymerase (PARP-1) contributes to loud sound induced cochlear lateral wall damage by triggering 'inflammatory effects' including upregulated intercellular adhesion molecule-1 (ICAM-1), P-selectin and platelet-endothelial cell-adhesion molecule-1 (PECAM-1) and NF-κB activation. Our findings show that in the wild-type (PARP+/+) mouse, ICAM-1 was expressed constitutively only at a low level in the vessels of the stria vascularis and of the spiral ligament. P-selectin was barely detected only in the vessels of the spiral ligament. No PECAM-1 labeling was detected in either the vessels of the stria vasculars or of the spiral ligament under unstimulated conditions. Following loud sound exposure: 1) a significantly elevated expression of the adhesion molecular proteins was demonstrated in some vessels of the stria vascularis and of the spiral ligament; 2) a significantly increased population and a small amount of emigrated leukocytes were observed in the vessels of the lateral wall or of cochlear basilar membrane; 3) an active form of NF-κB was expressed in the cytoplasm and nuclei of marginal cells. NF-κB immunoreactivity was not observed in the vessels of the stria vascularis; (4) In knockout (PARP-/-) mouse, no increased activity of these proteins and population of leukocytes were observed. We conclude 1) loud sound triggers an 'inflammatory-like' reaction in the lateral wall, which takes place in the absence of NF-κB mediation; 2) PARP has a modulatory role on the 'inflammatory-linked' protein expression.

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660 Afferent and Efferent Innervation in the Normal and Sound-damaged Bullfrog Amphibian Papilla

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Electrically resonant hair cells (HCs) in the bullfrog amphibian papilla (AP) form ion channel clusters and synaptic connections with auditory neurons. To study the relationship between channel clustering and synaptogenesis in repairing and regenerating HCs, we immunolabeled normal and sound-exposed APs (800 Hz tones, 160 db SPL for 20 hrs) cultured for 3 or 9 days post-sound exposure with antisera against myosin VI, a known HC marker, and against neurofilament proteins to label the terminals and synaptic endings of auditory neurons. We then used confocal microscopy and proprietary imaging software (*NeuroLucida, NeuroBrightField*) to reconstruct the terminal arbors of single auditory neurons.

The AP nerve branch approached the sensory epithelium from the lateral side, bifurcating into two branchlets that approached the rostral and caudal AP, respectively. In agreement with previous morphophysiological studies, the terminal morphology of individual neurons was highly restricted, with thicker fibers in the rostral branchlet providing 5-10 and thinner fibers in the caudal branchlet <5 endings to 1-3 HCs. We also immunolabeled APs with antisera against calbindin, as well as CGRP and ChAT, two known markers of efferent neurons, to identify efferent terminals and synaptic endings. Our results confirm that efferent synaptic endings are restricted to HCs in the rostral region and the lateral third of the caudal extension, implying that efferent

neurons are found only in the rostral branchlet of the AP branch. They also suggest that afferent and efferent neurons can be distinguished by calbindin immunocytochemistry. Preliminary studies in sound-exposed APs indicate that afferent synaptic connections, lost in the caudal region by 3-days after sound exposure, are partially or totally restored by 9-days post-sound exposure.

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661 The Effects of Ossicular Fixation in Human Temporal Bones

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The goals of this study were to: 1) investigate the conductive effects of ossicular fixations using human temporal bone preparations; 2) compare the accuracy and reliability of various clinical measures – laser vibrometry of the umbo, direct 'surgical' palpation, and tympanometry – for identifying and locating ossicular fixation.

Experiments were performed on fresh, normal temporal bones. The stapes and round window were accessed through the facial recess, and the malleus and incus through the attic. The umbo, stapes, and round window velocities (0.2–8 kHz) were measured with laser Doppler vibrometry in response to an acoustic chirp. Controlled fixations from soft to hard were made with dental impression material or dental cement on one or more ossicles. Measurements were made before and after fixations and after reversal of fixations. In addition, we assessed tympanometry and manual palpation of the ossicles by an otologist blinded to the state of fixation.

To date, measurements in 4 temporal bones have shown that: 1) Graded levels of malleus head fixation result in graded decreases in umbo and stapes velocities for frequencies below 1 kHz. 2) Graded levels of stapes fixation produce large graded changes in stapes velocity and small changes in umbo velocity. Since the changes in umbo velocity produced by malleus head fixation are large, fixation-induced changes in umbo velocity are not limited by mallear bending. Therefore, the small reductions in umbo velocity observed when the stapes is firmly fixed suggest relative motion in the ossicular joints. Generally, ossicular palpation correlated with the state of fixation, while tympanometry was not sensitive to the state of fixation.

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662 The Acoustic Evaluation of Stapedotomy Using a Temporal Bone Otosclerosis Model

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The effect on post-operative hearing results of different stapes piston diameters has been reported clinically and in analog circuit models. Generally, larger piston diameters produce better lower frequency post-operative hearing results. However the clinical data are confounded by the difficulties in interpreting results of non-randomized retrospective trials performed by different surgeons. Moreover, mathematical and circuit otosclerosis models are not accurate enough at this time to be reliable predictors of the clinical situation because of inadequate experimental data to validate the assumptions. We used a human temporal bone otosclerosis model to evaluate stapedotomy with pistons of different surface area. The model has the footplate cemented in the oval window with fast drying epoxy cement. The output measure for each pistons evaluation in this model is displacement of the round window membrane before and after piston insertion. The displacement measurement was made with a non-contacting laser Doppler vibrometer(LDV). The input was a constant sound pressure level at the tympanic membrane from 0.125 to 8kHz. In the present study, we evaluated three different size pistons. Two were 0.4mm and 0.8mm diameter commercially available stainless steel wire-Teflon pistons, the third piston was an experimental oval shaped large piston, which was piston whose area (1.3x0.8mm) was equivalent to a 1.05mm diameter round piston. For each piston, twelve temporal bones were studied. Comparison of the 0.4mm and 0.8mm diameter pistons showed slightly better sound transmission (3-8dB) with the larger piston at frequencies below 2kHz. The large experimental piston provided improved transmission from 0.5 to 4kHz, compared to the 0.4mm and 0.8mm diameter pistons. In further experiments, removing the head of the malleus plus sectioning the tensor tympani muscle tendon and removing the incus produced further increases in sound transmission.

663 Malleus-to-Stapes-Head vs. Malleus-to-Foot-Plate Hydroxyl-Apatite Ossicular Prosthesis: Pressure Gain Measurements

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Partial Ossicular Reconstruction Prostheses (PORPs) are widely used in clinical practice. However, hearing outcomes are highly variable, depending on the PORP properties (eg: biomaterial, mass, rigidity, length), surgical technique and PORP positioning. Also, some surgeons prefer malleus-to-stapes-head (MSH) PORPs, whilst others use malleus–to-foot-plate (MFP) PORPs, although there is little scientific data to support either selection. Our aim is to compare the mean pressure gain of both PORP designs. Cochleo-vestibular pressure measurements in human temporal bones for the hydroxyl-apatite MFP-PORP have been reported by Kunda et al [submitted to Otology & Neurotology, 2002]. Presently, the ear canal pressure (P_{ec}) and cochleo-vestibular pressure (P_v) were measured, in the 0.1 to 10 kHz range, in two frozen temporal bones with methods previously described (Puria et al, 1997, JASA). The following conditions were tested: 1. intact incus (normal ear) 2. incus removed (diseased ear) 3. with MSH-PORP placed at the neck of the malleus (reconstructed ear). The normal middle ear gain (G_{ME}) is defined as the ratio of the vestibular pressure for the normal ear $P_{v(n)}$ to the ear-canal pressure P_{ec}. The MSH-PORP relative gain (G'_{msh}) is defined as the ratio of the vestibule pressure with MSH-PORP, $P_{v(msh)}$ to $P_{v(n)}$. Similarly, the MFP-PORP relative gain (G'_{mfp}) is the ratio of the vestibule pressure with MFP-PORP, $P_{v(mfp)}$ to $P_{v(n)}$. For the 0.1 to 5 kHz range, G'_{msh} showed an average gain of –20 dB, whereas G'_{mfp} averaged a gain of –8dB, compared to the normal ear. In summary, the MFP-PORP showed better average pressure gain compared to the MSH-PORP, by about 12 dB. However, for G'_{msh} at frequencies above 6 kHz, $P_{v(msh)}$ approaches and exceeds $P_{v(n)}$, as is also seen for G'_{mfp} with an intact stapes suprastructure. More data will be collected in future MSH-PORP experiments, to confirm these preliminary results.

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664 LDV Assessment of Stapes Footplate Motion in PORP and TORP Reconstruction

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OBJECTIVE

Evaluate the performance of PORP and TORP reconstruction in the human temporal bone model at the nanometer level and relate this to clinically observed hearing outcomes.

METHODS

A canal wall up mastoidectomy and extended facial recess was performed on 6 fresh human temporal bones. The canal wall was drilled down to approximately 2mm above the tympanic membrane and replaced with a plastic coupler. The tympanic membrane and footplate were undisturbed. Three conditions were measured in each specimen:

intact chain, PORP reconstruction, and TORP reconstruction. Footplate displacement was measured with a Laser Doppler Vibrometer (LDV) for each condition. A small 3M reflector was placed slightly posterior of the center of the footplate to allow for TORP placement in the center.

Single point Laser Doppler Vibrometer (LDV) measurements were made using a swept sine stimulus of 100 dB SPL and scanning LDV measurements at octave frequencies of 94 dB SPL. Sound stimulus was delivered using an ER-2 insert earphone and monitored using an ER-7 probe microphone.

RESULTS

The mean TORP reconstruction loss was 5-10 dB relative to the intact chain stapes displacement. The mean PORP loss was 10-15 dB. No significant difference between PORP and TORP performance was observed due to the high degree of variability observed in the samples.

DISCUSSION

Reported clinical differences between PORP and PORP results cannot be accounted for in the present model. Differences may be due to healing effects or surgical approach (transcanal versus mastoid).

665 Ossiculoplasty: The Effects of Interposed Materials Between the Eardrum and the Prosthesis

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During Ossiculoplasty, cartilage is often used to cover the prosthesis to prevent extrusion. The interface of the prosthesis to the eardrum is complex. This interface will change substantially with healing, with scarring, eardrum thickening and tension changes. The effects of changes at the eardrum/prosthesis interface are largely unexplored. Cartilage could be replaced with softer materials such as fascia, or harder materials such as bone. If interposed material is detrimental, it may be better to take the slightly higher risk of extrusion. The size of the cartilage may have an effect

We used a fresh cadavaric middle ear model of ossicular reconstruction with a PORP (Partial Ossicular Replacement Prosthesis) which connects the posterior eardrum to the stapes head. The effects of changes at the eardrum/prosthesis interface were compared by measuring stapes footplate movements with a laser Doppler Vibrometer. Sound at 80-95dB in the external ear was the driving stimulus. The conditions tested were: direct contact PORP to eardrum, cartilage in interface, Merocel® (soft) in interface, and glass (rigid) in interface. The PORP was also glued to the eardrum.

The effects of insertion of material at this interface are significant. New resonances are introduced in the stapes response. The exact effect varies from bone to bone, but there is clearly a difference in response between soft and hard materials at this interface. Larger cartilage appears to reduce the low frequency response.

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666 A Comparison of Ossiculoplasty with Stapes to Malleus and Stapes to Eardrum Prostheses

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Many methods for surgical reconstruction of missing ossicles using prostheses are available. The optimal method is not clear, and often depends on the surgeon's preference. Two commonly used methods are reconstructions in which the incus has been removed and the middle ear reconstructed with either a prosthesis from the stapes head to the malleus (Incus Replacement Prosthesis-IRP) or a prosthesis from the stapes head to the eardrum (Partial Ossicular Replacement Prosthesis - PORP). The role of the malleus in acoustic function of the prosthesis is not clear. Our objective was to compare these reconstructions in a cadavaric human middle ear.

Fresh human cadaveric temporal bones were harvested within 48 hours after death. Measurements on the stapes footplate were carried out using a Laser Doppler Vibrometer. The intact temporal bone stapes footplate was first measured. The movements of the footplate were compared for the two types of reconstruction . We used commonly commercially available hydroxyapatite prostheses: the Goldenberg PORP and IRP (Gyrus, Memphis, USA). These are similar to many other prostheses of the same essential design. The reconstruction was performed through the facial recess The tympanic membrane was stimulated with a sound input of 80 – 95 dB SPL over a frequency range of 0.25 to 8kHz. Measurements were made for several lengths of each prosthesis to allow for the confounding effects of tension.

Our results show substantial differences in the performance of the IRP and PORP prostheses. The differences are complex and frequency dependant. These differences are also present in the 3-Dimensional movements of the footplate.

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667 Effects of Prosthesis Tension upon the Stapes Vibration Modes in Middle Ear Surgical Reconstruction

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In surgical ossicular reconstruction of the middle ear, the prosthesis can be placed under varying tensions. A common surgical practice is to insert the prosthesis under significant tension , both for stability and because it is perceived as a more "secure" connection. The effects of prosthesis tension on the performance of the prosthesis has not been systematically investigated. Our objective is to investigate stapes footplate vibration responses with prostheses from the eardrum to the stapes head (PORP), and from the malleus to the stapes head (IRP) of varying lengths. This results in varying resultant tensions.

Fresh human cadaveric temporal bones were harvested within 48 hours after death. A Laser Doppler Vibrometer was used to determine the change in behaviour of the centre stapes footplate and tympanic membrane. First the intact temporal bone was measured. Subsequently reconstructed conditions were measured. Three sizes of prosthesis are compared for each reconstruction. The tympanic membrane was stimulated with a sound input of 80 – 95 dB SPL over a frequency range of 0.1 to 8 kHz. Experiments were performed on 7 temporal bones.

In most temporal bones, the loosest prosthesis had the best low frequency response, and worst high frequency response, and vice versa. The low frequency responses were the most sensitive to changes in prosthesis length. The change in function was not a linear function of length.

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668 An Objective Measure of Conductive Hearing Loss

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The changes in pure-tone thresholds induced by positive and negative ear canal air pressures were compared to changes in conductance at the same pressures in three human participants with normal hearing. Pure-tone thresholds at 0.5 and 2 kHz were measured using a two-interval two-alternative forced-choice procedure at ambient pressure, and at ear

canal pressures of positive and negative 200 daPa. Immediately prior to and following each threshold measurement, measures of wideband conductance and reflectance were obtained without removing the probe from the ear canal. If the pre-threshold measurement of conductance change did not agree with the post-threshold measurement, the entire run was repeated for that frequency. Each set of measurements was obtained twice and the data were averaged for each measurement. Changes in the one-third-octave conductance measures at 0.5 kHz and 2 kHz were compared to the changes in pure-tone thresholds. Better agreement was found between threshold and conductance changes for positive pressure shifts than negative pressure shifts. When averaged across pressures, pure-tone threshold shifts for both frequencies were within 2 dB of shifts in conductance. The results of this study suggest that the change in middle-ear conductance provides an objective measure of conductive hearing loss for the conditions in this experiment.

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669 Demonstration and Objective Measurement of the Performance of Implantable Hearing Devices

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For more than five years the Symphonix Vibrant® Soundbridge has provided an alternative for patients who do not benefit from conventional hearing aids. Previously, however, it has not been possible to preoperatively demonstrate the middle ear implant or objectively determine the transmission properties of these devices.

Following the development of the direct drive simulator (DDS) it is now possible to directly stimulate the ossicular chain in a non-invasive manner using a small transducer placed on the tympanic membrane. This demonstrates the mechanical energy transfer and provides the patient with a sound impression that has proven to be comparable with the implanted system. The procedure is, overall, very comfortable for the patient. Different source signals such as music, speech or environmental sounds can be used for a comparison with conventional hearing aids.

The audiological results of the implanted patients are stable in the long-term but reveal an inter-individual variability owing to the differential coupling of the transducer with the ossicular chain. A set-up has been defined in order to measure the sound pressure level in the outer ear canal as the Reverse Transfer Function (RTF) when the ossicular chain is stimulated by the middle ear implant. The RTF represents the actual energy transfer to the inner ear and enables the surgeon to intraoperatively evaluate and optimize the coupling. The hearing aid acoustician can use the RTF during the fitting procedure of the external audioprocessor to take individual differences into consideration. Based on the measurements of more than 40 patients the RTF in combination with the standard audiometric tests and the subjective contentment, resulted in the establishment of a prescription rule for the audioprocessor allowing individual requirements to be met.

670 A New Electromagnetic Hearing Aid Using Coils to Vibrate the Ossicles

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Research and development over the past two decades has shown that implantable hearing aids can circumvent some of the problems found in conventional hearing aids, such as feedback and high distortion. The most prominent feature of the implantable hearing aid is that a magnet or piezoelectric bimorph is directly coupled to the one of the middle-ear ossicles. However, these devices have not yet been widely used because most vibrators are too heavy to obtain a good performance at high frequencies and it is difficult to apply them in children who are still growing.

In this study, as the first stage in the development of a non-invasive electromagnetic hearing aid, a new transducer was made for generation of high-excitation force to vibrate the ossicles via the tympanic membrane. This transducer is composed of a core, driving and induction coils, a rare earth magnet, and a vibrator coil. The core, the driving and induction coils, and the magnet are designed so that they can be installed in the external ear canal of humans. With regard to the vibrator coil, which is attached to the center of the tympanic membrane, its optimal mass was determined to be 18.3 mg by the finite-element method (FEM). *In vivo* experiments using guinea pigs showed that a prototype of the optimally designed transducer was able to generate an excitation force of more than 93 dB SPL in terms of sound pressure at frequencies between 0.8 and 10 kHz. This result indicates that the transducer developed in this study can be used to treat patients with a hearing loss up to 70 dB HL.

671 DPOAE Input/Output Functions in Children with Auditory Neuropathy/Auditory Dys-synchrony

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Otoacoustic emissions (OAE) have been widely used to test outer hair cell (OHC) function as they reflect the cochlear distortions produced by the motile properties of normal OHCs. OAEs are an important part of a complete audiologic test battery, particularly with respect to diagnosis of auditory neuropathy/auditory dys-synchrony (AN/D) in children. Current criteria for AN/D diagnosis include absent auditory brainstem response (ABR) and normal OAEs; however, OAEs are typically measured at only one stimulation level. OHC response characteristics to acoustic stimulation are dependent on the stimulus level, and therefore the current clinical method of OAE testing at a single level may not be sufficient to determine normal OHC function. OHC function in AN/D subjects include both abnormal and normal characteristics, with abnormally large cochlear microphonic and summating potentials, and normal tuning with distortion product OAE (DPOAE) suppression (Abdala et al., 2000). DPOAE input/output (I/O) functions show frequency-specific responses across stimulus level. I/O patterns have been well characterized in humans with normal hearing, sensorineural hearing loss (Dorn et al., 2002), and in animals with various otologic anomalies. In the case of AN/D, the DPOAE I/O function is unknown. The purpose of this study is to qualitatively characterize DPOAE I/O functions in AN/D children compared with age- and gender-matched normal hearing controls. Preliminary data indicate both normal and abnormal DPOAE I/O functions in the test group, which varied by frequency within subjects. Potential relevance of DPOAE I/O functions will be discussed with respect to the relationship with pure-tone thresholds and speech perception measures.

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672 The Effects Of Acoustic Trauma On Four Different Response Measures Of Cochlear Sensitivity

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We describe the effects of pure tone exposures on four different response measures: the cochlear whole-nerve action potential (CAP), distortion product otoacoustic emission (DPOAE), cochlear microphonic (CM), and electrically evoked otoacoustic emission

(EEOAE). Both the CM and the EEOAE were evoked by sweeping frequency (SR830 lock-in amplifier) and analyzed in terms of response amplitude, phase and time delay spectrum (TDS) (Ren and Nuttall, 2000). These experimental results are analyzed in view of the interaction of wave fixed or place fixed sources of reverse propagating waves.

Pigmented guinea pigs were anesthetized, tracheomtomized and surgically prepared for placement of a silver wire on the round window (RW) of the cochlea. The animal's heart rate, oxygenation and body temperature were monitored. The sound delivery system consisted of a custom made speculum mated with two Beyer earphones and an Etymotic microphone.

The CAP audiogram and DPOAE-gram produced comparable estimates of the hearing loss. The results from the CM measurements show that the amplitude spectrum is affected in a similar manner by both low and high frequency exposures. This is probably due to the site of the CM recording (the RW) and vectorial nature of the voltage summation.

The effects of sound exposure on the EEOAE showed more specific changes in the amplitude spectrum reflecting the exposure frequency. Low frequency exposures produced broad changes in EEOAE amplitude. High-frequency exposures produced changes restricted to the high-frequency region of the cochlea. The results will be discussed in terms of the frequency specificity of the EEOAE and multiple sites of generation.

673 Differential Diagnosis of Common Hearing Dysfunctions using a Comparison of Audiometric Status and Specific Distortion Product Otoacoustic Emission Measurements

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The two most common hearing dysfunctions in the human population appear to be: 1) cumulative noise damage, resulting in predominantly outer hair cell loss; and 2) strial deterioration, which often accompanies aging and results in a lowered endocochlear potential (EP). These two conditions cannot be distinguished purely by their effect on audiometric status. With continuing innovations in available treatments, however, it will be increasingly useful for clinicians to be able to distinguish between the two conditions simply and non-invasively. Recent experiments have compared auditory brainstem responses (ABRs) and distortion product otoacoustic emission (DPOAE) measurements before and after similar dysfunctions were induced in animal models. At a given audiometric status, acoustic damage typically resulted in a larger increase in DPOAE thresholds than was observed when EP was lowered. The distributions were found to be non-overlapping, so that the following rule was obtained: if the increase in ABR threshold, ΔABR, is 20 dB or more at a given frequency, then calculate the emission threshold "boundary value,"

$\Delta Em = 0.6\ \Delta ABR + 8$ dB.

If the observed emission threshold shift is larger than ΔEm, the underlying dysfunction is acoustic damage, otherwise it is due to a lowered EP. For example, if the audiometric threshold is found to be 40 dB above normal at a particular frequency, then the associated emission boundary value would be calculated to be ΔEm = 32 dB. If the elevation of the DPOAE threshold above normal at the same frequency is found to be greater than 32 dB for this subject, the underlying dysfunction is probably noise damage; if less, strial dysfunction would be the tentative diagnosis.

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674 The Application of Otoacoustic Emissions in Detecting Carriers of Autosomal Recessive Non-Syndromic Hearing Loss.

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ABSTRACT:

Objectives: To determine whether subclinical auditory anomalies are present in carriers of autosomal recessive non-syndromic hearing loss (ARNSHL) and whether such changes can be detected using otoacoustic emissions (OAEs), a test of outer hair cell integrity. The possible association of any OAEs abnormalities and the presence of mutations in the GJB2 gene (Cx26) will be explored.

Methods: The study was designed as a case-control study. OAEs were recorded in 10 obligate carrier parents who were positive for mutations of the Cx26 gene and 10 presumed carrier parents from families who satisfied criteria of ARNSHL but did not screen positive for the GJB2 gene. These were compared to 10 age and sex matched controls. In all subjects OAEs, including transient OAEs, distortion product OAEs, and a test of medial olivocochlear (MOCB) efferent suppression, were carried out.

Results: OAEs abnormalities were detected for both carrier groups. TEOAE anomalies were more predominant in those parents that did not carry mutations for the Cx26 gene. Of particular note is the finding of high prevalence of absent TEOAE responses for the mid and high spectral bands for both carrier subgroups. DPOAE data again showed greater significant reductions for the Cx 26 -ve carriers especially in the mid and high frequency spectral bands. Finally, the MOCB efferent test detected significant reduced suppressions only for those parents that did not carry mutations in the GJB2 gene.

Conclusions: The study provides further evidence for the value of OAEs measurements in unveiling subclinical cochlear dysfunction in autosomal recessive carriers of hearing loss. The feature of high OAEs absent responses at 2.0 and 4.0 kHz shows susceptibility of the mid and high frequency regions. Further evidence of genetic heterogeneity for those carriers that carried no mutations for the GJB2 gene, was shown by the finding of reduced MOCB efferent suppression test in these carriers only.

675 Predicting Pure-Tone Thresholds from DPOAE Input/Output (I/O) Functions

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Recently, Boege and Janssen (2002) fit linear equations to DPOAE I/O functions after DPOAE level (in dB SPL) was converted into μPa. Only I/O functions with at least 3 points having SNR exceeding 6 dB were included. After meeting additional inclusion criteria related to the linear fits, these equations were solved for the input level at which the DPOAE equaled 0 μPa, which was defined as DPOAE threshold. Significant correlations were observed between these DPOAE thresholds and audiometric thresholds. The present study extends their work by (1) evaluating the effect of frequency, (2) determining the behavioral thresholds of those ears that did not meet inclusion criteria, and (3) including a wider range of stimulus levels. DPOAE I/O functions were measured in as many as 278 ears of subjects with normal and impaired hearing. Nine f_2 frequencies (0.5 to 8 kHz in ½ octave steps) were used, L_2 = 10 to 95 dB SPL (5 dB steps), and $L_1 = 0.4\ L_2 + 39$ dB (Janssen et al, 1998) for L_2 levels up to 65 dB SPL, beyond which $L_1 = L_2$. For the exact same conditions as those used by Boege and Janssen, we observed a frequency effect such that correlations were higher for mid and high frequency threshold comparisons, with lower correlations at lower frequencies and at 6 and 8 kHz. In addition, a

larger proportion of ears not meeting inclusion criteria at mid and high frequencies had hearing losses exceeding 40 dB HL, compared to lower frequencies. These results suggest that DPOAE I/O functions can be used to predict audiometric thresholds with greater accuracy at mid and high frequencies, but only when certain inclusion criteria are met. When these inclusion criteria are not met, the expected amount of hearing loss increases. Increasing the range of input levels from 20-65 dB SPL to 10-85 dB SPL increased the number of functions meeting inclusion criteria and slightly increased the overall correlation between DPOAE and behavioral thresholds.

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676 DPOAE Suppression in Normal-Hearing and Hearing-Impaired Human Subjects

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DPOAE suppression measurements were made in 19 subjects with normal hearing and 24 subjects with mild-to moderate hearing loss. The probe consisted of two primary tones (f_2, f_1), with f_2 held constant at 4 kHz and $f_2/f_1 = 1.22$. Primary levels (L_1, L_2) were set according to the equation $L_1 = 0.4L_2 + 39$ dB (Janssen et al., 1998), with L_2 ranging from 20 to 70 dB SPL (normal hearing subjects) and 50-70 dB SPL (subjects with hearing loss). Thus, one difference between normal and impaired ears was the range of primary levels over which DPOAEs could be measured. Responses elicited by the probe were suppressed by a third tone (f_3), varying in frequency from approximately 1 octave below to ½ octave above f_2. Suppressor level (L_3) varied from 10 to 85 dB SPL. Prior to the presentation of each suppressor (f_3), responses were measured in a control condition in which only the probe (f_2, f_1) was presented. Responses in the presence of the suppressor were subtracted from the average response from the two closest control conditions in order to convert the data into decrements (amount of suppression). The slopes of the decrement versus L_3 functions were slightly less steep for lower frequency suppressors in the impaired ears, although the differences were small. Suppression tuning curves, constructed by selecting the L_3 that resulted in 3 dB of suppression as a function of f_3, resulted in tuning curves that were similar in normal and impaired ears when the two groups were compared at similar primary levels. Quantities such as Q_{10} and tip-to-tail difference were the same for normal and impaired groups. These results are consistent with the view that normal and mildly impaired human ears function similarly at moderate and higher SPLs.

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677 Suppression Tuning of Rabbit Distortion-Product Otoacoustic Emissions Following Permanently Damaging Acoustic Overexposure

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Psychophysical, basilar membrane (BM), and single nerve fiber tuning curves, as well as suppression of distortion-product otoacoustic emissions (DPOAEs), all give rise to frequency tuning patterns with stereotypical features. Similarities and differences between the behaviors of these tuning functions both in normal conditions and following various cochlear insults have been documented. While neural tuning curves (NTCs) and BM tuning curves behave similarly both before and after cochlear insults known to disrupt frequency selectivity, DPOAE suppression tuning curves (STCs) do not necessarily mirror these responses following either administration of ototoxins (Martin et al, 1998), or exposure to temporarily damaging noise exposure (Howard et al, 2002). However, changes in STC parameters may be predictive of other types of cochlear dysfunction such as cochlear immaturity in neonatal humans (Abdala, 1998). To determine the effects of noise-induced permanent auditory dysfunction on STC parameters, rabbits were exposed to high-level noise that led to long-lasting reductions in DPOAE level, and comparisons between pre- and post-exposure DPOAE levels and STCs were made. Statistical comparisons of pre- and post-exposure STC values at CF revealed consistent basal shifts in the frequency region of greatest cochlear damage, whereas thresholds, Q_{10dB}, and tip-to-tail gain values were not reliably altered. Additionally, a large percentage of high-frequency lobes associated with third tone interference phenomena, that were exhibited in some data sets, were dramatically reduced following noise exposure. Thus, previously described anomalous suppression above f_2 by interference tones may also be studied using this type of experimental manipulation (Martin et al, 1999; Mills, 2000).

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678 Vector Subtraction of DPOAE Interference Response Areas

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DPOAEs are generally assumed be generated in the region of primary-tone overlap on the basilar membrane. However, suppression and enhancement of emissions by a third tone (f_3) well above f_2 have been documented in a variety of mammalian species, which implicates a high-frequency DPOAE source. Fahey et al. (JASA 2000) proposed two possible mechanisms to explain this source: the harmonic mechanism mixes the harmonic of one primary with the other primary whereas the catalyst mechanism mixes intermediate products of f_3 with one or both primaries. The harmonic mechanism could be a component of two-tone DPOAE measurements under some conditions and may have an impact on their interpretation. A major weakness of the proposed harmonic mechanism is that f_3 often seems to have level effects well above the harmonic frequency. The present study describes a technique for performing vector subtraction between interference response areas (IRAs), which are contours of iso-suppression/enhancement plotted as a function of f_3 frequency and level. IRAs were taken before and after manipulations that preferentially affected high-frequency interference above f_2, including noise exposure, diuretic administration and DPOAE adaptation to stimulus on-time. The IRAs were converted to DPOAE vectors, subtracted and reconstituted into an IRA residual representing the high-frequency source. The residual IRAs were consistent with the harmonic mechanism in that f_3 generally produced level effects only at or below the harmonic frequency, and only phase effects above this region. Thus, when f_3 is well above the harmonic frequency it produces phase changes in the harmonic source that could account for suppression/enhancement above the harmonic place in the original IRAs.

679 Empirical Estimates of the Spatial Origin of Stimulus-Frequency Otoacoustic Emissions

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Stimulus-frequency otoacoustic emissions (SFOAEs) evoked by a probe tone can be revealed by suppressing them with tones more than an octave higher in frequency (cats: Guinan, J. J., 1990, in *Mechanics and Biophysics of Hearing*, pp. 170–177; guinea pigs: Souter, M., 1995, Hear. Res. 90: 1–11; humans: Siegel, J. H. and Badri, R., 2002, ARO Abst. 25: 84, 2002). We report here similar measurements in chinchillas.

SFOAEs were evoked with probe tones with frequency (f_{pr}) of 1, 2, 4, 6 or 8 kHz, presented at 10-20 dB SPL, and suppressed with 45-60 dB SPL tones stepped in 21.54 Hz increments over a wide range of frequencies (f_{su}) > f_{pr}. For all f_{pr}s, the magnitudes of suppression "residuals" generally declined gradually with increasing f_{su} but

exhibited 1-3 nulls (typically accompanied by π - phase shifts) for f_{su} - f_{pr} < 1 octave.

On the assumption that higher f_{su}s correspond to more basal cochlear regions, estimates of the spatial profile of SFOAE generators were obtained by differentiating the residuals with respect to f_{su}. The plots of differential magnitude-vs.- f_{su} were generally devoid of nulls. The plots of differential phase-vs.- f_{su} were free of π - phase shifts and resembled phase-vs.-frequency plots of basilar-membrane responses.

Moderate (< 30 dB), long-lasting and sharply-localized TTS induced by pure tones greatly reduced the magnitude of the residuals for f_{su}s equal to the frequencies of CAP threshold elevation. This was true even when the TTS was confined to frequencies well above that of f_{pr}. The present evidence supports Guinan's contention (op. cit.) that SFOAEs originate over a wide region heavily weighted toward the cochlear base (due to the slowly varying phase of local SFOAE generation). Our data do not support the notion that the peak region of the basilar-membrane traveling wave dominates the generation of SFOAEs (Zweig and Shera, JASA 98:2018-2047, 1995).

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680 Comparison of SFOAE Suppression Patterns in Humans to Basilar Membrane Two-tone Suppression in Animals.

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We reported the recovery of stimulus frequency otoacoustic emissions (SFOAE) using suppressors extending over an octave above the probe frequency in normal hearing human subjects (Siegel, et al., ARO Abst. 25:84, 2002). The SFOAE phase varied with suppressor frequency. These results imply that the SFOAE generation region is extended and that there must be phase cancellation of SFOAE sources. We seek to evaluate how closely the SFOAE suppression patterns correspond to analogous basilar membrane (BM) two-tone suppression studies in animals (i.e., Rhode and Cooper, Hear Res, 66(1); 31-45,1993; Cooper, J Acoust Soc Am 99(5); 3087-98,1996). The SFOAEs evoked by a probe tone at 1, 2 or 4 KHz at 30 dB SPL were measured using suppressor tones at 50 dB SPL varied in frequency increments of 21.5 Hz, starting just above the frequency of the probe tone. The residual was measured as previously described (Siegel, et al., ARO Abst. 25:84, 2002). To provide a measure of SFOAE suppression directly comparable to BM data we expressed the SFOAE data as the part of the emission that has *not* been suppressed (the ***remainder SFOAE***). When the probe and the suppressor frequencies were similar, the residual reached a plateau indicating complete suppression of the SFOAE. This provided our measure of the ***total SFOAE***. The remainder SFOAE was then obtained by vector subtracting the residual at each suppressor frequency from the total SFOAE. Suppression of the remainder SFOAE was consistently evident for suppressors nearly an octave above the probe frequency. In contrast, suppression in the BM with comparable stimuli has been reported to be restricted to 0.16 to 0.33 octave above probe frequency. The extent and the direction of the change in phase of the remainder SFOAE as a function of suppressor frequency varied between subjects and did not resemble the phase variation in BM suppression studies. Our findings support the hypothesis that the SFOAE suppression patterns measured in the ear canal do not accurately reflect the suppression patterns measured at a single point on the cochlear partition.

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681 Two-tone Suppression of Stimulus Frequency Otoacoustic Emissions

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Stimulus frequency otoacoustic emissions (SFOAE) can be measured using an additional suppressor tone, and the resulting paradigm is analogous to two-tone suppression, which has been measured neurally and mechanically. Two-tone SFOAE suppression has been related to cochlear mechanics (e.g., Brass and Kemp, 1993), but these data are limited to 3 ears tested over a 1-octave range of probe frequency. The present research reports two-tone suppression of SFOAE responses obtained in 20 normal-hearing adult ears at 5 octave frequencies (fp=0.5-8.0 kHz) over a ~40 dB range of probe levels (Lp), in which suppressor frequencies (fs) were varied from -2.0 to 0.7 octaves relative to fp. Suppressor levels (Ls) ranged from just detectable up to full suppression. The use of multiple fs's provided independent measurements of full suppression of the SFOAE present in the no-suppressor condition. The resulting total SFOAE input/output (I/O) functions are monotonic and compressive. Consistent with past research, the lowest suppression thresholds occur for a "best" fs slightly higher than fp. The I/O functions relating SFOAE decrement to Ls have a steep slope at frequencies much lower than best fs, and a shallow slope near best fs. Suppression tuning curves (STCs) constructed from these I/O functions are well defined at 1, 2, and 4 kHz, but less so at 0.5 and 8.0 kHz. Individual and group analyses show that tuning is sharper at lower Lp. STCs vary with Lp with slopes close to 1 dB/dB for fs near best fs and reduced slopes at lower fs, consistent with compressive response near best fs and more nearly linear response at lower fs. These data are broadly consistent with basilar-membrane measurements, neural recordings, behavioral masking, and other OAE measurements.

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682 Suppression of Transient Otoacoustic Emissions at Spontaneous Otoacoustic Emission Frequencies.

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Sharper psychoacoustic tuning curves have been demonstrated at frequencies where spontaneous otoacoustic emissions (SOAE) are detected. Central auditory mechanisms were hypothesized to be responsible for the greater frequency selectivity at SOAE frequencies. Previous findings from this laboratory have demonstrated that enhancement of frequency selectivity at SOAEs is also found when estimated by suppressing toneburst-evoked otoacoustic emissions (TEOAE). This lends support to a peripheral mechanism for sharper frequency selectivity at an SOAE frequency. To date, these findings are limited to tuning measurements made at 4000 Hz.

The purpose of this study is to extend the analysis of TEOAE STCs to SOAE frequencies other than 4000Hz. Eight-cycle, 50-dB SPL tonebursts presented in linear stimulus blocks were used to evoke TEOAEs centered on an SOAE. These TEOAEs were then suppressed using puretones presented ipsilaterally. The suppressor level that reduced the OAE amplitude by -6 dB was plotted as a function of suppressor frequency to yield suppression tuning curves. Suppression tuning curves at SOAE frequencies were compared to tuning curves generated at the same frequency in the opposite ear of subjects that had no measurable SOAEs.

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683 Evidence for a Pitch Helix in the Ventral Nucleus of the Lateral Lemniscus in the Gerbil.

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In humans acoustic signals with periodic envelope modulations may elicit the percept of pitch. As a neuronal basis, we suggest coincidence of delayed and undelayed responses to the modulations in the inferior colliculus (ICC; Langner et al., Hearing Res. 168, 2002). Accordingly, units in the ICC were found to be tuned to different modulation frequencies and to be arranged in periodicity maps.

In line with the model we observed comb-filters in neurons of the ICC during the first 30 ms of their responses to periodic signals. However, after this initial phase the filter curves were transformed into band-pass filters. Since we assume that the source of the underlying inhibition is the ventral nucleus of lateral lemniscus (VNLL) and that it should receive input from the ICC, we investigated the VNLL for a possible periodotopic map.

For that purpose we used the 2-deoxyglucose technique. Stimuli were harmonic complexes with fundamental frequencies ranging from 40 to 800 Hz and cut-off frequencies at the low (0.4 - 5 kHz) and the high frequency border (2 - 8 kHz) of their broad band spectra. To avoid distortions an electrostatic speaker (STAX) and low intensities (0 - 55 dB SPL) were used. Animals were placed singly in a soundproofed chamber and were stimulated for 1h.

Our results show that the VNLL is weakly but distinctly labelled by different harmonic sounds in line with the assumption of activation by descending projections from the ICC. Moreover, along the longer axis of the VNLL low periodicities (pitches) are mapped dorsally and high periodicities ventrally with a gradient of about 0.28 mm/octave. Taken together with the analysis of the fine structure of the glucose labels this suggests a helical periodicity map in the auditory system of the gerbil reminding of the well-known pitch helix of music psychologists.

The results are in line with a similar helical organization of the VNLL of rat (Merchán and Berbel, J. Comp.Neurol. 372, 1996).

684 Modeling Spectral Integration that Underlies IC Responses to Complex Tones

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Harmonic complex tones with a mistuned component elicit distinctive temporal discharge patterns from inferior colliculus (IC) neurons, and the pattern changes in a complex manner with changes in stimulus parameters (Sinex et al, Hear. Res., 168, 150-162, 2002). The periodicity of the discharge patterns suggests that IC neurons receive discrete inputs that are phase-locked to the envelope of the stimulus in multiple narrow bands. A simple computational model was developed to examine the way in which temporally-patterned inputs might be extracted in the lower brainstem, then combined in the IC to produce the observed response patterns. The model included three stages. In Stage 1, which represents processing in the cochlea, the stimulus was bandpass-filtered and rectified. In Stage 2, assumed to represent processing in the cochlear nucleus and/or superior olivary complex, the envelope of the narrowband waveform from Stage 1 was extracted. In Stage 3, representing the IC, these envelopes were combined across frequency channels. Model parameters such as bandwidth of integration and strength of inhibition were varied. Model outputs could be made to resemble the responses of real neurons if two conditions were met. First, the input to Stage 3 had to include responses of Stage 2 neurons across a wide range of characteristic frequencies (CFs). Second, it was necessary to include both inhibitory and excitatory inputs. It was possible for a single set of model parameters to reproduce the disparate discharge patterns elicited by variants of the complex tone. The implications of these results for processing at and below the level of the IC will be discussed.

685 Simultaneous Single-Unit Recording from Local Populations in the Inferior Colliculus

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Simultaneously recording from multiple neurons offers a chance to study neural correlations while increasing data yield, yet these techniques have rarely been applied to auditory brainstem nuclei. Here, we use 'tetrodes,' four channel electrodes with closely spaced recording sites (<35μm), to investigate the response properties of local, neural populations in the inferior colliculus (IC). We used both custom made wire tetrodes and silicon based arrays with tetrode-like geometries to record from the IC of anesthetized cats.

Tetrodes acquire multi-unit activity and exploit the spatial sampling of the four recording sites to reconstruct single-unit spike trains. The joint information in the four channels increases the probability of identifying a neural event over using a single channel, thereby improving spike detection. After detecting the times of neural events, we create a four-dimensional representation of each event by applying principle component analysis to the associated waveforms on all channels. Each cluster of neural events in the four-dimensional space defines an individual neuron of our population.

We extracted single unit spike trains for up to four simultaneously recorded neurons from a recording location. In response to pure tones, all units within a local group exhibited similar best frequencies, but could differ in thresholds and temporal discharge patterns. Further, locally recorded units showed no significant correlation beyond that induced by the stimulus. We plan to extend this technique to study the properties and interactions of local populations within iso-frequency laminae of the central nucleus of the IC.

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686 Species Differences in the Spectral Processing Pathways of the Inferior Colliculus

*Bradford J. May

The inhibitory features of frequency response maps (FRMs) reveal the dominant ascending influences on single-unit activity in the central nucleus of the inferior colliculus (ICC). In decerebrate cats, this system of functional classification has been used to describe abundant inputs from the dorsal cochlear nucleus (DCN). The pathway linking the DCN to the ICC appears to enhance the auditory processing of spectral information and is critical for accurate sound localization behavior. This poster presentation describes a similar analysis of single-unit response patterns in the ICC of awake mice and decerebrate guinea pigs. These comparison species were selected because they are commonly used in electrophysiological studies of the inferior colliculus but do not share the predatory lifestyle of the cat. Our investigations indicate that the major FRM response types are conserved across species, but the relative distribution of response types is substantially altered. In relation to cats, mice and guinea pigs exhibit a conspicuous under-representation of the so-called type O units that are assumed to reflect DCN inputs to the ICC. Since sources of brainstem innervation are similar in cats, mice, and guinea pigs, species differences in spectral processing pathways of the ICC suggest that ascending auditory inputs are shaped by intrinsic properties of the auditory mid brain to promote the effective transmission of biologically relevant information.

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687 Immediate Changes in Response Properties of Cat Inferior Colliculus Neurons Following Sequential Spiral Ganglion Lesions.

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Tone-evoked responses were recorded from the cat inferior colliculus (ICC) before and immediately after progressively larger mechanical lesions of the contralateral spiral ganglion (SG). Previously, we demonstrated that acute and spatially restricted lesions of the SG changed the central representation of sound frequency in both the ICC and primary auditory cortex. In those studies, we recorded frequency response areas (FRAs) of single neurons and multi-neuron clusters using tungsten microelectrodes and silicon microprobes (Center for Neural Communication Technology, U. of Mich.). These FRAs were recorded one site at a time over several hours. In this study, cluster responses were recorded simultaneously at all 16 site of probe, which were fixed in place prior to any lesion. Several probe designs were used and their design determined separation of adjacent recording sites, which varied from 50 to 200 μm. Single neuron responses were sorted from multi-neuron clusters by off-line principal component analysis using Bayesian statistical inference to resolve spike overlaps. FRAs were constructed from sorted spikes and represented the frequency tuning of the same neurons before and after each lesion. Pre- and post-lesion CAP audiograms were used to document peripheral effects of sequential lesion enlargement. Restricted SG lesions generally caused threshold increases over a restricted frequency range corresponding to the lesion frequencies. Thus, post-lesion FRAs had threshold notches, silent areas within the excitatory areas. Sequentially enlarged lesions produced FRAs with progressively larger silent areas corresponding to the larger range of lesion frequencies. Only in cases of very large lesions were neurons completely silenced. These acute changes in ICC neural sensitivity produced immediate shifts in CF and immediate alterations in the tonotopic organization of the ICC.

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688 Intensity Dependence of the Auditory STRF

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Studies using pure-tone tuning curves have long demonstrated a dependence on receptive field bandwidth and sound pressure level (SPL). These studies demonstrate that auditory neurons can respond exclusively to a restricted range of frequencies at low intensities but typically exhibit expanded integration areas at louder levels. According to these results, the representation of a complex sound assumes a level-dependent representation. We investigate the intensity dependence of the auditory spectro-temporal receptive field (STRF) from single unit recordings in the inferior colliculus. Following pure-tone tuning curve recordings, we presented dynamic ripple stimulus sequences at two, three or four intensity cross-sections. We then quantitatively evaluated structural characteristics of the STRF at each SPL. Specifically, we extracted various spectral and temporal parameters (e.g. bandwidth, duration, energy, etc.) from the STRF and studied their intensity dependence. Initial results suggest that the excitatory receptive field structure is relatively unchanged with increasing level, although the receptive field energy is significantly affected. Such findings indicate that broadband stimulation may stabilize excitatory integration areas thus leading to an invariant or level-independent representation for spectro-temporal stimulus features.

689 Encoding of Second-order Amplitude Modulation in the Inferior Colliculus

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Most studies of neuronal responses to amplitude-modulated sounds have employed sinusoidal modulation waveforms. Unlike these simple modulations, however, the modulation frequency and depth of natural sounds often vary with time. Such fluctuations are termed second-order modulations. We used tones modulated by bands of noise to test the hypothesis that neurons in the IC encode information about varying modulation frequency and depth.

We recorded responses of neurons in the IC of the anaesthetised (urethane 1g/kg, i.p. and Hypnorm 0.2 ml i.m) guinea pig to tones presented contraurally at best frequency that were amplitude-modulated by bands of frozen noise. Histograms generated from the spike times were cross-correlated with the noise modulator to determine the extent to which firing synchronised to the first-order modulation envelope. The ability of units to follow second-order modulations was measured by finding the envelope of the noise using a Hilbert transform and cross-correlating it with the histogram.

Neuronal firing synchronises to the first-order and second-order modulation envelopes when the noise modulator contained low modulation frequencies (0-0.5 kHz). When the noise modulator encompassed a higher frequency range (2-3 kHz) there was no correlation between firing and the first-order modulation, but the firing did correlate with the second-order modulation waveform of the stimulus. As with responses to first-order modulations, neurons responded best to low-frequency components (< 0.5 kHz) in the second-order modulation.

The results demonstrate that neurons in the IC are sensitive to the second-order components of amplitude modulations that are absent in sinusoidal stimuli. Furthermore, neurons encode the second-order modulation in their firing even when the first-order modulation frequencies of the stimulus exceed the range to which the neurons are sensitive.

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690 Responses of Inferior Colliculus Neurons to SAM Stimuli with Varied Modulation Depth

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Responses to sinusoidally amplitude modulated (SAM) stimuli were recorded from neurons located in the central nucleus of the inferior colliculus (ICc) of chinchillas. The modulation depth was varied from 0 to 100% by a step of 20%, and the modulation frequency was varied up to eight octaves by a step of one octave or less. Discharge rate (rMTF) and vector strength (tMTF) modulation transfer functions were obtained with different modulation depth. The region of suppression where the discharge rate decreased with increasing modulation depth was observed in rMTFs. In addition the region of enhancement where the discharge rate increased with increasing modulation depth was also observed. Suppression region and enhancement region could be seen in the same neuron. We found that while suppression region was more common in pause and sustained neurons, enhancement region was dominant in onset neurons. The Rayleigh statistic ($a<0.001$) was used to determine the modulation threshold for vector strength. The vector strength score rarely decreased with increasing modulation depth, except for pause neurons at low modulation frequencies. Also bandpass tMTF was always obtained at shallow modulation depth, even if low-pass tMTF was observed at full modulation (100%). Comparing the modulation threshold across difference peristimulus histogram groups, pause and sustained neurons showed all modulation thresholds below

40% while onset neurons showed all thresholds above 20%. It suggests that the modulation threshold is mediated by pause and sustained neurons rather than onset neurons in the ICc.

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691 Effects of Noise Bandwidth and Modulation on Signal Detection for Single Neurons in the Frog Auditory Midbrain

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Natural auditory scenes, like frog choruses, are complex. Detection of sound in a noisy background is a challenging task, and depends on the bandwidth and modulation depth of the background noise. In this study, we examined how these noise attributes influenced the detection threshold for a synthetic mating call (i.e., a series of tone pulses at CF with a modulation rate of 20 Hz) for single units in the frog auditory midbrain. The noise was centered at CF with bandwidth ranging from 100-4000 Hz at constant spectral level, 15 dB above the threshold for the signal in quiet. Both signal and noise were broadcast from a loudspeaker in the freefield. Unmodulated noise generally increased the signal detection threshold; sinusoidally modulated noise with a 7-9 Hz modulation rate at equal peak intensity and/or equal overall energy also elevated this threshold, but to a lesser extent. For both types of noise, an increase in noise bandwidth typically increased the detection threshold, but a decreased detection threshold was also regularly encountered. A number of cells responded only to the tone pulses placed in the troughs of modulated noise; the coincidence with peaks in the modulated noise suppressed the response to the signal. In some cells, noise strengthened the response to tone pulses; they responded strongly when the tone pulses coincided with the peaks of the modulated noise. These results suggest that frog auditory midbrain neurons are heterogenous and the response of a neuron to complex signals cannot be predicted from its responses to the components.

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692 Spectral and Temporal Response Properties of Single Neurons in the Inferior Colliculus of the Mouse

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Many animals emit spectrally and temporally complex social vocalizations, yet little is known regarding the neural processing of such calls. We are currently examining the behavioral meaning of social calls and the auditory processing of these calls in mice. We recorded vocalizations from groupings of C57BL/6 mice while recording their behavior. The component frequencies and temporal structure of the vocalizations recorded during copulatory behaviors were very different from those recorded during same-sex pairings. We then tested whether response features of neurons in the central nucleus of the inferior colliculus (ICC) are suited for the analyses of these vocalizations. Excitatory frequency response areas of single neurons were tested with tones of different frequencies. Facilitatory and inhibitory areas were tested by holding one tone at the unit's best frequency while varying the frequency and intensity of a second tone. Duration tuning was also tested. Most neurons had one excitatory response area whereas twenty percent of the neurons had multiple response areas. Half of these had their second excitatory peak in the frequency range of the copulatory calls (35 - 80 kHz). An additional ten percent of neurons showed facilitation between two different tones. Forty-two percent of neurons showed selectivity to durations that were similar to durations of the recorded calls. These spectral and temporal response characteristics suggest that some neurons in ICC may show selectivity to social vocalizations. In particular, inhibitory and facilitatory response areas and duration selectivity may increase neuronal selectivity to social calls. The actual selectivity of mouse ICC neurons to social vocalizations remains to be tested.

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693 Masked Thresholds in the Inferior Colliculus: Relationships to Gap Encoding and Frequency Selectivity

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Previously, we reported that a subset of phasic inferior colliculus (IC) neurons are specialized (SP) in that they encode gaps even when the gap is filled with background noise. In contrast, gap encoding is degraded in nonspecialized (NS) units in the presence of background noise. Here we test the hypothesis that aspects of the masked rate-intensity function (RIF) could predict whether a unit is SP. RIFs (3 dB steps; quiet (Q) and masked (M) with wideband (WB) noise 15 dB above threshold) obtained from single units in 1.5-4 mo CBA mice were characterized by tone threshold (TT; lowest tone level eliciting an increase in spike count); minimum (MIN) count; maximum (MAX) count; and dynamic range (DR) slope, width, and midpoint. TTs of both unit types increased with masking, but NS units had lower TTs (14 dB Q; 24 dB M) than SP units (19 dB Q; 31 dB M). Both the MIN and MAX spike counts of SP units were less than that of NS units in the Q (MIN: 7.67 SP, 16.47 NS; MAX: 98 SP, 143 NS) and M (MIN: 10 SP, 21 NS; MAX: 81 SP, 130 NS). The DR midpoint shifted to higher levels with masking, but occurred at lower levels for NS (26 Q; 35 M) than SP (31 Q; 41 M) units. Thus, SP units may be less sensitive (higher TT and DR midpoint, lower spike counts) to masked tones. In addition to WB noise, we used narrowband (NB) noise centered on the best frequency (BF) and adjusted to compensate for spectrum level to investigate the effects of on-BF and off-BF masking. NB noise increased the amount of masking: the TT was higher (42 dB WB; 63 dB NB), the DR was smaller (17 dB WB; 11 dB NB), and the DR midpoint was shifter to higher levels (50 dB WB; 68 dB NB). On-BF NB noise also had a more severe effect than WB noise on the MAX spike count (86 spikes WB; 61 spikes NB). Thus, reducing off-BF energy served to increase masking, both for SP and NS units, and preliminary analysis suggests that specialization can be predicted by the masked RIF.

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694 Within and Across Channel Gap Detection from the Inferior Colliculus of the Chinchilla

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In a series of perceptual studies, Phillips and colleagues have studied gap detection across channels. Using pre-gap and post-gap stimuli that vary across ears, they reported higher gap thresholds across channels than within channels. The present investigation studies across-channel gap detection while recording from the inferior colliculus (IC) of the unanesthetized chinchilla.

Seven chinchillas had electrodes chronically implanted in both IC. Following a recovery period, each animal was passively restrained, with insert earphones placed in each ear. Recordings from each IC were made to 80 dB SPL noisebursts. The first noiseburst (NB1) varied in duration (5, 10, 30, 50 ms) and was always presented to the left ear, and the second (NB2) was 50 ms, and was presented to the left or right ear. Gaps ranged from .5 to 32 ms.

For within-channel studies, the onset response from the IC to NB2 increased in latency and decreased in amplitude with decreasing gap (i.e., showed forward masking). Most animals showed onset responses to NB2 at 0.5-1 ms gaps for the 10, 30 and 50 ms NB1 durations, but threshold increased to 2 ms for the 5 ms NB1 duration. For across-channel studies, most animals showed responses to gaps of 0.5 ms for NB1 durations of 10, 30, 50 ms, and 1 ms for the 5 ms duration NB1. Across-channel IC onset response latency did not vary across gap, while IC onset response amplitude decreased for gaps below 4-8 ms. The offset responses to NB1 of 5 ms duration are absent for both within and across channel studies.

Although these findings do not directly parallel those reported by Phillips in human perceptual studies, these results demonstrate some

differences between within-channel (same ear) and across-channel (across ear) effects in gap detection.

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695 The Effect of Continuous Masking Noise on Chinchilla Inferior Colliculus Responses to Noisebursts Varying in Plateau Level and Risetime

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Responses from chinchilla inferior colliculus (IC) are affected by noiseburst level and risetime. Respecifying stimulus level as onset slope (in Pa/s) results in a superimposition of the latency and amplitude functions for a range of noiseburst risetimes. For latency, this normalization occurs even for short risetimes; for response amplitude, this normalization fails for brief risetimes. The present investigation evaluates whether this feature of the amplitude data is affected by the presence of a continuous noise background.

IC responses were recorded from the left IC in unanesthetized chinchillas to right-ear stimulation. Stimuli were noisebursts that varied in level (78 to 0 dB SPL in 6 dB steps), with risetimes of .125, .25, .5, 1, 2, 4, 8 and 16 ms, both in quiet and in the presence of a 40 dB SPL noise. Responses showed the expected latency decrease and amplitude increase with increasing noiseburst level and decreasing risetime. Masking noise produced a modest increase in noiseburst threshold. Respecifying stimulus magnitude as onset slope (Pa/s) caused the latency/intensity functions for stimuli of different risetimes to superimpose; the continuous masking noise produced a shift in the latency functions towards higher stimulus values. Amplitude/intensity functions, plotted in Pa/s, superimposed for risetimes greater than 2 ms. At shorter risetimes, the amplitude data fell below those for longer risetimes, and this departure occurred at progressively larger stimulus slopes (in Pa/s) with decreasing risetime. This same pattern was observed in the presence of the masking noise, although the functions were displaced towards higher stimulus values. Moderate-level masking noise does not appear to qualitatively change the effects of noiseburst risetime and level on responses from the chinchilla IC.

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696 The Effect of Risetime on Chinchilla Inferior Colliculus Responses to Ultrasonic Air- and Bone-Conducted Tonebursts

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Auditory evoked potentials (AEPs) are affected by stimulus risetime and level. In this study we investigated the effect of risetime when using ultrasonic stimuli. We presented 22 kHz tonebursts to seven chinchillas by either bone- or air-conduction (BC, AC) and measured the evoked response via an electrode chronically implanted in the contralateral inferior colliculus (IC). Stimulus risetimes included: 0, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16 and 32 ms; and stimulus level varied over a 90 dB range. IC onset response amplitude and latency were studied.

For AC, amplitude increased and latency decreased with decreasing risetime and increasing level. BC evoked responses behaved similarly with several important differences. First, even for high stimulus levels BC responses were smaller than AC responses. Second, small increases in the shortest risetimes resulted in larger decreases in amplitude for AC than BC at moderate and high stimulus levels. Third, the slope of the latency-intensity function was steeper for AC than BC at longer risetimes for moderate to high stimulus levels.

Ultrasonic AC and BC stimuli varying in level and risetime produce responses from the chinchilla IC that are similar qualitatively, but differ quantitatively in several ways.

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697 Physiology of the Tectal Commissural Column

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The long and narrow tectal commissural column (TCC; Vinuela et al., 2000, ARO Abstr. 23:179) is located in the rat's midbrain tectum between the commissures of the inferior and superior colliculi (IC and SC). It is innervated by the IC, nucleus of the brachium of the IC, and auditory cortex and innervates the ipsilateral superior olivary complex, which suggests a role in the auditory system. Moreover, its significant connections with non-auditory nuclei suggest a multi-sensory function. We have begun the analysis of the functional properties of the TCC.

Using anesthetized albino and pigmented rats, we have recorded the activity of TCC neurons in response to sound. Vertical electrode penetrations were made rostral to the IC and within 200 μm of the midline. Stimuli to each ear were tones, amplitude-modulated tones, and noise.

Past the cerebral cortex, a typical penetration crosses the light-sensitive and sound-insensitive medial SC, and then a narrow region with no responses to sound or light before entering the TCC, whose neurons are responsive to sound, but not to light. TCC recording sites were verified histologically.

Although TCC neurons are functionally varied, most neurons: 1) have a clear best frequency (BF), a high threshold, and a Q10dB value similar to that of typical nerve fibers; 2) show an excitatory, sustained response to suprathreshold stimuli near the BF; and 3) follow amplitude modulations very poorly. Fewer neurons are broadly tuned, have low thresholds, or are phasic responders. Most neurons are best driven contralaterally; ipsilateral effects are rare, as are examples of strong binaural interactions. These data suggest that the TCC participates in important, yet unknown, aspects of auditory processing related with frequency rather than with sound localization or stimulus timing.

698 Responses of Neurons in the Medial Geniculate Body to Vocalizations in the Guinea Pig

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Single unit activity was recorded extracellularly in the medial geniculate body (MGB) of anaesthetized guinea pigs. Acoustical stimuli - four typical guinea pig vocalizations (purr, chirp, chutter and whistle) and temporally and spectrally modified versions of the whistle - were presented in free-field conditions. Neuronal responses reflected the spectral and temporal features of the acoustical stimuli, but showed significant differences. The firing pattern typically displayed an onset character with enhanced activity at the beginning of the call or individual call components, followed by weak or no activity during the rest of the call or call components. Such a response type poorly represented slow fluctuations in the sound envelope for longer vocalizations, particularly for whistle. A significant portion of units failed in repetitive firing for rhythmically repeated components of purr, which has a repetition frequency of ~12.5 Hz. A time-reversed whistle elicited a slightly, but significantly weaker (by 13% on average) response than did a natural whistle. The spectral envelopes of vocalizations were poorly represented by the mean firing rate vs. CF profiles. If the natural whistle was spectrally modified such that the near-CF spectral band remained unchanged but side bands were eliminated, the neuronal response did not show a uniform effect: it could be either suppressed, enhanced or unchanged in comparison with the natural whistle. In summary, our results demonstrate that vocalizations are represented in a population of MGB neurons in a distributed and abstract way, and also that these communication sounds are processed in the MGB in a different way than in the inferior colliculus.

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699 Real-Time, High-Resolution Simulation of the Auditory Pathway

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Recent advances in computing and auditory neuroscience have now made it possible to produce high-resolution, real-time simulations of major portions of the human auditory pathway. I will demonstrate high resolution, real-time models of the cochlea, major cell-types of the cochlear nucleus, lateral and medial superior olivary complex, as well as polyphonic pitch perception based on combination-sensitive cells measured in inferior colliculus and auditory cortex, all running live on a notebook computer. In addition, this system has been integrated into a modern speech recognizer and has been demonstrated to provide higher phoneme-accuracy performance than standard frame-based Fast Fourier Transform (FFT) methods. The performance difference is attributable to various aliasing artifacts which are produced by the frame-based FFT methods, and are not produced by the biologically correct models.

700 Behavioral Discrimination of Auditory Space Is Predicted by Average Neuronal Discrimination

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The barn owl, *Tyto alba*, is a nocturnal predator, renowned for its sound localization ability. Using a habituation-recovery paradigm, we have shown that the minimum audible angle (MAA) of the owl is 3°(Bala and Takahashi, Abstract #4614, ARO Midwinter Meeting, 2000). How does this behavioral acuity relate to acuity of space-tuned neurons in the barn owl inferior colliculus? We assessed the ability of space-specific neurons to discriminate small spatial separations by changes in their rate of discharge. Neuronal acuity was assessed in 92 isolated space-specific neurons at a resolution of 1°, using virtual auditory stimuli presented through individualized head-related transfer functions (HRTFs). Behavioral and neuronal discrimination were quantified using methods based on signal detection theory, yielding directly-comparable indices. We show that 1) A significant proportion of neurons are able to discriminate azimuthal separations at performance levels that match, or exceed, behavior. Thus, many neurons were able to discriminate a separation of 3° or less. Mechanisms requiring pooling across neurons in order to improve neuronal performance are thus not necessary in the barn owl. 2) Spatial discrimination within a neuron's receptive field is location dependent: discrimination is optimal at the slope of the receptive field, or where the change in firing rate is maximal. 3) Averaging neuronal discrimination across our entire sample of neurons yields a very close match to behavioral performance, indicating that behavior is driven not by the performance of a few optimally tuned neurons, but by the response of a population of neurons that form an image across the space map.

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701 Physiological Correlates of the Precedence Effect: The Role of GABA-ergic Inhibition

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The 'precedence effect' can be described as the dominance of the first arriving wave front on the perception of sound. This dominance allows sounds to be accurately located in a reverberant environment despite the presence of conflicting cues to sound-source location. When listeners are presented with a direct sound and a single reflection with conflicting directional information, they assign the location to that indicted by the direct sound, seemingly ignoring the directional information in the reflection. Auditory neurons, including those in the inferior colliculus (IC), respond to a range of psychophysical stimulus configurations respond robustly to the first (direct) sound but show little or no response to the second (reverberated) sound up to times when two distinct sounds would be heard by human listeners, i.e. 8-10ms. One suggested mechanism to account for the suppressed neural response of IC neurons to the second sound is neural inhibition mediated by GABA, possibly via neurons that project to the IC from the dorsal nucleus of lateral lemniscus. To test the hypothesis that GABA-ergic inhibition reduces neural output to reverberated sound, single neuron responses to simulated reverberant sounds were made in the IC of anaesthetized guinea pigs. Stimuli consisted of two binaural clicks of equal intensity, presented at a neuron's best interaural time difference, and separated in time by 2-20ms. A total of 11 conditions, including a single binaural click, were presented randomly with 40 repeats. The GABAA antagonist gabazine was applied iontopheretically during recording using multi barrel pipette electrodes. On application of gabazine, the response of all neurons to the second click increased to a greater extent than the response to the first click. However, no neuron showed a response to the second click that was equal to its response to the first. Complete recovery following termination of the gabazine current was achieved in all cases.

702 Inhibition in the Inferior Colliculus Regulates Neural Response Gain.

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Inhibitory mechanisms play a critical role in the generation of neural sensitivity to interaural time disparities (ITDs) in the medial superior olive (MSO), the primary site of low-frequency binaural interaction (Brand et al. 2002; Nature 417: 543-547). Recently, it was suggested that inhibition facilitates sharpening of ITD tuning in the inferior colliculus (IC, Sterbing et al. 2002; ARO Abstracts 25: 13.). We recorded single-neurone extracellular responses in the anaesthetised guinea pig IC to interaurally-delayed tones. Using micro-iontophoresis, we added tonic inhibition (by application of GABA) or tonic blockade of inhibition (by application of bicuculline or GABAzine). Comparison of neural responses recorded under control and drug conditions revealed that neural discharge rates were closely related by relationships indicative of additive gain, multiplicative gain, or by a combination of both. Using these relationships, the ITD functions recorded under drug conditions were scaled to the equivalent spike rate of the control condition. Tonic addition of GABA should have an "iceberg" effect on discharge rate, reducing the number of spikes evoked in a non-ITD dependent way. However, blockade of inhibition has the potential to unmask any ITD-dependent inhibitory input onto the neurone. Using the scaling technique, no consistent change in the ITD tuning of these neurones was observed with drug application. We hypothesise that inhibition in the IC does not adjust ITD tuning but, instead, regulates the gain of its output projections to higher centres, maintaining a neurone's response within the dynamic range of its input / output function. Thus, our data do not support the conclusions of Sterbing et al. (2002) and suggest that inhibition in the IC does not play a role in sharpening ITD tuning. Rather, inhibition appears to control the output gain of IC neuronal responses, potentially by modulation of the summated excitatory synaptic input currents.

703 Responses of Inferior Colliculus Neurons to Interaural Time Differences of Amplitude Modulated and Tranposed Tones

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Sensitivity to interaural time differences (ITDs) is conveyed by the waveforms (i.e., fine-structure and envelope) of low-frequency sounds and by the envelopes of high-frequency sounds. Sensitivity to ITDs within high-frequency sounds, however, is relatively poor compared with sensitivity to ITDs within low-frequency sounds. One explanation

for this difference is that high-frequency binaural neurons are less specialized for interaural temporal processing than are low-frequency binaural neurons. Recently, however, it has been demonstrated, using high-frequency "transposed" tones, that just noticeable differences in envelope-based ITDs can be as small as those measured for low-frequency tones. In order to examine the neural basis for the enhanced sensitivity to transposed, compared with sinusoidal amplitude modulated (SAM) tones, we recorded extracellular, single-neuron responses in vivo from the inferior colliculus of anaesthetised guinea pigs to SAM and transposed tones presented in closed-field. Transposed tones were produced by multiplying a half-wave rectified, low-pass filtered low-frequency pure tone with a high-frequency carrier (normally at the neuron's characteristic frequency or CF), This produced a signal whose envelope mimicked that of a rectified low-frequency sine-wave, the form of the presumed output of auditory nerve fibres to low-frequency tones. Responses to a range of interaural phase differences (+/-0.5 cycles) and envelope modulation rates (10-720 Hz) were obtained for 43 high-CF (>1.5 kHz) neurons. Of 13 neurons sensitive to envelope ITDs, both the absolute number of discharges and the modulation of the discharge rate with ITD was greater for transposed tones than for amplitude-modulated tones. We hypothesize that the increased sensitivity of IC neurons to the envelope ITDs of transposed tones can be explained by the increase in phase-locked input to high-frequency binaural-integrator neurons afforded by the transposed tone.

704 The Relative Contribution of Time and Phase Delays to Interaural Delay Sensitivity of Inferior Colliculus Neurons

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Neurons in the medial superior olive (MSO) of the brainstem receive input from both ears via the cochlear nucleus on each side of the brain, and have long been known to be sensitive to interaural time differences (ITDs) of sounds presented to the two ears. It is generally assumed that such sensitivity arises due to differences in axonal conduction delay between the two inputs to MSO neurons. However, the recent demonstration that glycinergic inhibition is responsible for the ITD tuning of mammalian MSO neurons suggests that precisely-timed glycinergic inhibition at the level of the MSO might be responsible for the introduction of delay in the system, not differences in axonal path length (Brandt et al. 2002, Nature 417, p543). Computer simulations reveal that this newly discovered mechanism produces a phase delay; i.e. all spectral components of a broadband stimulus are delayed by a constant phase value. In order to ascertain the extent to which conduction (i.e. time) delays and phase delays contribute to the ITD-sensitivity of low-frequency neurons, broad-band noise delay functions were recorded from the IC of anaesthetised guinea pigs and modelled with a Gabor function - the product of a Gaussian and a sinusoidal function. The parameters of the fitted function can be used to provide measures of both the time and phase delays, as well as other neural filter properties. Data from preliminary experiments indicate neither a constant phase nor a constant time system, but a mixture of the two. Such a mechanism could permit tuning of ITD sensitivity by means of modulating the inhibitory conductance at the level of the MSO.

705 Tuning to Interaural Time Difference in the Cat Inferior Colliculus: Dependence on Characteristic Frequency

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Neural tuning to interaural time differences (ITD) in the inferior colliculus (IC) of the cat was studied as a function of characteristic frequency (CF). This relationship is of interest because CF-dependence of ITD tuning in the guinea pig IC casts doubt on the conventional labeled line model for ITD representation (McAlpine et al. Nat. Neurosci. 4:396-401). Cats have larger heads than guinea pigs, and so may provide a better model for ITD coding in humans.

We obtained rate-ITD functions of single units for interaurally delayed broadband noise. Most neurons had best ITDs in the contralateral hemifield, and not near the midline, where spatial acuity is finest. For low-CF units (< 1 kHz), the distribution of best ITD exceeded the physiological range experienced by the cat. For high-CF units, the distribution was much narrower, and may not even span the physiological range. The ITD functions became more sharply tuned as CF increased. The halfwidth expressed in terms of interaural phase difference (IPD) was nearly independent of CF, with an average value of about 0.4 cycles. Because the best IPD averaged about 0.2 cycles, the ITD functions were positioned such that their maximum slopes occurred near the midline.The sharpness of frequency tuning, inferred from the ITD function, improved with CF. This trend closely parallels that seen in the auditory periphery.

These results are consistent with those from the guinea pig suggesting that ITD is computed on the basis of a population code. The lack of a labeled line code has consequences for models of dichotic pitch perception, which rely on the ability to scan activity across a wide range of ITDs. One interpretation of a population code is that the auditory system may have the ability to compute what the ITD *is*, without retaining the ability to resolve individual features within a map of ITD.

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706 A Population of ITD-Sensitive Units in the Cat Inferior Colliculus Shows Correlates of Spatial Release from Masking

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Spatial release from masking (SRM) is the improvement in detection obtained when a signal is separated in space from a masker. SRM is likely to be involved in the ability to listen in noisy environments. We are studying the neural mechanisms underlying SRM; here we concentrate on interaural timing differences (ITD). Because ITD-sensitive units are thought to act as Jeffress-type cross-correlators, we hypothesize that a population of these units tuned to different ITDs will show a correlate of SRM.

We record from single units in the anesthetized cat inferior colliculus (IC), focusing on ITD-sensitive units. Stimulus azimuth is simulated using head-related transfer functions; the stimulus is a 40-Hz chirp train in continuous broadband noise. For each unit, the signal-to-noise ratio (SNR) at threshold is measured at several signal and noise azimuths. Threshold is defined as the SNR at which the signal can be detected for 75% of the trials. The population threshold is defined as the best threshold across all the units for each signal and masker combination.

The population threshold varies slowly with noise azimuth; the worst thresholds occur when the signal and masker are close. The threshold improves by about 10 dB when the signal and noise are separated by 90°. The masking mechanism for individual units depends on the masker ITD. Placing the noise at a favorable ITD excites the unit so much that the response to the signal is overwhelmed; placing the noise at an unfavorable ITD suppresses the response to the signal without producing any excitation itself. A cross-correlator model produces similar results. Overall, ITD-sensitive units in the IC show a correlate of SRM, and the mechanism is consistent with a Jeffress-type cross-correlator.

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707 Binaural Processing Of Interaural Level Differences In The Inferior Colliculus Of The Cat

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Cells in the mammalian inferior colliculus (IC) have been shown to be sensitive to interaural level differences (ILDs), the binaural cue formed by the difference in the level of a sound reaching the two ears due to the head shadowing effect. The IC receives converging inputs from many peripheral nuclei, including the lateral superior olive (LSO) and the dorsal nucleus of the lateral lemniscus (DNLL), both of which already show ILD sensitivity. It is not known, however, whether the converging inputs from the LSO and the DNLL are at matched frequencies.

To explore this further, we have investigated the effects of changing frequency and contralateral sound level on the ILD sensitivity of IC cells in barbiturate anesthetized cats. The ILD functions of all the cells were sensitive to both parameters. In general, when frequency was held constant and contralateral level was varied, the shape of the overall ILD function remained similar, though it could shift on either axis. However, when frequency was varied and contralateral level was held constant, the shape of the ILD function changed more dramatically, which may reflect differing ipsilateral and contralateral contributions at different frequencies. Using a computer model, we also explore the possibility that this effect is due to contributions from LSO and DNLL inputs that are not matched in frequency.

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708 The Intermediate Nucleus of the Lateral Lemniscus Responds to Complex Properties of Species Specific Calls

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Previous studies have shown that the inferior colliculus (IC) and dorsal nucleus of the lateral lemniscus (DNLL) process species specific calls in fundamentally different ways. Where IC neurons are highly selective and respond only to some calls and not others that encroach upon their tuning curves, DNLL neurons respond to any call so long as the call has energy in the neuron's tuning curve. Here we evaluated how one of the other nuclei of the lateral lemniscus, the intermediate nucleus (INLL), responds to species-specific calls. We evaluated these features in Mexican free-tailed bats, the same species that was used previously to study responses to species-specific calls in the IC and DNLL. Tuning curves and rate level functions, generated with tone bursts, were used to determine the cell's response preferences. Communication and echolocation calls were presented in the forward and reverse directions to examine cell specificity for complex sound features.

Many INLL cells were tuned narrowly at low intensities but broadened substantially at higher intensities. INLL cells responded to most or all of the calls that we presented. In this regard INLL neurons were similar to DNLL neurons but were different from IC cells. Commonly, INLL cells responded to calls played in the forward and reverse directions with different response magnitudes. Some cells responded to a different subset of calls in the forward direction than they did to the same calls played in reverse, whereas in others different response patterns were evoked by calls played in the forward versus the reverse direction. These differences in response magnitude and/or selectivity to forward and reverse versions of each call are in contrast to DNLL cells, which respond to forward and reverse versions of calls with the same response magnitudes and selectivities. Thus the processing of species-specific sounds by INLL neurons is more complex than the processing in the DNLL but is far simpler than the processing in the IC.

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709 On the Relationship Between Spectrotemporal Response Areas and Selectivity for Rate and Direction of Frequency Modulation (FM) in the Mustached Bat Inferior Colliculus (IC).

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Facilitatory and inhibitory interactions between paired pure tones have been shown to be sensitive to the frequencies and relative timing of the two tones. Single units in the IC have been shown to be selective for rate and direction of FM. To investigate the relationship between these phenomena, we presented FM sweeps of three bandwidths (6, 12 and 18 kHz) and six durations (64, 32, 16, 8, 4, and 2 ms) and recorded responses of single units in bat IC. For units that demonstrated FM directional preferences, we then generated spectrotemporal response areas (STRAs) by using a two-tone forward masking paradigm. To relate the STRA to the frequency range of the FM in the biosonar signal, we paired a 4 ms BEF probe with a 4 ms masker that varied by +/- 6 kHz around BEF. Tone intervals varied from 0 - 32 ms. Contour plots of the resulting spike count matrix often showed distinct regions of suppression and/or facilitation within this narrow 12 kHz bandwidth. We then analyzed the relationship between FM response and STRAs by superimposing on the STRA frequency sweep trajectories matching those tested empirically. Linear summation of facilitation and suppression along the trajectories resulted in an estimate of the unit's response to FMs of different rates and directions. A preliminary comparison of actual to STRA-predicted FM responses revealed that the STRA could reliably predict overall directional selectivity in most cases (83%). However, the STRA also successfully predicted certain aspects of rate selectivity in only about half of the units (42%). The surprising finding that the STRA was only partially able to predict rate selectivity indicates that there may be additional non-linearities in the response to FM sweeps that are not related to two-tone spectrotemporal facilitation and inhibition.

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710 The Effect of FM Bandwidth on Modulation Rate Selectivity in the External Nucleus of the Bat IC

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Previous experiments showed that neurons in the ventral ICX (ICXv) of the mustached bat are directionally selective for upward linear frequency modulations (FM). ICXv was also found to be a component of the central acoustic tract, receiving input from the brainstem and projecting to the superior colliculus, suprageniculate nucleus, and pretectal area. In this experiment we examined the effect of FM bandwidth on modulation rate selectivity. We presented linear FM with bandwidths of 6, 12, and 18 kHz centered on unit best frequency at 8 modulation rates between 0.094 and 9 kHz/ms. Up and down sweeps were presented at best pure tone amplitude. Frequency response areas were collected to relate effects of FM bandwidth to the shape and extent of excitatory and inhibitory spectral regions. We also verified the connectivity of the ICXv by iontophoretically injecting biotinylated dextran amine.

Of 76 units isolated, 47 units were located in ICXv. We classified the modulation rate functions as high pass (HP), low pass (LP), band pass (BP), and all pass (AP). The great majority (81%) of ICXv units were either low pass or broadly tuned to modulation rate. Specifically, of 42 fully characterized units, 54.8, 26.2, 14.3, and 4.8% were LP, AP, BP, and HP, respectively. Rate selectivity was nearly the same for all 3 FM bandwidths, which suggests that modulation rate and not bandwidth *per se* is the critical stimulus feature. Tract tracing corroborated prior

experiments but also revealed a significant input from the dorsal nucleus of the lateral lemniscus and a minor input from the central nucleus of the IC.

In conclusion, modulation rate is more important than FM bandwidth in determining response to FM sweeps in the ICXv.

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711 Functional Architecture and Cortical Projections of the Internal Division of the Rabbit Medial Geniculate Body

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Although an internal division has not been identified as a distinct component of the medial geniculate body in most mammals, cytoarchitectonic studies, histochemical stains and calcium-binding protein expression clearly demarcate a wedge shaped internal nucleus (MGI) that separates the ventral (MGV) and dorsal medial geniculate subdivisions in the rabbit. In contrast to the main lemniscal pathway through the parvalbumin-immunoreactive MGV, MGI neurons are calbindin (CB) immunoreactive and the MGI neuropil is filled with CB-positive fibers and terminals. In the present study, traditional electrophysiological mapping techniques, juxtacellular labeling of single cells and anterograde labeling of cortical projections were used to study the functional architecture of the MGI. Mapping studies revealed that MGI neurons had labile responses to acoustic stimuli. MGI cells were only weakly responsive to (mostly low frequency) pure tones and responded best to noise (or more complex) stimuli. No frequency progression was seen during electrode penetrations through the MGI. A total of 15 MGI neurons were juxtacellularly labeled with biocytin and fully reconstructed with a computer microscope. MGI neurons exhibited a number of different morphologies including ventral-like tufted cells, multipolar neurons as well as wide-fields cells. Spatial analyses revealed a horizontal bias of the dendritic trees in the coronal plane, an orientation which paralleled the predominant orientation of Nissl-stained somata and calbindin-immunoreactive fibers. The cortical projections of MGI neurons were examined using iontophoretic injections of biotinylated dextrans into the MGI. Temporal cortices ipsilateral to the injection sites were flattened between glass slides and cut in the tangential plane. MGI projections were characterized by widespread multiple terminal fields. In contrast to MGV projections, the majority of MGI axons terminated in a belt region anterior, dorsal and posterior to AI.

712 Effects of Rise-Time and Duration on Combinatorial Interactions in the Inferior Colliculus.

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In the mustached bat's inferior colliculus (IC), many neurons show facilitatory or inhibitory interactions in response to combinations of distinct spectral elements in sonar or social vocalizations. Using single-unit recording, we studied how such interactions were affected by changes in rise-time or duration of the lower facilitating or inhibiting frequency. Of 31 facilitated units, 22 were facilitated under both short (0.5 ms) and long (5.0 ms) rise-time conditions. Of the remainder, units could either lose or gain facilitation with longer rise-time. The change in low frequency rise-time had no consistent effect on the neurons' facilitated response magnitude. Changes in duration of the lower frequency signal (from 4 to 13 ms) also did not change the facilitated response magnitude. Further, neither signal manipulation (rise-time or duration) altered the best facilitatory delay. These observations suggest that low frequency facilitating input to IC neurons is insensitive to rise-time, and has a phasic response. Among 29 units showing inhibitory interactions, both signal manipulations had significant effects. Longer rise-times did not eliminate inhibitory interactions but they evoked somewhat less inhibition, and the best delay of the inhibitory signal increased. Low frequency signals with longer durations evoked stronger inhibition, with the best delay of inhibition increasing. These results suggest that low frequency inhibitory input is sensitive to energy changes associated with changing rise-time. Further, low frequency inhibitory inputs appear to be tonic. The functional properties of low frequency facilitating and inhibitory inputs to IC combinatorial neurons thus appear to differ.

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713 Timing of Inhibition Shapes FM Sweep Selectivity

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This report focuses on the timing of the excitatory and inhibitory inputs that create the spectrotemporal fields of inferior colliculus neurons that respond selectively to the direction and velocity of the downward FM sweeps of the echolocation pulse of the pallid bat, subject of this study. A two-signal paradigm was used to determine the arrival times, the durations, and the persistence of excitatory and inhibitory inputs, and then used to predict how neurons would respond to changes in sweep direction and velocity. Neurons with no selectivity for FM sweeps were also tested to assess the validity of this approach.

At one extreme, neurons that did not respond to short-duration FM sweeps would respond if duration was increased. The point at which they responded was predicted by the locations of the inhibitory receptive fields, and the arrival times, durations and persistence of their inhibitory inputs. At the other extreme, neurons that responded only to downward FM sweeps had opposite requirements. They preferred short-duration FM sweep rates, and had preferred sweep velocities. All frequencies within an excitatory sweep were inhibitory when presented as single tones. What remains is the timing of inputs. There was a frequency-dependent difference in the arrival times of inhibitory inputs, with lower frequency input arriving earliest, and high frequency inhibition arriving after excitation. Arrival times of inhibition varied as much as 4 msec across frequency. These shaped selectivity for sweep direction and velocity, respectively. An upward FM sweep gives a temporal advantage to the fast low-frequency, inhibitory input, and suppressed the respond. The late high-frequency inhibition in the preferred downward direction, if given enough temporal advantage in a slow downward sweep, will eventually arrive before the excitatory input, thus determining the preferred sweep velocity. How this asymmetry in arrival times is created remains to be determined.

714 Role of Leading Inhibition in Time Domain Processing in the Auditory Midbrain

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Many central auditory neurons in echolocating bats exhibit delay-tuned responses showing selectivity to the time interval between an intense pulse and a delayed "echo". Sullivan [1982] proposed that this selectivity is created by a coincidence mechanism with paradoxical latency shift (PLS) as a vital building block. PLS is characterized by longer response latency to more intense sounds. In the auditory cortex, 55% neurons showing PLS also exhibit delay-tuned responses, and the unit's best delay corresponds to the amount of PLS. Recent studies show that, in the inferior colliculus (IC), PLS is attributed to GABAergic inhibition that is most prominent at high sound levels; this inhibition suppresses the early component of unit's response [Klug et al., 2000; Galazyuk and Feng 2001]. We investigated the robustness of PLS and its role in processing patterned sounds in the bat IC. The responses of PLS and non-PLS neurons were studied to paired sound pulses of unequal amplitudes having different inter-pulse intervals. Unlike the cortex, PLS neurons in the IC did not exhibit delay-tuned response. Instead, they showed either no response or robust inhibition at the echo delay corresponding to the unit's PLS. This interaction pattern can be explained by a leading inhibitory mechanism. For all PLS neurons, there was an early inhibition preceding excitation by a few ms, and it had a higher threshold than excitation; these findings were supported by preliminary data from intracellular recording from PLS

neurons (at high sound levels they showed an early IPSP followed by an EPSP/spike). In contrast, non-PLS neurons showed essentially purely excitatory interactions with occasional display of post-excitatory inhibition. PLS neurons in the IC of leopard frogs also showed similar inhibitory interactions to paired pulses suggesting a common auditory mechanism.

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715 Sonar Signal Temporal Patterning Shapes Echo-Delay Tuning in the Bat Midbrain

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Echolocating bats transmit ultrasonic vocalizations and use information in returning echoes for spatial orientation and insect capture. Analyses of signals produced by the FM bat, *Eptesicus fuscus*, during the approach phase of insect pursuit reveal plateaus at repetition rates of 50-60 Hz for time periods as long as 700 ms. Stable periods of sound repetition rate (sonar "strobe groups") occur when the bat is selecting a target, changing the direction of its flight path and in the presence of obstacles (Moss & Surlykke, 2001, *JASA, 110*: 2207-2226). We hypothesize that sonar "strobe groups" shape the spatial-temporal response profiles of neurons in the bat auditory system. Our current experiments directly test this hypothesis.

Here, we report on single unit extracellular recording experiments in the bat midbrain superior colliculus (SC), a brain region where we have previously identified a class of combination-sensitive auditory neurons that exhibits spatial selectivity in azimuth, elevation, and range (Valentine & Moss, 1997, *J. Neurosci., 17*: 1720-1733). Range tuning is quantified using simulated pulse-echo pairs with variable delays, and the spatial response profiles of these neurons are broad when measured with slow presentation rates (5 Hz) of stimulus pairs. In this study, we measured echo-delay tuning across a range of signal repetition rates that approximate those used by the bat during the search and approach phase of insect pursuit (5-50 Hz). Echo-delay tuning curves sharpened when sound stimuli simulated the sonar "strobe groups" observed during insect capture. In addition, responses at a neuron's "best echo delay" could be attenuated by stimulation with changing intervals between pulse-echo pairs. These data suggest that sonar signal temporal patterning directly impacts the spatial response profiles of SC neurons in the bat.

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716 The Parabrachial Nucleus is Directly Involved in the Control of Echolocation Call Parameters in Horseshoe Bats.

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In mammals, the parabrachial nucleus (PB) is believed to play a complex role in the coordination of breathing with the onset of vocalization. Single-unit recordings in cats and monkeys have revealed neuronal activity correlated to both vocalizing and respiration. We have previously reported that pharmacological stimulation of a midbrain tegmental region caudal and medial to the DNLL can cause rapid and pronounced changes in the call frequency of echolocating horseshoe bats. A detailed functional and anatomical mapping of this brain area has now revealed that the ventrolateral section of the PB appears to be centrally involved in the control of call frequency in these bats. Iontophoretic injections of the $GABA_A$ antagonist Bicuculline methiodide (BMI) were repeated in steps of 250 µm throughout an area covering approximately 1750 µm rostrocaudally, 800 µm mediolaterally, and 1100 µm dorsoventrally around PB. The injection sites in three animals were then reconstructed based upon histological verification of biotinylated MUS or electrolytic lesions as a reference using a stereotaxic approach. Since BMI is known to elevate call frequency in this region, the magnitude of subsequent changes in call frequency were plotted within a semi three-dimensional map of the transected area, revealing that the majority of BMI's effects could be localized to the PB. On some occasions pharmacological injections altered other call parameters, such as call duration and call rate, however the effects of BMI on call frequency appeared to be independent of these other changes. Single-unit recordings and anatomical tracer injections were used to further explore the physiological and anatomical characteristics of this nucleus in bats. The results suggest that in addition to respiratory control the parabrachial nucleus may also be directly involved in the control of multiple parameters of mammalian vocalizations.

717 Effects of Age on Susceptibility to Noise-Induced Cochlear Damage

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Studies evaluating the influence of age on susceptibility to noise-induced hair cell loss (NIHL) have produced conflicting results. Some report no effect of age whereas others report greater loss of hair cells in older animals. Recently, we showed that young and aged gerbils were equally susceptible to NIHL at 1-8 kHz, but that older animals were significantly more susceptible than young gerbils to NIHL at 16 kHz. Here we examine the influence of age on the susceptibility of hair cells to noise trauma. Mongolian gerbils aged 4-8 months (n=7) or 34-38 months (n=7) were exposed monaurally to a 3.5 kHz tone at 113 dB SPL for one hour. The contralateral ear was plugged and served as a control ear. Hearing was again tested one month post-exposure and harvested and processed for histopathological studies. Cytocochleograms were obtained for each ear from both age groups. Hair cell loss was normalized within each age group by subtracting the number of cells lost in the unexposed ears from that in the exposed ears. This normalization allowed a comparison of the effects of noise on hair cell loss with the effects of aging under control. Results for the three rows of outer hair cells were averaged. Little inner hair cell loss was observed and results are not included here. Noise-induced hair cell loss was greatest at approximately 5-8 kHz (15-25%). In 5/7 aged gerbils, the hair cell loss pattern was very similar to that in young subjects, suggesting minimal influence of age on susceptibility to noise-induced hair cell loss. However, 2 aged gerbils which were littermates had a secondary peak of hair cell loss at 16-20 kHz, suggesting a possible genetic influence on the susceptibility to noise-induced hair cell loss in aged animals. Interestingly, all aged gerbils showed increased hearing loss at 16kHz, suggesting that all aged animals may have had sub-lethal damage to hair cells in the basal region of the cochlea which was not observable with light microscopy.

718 The Lateral Superior Olive of Young and Old Gerbils: GABA and Glycine Immuno-Histochemistry.

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In an effort to better understand central auditory processing deficits that occur in aging humans we use the gerbil as a model for studying age dependent changes. Here we present data on the lateral superior olive (LSO) that is crucial for processing binaural acoustic stimuli. Alternating serial sections through the LSO from a total of 27 gerbils (1- 54 months of age) were processed for the immuno-histochemical demonstration of the inhibitory neurotransmitters GABA and glycine and analyzed using a custom video microscopy- and image-analysis-system. One section near the middle of the rostro-caudal extension of the left and right LSO was selected in each animal for detailed analysis. Data from the lateral (low frequency) inter-mediate (medium frequency) and medial (high frequency) portion of the LSO were collected

separately to detect potential frequency specific changes. The mean of the left and right LSO from each animal was calculated and used for the statistical analysis of age dependent changes.

Gross dimensions of the LSO (rostro-caudal extension and cross-sectional area) showed no significant age dependent changes. In addition, the number and density of GABAergic and glycinergic cells did not change with age, there was no loss of inhibitory neurons in old gerbils. However, in young and old gerbils, there was a strong gradient in the density of inhibitory neurons, being high in the low frequency area and low in the high frequency portion of LSO. The cross sectional area of inhibitory neurons was not affected by age in the low frequency portion of LSO. However, GABAergic neurons were significantly smaller in the medium and high frequency segments of LSO in old gerbils, while a significant difference was only present in the high frequency portion for glycinergic neurons. Contrary to our expectations, the staining intensity was higher in old gerbils. These morphological alterations in the inhibitory system of the LSO may be associated with difficulties of binaural processing that have been described in old humans and rats.

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719 Age-related Behavioral, Physiological, and Biological Changes in the Rat Auditory and Cognitive Systems

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Presbyacusis is the progressive deterioration of hearing with aging. Although the mechanisms contributing to the loss of hearing sensitivity with age are not entirely understood, there is mounting evidence that aging results from the persistent attack of reactive oxygen species (ROS) on cellular and mitochondrial structures.

The purpose of the current study was to describe age-related changes in the auditory system and cognitive function. Auditory changes were assessed using cochlear histology and auditory brainstem responses (ABR). The cognitive function of spatial learning patterns was evaluated using the Morris Water Maze (MWM). To determine ROS mechanism involvement in damage, we investigated membrane stability using mitochondrial membrane potentials (MMP)and ROS formation using flow cytometery.

The experimental subjects consisted of two groups of F344 rats (young rats, 2-3 months[n=6] : aged rats, 21-24 months [n=6]). Each subject was trained for seven days on the spatial learning task and tested in the apparatus following the last trial. ABRs were evaluated at 3, 6, 9, 12 and 18 kHz. Samples of blood were obtained for flow cytometry and mononuclear cells obtained for assessment of mitochondrial membrane potentials.

Results showed significant differences between groups for behavioral, physiologic, and cellular measures. Specifically, the latency measures for spatial location were significantly longer in the aged group. ABR measures showed significantly higher thresholds in the aged animals for each frequency. Surface preparation of the organs of Corti revealed more hair cell loss in the aged group. Mitochrondrial membrane potentials of the aged subjects were 60% lower than the young group. Free radical formation, measured by flow cytometry, was 65% higher than the young group.

These findings support that there is subcellular structural and functional decline which results in reduced auditory sensitivity and cognitive function in aged animals.

720 Layer-Specific Age-related Changes in GAD Protein Levels in Rat Primary Auditory Cortex

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GABA has been shown to play a key role in processing complex acoustic signals at many levels of the auditory neuraxis. Primary auditory cortex (A1) contains a network of GABAergic neurons and extrinsic GABAergic inputs throughout its layers. Normal adult inhibitory processing appears affected by altered peripheral input to the auditory pathway. Damage to the adult cochlea results in an increase in the amplitude of superthreshold cortical-evoked potentials. Aging, which can be modeled as a slow peripheral degradation of the auditory input to the brain, alters GABA and glycinergic systems in auditory brainstem. The present study examined age-related changes in the GABA synthetic enzyme glutamic acid decarboxylase (GAD) in the heteromorphic layers of the rat A1. GAD protein levels were determined using quantitative cellular immunocytochemistry for GAD_{67} in A1 of young (4-6 mos), middle (20-22 mos), and aged (31-33 mos) rats. All but layer 5 showed significant age-related decreases in GAD_{67} protein levels. Significant age-related decreases in GAD_{67} protein levels were measured (deviation scores in std. error units) in layers 2 (-8.2), 4 (-5.5) and 6 (-6.0) with a smaller change in layer 3 (-3.3) and no significant change in layer 5 (-0.1). These data are suggestive of age-related layer-specific presynaptic changes in GABA function. These findings are similar to presynaptic age-related GABA changes observed for GABA in the inferior colliculus. Preliminary postsynaptic $GABA_A$ receptor data suggest that selective age-related layer-specific $GABA_A$ receptor subunit switches occur in rat A1. These receptor changes could be in response to the presynaptic GAD protein changes in A1 observed in the present study. Age-related layer-specific changes in GABA neurochemistry would likely result in altered coding of acoustic signals in A1 of older individuals.

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721 Auditory Brainstem Neuronal Activity Decreases Following Cochlea Removal in Aging Broiler Chickens with Normal Cochleae

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In the chicken auditory system, cochlear nucleus (nucleus magnocellularis, NM) neurons receive their only excitatory input from the ipsilateral cochlea. Cochlea removal (CR) results in an immediate decrease in NM neuronal electrical activity, followed by death of 30% of NM neurons. Previous work showed the decrease in NM activity and subsequent loss of NM neurons occurs in all hatchling chicks. Adult egg layer birds showed neuronal loss after CR. Neuron number remained stable in adult broiler birds. This suggested that effects of CR on NM were breed-dependent. We now know that aging egg layer birds maintain largely normal cochleae. Most aging broiler birds display age-dependent cochlear degeneration, which may alter the effect of CR on NM.

Recent work showed that adult broiler birds with normal cochleae exhibit NM neuronal loss after CR similar to hatchling chicks and adult egg layer birds. This suggests that the response to CR is dependent on cochlear integrity, not breed. This study investigates whether these differences in neuronal death in adult broiler birds are due to differences in activity changes occurring with CR. We examined NM neuronal activity after CR in 2, 30, 39, and 52 week-old birds with normal cochleae. Glucose uptake is indicative of changes in neuronal energy demands, so measurement of glucose uptake indirectly measures neuronal electrical activity. Using the 2-deoxyglucose method to examine NM neuronal activity, we found decreased activity ipsilateral

to CR in all ages examined. This suggests that when a normal cochlea is removed, neuronal activity decreases and neuronal death occurs. Future studies will investigate neuronal activity changes in adult birds with damaged cochleae.

722 Effects of Oxidative Stress on Hair Cell Mitochondria In Vitro

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Oxidative stress resulting in induction of apoptosis has been demonstrated in several different types of cochlear pathology. Ototoxic drugs such as aminoglycosides or cis platin produce free radicals resulting in apoptosis. Noise trauma also appears to partially mediated through oxidative damage. Some studies now suggest that the aging in the nervous system as well as in the organ of Corti may be the result of prolonged recurrent oxidative stress resulting in eventual apoptosis of the damaged cells. Although the stria vascularis, auditory neurons and inner hair cells are all susceptible to oxidative damage, the outer hair cells appear particularly vulnerable to oxidative damage and loss of outer hair cells is seen in all of the disease processes outlined above. The mitochondrion has been shown to play a pivotal role in apoptosis in a variety of experimental system and auditory diseases. Using P3 mouse oragnotypic cultures we demonstrate that low dose oxidative stress results in damage to mitochondrial DNA, changes in mitochondrial gene expression and ultimately collapse in of the mitochondrial membrane potential in OHCs leading to apoptosis. Prior inhibition of mitochondrial protein production through pretreatment of cultures with chloramphenicol, increased the cytotoxic effect of 0.01 mM hydrogen peroxide treatment. Pretreatment of cultures with cyclosporin A prevented loss of mitochondrial membrane potential and induction of apoptosis.

723 The Effect Of Growth Factors (VEGF, PDGF, b-FGF, TGF-ß1), Stress Hormones (Epinephrine, Norepinephrine, Cortisol) and Sexual Steroids (Estrogen, Testosterone) On Ageing Cochlear Vessels.

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Proliferation and contractility of inner ear vessels are significantly reduced during ageing.

In the present study the effect of vascular growth factors (VEGF, PDGF, b-FGF, TGF-ß1), stress hormones (epinephrine, norepinephrine, cortisol) and sexual steroids (ß-estradiol, 5-a-dihydrotestosterone) on proliferation, contractile proteins and contractility of young (10 weeks in culture) and old (14-16 weeks in culture) cochlear vascular smooth muscle cells (VSMCs) and arteriolar pericytes (PCs) were examined.

Cell contractility was measured after stimulation with vasoactive agents using digital fluorescence microscopy (Photometrics-PMIS®). Concentration of contractile cytoskeletal proteins was determined using the protein assay according to Bradford (BioRad®), and cell proliferation was measured using a cell titer assay (Promega®). For treatment culture medium was supplemented with the aforementioned growth factors and hormones during the 11th to 12th week in culture.

Reduction of proliferation during cellular ageing was only partly antagonized in both cell lines with epinephrine (44 ng/l), norepinephrine (220 ng/l), PDGF (25 ng/ml) and b-FGF (25 ng/ml), while cortisol (108 µg/l), VEGF (50 ng/ml) and TGF-ß1 (10 ng/ml) as well as ß-estradiol (0.2 µg/l) and 5-a-dihydrotestosterone (10 µg/l) had no effect. Reduction of contractile cytoskeletal proteins during cellular ageing was fully antagonized in both cell lines with epinephrine and norepinephrine and was partly antagonized with all growth factors tested, while cortisol and the sexual steroids had no effect. Loss of contractility was fully restored in old VSMCs and PCs with all tested growth factors, stress hormones and sexual steroids, indicating powerful treatment modalities against vascular ageing.

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724 Unbiased Stereological Type I and Type II Hair Cell Counts in the Human Crista Ampullaris and Utricular Macula.

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Estimates of type I and type II hair cell counts in the human horizontal crista ampullaris and utricular maculae normal individuals were obtained using the physical fractionator technique (N=16, age range 26-98 years). In subjects under age 70 (range from 26-67 years old, n=5), there was an average of 6998 total hair cells: 3575 ± 187 type I hair cells, 3424±150 type II hair cells, and 8250 ± 475 supporting cells. The type I: type II ratio was near unity: 1.04: 1. The average hair cell: supporting cell ratio was 0.85. The type I and type II hair cells, and supporting cells were packed more tightly in the peripheral (P) > intermediate (I) > central (C) regions. The type I hair cell distribution was P=42%, I=30% and C=28%. Type II hair cell distribution was similar: P=42%, I=31%, and C=27%. The supporting cell distribution was P=43%, I=30%, C=27%. The type I: type II ratio was highest in the central region: 1.14 vs. 0.98 in the periphery. There was a significant effect of age on decreasing hair cell counts: both type I ($p= 0.0006$), type II ($p<.0001$), and the type I: type II hair cell ratio ($p=.0005$). In the 9th decade, there were 2856 ± 195 type I hair cells, 2602 ± 440 type II hair cells, and 7731 ± 507 supporting cells. In the 10th decade, there was an average of 2607 ± 249 type I hair cells, 1628±124 type II hair cells and 8055±194 supporting cells. The type I: type II ratio increased from 1.044 in younger to 1.098 (9th decade) and 1.601 (10th decade). Type I hair cell counts were relatively preserved with age in the periphery, and type II hair cell counts diminished greatly with age in all regions. In the utricular maculae (N=10, age range 42 to 96, X = 82 years old), the average hair cell count was 27,508, consisting of 17,326 type I hair cells and 10,182 type II hair cells. The type I: type II hair cell ratio was 1.70:1. In the age range of this study, there was no statistically significant correlation between utricular hair cell counts and age.

725 The Effects of Primary Tone Relative Level on DPOAEs from Normal Hearing Young and Aged Adults

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Sims and Burkard (ARO, 2001) showed that older subjects had smaller distortion product otoacoustic emission (DPOAE) amplitudes, for a range of F2/F1 frequency ratios. Although the older subjects had normal hearing through the conventional audiometric range, their hearing was very elevated (re: young adults) for the ultrahigh frequencies (UHF). The present study looked at DPOAEs for a range of relative primary tone levels in normal hearing young and older adults.

All subjects had audiometric thresholds of 20 dB HL or less (0.25-8 kHz) in the test ear. Thresholds were also obtained to UHF tones of 9 through 20 kHz. Older subjects were 60 years or greater in age, young adults were 18-26 years old. DPOAE input/output (IO) functions used an F2/F1 ratio of ~1.22, with F2 frequencies of 2002 and 4004 Hz. L1-L2 levels ranged from 30 to –15 dB in 10 dB steps. For the subjects evaluated to date, older subjects, while similar in threshold through 8 kHz, typically showed very elevated UHF thresholds, compared to the young adults. Suprathreshold DPOAE conditions typically yielded

larger mean amplitudes in the young adults than the older adults, for a wide range of L1-L2 relative levels. These data suggest that ultrahigh frequency cochlear regions might contribute to DPOAE amplitudes, or that the manifested basal hearing loss in the older subjects correlates positively with subclinical damage in more apical (conventional audiometric frequency) damage.

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726 Aging and Suppression of Otoacoustic Emissions: The Blue Mountains Hearing Study

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Decreases in the magnitude of efferent suppression of transient-evoked otoacoustic emissions (TEOAEs) with age are reported in some studies (Castor et al., 1994; Hood et al., 1997; Parthasarathy, 2001), but no change has been reported by others (Quaranta et al., 2001). To address this issue in a large number of subjects, we included measurement of TEOAE suppression in the Blue Mountains Hearing Study (BMHS) in Australia. The BMHS is a population-based survey of age-related hearing loss that commenced in 1997 and is the hearing component of the Blue Mountains Eye Study (Mitchell et al.) that began in 1992. For the hearing segment, 2015 residents (75% of those eligible) over 50 years of age in two adjacent postal districts in the Blue Mountains region of Australia were evaluated. Along with participating in eye and nutrition studies, subjects responded to comprehensive hearing and medical questionnaires and received extensive behavioral and physiological measures of peripheral and central auditory function. TEOAE suppression was tested in over 900 participants, excluding persons with significant hearing loss or lack of OAEs. TEOAEs were obtained using 65 dB peak sound pressure linear clicks and contralateral broadband noise at 65-70 dB SPL. Two without noise and two with noise conditions were acquired. Signal-noise characteristics and amplitude and spectral characteristics of the OAEs and suppression were analyzed using the Kresge EchoMaster program. Results indicate decreases in suppression amplitude in older subjects. Characteristics of suppression, signal-noise analyses, and relationships to other patient characteristics and test results will be presented.

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727 Effect of Age on Binaural Sentence Intelligibility in Noise in Normal Hearing Listeners

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The purpose of this study was to evaluate the effect of age on speech intelligibility in noise with adult subjects whose audiometric thresholds were in the normal hearing range. The Hearing in Noise Test (HINT), designed to simulate everyday listening in noise, was used to measure speech intelligibility in quiet and in noise, as well as to measure release from masking (RFM). Speech intelligibility in noise improves when the speech and noise sources are spatially separated. This gain in intelligibility reflects the extent to which the individual benefits from spatial separation of speech and noise and is often referred to as RFM. Speech was presented from a loudspeaker located at 0° azimuth in four conditions: without noise and with noise at 0°, 90°, and 270° azimuths. The subjects with normal hearing (N=54) were drawn from a pool of 256 adults with varying degrees of hearing loss collected over a five-year period. The subjects were grouped on the basis of age: young (18-37 years), middle-aged (38-57 years), and old (58+ years). Sentence thresholds without noise were better for the young and middle-aged groups as compared to the old. All age groups performed better when the speech and noise were separated by 90 degrees; however, speech intelligibility was highest when noise was produced at the left side (270° azimuth). This has been termed right-ear advantage in previous studies. The results indicated that RFM declined with age, especially in the middle-aged and old groups. In summary, these findings revealed that age degrades the ability to benefit from the separation of speech and noise. In addition, the reduced ability to benefit from spatial separation of speech and noise begins in middle age.

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728 Effects of Stimulus and Noise Rate Variability on Speech Perception by Young and Elderly Listeners

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These experiments measured the influences of stimulus temporal complexity on speech recognition by older listeners. Two hypotheses were evaluated: 1) older listeners exhibit poorer recognition scores for variable-rate speech, compared to younger listeners; and 2) older listeners experience greater masking effects of noise when the temporal characteristics of the speech and noise are the same compared to when they are different. The effects of varying the linguistic cues were also evaluated. Listeners were young and elderly adults with normal hearing or sensorineural hearing loss. Three syntactic forms of the SPIN sentences were presented in quiet in various speech-rate conditions: natural rate, uniformly time-compressed, or time-compressed for one phrase. Conditions with noise involved presentation of the uniformly time-compressed speech stimuli (50% TC ratio) together with normal-rate or time-compressed babble. In quiet, listeners generally showed best performance for normal-rate speech, poorest performance for uniformly time-compressed speech, and moderately depressed scores for variable-rate speech. Age-related deficits were observed, but varied somewhat with the syntactic structure of the speech stimulus. In noise, all groups exhibited poorest scores in the 50% time-compressed babble condition and better but equal scores in the normal-rate and moderately time-compressed (25% TC ratio) babble conditions. Older listeners performed more poorly than younger listeners in all noise conditions. Overall, the findings support the hypothesis that older listeners are at a disadvantage, compared to younger listeners, for recognizing speech stimuli presented at a variable speech rate. Moreover, all listeners, regardless of age, perform better in noise when the temporal characteristics of the target speech signal and background babble are distinct than when they are matched.

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729 Improvements in Trunk Sway Observed for Stance and Gait Tasks during Recovery from an Acute Unilateral Peripheral Vestibular Deficit

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To track improvements in postural control during recovery from an acute unilateral peripheral vestibular deficit (UVL) and to determine if recovery rates were different for stance and gait tasks, trunk sway was quantified for these tasks at 3 time intervals (at onset of UVL, after 3 weeks and after 3 months). Peak-to-peak trunk sway amplitudes of angle and angular velocity in the roll and pitch directions, as well as task duration, were examined in a total of 28 UVL patients. Stance tasks

involved standing on 1 or 2 legs with eyes open or closed. Gait tasks consisted of tandem gait, walking normally with eyes closed, or with the head rotating or pitching, walking up and down stairs, and walking over a series of low barriers. Stance and tandem gait tasks were repeated using a foam support- surface instead of a normal floor.

The amplitudes of pitch trunk sway for 2-legged stance tasks with eyes closed underwent the greatest reduction 3 weeks after UVL onset. At 3 months, trunk sway was normal for all 2-legged stance tasks. One-legged stance tasks with eyes open showed a slower improvement, except for task duration which improved rapidly. Trunk sway for the simple gait tasks was nearly normal at 3 months, however, task duration was still longer than normal. More complex gait tasks, such as walking 8 tandem steps on foam, or walking up and down stairs showed no improvement in trunk roll sway at 3 months. A mix of variables from mainly gait tasks best identified a balance deficit due to UVL, with complex gait tasks becoming more important for identification purposes as compensation progressed. These data suggest that recovery of normal trunk control following vestibular hypofunction is more rapid for stance tasks than gait tasks. Even at 3 months, trunk sway for complex gait tasks was not normal. Thus, trunk sway for gait tasks provides a better insight into remaining deficits in balance control of vestibular-loss patients than the sway of stance tasks.

730 Cervical-Vestibular Interaction In Patients With Cervicogenic Dizziness

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The purpose of this study was to determine if the direction of postural sway during galvanic vestibular stimulation (GVS) is altered in patients with cervicogenic dizziness as compared to healthy adults. Eight adults, aged 21-65 years, diagnosed with cervicogenic dizziness (CD) were matched by age and gender to healthy control subjects. Seven continuous cycles of sinusoidal, binaural, bipolar GVS were applied over the mastoids (peak intensity 0.5mA, frequency 0.25 Hz). Each of 2 sessions consisted of 6 trials for each of 3 yaw head-on-trunk positions (0,±30°): 3 with and 3 without GVS. Anterior-posterior (A-P) and medial-lateral (M-L) center of pressure (COP) were recorded continuously during each trial and were bandpass filtered at the stimulus frequency. For cycles 2-7, the resulting transverse plane scatterplot was fit with an ellipse, which provided parameters of major and minor axis length and direction of sway. The median angle of the direction of sway was calculated for each trial. Center of pressure sway direction was regressed with head position. The subjects with CD demonstrated significantly greater magnitude of sway (controls: 1.83±1.30; CD:2.26 ±1.20). The slope of the relationship between head position and sway direction during the GVS trials was reduced in the subjects with CD (controls 1.39±0.25; CD 1.13±0.18). These results suggest that sway induced by GVS may form the basis of a diagnostic test for cervicogenic dizziness.

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731 Intermittent Transtympanic Micropressure Applications Control the Symptoms of Meniere's Disease

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Context: Meniere's disease results in life-disruptive vertigo in 30% of cases, requiring surgical therapy for control. Non-invasive therapy using the Meniett™ device has been suggested as an alternative to surgery for these cases.

Objective: To delineate the efficacy of Meniett™ device therapy in people with classic, unilateral audiovestibular Meniere's disease unresponsive to traditional medical treatment.

Design, Setting, and Participants: Sixty–two study participants with active unilateral cochleovestibular Meniere's disease used the Meniett™ device three times daily for four months in a randomized, double-blinded placebo-controlled clinical trial. Half the participants used an inactivated placebo device (control group) and half used a functioning device (active group).

Intervention: All participants were advised to adhere to a low sodium diet and all had a tympanostomy tube placed in the tympanic membrane on the affected ear. Diuretic and vestibular suppressant medications were used as needed.

Main Outcome Measures: Control of vertigo, functionality and hearing were compared across the two study groups. Outcomes were based on the participants' daily diary. The null hypothesis was no significant difference in vertigo control, functionality, and hearing between the active and control groups at 2 and 4 months.

Results: As of Sept. 27, 58 subjects were enrolled in the clinical trial. Followup will be complete by Jan 15, 2003. The final results can not be ascertained until followup is complete and the investigators are unblinded. Thus, the study results, which are not known yet, will be presented at the meeting.

732 The Influence of Physical Therapy Intervention on Individuals with Central Vestibular Dysfunction

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Forty-eight patients with a diagnosis of a central vestibular disorder (31 F; 17 M) were identified through a retrospective chart review. Diagnoses included central vestibulopathy – not otherwise specified (n=13), cerebellar dysfunction (n=11), stroke (n=10), mixed central and peripheral vestibulopathy (n=9), and post-traumatic central disorders (n=5).

At initial and discharge visits, patients completed the Dizziness Handicap Inventory (DHI), the Activities-specific Balance Confidence scale (ABC), the Dynamic Gait Index (DGI), the timed "Up & Go", the five times sit to stand (5TSS), and the Berg Balance Scale (BBS). For each outcome measure, a Wilcoxin Signed-Rank test was calculated between the initial and final visit for the DHI, ABC, DGI, and the BBS. Paired t-tests were calculated between the initial and final visit for the timed "Up & Go" and 5TSS.

Patients were treated for a mean of 5 visits. Each patient was provided with a customized home exercise program at the end of each physical therapy session. Significant improvement was observed in all outcome measures. The DHI (n=44) decreased by an average of 13 points

(p<.001). The ABC (n=45) improved an average of 13% (p=.001). DGI (n=38) improved by an average of 3.8 points (p=.001). The timed "Up & Go" (n=34) improved by an average of 3.2 seconds (p=.001). The BBS (n=12) improved an average of 6 points (p=.014). The 5TSS (n=12) improved an average of 6.8 seconds (p=.003)

Patients with central or mixed peripheral and central vestibular disorders significantly improved regarding their balance, gait, self-perceived balance, and dizziness handicap. Patients with central vestibular disorders benefit from an individualized vestibular physical therapy exercise program.

733 Vestibular Evoked Myogenic Potential (VEMP) Dynamics Are Altered in Meniere's Syndrome.

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Objective: The VEMP in the sternocleidomastoid muscle results from an inhibitory reflex response to acoustic stimulation of the ipsilateral saccule. We have shown previously that normal subjects have VEMP interaural amplitude symmetry equivalent to intra-aural test-retest symmetry, and elevated thresholds in ears with Meniere's syndrome. In this study we have refined our methods and further characterized the VEMP response of affected and unaffected ears of subjects with unilateral Meniere's syndrome.

Methods: VEMP was evoked by ipsilateral clicks and tonebursts at 250, 500, and 1000 Hz at a rate of 13/sec in normal and unilateral Meniere subjects. Responses were recorded at decreasing signal intensities until response threshold was obtained. Response amplitude, latency, and threshold were recorded.

Results: VEMP was present in all ears tested. Compared to normals and to unaffected ears of Meniere subjects, affected Meniere ears had significantly increased VEMP thresholds. Affected Meniere ears showed threshold shifts at all frequencies and there was less tuning apparent at 500 Hz. Unaffected ears of Meniere subjects also showed significantly elevated VEMP thresholds compared to normals. Analyses of VEMP thresholds for effects of age, hearing loss, and audiometric configuration showed no significant differences.

Conclusions: Meniere ears display alterations in VEMP threshold and tuning, supporting our hypothesis of altered saccular motion mechanics arising from hydropic distention. Unaffected ears of unilateral Meniere subjects show similar changes, though to a lesser degree. This finding may be due to occult saccular hydrops in the asymptomatic ear, or to binaural interactions in the VEMP otolith-cervical reflex arc.

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734 VEMPs in the Monitoring of Gentamicin Treatment for Meniere's

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Saccular function assessed by VEMPs gives an independent metric of peripheral vestibular function. We have verified (ARO midwinter meeting 2002) that this measure varies independent of caloric function in a wide range of peripheral disorders. In a prospective study we have collected this information together with caloric and rotational chair data on patients undergoing gentamicin treatment for Meniere's disease. This report will provide the results from 30 patients, a minimum of 6 months from the end of their treatment. Comparison of VEMP data to caloric results will show independent changes during treatment. Correlation of VEMP data with rotary chair changes during treatment will be presented. Patient survey data on recovery post treatment as a function of saccular performance via VEMPs will be discussed.

735 Factors Affecting Vestibular Evoked Myogenic Potentials in Human Subjects

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Vestibular evoked myogenic potentials (VEMPs) were examined in a series of otologically normal human subjects to identify various signal parameters and test conditions which might affect VEMP amplitude and latency. Click-evoked VEMPs were studied in the context of changes in head position and contralateral masking noise levels in an effort to identify suitable bilateral recording parameters. The effect of contralateral noise is reported in intensity series (50-70 dB EM) on the stimulated-side VEMP amplitude and latency. Variations in amplitude and latency of the VEMPs were found as a function of contralateral noise level. Binaural stimulation and bilateral recording protocol is described in the context of various stimulus parameters and subject positioning. Standard VEMP electrode montage is used referencing each sternocleidomastoid muscle to the upper sternum notch. Filter settings (10-2k Hz BP), stimulus intensity (95 dB nHL) and stimulus repetition rate (5.1/sec) are also standard. Results indicate that contralateral recordings (non-stimulated side) are reliable, but may or may not represent evoked electrical activity generated from the contralateral side. If indeed the lower amplitude contralateral recording reflects myogenic activity from that side, a theoretical explanation is given for the probable neural mechanism. Also, contralateral masking noise changes the VEMP recorded contralaterally only slightly but affects the stimulated-side VEMP significantly. These findings are discussed with reference to cases of sensorineural hearing loss and some with vestibular pathology.

736 Frequency Sensitivity of Vestibular Evoked Myogenic Potentials in Meniere's Disease

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Although the frequency sensitivity range of human vestibular evoked myogenic potentials (VEMP) had been reported to be less than 1000 Hz, the frequency dynamics of VEMP involved in pathogenesis is still unknown. We studied human frequency dynamics of VEMP and its clinical use. The p13-n23 amplitude of VEMP was dependent on the muscular tonus, so repeated recording may cause muscular fatigue and unreliable results. To overcome this, we normalized amplitudes, such that they were not dependent on muscular tonus. Normalization involved dividing the value of each amplitude by the pre-stimulated root mean square of the myogenic potential.

The frequency dynamics of VEMP were measured in twenty patients with unilateral Definite Meniere's disease. Sixteen healthy volunteers (32 ears) were used as controls. In both groups, normalized amplitudes of VEMP were recoded after a tone burst at 250, 500, 700, 1000, 1500, 2000 and 4000 Hz. We considered results to be abnormal where the maximum normalized amplitude was at 1000 Hz and higher frequencies, in accordance with a previous report. Five patients with Meniere's disease who showed abnormal results were rerecorded 1 hour after intravenous injection of furosemide (20 mg).

In the control group, 24 of 32 (75%) ears indicated maximum amplitude lower than 1000 Hz. In patients with Meniere's disease, abnormal results were found in 13 of 20 (65%) patients. The frequency sensitivity range in Meniere's disease was significantly higher than that in the control group ($P < 0.01$). After furosemide injection, maximum amplitudes moved to a lower frequency.

These findings suggested that the change in the acoustic impedance of the saccule is affected by the endolymphatic hydrops and that the frequency sensitivity range of VEMP is useful for the diagnosis of endolymphatic hydrops.

737 Transtympanic Gentamicin: Perilymph Concentration Peaks

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Transtympanic gentamicin administration has become a popular modality for the treatment of Meneire's Disease. This modality and other "inner ear medical therapy" are gaining increased clinical and scientific attention. We have previously described the kinetics and effects of gentamicin uptake in the inner ear after delivering the medicine into the middle ear using a variety of different techniques and sustained release modalities. In this work we focus on the peak perilymph gentamicin concentrations of three different administration techniques. In all three techniques we describe an initial early peak perilymph concentration in the 2 to 6 hour post-installation range followed by a reduction in the perilymph concentration and a second peak in the 18 to 30 hours post-installation range. The significance and explanation of this bimodal pattern of uptake are examined. In addition, the morphological and functional correlates of these peaks are detailed and analyzed. The information gained from this study will increase our scientific understanding about the effects of gentamicin on the inner ear and allow clinicians to more effectively treat patients with inner ear disorders.

738 Angular VOR Gain Predicts Vertigo Control After Intratympanic Gentamicin Treatment for Meniere's Disease

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We measured three-dimensional angular vestibulo-ocular reflexes (AVOR) elicited by rapid rotary head thrusts in 19 subjects with Meniere's disease before and after a single intratympanic injection of gentamicin. Eight subjects required a second gentamicin injection for recurrent vertigo at a mean of 223 days after the first treatment. AVOR gain was defined as eye velocity/head velocity during the 30 ms before peak head velocity. Head thrusts exciting the ipsilateral horizontal (IHC) and anterior canals (IAC) elicited significantly higher AVOR gains in the 8 subjects whose vertigo would recur than in the 11 who enjoyed control with one injection alone. The multiple-treatment group had IHC and IAC gains of 0.66 ± 0.19 and 0.61 ± 0.10 (mean ± SD) after the first treatment. In contrast, the single-treatment group had IHC and IAC gains of 0.42 ± 0.14 and 0.47 ± 0.13 after their treatment (IHC: $p = 0.009$; IAC: $p = 0.01$). In addition, the asymmetries in gain between the IHC and IAC and their contralateral coplanar mates were significantly greater in the single-treatment group than in the multiple-treatment group. Seven of the 8 subjects in the multiple-treatment group had post-treatment IHC gains > 0.55, whereas only 2 of the 12 subjects in the single-treatment group had such high gains. A refixation saccade after a rapid head thrust (head thrust sign) is the clinical correlate of diminished gain in the IHC. For subjects who had no head thrust sign before treatment, this sign developed after treatment in all 9 patients in the single-treatment group versus in only 4 of the 7 patients in the multiple-treatment group. The results suggest that successful treatment of Meniere's disease with intratympanic gentamicin correlates with attenuation of semicircular canal function and that control of vertigo might be predicted when IHC gain is reduced to < 0.55.

739 Nystagmus Measured With Video-Oculography And Vertigo After Stapedotomy

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Hearing results have been extensively documented after stapes surgery, whereas vestibular symptoms and signs with quantification of nystagmus by video-oculography (VOG) has not been emphasized. Postoperative nystagmus and vestibular symptoms were recorded one week and one month after operation for ten patients, who underwent primary stapedotomy.

The eye movements of the dominant eye were recorded with a rubber mask-mounted small video camera.The VOG recordings were stored as eye position curves in horizontal and vertical planes, and also to a standard VHS-videotape, and analyzed off-line for spontaneous nystagmus, gaze evoked nystagmus, and head shaking nystagmus. Any nystagmus detected was characterized by calculating maximal slow phase velocity and direction.

Slight sensation of vertigo and nystagmus with low (< 3 deg/s) slow-phase velocity were commonly found. Nystagmus did not always obey Alexander's law. Postoperative nystagmus may be due to the perilymphatic leak, mechanical trauma, or chemical irritation of the membraneous labyrinth after stapedotomy. Symptoms and signs of vestibular disturbance after stapedotomy seem to be temporary and those with clinical significance are rare.

740 Validation of Five Times Sit to Stand in Persons with Vestibular Disorders

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The purpose of this study was to determine the concurrent validity of the five times sit to stand test (FTSS) in persons with vestibular dysfunction and the ability of the FTSS to discriminate between persons with and without vestibular dysfunction. A retrospective chart review of 93 patients seen for vestibular rehabilitation was completed (mean age=61). The control group consisted of 81 healthy control subjects (mean age=60) whose data were collected as part of a previous study. All participants performed the FTSS from a 42.5 cm chair. Time required to stand and sit five times as quickly as possible with arms folded was recorded. They also performed the Dynamic Gait Index (DGI) and completed the Activities-specific Balance Confidence (ABC). Concurrent validity of the FTSS with the DGI and the ABC in the patient group was determined using the Spearman correlation. Multivariate linear discriminant analysis was used to determine the ability to predict group (vestibular vs. control) membership with the FTSS, the DGI and the ABC. The patient group demonstrated significantly higher FTSS times than the controls (15.7 vs. 10.5 sec; $p<.01$). In the patient group, the FTSS displayed moderate correlation with the DGI ($r=-.55$) and weak correlation with the ABC ($r=-.25$). A discriminant function equation including the FTSS, the ABC, and the DGI correctly predicted patient group membership with 85% accuracy. The FTSS is a valid measure for use with patients with vestibular dysfunction and is able to discriminate those with vestibular dysfunction from those without.

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741 Driving Disability in Patients with Vestibular Disorders

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Driving is one of the most important daily life tasks performed by people in industrialized societies around the world. In most communities in North America the ability to drive a motor vehicle is essential for mobility within the community, to go to work, run errands, and participate in community activities. The ability to drive can be affected by sensorimotor impairments, including vestibular disorders.

Although the literature includes several papers about physicians' beliefs about the driving skills of patients with vestibular disorders, no studies have examined patients' experiences driving. We surveyed patients with several vestibular impairments, including benign paroxysmal position vertigo (BPPV), chronic peripheral vestibulopathy, Meniere's disease and post vestibular nerve section or acoustic neuroma, and compared them to a sample of normals. All subjects were interviewed using a well-normed instrument used previously to evaluate elderly patients with vision impairment. Normal people report no significant deficits in driving skill. BPPV patients report few problems, as well. The other groups vary considerably but report more problems. In particular patients with chronic, uncompensated labyrinthitis have difficulty responding to many driving challenges. Post-operative and Meniere's disease patients are intermediate. These results differ somewhat from physicians' beliefs about patients' driving skill.

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742 Vestibular Testing in Children

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Background. Normative age appropriate vestibular and balance data obtained in a large cohort of children with a well documented history of middle ear status and with no middle ear effusion at time of testing have not been reported previously.

Objectives. To obtain longitudinally normative data in a cohort of children at ages 3,4,5,6 and 7 years using standard vestibular and balance test protocols and to determine, in the absence of concurrent middle ear effusion (MEE), possible effects of a history of otitis media (OM).

Methods. Children were evaluated using pneumatic otoscopy, tympanometry, audiometry, and vestibular (rotational and moving platform posturography) testing at yearly intervals. Rotational testing was performed during sinusoidal rotation at different frequencies and velocities as well as constant velocity rotation. In addition, middle ear status was assessed every 6 weeks by pneumatic otoscopy and tympanometry.

Results. Normative age appropriate rotational and moving platform posturography data will be presented for children ages 3, 4, 5, 6 and 7 years. During posturography testing the children performed well for conditions I-III, but had increased sway during conditions IV-VI at all ages. Children age 3 years with a significant history of OM demonstrated a higher gain on rotation than children with no or minimal history of OM.

Conclusions. Vestibular testing can be performed in children. However, rotational testing can be performed more easily than moving posturography testing in younger children. In most children the effect of previous OM on balance seems to be minimal.

743 The Effects of Vestibular Physical Therapy Intervention on Balance Normal Individuals who have been Artificially Spatially Disoriented

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The purpose of this study was to investigate the effects of vestibular physical therapy on adaptation of normal subjects who had been artificially spatially disoriented. Forty male and female subjects who were identified as balance normal based on the results of computerized dynamic posturography (CDP) and the dynamic gait index (DGI) were randomly assigned to two experimental groups. Both groups of subjects were fitted with diagonally shift lenses. Both groups were retested by CDP and DGI. The control group was required to sit still for twenty minutes and view a video. The active physical therapy group was required to perform vestibular physical therapy tasks. All subject were again reassessed by CDP and DGI after control or active intervention. Lenses were then removed and subjects were retested to assure return to normal baseline status. There were no significant differences in the CDP scores within or between groups while wearing the distortion lenses. There was a significant difference in the DGI of both groups upon donning the lenses. However, the active group with lenses displayed less spatial disorientation after twenty minutes than the control group with lenses in place as assessed by a better score on the DGI. All subjects returned to pre-test normal by twenty minutes after removal of the goggles. The results of this study indicate that vestibular physical therapy enhances adaptation to artificially induced states of visual spatial disorientation.

744 "Quick Spins" After Vestibular Nerve Section Respond to Anticonvulsant Therapy

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Selective vestibular nerve section is an effective surgical treatment for unilateral Meniere's disease that can preserve hearing. However, some patients experience continued or even worsened vertigo after vestibular neurectomy. We report 4 patients who were afflicted with frequent brief spinning spells, or "quick spins" following vestibular neurectomy. In all four cases there was a symptom free period ranging from 1year to 3years. All cases had an excellent therapeutic response to carbamazepine or oxcarbamazepine, agents conventionally used for neuralgia, suggesting that these symptoms are caused by a hyperexcitable vestibular nerve. These spells may be induced by irritability in remaining vestibular nerve fibers or due to neuroma formation. Treatment for neuralgia should be considered in the context of "quick spins" following vestibular neurectomy.

745 Sway on a Randomly Moving Platform with Vibrotactile Tilt Feedback

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Responses during standing have been characterized in 5 vestibular deficient subjects by measuring the root mean square (rms) center of pressure and also by the use of stabilogram diffusion analysis. Subjects wore a 1-axis version of a vibrotactile balance prosthesis that measured A/P body tilt and displayed the tilt estimate with columns of tactors mounted on the front and rear of the torso. Subjects were tested eyes-closed with and without the balance aid.

Analysis of the rms body tilt estimate from the inertial instrumentation showed that all vestibulopathic subjects had their anterioposterior tilt significantly reduced ($p<0.05$) during runs featuring 2-axis random noise platform perturbation when the vibrotactile feedback condition was compared to the no-balance aid condition. This was also true for all but one subject during quiet standing without platform motion (this subject had very small tilt even with no-balance aid).

Diffusion Analysis showed that the vibrotactile feedback of body tilt allowed the subjects to control posture more quickly than without feedback (Fig. C-9). During conditions that induced a mild 2-axis random platform motion, there was a significant change ($p = 0.04$) for all subjects for anterioposterior sway with anterioposterior tilt displayed. The change in mediolateral sway was not significant. This is evidence of direction-specific control.

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746 Frequency Analysis of a Vibrotactile Balance Prosthesis.

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The purpose of this study was to determine if an experimental vibrotactile balance prosthesis can substitute for vestibular orientation information in bilateral vestibular loss (VL) subjects and enhance balance control in subjects with normal sensory function. Quiet stance in 6 normal and 4 VL subjects was perturbed by support surface (SS) rotation about the ankle joint axis according to a pseudorandom motion profile with wide-bandwidth, white-noise properties. The anterior-posterior body sway response and SS stimulus were analyzed using spectral analysis methods to compute a transfer function describing the stimulus-response relationship over a 0.017-2.2 Hz frequency range. A comparison was made between transfer functions obtained both with and without vibrotactile orientation cues applied through an array of 12 tactile vibrators (tactors) held against the surface of the torso. Tactor vibration was designed to provide body orientation feedback related to body sway angular position and velocity.

Results showed a frequency dependent reduction in stimulus-evoked body sway associated with tactor vibration. Body sway reduction was always greatest at lower frequencies and was dependent on the SS stimulus amplitude. At 2° peak-to-peak SS stimulus amplitude, body sway was reduced at frequencies below 0.8 Hz. At SS stimulus amplitudes of 4° in VLs and 8° in normals, body sway reductions occurred below about 0.1 Hz. This frequency dependent sway reduction indicates that VLs were unable to fully substitute orientation cues from this particular vibrotactile stimulus for their absent vestibular cues. However, the vibrotactile stimulus did provide a functionally useful improvement in balance control.

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747 Specific Space Motion Sickness Related Drugs Affect Only the Horizontal Semi Circular Canal Function but not the Utricular Function.

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A mismatch between utricular and semi-circular canal output can provoke space motion sickness. To assess the utricular and the horizontal canal functions, we apply the unilateral otolith test and ENG tests. During constant rotation of 400 degrees per second the subject us 4 cm translated along an interaural axis to the right and to the left. When the axis of rotation is positioned through one utricular system, only the contralateral utriculus is stimulated. Consequently, the centrifuged utricle “feels” a GIA tilt of 21 degrees what induces an ocular counter rolling (OCR), measured on-line using three dimensional video-oculography. For analysis of the experimental data, we use a theoretical model proposing a linear relationship between the OCR and the head centre GIA. The slope is a measure of the utricular sensitivity and the intercept represents utricular preponderance.

These results are presented in the framework of a study ‘Pharmacological countermeasures for space motion sickness (NSBRI / NASA grant: #NCC9-58)’, the aim of which is to assess the effect of promethazine, scopolamine, lorazepam and meclozine in healthy subjects.

Nine healthy volunteers were recruited, having three control sessions and 5 medications (placebo included), separated each by a week.

Wilcoxon paired analysis indicates a significant decline in utricular sensitivity only after intake of promethazine ($p = 0.044$) and lorazepam ($p=0.012$). All medications decreased the horizontal canal responsiveness as determined by the caloric sum (promethazine $p=0.011$, lorazepam $p=0.049$, scopolamine $p=0.021$ and meclozine $p=0.036$). Also the gain was reduced ($p=0.008$, 0.036, 0.011 and 0.012 respectively).

Our results indicate that intake of promethazine and lorazepam lead to a suppression of the entire vestibular sensory organ, whereas scopolamine and meclozine only induce central inhibition and horizontal semicircular canal function.

748 Tullio Phenomenon Revisited

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The development of nystagmus and vertigo, in response to acoustic stimuli, is known as Tullio phenomenon (TP). According to the literature, the highest prevalence of this was noted in cases of congenital syphilis. Schuknecht and Nadol postulated that an abnormal transduction of sound to the vestibular system was via stapediovestibular fibrosis. Our experience regarding TP, via a case of superior canal dehiscence, provides a new perspective to this symptom.

A 44 year-old woman developed TP and left-sided hearing. Serial audiometry, clinical evaluation, videonystagmography and temporal bone CT prompted surgical intervention. Preoperatively, a low frequency oscillating torsional nystagmus was seen with Barany noise apparatus presentation to the left ear and TP. Upon surgical exploration, the left superior canal dehiscence was confirmed and repaired. Postoperative evaluation failed to demonstrate TP or the torsional nystagmus. Incidentally, a depression of waves I, III and V of the ABR were noted as the middle cranial fossa was manipulated, prior to the actual patching of the dehiscence. The patient had a left hearing deficit after surgery.

Further review of congenital otosyphylis literature indicates that it causes a resorptive osteitis of the temporal bone. It is plausible then to think that the mechanism of TP in congenital otosyphylis may be via canal dehiscence. The resolution of TP strongly suggests that mechanisms aside from stapediovestibular fibrosis may cause similar problems. The dehiscence allows for a change in endolymphatic hydrodynamics as pressure shifts occur along the newly formed perilymphatic-arachnoid interface and are then transmitted to the perilymphatic-endolymphatic interface. In our patient, the sensorineural hearing loss suggests that usual endolymphatic flow has been compromised.

The pathogenesis of TP may be a consequence of distorted endlolymphatic fluid mechanics and less likely by stapediovestibular-level disease.

749 The Effects of Varying Hearing Aid Input-Output Function on Speech Recognition and Overall Sound Quality Preferences

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The input-output (i/o) function of a hearing aid reflects the amount of gain provided at different input levels. It affects the audibility of the sounds and also the comfort of hearing aid use. Few researchers have systematically explored whether any i/o function is better for speech recognition and more preferred at different input levels, or whether certain i/o functions are more suitable for listeners with different configurations of hearing loss. The purpose of this study was to explore the effects of varying hearing aid i/o function on speech recognition and overall preference ratings for listeners with sensorineural hearing loss.

An experimental hearing aid was built to simulate six i/o functions: 1) linear peak-clipping, 2) linear compression limiting, 3) curvilinear wide dynamic range compression (WDRC), 4) high-level-linear WDRC, 5)

high-level-limiting WDRC and 6) Mid-level-linear WDRC. All other hearing aid characteristics were kept constant. Seven hundred and twenty IEEE sentences mixed with a four-talker babble were processed by these six i/o functions at input levels of 45, 65 and 90 dBSPL. The sentences were then played back to: 1) subjects with normal hearing, 2) subjects with flat loss, and 3) subjects with sloping loss. Two signal-to-noise ratios were used for each group in order to bracket their signal-to-noise ratios for 50% speech recognition. The gain of the i/o functions was matched at 65 dBSPL. Sentences processed at 65 dBSPL hearing aid input were presented to the subjects at their comfortable listening levels and sentences processed at 45 and 90 dBSPL input levels were presented at levels as if the volume control was fixed. The experimental conditions were counterbalanced. Results suggested that a good hearing aid, which yields low SNR-50s and high preference ratings, makes soft sounds audible, and loud sounds comfortable and free of distortions. The exact i/o function implemented to achieve these goals is less important.

750 Probe Level Effects in Clinical Admittance Testing

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A crucial component of the audiological test battery is the measurement of the acoustic reflex threshold. Recent studies have shown that a wideband reflectance method yields lower reflex thresholds than traditional methods using a 226 Hz probe tone. One possible explanation for this increased sensitivity is that the level of the 226 Hz probe tone used in the clinical method (85 to 90 dB SPL) is high enough to elicit an acoustic reflex prior to the presentation of the activator signal. If this occurs, the sensitivity of reflex threshold measurement may be compromised due to possible adaptation of the reflex; or effects related to the reflex input-output function. This study was designed to determine if the acoustic reflex is induced by a 226 Hz activator tone at the levels used clinically, and if so, to determine an average acoustic reflex threshold for the 226 Hz probe tone in 20 young adults with normal hearing. The acoustic reflex threshold was evaluated using two methods: a magnitude method that determined if the activator condition surpassed baseline variability and a correlation method to determine if there was a significant cross-correlation between responses at adjacent activator levels. The 226 Hz tone, presented at a level recommended for clinical testing, resulted in an acoustic reflex in over 50% of the ears tested. Of the 23 ears that had an acoustic reflex to the 226 Hz tone, the average reflex threshold was 80.7 dB. This suggests that the level of the 226 Hz probe tone used in clinical admittance testing is excessive, and may compromise reflex threshold sensitivity.

751 ASSR Threshold and Response Growth: Relationship to Audiometric Configuration

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The auditory steady-state response (ASSR) is receiving attention as potential alternative to the auditory brainstem response (ABR) for frequency-specific estimation of audiometric thresholds in infants. Recent studies have reported ASSR thresholds to be correlated with behavioral thresholds (e.g. Dimitrijevic et al 2002; Herdman & Stapells 2001; Perez-Abalo et al 2001), although there has been little focus on whether this is true for all audiometric configurations. The specific aims of this study were (1) to investigate whether the shape of the behavioral audiogram (flat vs. sloping) affects how accurately ASSR thresholds estimate behavioral thresholds and (2) to explore the form and potential clinical utility of suprathreshold ASSR measures. ASSR testing was completed on 30 adults: 10 normal hearing, 10 flat SNHL, and 10 sloping SNHL. Thresholds and growth functions were obtained using 100% AM stimuli for the carrier frequencies 0.5, 1, 2, and 4 kHz presented simultaneously.

Results showed that ASSR thresholds were highly correlated to pure-tone behavioral thresholds at all frequencies in all 3 groups. The difference between ASSR and behavioral threshold was larger in the normal-hearing group than in either hearing-impaired group, especially for the low frequencies. However, a simple linear correction may be in order for converting ASSR thresholds to behavioral threshold estimates. ASSR amplitude response growth was variable across individuals, but was steeper in the hearing-impaired groups than in the normal-hearing subjects. Growth functions were not steeper in the sloping loss group than the flat loss group in the high frequencies, suggesting little contribution of the neighboring hair cells in normal regions of the cochlea as intensity is increased.

752 ECochG (Electrocochleography) to Toneburst with Ear Canal Electrode and Its Advantages

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ECochG can be used in estimating conditions for both cochlea and auditory nerve, while ABR is for nerve and otoacoustic emission (OAE) is only for cochlea. Further, ECochG is affected by forward transmission, resulting in lower threshold than OAEs (Zhang & Abbas, 1997). ECochG, obtained from the electrode closer to the generator, has an action potential greater than that in ABR. Therefore, ECochG is important in clinical audiology and deserves more studies. ECochG electrodes vary in their configurations. TMtrodes (Tympanic Membrane electrodes, Ferraro & Durrant, 2002 in Katz) can be better in many aspects than invasive TTtrodes (TransTympanic); while ECtrodes (Ear Canal) could generate usable responses based on the previous reports. However, the advantages of ECtrode and the recordings with it in response to tonebursts have not been completely addressed. We recorded the responses with ECtrode. In comparison, the N1 amplitude with ECtrode could be up to half of that with TMtrode. Using clicks, wave III & V were also displayed, with amplitude ratio of V/N1 smaller than V/I in ABR. Using a 14-ms 2000-Hz toneburst, the CM (cochlear microphonic) waveform and N1 were clear, while wave III and V were not. Based on the latency of SP, envelope of toneburst, and potential effect of wave III-V, we selected SP analyzing time within a period between the beginning of the toneburst plateau and pre-trough of N1. We define the SP amplitude by averaging the data points of that period instead of one point because of CM waveform. Since the signal to noise ratio obtained with ECtrode is small, it is critical to avoid pitfalls such as cross-talk. Compared with TMtrode, we found that ECtrode advantages include no risk to TM, no load on TM (perhaps good at some frequencies such as 2000 Hz), low impedance, stable impedance, existence of wave V & V/N1, easy application, no stress on patient, applicable to young ages, applicable to minor TM conditions, simultaneous OAE recordings, etc.

753 Electrically Evoked Auditory Responses to Differential Stimuli

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Speech intelligibility seemed to be affected by specific coding strategies and electrode arrays in electrical stimulation of auditory nerves. A great deal of these approaches were based on scala tympanic electrical stimulation where cochlear tonotopy is an important principle for better discrimination. In this study, electrically evoked auditory responses were evaluated to analyze the effects of current distribution and channel interaction in response to differential stimuli in the scala tympanic electrode array. Electrically evoked compound action potential and E-ABR responses were recorded in cochlear implantees in response to referenced and off-referenced parameter values to estimate reflection coefficients in given coding strategies and electrode arrays. Within limited ranges, current levels were systematically varied and paired channel distances were adjusted in both mono and bipolar electrode arrays. Subjective responses were also compared with these differential response data to analyze the outcomes. In this result, differential

response curves were steeper at lower stimulation levels and then terminally reached saturated states. Monopolar and bipolar stimulation arrays also showed wide ranges in differential effects. These effects were varying and depending on electrode arrays and channel arrangements. Distinct differential effects of stimulation were observed in terms of current levels and electrode arrays. The proposed approach may be useful in objectively determining effective differential current levels and estimating channel interactions in the scala tympanic electrode array for better speech intelligibility.

754 Mismatch Negativity in Children with Auditory Neuropathy and Sensorineural Hearing Loss

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The purpose of this study was to determine if the cortical event related potential (ERP) "mismatch negativity" (MMN) was present in children with auditory neuropathy (ANHL) or sensorineural hearing loss (SNHL). Our group has previously demonstrated that the presence of obligatory-ERP components P1, N1 and P2 are related to speech perception abilities in children with ANHL (Rance et al 2002). We hypothesised that MMN presence would be related to speech perception abilities. Speech perception abilities are generally quite poor for children with ANHL, even when pure tone sensitivity is only mildly to moderately impaired. Two groups were tested:1)children with ANHL (N=18)and 2)children with SNHL matched on the basis of pure tone sensitivity and age to members of the ANHL group. MMN were evoked by tone(400 vs. 440 Hz) and word(/dæd/ vs. /bæd/)contrasts. MMNs were obtained from a significant majority of children with SNHL, and for only half of the children with ANHL. High variability in MMN onset latency, duration and magnitude were noted in both groups. When additional analyses were completed, it was seen that commonly used criteria for defining MMN were not powerful enough to distinguish a test (deviant vs. standard response) from a control (standard vs. standard response) condition.

We hypothesised that children with AN would have abnormal or absent MMN indicative of their auditory disability which results in poor speech perception. MMN was consistently obtained in children with SNHL who had speech perception abilities commensurate with their degree of hearing loss. MMN were inconsistently obtained in children with ANHL and poor speech perception abilities, even when these children demonstrated normal obligatory ERP components, P1 and N1. The methods of ERP data collection and analyses that are currently used in clinical contexts, however, are not sufficiently robust for employing MMN as a prognostic measure for speech perception ability.

755 Auditory Neuropathy/Dys-synchrony (AN/AD): Management and results in 193 patients

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We have confirmed the diagnosis of AN/AD by reviewing records of ABRs, emissions, reflexes and other tests on 193 patients of whom we have seen 60 at LSU. Only one patient showed a small remnant of a middle ear muscle reflex, which has otherwise been absent or grossly abnormal in all of these patients. We suggest that middle ear muscle reflexes be one of the screening tests for AN/AD along with emissions and ABR. The latter should consist of separately obtained averages to a positive and negative polarity click to separate CM from neural responses. We saw our first patient in 1982 and from 1985 until 1992 we tried carefully fit hearing aids with real ear measurements and Auditory Verbal Therapy. This strategy has NOT been successful in helping a single one of these 193 patients learn language solely by auditory means (although 7% developed language and speech spontaneously with NO intervention).While hearing aids improved detection thresholds, the long term value of hearing aids in understanding speech is far poorer than predicted based on the audiogram and/or articulation index alone. FM systems to enhance signal-to-noise ratio and facilitate lip-reading had only modest success. Therefore, we now opt to by-pass the hearing aid trial before implantation. Because outcomes can range from total deafness to normal language development, we recommend consultation with SLPs, visual language support (Cued Speech and/or signs) along with spoken language and lip reading to ensure language acquisition, while educational decisions are made based on development and parents' desires. If cochlear implantation is chosen, (clearly successful in 28/35 of our patients, and too early to tell in the other 7) auditory verbal therapy is ideal post-implant but has been most successful when supplemented by some visual language (Cued Speech and/or signs) for use when the implant is off.

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756 Neonatal ABRs In Meconium Staining And Meconium Aspiration

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Meconium is an infant's first stool. It is normally released after birth. Sometimes it is released before birth into the amniotic fluid following fetal distress. This "meconium staining" of amniotic fluid occurs in about 5-15% of births, mostly full-term births. Rarely does it cause serious problems. However, if meconium is aspirated by the fetus or newborn, it can cause respiratory distress, pneumonia, asphyxia, pneumothorax, and CNS injury. Respiratory distress and CNS injury are risk factors for hearing loss. Consequently, we recorded ABRs from 32 full-term infants with heavy meconium staining. In addition, we collected ABRs from one infant with meconium aspiration syndrome (MAS). Controls were 159 full-term infants with no heavy meconium staining. We predicted poorer ABR results from heavy meconium-stained infants. Exclusion criteria were maternal substance abuse, chromosomal anomalies, structural defects, and infections. ABRs were typically recorded within 24-48 hr after birth, using 70 and 40 dBnHL clicks. Each ear was tested separately. "Pass" signified the presence of scorable ABRs from both ears at the 40 dB intensity as well as wave amplitudes, latencies and interpeak latencies within normal limits. There was a trend for a lower Pass rate among the heavy meconium-stained group versus the control group (72% vs 82%, $p = 0.15$). The one infant with MAS had bilaterally elevated ABR thresholds, greatly prolonged wave I latencies, and absent waves III and V. These results suggest that heavy meconium staining is associated with a moderately lower Pass rate on neonatal hearing-screening tests. In addition, MAS can be associated with peripheral and central hearing losses and lower brainstem damage that can be detected early with diagnostic neonatal ABRs.

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757 Idiopathic Sudden Sensorineural Hearing Loss-Will a National Database Solve the Mystery?

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Idiopathic SuddenSensoneural Hearing Loss (ISSHL) has an incidence of 5-10/100.000/year. Different treatment policies have been developed according to different hypotheses about the etiology. The number of treated cases at any given clinic in Sweden is too low to provide a basis for studies to demonstrate an effect on outcome due to treatment. A

national database for ISSHL is started Sept 1 2002 and 90% of the ENT-clinics are participating. Demographic data, type of audiogram, time before treatment, treatment, hospitalization, sick leave and final outcome (audiogram) after three months are requested. The data will help to clarify the disease both with respect to whether any of the present forms of treatment help, and which variables (audiogram type etc) can be used to predict the outcome. Data from at least 400 patients/ year will be available. The clinics will be clustered according to their preferred treatment policy and controlled double blind studies will be planned. Knowing the local incidence in advance would make it possible to estimate how long it will take to perform the studies with sufficient statistical power. To prepare for the start of the database the questionnaire to be used has been tested retrospectively on all patients who has got the diagnosis ISSHL between 1997-2002 at their initial visit to the ENTclinic at the university hospital in Linköping. Out of the 153 patients with an initial diagnosis of ISSHL 15 developed Menieres disease, six were diagnosed with acustic neurinoma. Nine were caused by trauma (acoustic and baro-). 93 of the remaining patients were possible to evaluate: 40 completely recovered or were markedly improved (>30dB), 18 partly recovered (10-30dB) and 35 had no restitution. 20 were treated with steroids, surgery in 3 cases and different degrees of rest for the remaining. Upsloping och midfrequency loss had 5 times chance of recovery compared to others. Most patients who did not improve at all had an initial audiogram of flat loss. A database, which after four years should encompass 1600-2000 patients will be extremely valuable for the present and future research.

758 Decreased Sound Tolerance and Tinnitus Retraining Therapy

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Decreased sound tolerance (DST) consists of two components: 1) hyperacusis, which results from abnormally strong reactivity of the auditory pathways to sound, only secondary yielding activation of the limbic and autonomic nervous systems; and 2) misophonia (dislike of sound), which results from enhanced connections between the auditory, limbic and autonomic systems.

Tinnitus Retraining Therapy (TRT) aims at habituation of activation of the autonomic nervous system evoked by signals present in the auditory pathways, i.e., the tinnitus-related neuronal activity, or activity evoked by external sounds. The same systems in the brain are involved in tinnitus and DST, and TRT treats both tinnitus and DST.

Results from 149 consecutive patients seen at Emory Tinnitus & Hyperacusis Center confirmed high prevalence of DST in tinnitus patients. For each patient, during the initial visit the pure tone Loudness Discomfort Levels (LDLs) were evaluated for frequencies from 0.5 to 12 kHz. Then, the average LDL for all frequencies and both ears, was calculated. 62.4% of patients have LDL <100 dB HL, 58.4% <95 dB HL, 38.3% <90 dB HL, and 22.8% <80 dB HL. 65.8% of patients stated that they experience DST. The average LDL for patients reporting DST was 81.7 dB HL, while for patients not perceiving a problem the average LDL was 102.0 dB HL.

There is a common opinion that hyperacusis is presented predominantly in people with normal hearing. Our results, however, showed that 55.5% of patients requiring treatment for hyperacusis have hearing loss as well. At the same time, in the population of patients with hearing loss (N=91) 62.6% reported DST, 57.0% were diagnosed with misophonia, and 29.7% with hyperacusis.

As 30% of tinnitus patients have hyperacusis, 86% of hyperacusis patients have tinnitus, and 4% of general population have clinically-significant tinnitus, thus significant hyperacusis probably exists in at least 1.4%, and DST affects about 3% of the general population.

759 Attenuation of Tinnitus by NMDA Receptor Agonist (Memantine) in Rats.

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The search for methods for tinnitus alleviation led to testing potential mechanisms of its generation and processing of the tinnitus-related neuronal activity within the brain. One of possible mechanisms involve glutamate NMDA receptors. To assess involvement of NMDA receptors in tinnitus, memantine, a strong inhibitor of NMDA-type glutamate receptor has been tested utilizing an animal model of tinnitus on a total of 42 rats.

Tinnitus was induced by daily subcutaneous injection of sodium salicylate (321 mg/kg/day) starting before or after Pavlovian suppression training. The control group received injections of saline. Memantine administration started at the beginning of behavioral procedures and lasted until the end of the experiment. The area between curves depicting process of extinction for groups with daily salicylate administration starting before and after Pavlovian training reflected the behavioral manifestation of tinnitus.

Control groups with salicylate alone showed a clear behavioral manifestation of tinnitus. Memantine in doses 5 and 10 mg/kg/day showed a statistically significant suppression of salicylate-induced tinnitus. Furthermore, the drug-induced tinnitus attenuation depended on the dose of memantine in statistically significant manner ($r=-.9499$, $t[5]=6.16$, $p<0.005$).

These data suggest potential clinical usefulness of memantine in tinnitus treatment, however, the mechanisms of memantine action are still not clear. The drug might act by its documented anticonvulsive property, as it seems that the tinnitus-related neuronal activity might resemble the epileptic activity. Alternatively, the drug might work directly on the medial olivocochlear efferent system innervation of the outer hair cells.

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760 Relationship Between Hearing Loss and Tinnitus in Hamsters Exposed to Intense Sound

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A relationship between hearing loss and tinnitus caused by exposure to intense sound was described by Hallowell Davis and his colleagues in 1950 (*Acta Oto-Laryngol. Suppl.* 88, 1-57). As a first step in determining if the relationship they found in humans can be studied in animals, the degree of tinnitus in hamsters exposed to an intense sound was compared with their hearing loss.

Tinnitus was assessed using a two-choice procedure in which hamsters were trained to go left to left sounds and right to right sounds. Next, their response on trials when no sound was presented (silent trials) was determined. The hamsters were then exposed to a 10-kHz tone at 125 dB for 1 hr and retested. A shift in an animal's responding on silent trials to the side of the exposed ear was interpreted as a sign that the exposure caused the animal to perceive a sound in that ear (i.e., tinnitus). An estimate of hearing loss was obtained using the auditory brainstem response to a noise band that spanned the frequency range of the hearing loss known to follow exposure to an intense 10-kHz tone.

The results revealed a significant correlation between the degree of hearing loss and the number of post-exposure sessions in which an animal showed tinnitus–that is, the larger the hearing loss, the longer the animals demonstrated tinnitus. To further explore the relationship between hearing loss and tinnitus, it will be necessary to determine if the pitch of an animal's tinnitus corresponds to the frequency of their hearing loss as noted by Davis et al.

761 The Therapeutic Effect of Chinese Traditional Medical Formulas on the Treatment of Sensorineural Tinnitus

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Objective: To observe the therapeutic effect of Chinese traditional medical formulas on the treatment of sensorineural tinnitus. Method: Selected 150 cases with chief complaint of sensorineural tinnitus. They were randomized to divide into the treating group of 100 cases with Chinese traditional medical formulas, and the controlling group of 50 cases with masking and Flunarizine. The treating group chose tow kinds of traditional Chinese medical formulas: (1)Tinnitus-‡T formula. Its indication is that occurrence of the tinnitus is not beyond one month, loudness of tinnitus is larger and pitch is generally lower, picture of the tongue is pink or red, feeling of the pulse is rapid or slippery, or namely acute stage. (2)Tinnitus-‡U formula. Its indication is that tinnitus has been beyond one month, loudness of tinnitus is smaller and pitch is usualler higher, picture of the tongue is pale or corpulent; feeling of the pulse is slight or feeble, or namely chronic stage. Dosage and administration: a dose of medicine of formula per one day is decocted in water for oral. The controlling group used masking method and took orally Flunarizine. Result: (1) Full recovery: 40 cases in TG, 7 cases in CG. (2) Improvement: 44 in TG, 17 in CG. (3) Invalidation: 16 in TG, 26 in CG. The results see table. The total effective rate or cure rate was significantly higher in treating group than in controlling group ($p<0.01$). Conclusion: It is obvious, based on the study, that Traditional Chinese Medicine can help to bring the advantages of treatment into full play. The traditional Chinese medical formulas are more effective on the treatment of sensorineural tinnitus.

762 A Sensitive Period for the Development of the Central Auditory System in Children with Cochlear Implants

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We are investigating the time course of deterioration, development and plasticity of the human central auditory system. Our measure of maturation of central auditory pathways is the latency of the P1 cortical auditory evoked response. We have recorded the P1 cortical potential in response to a speech sound /ba/ in 104 congenitally-deaf children who are fit with cochlear implants. Previously, we have reported that that for these children (who for the most part had profound hearing losses) there is a sensitive period of about 3.5 years during which the central auditory pathways remain maximally plastic [Sharma et al., Ear. Hear., 2002]. Cochlear implantation within this sensitive period allows for age-appropriate development of cortical response latencies within 6-8 months of implant use. On the other hand, in children who were implanted after age 7 abnormal P1 latencies were observed even after years of implant use, suggesting that too long a period of deprivation may result in reduced plasticity in auditory thalamo-cortical pathways. In the present investigation we examined the effect of pre-implantation hearing thresholds on central auditory development in late-implanted children. We compared P1 response latencies of: 1) congenitally profoundly deaf children who had very poor aided performance prior to implantation and, 2) children who had better aided hearing thresholds and speech perception scores prior to implantation. Preliminary results show that children with significant aided benefit prior to implantation evidenced age-appropriate cortical response latencies after implantation. These results suggest that auditory stimulation preserves the plasticity of central auditory pathways.

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763 Perioperative, Transtympanic Electric ABR in Pediatric Cochlear Implant Candidates

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Study design: This was a retrospective study including 58 pediatric patients implanted at our facility.

Results:

EABR latencies and thresholds were analyzed as a function of 1) age at implantation, 2) etiology of hearing loss, and 3) preoperative pure-tone thresholds. Wave V (4.44 ms/ 0.53) was present in all implanted ears. Thresholds were relatively elevated in children with post-meningitic deafness. Thresholds for children with congenital cochlear malformations were similar to those obtained on children with normal cochleae. A unilateral absence of response was more prevalent in patients with congenital malformations. There were no significant differences in perioperative, transtympanic wave V latency between younger and older groups In general, lower EABR thresholds were associated with lower preoperative pure-tone thresholds. Comparisons were also made between perioperatively obtained EABR wave V latencies and wave V latencies obtained at different post-implantation time intervals: there were no substantial differences.

Conclusions:

The presence of EABR components indicates that the most peripheral portion of the auditory pathway, extending from the cochlear nucleus complex to the level of the nucleus of the lateral meniscus, is functional in most pre-lingually deafened children, despite their history of reduced or absent auditory input. The presence of a wave V in these patients along with an absence of age effect on wave V latency, confirms that the human auditory pathway is in a high state of maturation at birth. Transtympanic EABR is a useful tool for pre-operative assessment of electric stimulability, particularly with very young children.

764 N1 latencies in NRT measurements of 36 Nucleus 24 Cochlear Implant Patients

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The absence of neuronal stimulation is known to induce neuronal degeneration. The same holds true for the auditory pathway and lacking auditory stimulation.

Since the implementation of neural response telemetry (NRT) in Nucleus 24 cochlear implant system electrically evoked auditory nerve compound action potentials (EABR) can easily recorded. Thus, NRT recordings can be used to monitor changes of the auditory pathway.

In our retrospective study of 36 patients implanted between 2000 and 2002 with a Nucleus 24 device, we analysed perioperative NRT measurements in relation to age of the patient, duration of deafness (or hearing loss) and origin of hearing loss.

We found primarily changes in N1 latency. These findings could be indicative of an ongoing neuronal degeneration. In our view, NRT recordings can contribute substantially to a more objective monitoring of neuronal changes in cochlear implantation which goes far beyond a testing of the integrity of the system.

765 The Effect of Recording Electrode Position on the Electrically Evoked Compound Action Potential

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The electrically evoked compound action potential (ECAP) can be recorded from human cochlear implant users and research animals. In humans, the ECAP is recorded from intracochlear electrodes using clinical telemetry systems. With animals, the ECAP can be recorded from intracochlear sites or electrodes positioned on the nerve trunk. The latter position provides superior recordings, enhancing the research utility of the ECAP. As part of our overall research program, we were interested in unifying animal and human data by recording ECAPs from both intracochlear and extracochlear sites using a within-animal design. While it is known that recording electrode location influences ECAP amplitude (Finley et al., 1997; Abbas & Brown, 2000), the effect of stimulus level has not been systematically examined. We hypothesize that, given the spatial attenuation of both stimulus and response currents, stimulus level will interact with the effects of recording electrode location.

We recorded ECAPs from acutely deafened cats using an 8-band Nucleus-type array. ECAPs were recorded from various intracochlear electrodes and sites located on and near the nerve trunk. Our present findings indicate that recording electrode position strongly affects both ECAP amplitude and growth. Specifically, the intracochlear sites produce ECAPs that, relative to ECAPs from nerve-trunk sites, overemphasize ECAP potentials recorded at low stimulus levels. We also have observed, in some preparations, that the degree of this emphasis varies in a graded manner with the intracochlear separation of the stimulating and recording electrodes. In addition to these findings, we will compare how ECAP amplitude profiles across recording sites vary with stimulus electrode configuration, as we hypothesize that site-related differences in ECAP amplitude and growth depend on the degree to which stimulation is focused within an intracochlear region.

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766 Effect of Stimulus Level on Cross-Turn Stimulation in Clarion CII Cochlear Implant Users

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There is evidence that, in cochlear implant users, high level electrical stimulation of intracochlear electrodes near the middle of the array may result in the stimulation of axons from more apical turns of the cochlea, or cross-turn stimulation (Frijns et al., 2002). Responses from these stimulated axons are reflected in the electrically evoked compound action potential (ECAP) amplitude recorded from apical electrodes. The Clarion CII allows for recording of the ECAP from the intracochlear electrodes via Neural Response Imaging (NRI). NRI uses a single electrode for stimulation and a second electrode for recording the ECAP response. In this study, we examined the potential for cross-turn stimulation at stimulus levels within the behavioral dynamic range of adult cochlear implant users. Medial intracochlear electrode 7 was stimulated with 32 μs biphasic pulses. Stimulus artifact was rejected using the alternating polarity paradigm. Three stimulus levels were chosen (high, medium and low) relative to the subjects' behavioral dynamic range and ECAP amplitude. As expected, larger ECAP amplitudes were recorded from electrodes near the stimulating electrode. Within subjects, the ECAP response configuration was consistent across stimulus levels. A comparison of the apical ECAP response patterns and stimulus level indicated that cross-turn stimulation did not occur within the subjects' listening ranges for this sample.

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767 Long-Term Deafened Gerbils: Relationship between Cochlear Physiology and Spiral-Ganglion-Cell Survival

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The overall focus of our research is to describe the electroanatomy of the cochlea, and to determine the effect of that electroanatomy on current-field distributions in the electrically stimulated cochlea. The literature suggests that neural differences exist among normal animals, acutely deafened animals, and long-term deafened animals. We hypothesize that cochlear electroanatomy also may be significantly different in the three groups as a result of varying patterns of tissue degeneration.

As a prerequisite to studies of current flow in electrically stimulated cochleae, cochlear potentials and spiral-ganglion-cell densities were assessed in normal-hearing gerbils and in gerbils four weeks after narrowband noise exposure. Round-window electrodes were used to measure compound-action potential (CAP) thresholds and tuning curves, summating potentials (SP) and cochlear microphonics (CM). After each physiologic experiment, cochleae were fixed, embedded, and sliced along the modiolar axis in four-micrometer sections.

Results show a 40-dB elevation of CAP thresholds from 8 to 16 kHz and above 30 kHz in noise-damaged animals compared to normal animals. Significant changes in SP and CM amplitude and polarity also are evident in the deafened animals. Histology shows a 50% reduction in spiral-ganglion-cell (SGC) density in the upper basal turn, which corresponds to the region of threshold shift. Further experiments will examine the relationship between electrically evoked CAP responses and SGC survival in normal and noise-damaged gerbils.

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768 Optimizing Multichannel Cochlear Implant Electrodes

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Studies conducted in neonatally deafened cats have demonstrated significant neurotrophic effects of electrical stimulation delivered by a cochlear implant. Several months of chronic stimulation promotes significantly increased survival of cochlear spiral ganglion neurons. The extent of neurotrophic effects depends upon variables such as duration of deafness and of stimulation, and frequency and/or complexity of electrical signals. In contrast, intracochlear trauma due to electrode insertion causes neural degeneration in damaged areas and offsets neurotrophic effects. Thus, an important goal for continued improvement of clinical cochlear implants is design of intracochlear electrodes that can be reliably inserted deeply into scala tympani without trauma.

The newer perimodiolar electrodes are designed to place stimulating contacts closer to the spiral ganglion, thereby reducing thresholds and

channel interactions. Our temporal bone studies show that the new Contour™ electrode inserts more deeply (mean depth, 18.0 mm or 419°) than the original banded electrode (mean, 15.3 mm or 285°) and positions contacts closer to the modiolus; but excursion into scala vestibuli occurs in about 30% of insertions with both these designs. The HiFocus II™ electrode also consistently places contacts closer to the modiolus than its predecessor, the Spiral Clarion™ electrode. Interscalar excursion is rare with the HiFocus II™ but severe insertion trauma occurs if it is fully inserted. In contrast, when the surgeon feels resistance and stops insertion at about 70% (mean, 16.3 mm or 333°) of full depth, the HiFocus II™ is virtually atraumatic. Findings have identified several key factors for optimizing results, including position and size of the cochleostomy, size/shape of the intracochlear electrode, and ratio of stiffness in the vertical vs. spiral axes of arrays.

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769 Experimental Prototype of a Modified Cochlear Implant Electrode for Drug Delivery to the Inner Ear

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Cochlear implants (CI) represent the treatment of choice in most cases of deafness. However, there is still great variability for the individual success. Following deafness, reduced numbers of spiral ganglion cells (SGC) most likely account for the decreased benefit of CI. Preserving the number of SGCs is thus a promising approach towards enhancing the effectiveness of CI treatment. Recently, several animal studies demonstrated the protective effect of neurotrophic factors (NF) on SGCs. We therefore investigated the possibility of modifying a CI electrode for the purpose of drug delivery to the cochlea. We used a modified Contour™ electrode array because this device already has an inbuilt lumen for the stylet, enabling the device to be straightened prior to insertion. To determine the basic requirements for a local drug-delivery device for the human inner ear, we cut the tip of the Contour™ electrode array to open the lumen of the array, and a connecting piece was developed to connect the electrode to a pump. The feasibility of using the array for drug delivery was tested using both an Alzet mini-osmotic pump and a mechanical pump. Results indicated that both the device and the connector element were fluid-tight and the diameter of the electrode lumen was sufficient to transport water-soluble substances through the electrode. The connection was also tested for its stability in terms of leakage and resistance to tractive forces. The connection to the pump was sealed for all tested pump rates and resisted tractive forces of up to 50 N. The system was also applied to temporal bones to evaluate its applicability to the human cochlea, showing that the modified Contour™ electrode is easy to handle in temporal bones and can be used to simulate drug delivery to the inner ear. The modified electrode described could provide a safe and easy-to-handle means of combining electrical stimulation with the beneficial effects of a local drug therapy applied to the inner ear.

770 Protective Effects of Electrical Stimulation and Neurotrophin Delivery on Auditory Neurons *in vivo*: Implications for Cochlear Implants

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A sensorineural hearing loss initiates ongoing degeneration of spiral ganglion neurons (SGNs). Reducing or preventing SGN degeneration has important clinical implications for cochlear implant subjects. We describe an electrode array capable of simultaneously delivering neurotrophic agents into the cochlea while electrically stimulating SGNs. In this study we examine the effects of chronic electrical stimulation and neurotrophin delivery on SGNs. Adult guinea pigs were deafened using an aminoglycoside and loop diuretic. Five days later, after confirming the animal was deafened, a scala tympani electrode array and drug delivery system was unilaterally implanted. An osmotic pump, designed to deliver 200 µl of either brain derived neurotrophic factor (BDNF) or artificial perilymph (AP) over a 28 day period, was connected to the electrode delivery system. Electrically evoked auditory brainstem responses (EABRs) were recorded post-operatively and the animal commenced a chronic electrical stimulation (ES) program. Charge balanced biphasic current pulses were delivered to bipolar electrodes at an intensity of 6 dB above EABR threshold. Thirty-three days after deafening, EABRs were again recorded and the cochleae harvested for histology. The extent of neural survival was determined by measuring SGN density and soma area in each cochlear turn. SGN survival in BDNF/ES cochleae were typically twice that observed in AP/ES treated or deafened control cochleae ($p<0.001$). Importantly, we observed no advantage of SGN survival in AP/ES treated cochleae over deafened, unstimulated controls. Finally, BDNF/ES treated cochleae typically exhibited significantly greater SGN soma area compared with AP/ES or deafened controls. This work demonstrates a significant advantage of ES combined with neurotrophin administration, over ES alone.

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771 Effects of Combination Treatment with Antioxidants and GDNF on Auditory Function in Deafened Guinea Pigs

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The benefit of cochlear prostheses depends largely on the survival and responsiveness of auditory neurons in the deaf patient. We have recently shown that local treatment with neurotrophins (BDNF and CNTF, Shinohara et al., Proc. Natl. Acad. Sci. USA 99:1657-1660, 2002) and systemic treatment with Trolox (vitamin E) and ascorbic acid (vitamin C; Maruyama et al., ARO, 2002) significantly preserved the electrical sensitivity of the auditory nerve and spiral ganglion cell (SGC) survival in the deafened guinea pig. The purpose of this study was to investigate effects of a combination therapy with antioxidants (Vit C + E) and the neurotrophic factor GDNF against auditory nerve degeneration and responsiveness in deafened guinea pig.

Guinea pigs (n=32) were divided into 4 groups. All were implanted with scala tympani cannulae-osmotic pumps and intracochlear Pt-Ir stimulating and epidural recording electrodes. Three groups were deafened by intracochlear neomycin, while the normal hearing group received artificial perilymph (AP). After deafening, the animals received 4-week treatments of either GDNF alone, GDNF + antioxidants, or AP; (GDNF, 10µg/ml, 0.5µl/hr via osmotic pump; Trolox, 1mg/kg + ascorbic acid 20mg/kg, IP.). Electrically-evoked auditory brainstem responses (eABR) were recorded on day 3 and then weekly to 6 weeks.

The eABR thresholds increased and remained elevated in untreated deafened subjects, while in the treated, after an initial increase, the eABRs decreased 2 weeks after deafening. There were statistical significant differences between the treated groups and the untreated group at 1 and all subsequent weeks after deafening. The GDNF + antioxidants group showed significantly lower thresholds than the group receiving GDNF alone at 1, 3, 4, 5 and 6 weeks after deafness. Thus, a combination therapy with antioxidants and GDNF demonstrated excellent positive effects on the electrophysiological responsiveness of the inner ear.

772 Cochlear Implant Electrodes can be Coated with Cells Expressing Transgenes

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Cochlear implantation is an important method of rehabilitating patients with severe sensorineural hearing loss. The satisfactory function of cochlear implants depends in part on a healthy population of spiral ganglion cells. In an attempt to reduce neural structure degeneration in the deafened cochleae we have coated the electrodes of cochlear implants with cells that can be used to express and secrete transgenes. This will provide a tool allowing targeted delivery of neurotrophic growth factors within the cochlea. Using the guinea pig animal model we have developed two methods of coating cochlear implant electrodes. In method one, cultured fibroblasts are attached directly to the electrode (made of a platinum iridium alloy). When using primary cultured cells (autograft), reporter gene expression for at least 22 days has been demonstrated *in vivo*. In method two the cells are suspended in agarose. The agarose is coated on to the electrode. The advantages of the second method include the ability to coat the electrode with a larger number of cells (thousands rather than hundreds). In addition the agarose provides a degree of immunoprotection allowing allogeneic cell transplants and precluding the need for individual primary culture in each recipient animal. With the second method using allogeneic cells, we have demonstrated reporter gene expression for at least 7 days and cell survival for at least 19 days *in vivo*. The above system will allow directed delivery of *ex-vivo* gene therapy with growth factor over-expression in combination with electrical stimulation, thereby enhancing the function of the cochlear implant.

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773 Effects of Semiconductor Substrate on Cochlear Nucleus Neurons in vitro

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Implantable hearing aids such as cochlear implants (CI) and auditory brainstem implants (ABI) provide auditory information by electrical stimulation of auditory neurons. The combination of microelectronics with auditory nerve cells may lead to further progress for the hearing quality by these devices. Whereas several kinds of neurons are known to grow on semiconductor substrates, interactions of cochlear nucleus (CN) neurons with such materials have yet to be described.

To investigate survival and growth behaviour of CN neurons on different semiconductors, CN explants from post-natal day 10 sprague-dawley rat were cultured for 96 h in neurobasal media on polished and unpolished silicon wafers (p-type Si {100} and p-type Si3N4 {100}) as well as plastic surface. These surfaces had been coated with poly-L-lysine and laminin. Neuron outgrowth was investigated after immunohistological staining for neurofilament by image analysis to determine neurite extension and directional changes.

No neurite growth was observed on unpolished silicon wafers (Si and Si3N4). On laminin coated, polished Si3N4, CN neurons reached the highest extent of outgrowth, with an average length of 290 µm and a mean of 8 neurons per explant, compared to 251 µm and 5 neurons on laminin coated pure silicon, 209 µm and 3 neurons on plain Si3N4, 132 µm and 5 neurons on plain silicon and 181 µm and 6 neurons on plastic.

The results of this study show the general possibility of CN neuron growth in culture on semiconductors. The differences in neuron length and counts per explant indicate that the growth of CN neurons is influenced by the semiconductor substrate as well as extracellular matrix proteins with laminin coated p-type Si3N4 {100} being the preferable material for future hybrid experiments on auditory-neuron-semiconductor chips.

774 Auditory Nerve Responses to Electric Pulse Trains following Treatment with Furosemide

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The influence of functional hair cells on electrical stimulation of the auditory nerve is an important issue, as individuals with significant residual hearing are now cochlear implant candidates. Our previous work has shown that animals treated with furosemide or a combination of kanamycin and ethacrynic acid demonstrate similar changes in auditory nerve responses to electrical stimulation. This study focused on the time course of such changes as well as the recovery process following injection of furosemide, which is known to have reversible effects on hearing. Hair-cell functionality was continuously monitored with the click- evoked compound action potential recorded by an electrode positioned near the auditory nerve. Auditory nerve responses to single biphasic electric pulses and biphasic electric pulse trains delivered by a monopolar intracochlear electrode were assessed over time. These measures demonstrated a clear relationship between the state of hair-cell function and electric auditory responses, as changes in electric responses coincided with the loss or recovery of acoustic sensitivity. Electric growth functions demonstrated an increase in slope, and the magnitude of saturated response tended to increase. Both trends were reversible with hearing recovery. Responses to electric pulse trains showed several reversible trends with deafening: (a) the magnitude of first-pulse response increased, (b) the magnitudes of response alternation tended to increase, (c) the degree of adaptation increased, and (d) the degree of refractoriness tended to increase. These results suggest that, with the presence of functional hair cells, auditory nerve responses to electric stimuli are less synchronized. Viable hair cells may therefore provide positive effect on auditory response to electric stimuli for implant patients with residual hearing, as they may enhance stochastic properties of the stimulated nerve.

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775 Adaptation in the Auditory Nerve in Response to a Continuous Electric Pulse Train

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Cochlear implants typically use modulated pulse train stimuli. Such stimuli may cause adaptation of the auditory nerve's response and thus influence the encoding of speech information. Using acutely deafened guinea pigs and the electrically evoked compound action potential (ECAP), we have investigation adaptation in response to pulse train stimuli. The use of a deaf animal model allowed us to investigate neural adaptation independent of effects produced by the hair cell and synapse. A single electrode was placed within the cochlea for stimulation and a second electrode was placed on the auditory nerve to record the electrically evoked action potential (ECAP). Stimuli consisted of trains of biphasic pulses where the interpulse interval (IPI) and the amplitude were varied. Results showed a decrement in the ECAP for IPIs of 12 ms to 1 ms over a recording time of 30 to 120 seconds. For IPIs of 50-100 ms, no adaptation was observed. The data obtained were fit by function with two decaying exponential

components, one fast (approximately 0.05 to 1 sec) and a second slower component (approximately 5 to 20 sec). Since the animals are deafened, the hair cells are not functional, therefore the adaptation observed likely stems from processes within the spiral ganglion neuron.

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776 Effects of Simultaneous Electric and Acoustic Stimulation (EAS) on Temporal Response Patterns of Cat Single Auditory Nerve Fibers

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In a previous study auditory nerve fiber responses of normal hearing cats to combined electrical and acoustical stimulation were studied with respect to spectral response properties (ARO Abstr.2001, 908). The present study is focused on the question whether temporal response patterns evoked by electrical stimulation are influenced by a simultaneously applied acoustical stimulus and vice versa.

Acute experiments were performed in adult normal hearing cats implanted with round window electrodes used for extracochlear electrical stimulation. Electrical stimuli were continuous sinusoidal low frequencies (125-250 Hz). For acoustic stimuli tone-bursts of 50-100 ms duration and different frequencies and intensities were used. After determination of the CF of a fiber a combination of acoustical tone bursts at CF and electrical sinusoids (phase locked to acoustic burst onset) 6dB above single fiber threshold served as stimuli. The PSTH, interval histogram and synchronization index (SI) were calculated for each EAS response and compared to responses evoked by single stimulation condition.

Evaluation of SIs generally suggests a slight to prominent decrease of synchronization to the electrical sinusoid with EAS. While in most cases an electrically evoked response still exists with EAS some fibers showed a total suppression of the electrical response by the acoustic stimulus. Switching off the acoustical stimulus led to a highly synchronized response pattern to electrical stimulation. Synchronization to the acoustical stimulus of phase-locking fibers was less deteriorated by the electrical stimulus. These results suggest that the acoustic response is less affected by the electrical stimulus than vice versa. The activity of hair cells may play the dominant role in generating the EAS responses.

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777 Binaural Interactions with Bilateral Electric Stimulation of the Cochlea: Evoked Potential and Single-Unit Measures

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While bilateral cochlear implantation is becoming increasingly common, little is known about binaural interactions in auditory neurons with such stimulation. To investigate this problem, we recorded evoked and single-unit responses in the inferior colliculus (IC) of acutely deafened, anesthetized cats to electric stimulation through intracochlear electrodes implanted bilaterally. Electrically-evoked auditory brainstem responses (EABR) to a biphasic pulse (50 μs/phase) were measured for both binaural stimulation and monaural stimulation of each ear. The binaural interaction component (BIC) was computed by subtracting the sum of the monaural responses from the binaural response.

BIC amplitude varied systematically with both interaural time and level differences (ITD and ILD). The BIC was maximal with zero ITD and an ILD close to the interaural difference in EABR thresholds. When pulse thresholds of single IC neurons were measured in the same animals, the average interaural threshold difference also matched the ILD that maximized the BIC.

Single-unit responses in the IC to low-rate (< 80 Hz) trains of biphasic pulses were strongly phase-locked and were sensitive to ITD over a limited range of current levels. ITD tuning broadened with increasing level and could saturate at 2-3 dB above threshold for some units. Higher-rate pulse trains typically only elicited onset responses. Responses to sinusoidally amplitude modulated pulse trains with a carrier rate of 1 kHz and modulation frequencies of 10-100 Hz were also recorded in some neurons. Most cells showed sensitivity to interaural differences in modulation phase over a range of modulation frequencies.

Overall, electric ITD tuning in some IC cells was as sharp as that seen in acoustic responses. However, dynamic range and maximum frequency of effective electric stimuli were limited.

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778 *In Vivo* Analysis of Intracochlear Electrode Placement Using the TACT® System

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In cochlear implants, variation in placement of the electrode array relative to the target spiral ganglion cells is thought to play a significant role in accounting for the widely reported variations in inter-subject performance. Current imaging protocols permit estimation of the longitudinal location of electrode contacts, but provide little information concerning the location of the electrode contacts relative to the modiolar wall. Tuned-Aperture Computed Tomography (TACT®) generates 3-D images from two-dimensional projections of unknown geometries. The present study employed the TACT® system to image intracochlear electrode arrays in a group of long-term implanted behaviorally-trained cats. The goal of this work was to determine the extent to which 3-D TACT-generated images could be used to estimate the placement of intracochlear electrode contacts relative to various cochlear structures, *in vivo*. Using TACT®, significant electrode and anatomical detail could be visualized, including the course the electrode wires and location of the all electrode contacts within scala tympani. Manipulation of 3-D images allowed estimation of the relative location of each contact within the scala. Estimations of contact position will be compared with psychophysical thresholds from the same animals.

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779 Pitch Judgements Made by Auditory Brainstem Implant Users

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An important goal when fitting an Auditory Brainstem Implant (ABI) is to obtain an appropriate rank ordering of electrodes, such that higher-frequency bands of speech are encoded on electrodes that evoke progressively higher pitch percepts. Unfortunately, the clinician does not know in advance what the correct ordering should be. As more than 3 million orderings are possible when 10 or more electrodes are active, this presents the clinician and patient with something of a challenge.

We are investigating methods of helping the clinician to find the appropriate ordering quickly and accurately. The Steinhaus procedure is designed to determine the proper ordering of an initially unordered set of items in a highly efficient manner. Our computer simulations of the responses of ABI users indicate that the Steinhaus procedure could provide both a greater degree of accuracy and require many fewer patient responses than a procedure presently used in clinical practice. Examination of the model parameters and the real-world validation of the procedure are ongoing. In addition, data from a large number of users (Otto et *al.*, 2002) reveal a modest correlation between electrode position and perceived pitch, and we are evaluating ways of incorporating this information into the fitting strategy.

780 Enhancing Temporal Cues To Voice Pitch Available Through Cochlear Implant Speech Processors

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Uncertainty surrounds the ability of users of continuous interleaved sampling (CIS) cochlear implants to perceive the changes in speech fundamental frequency (F0) that represent intonation. In principle, temporal envelope cues to pitch are available in CIS processed speech. However, such cues may be of little utility for F0s at the higher end of the voice pitch range, and, regardless of F0, may be limited due to the complexity of the stimulating waveforms. An acoustic simulation of CIS processing was used to investigate possible ways of clarifying temporal cues to voice pitch. Synthetic diphthongs with monotonic glides in F0 were processed by a noise-excited vocoder. Overall channel levels were controlled by the slow-rate spectral dynamics of the diphthongs, while the temporal structure representing F0 was simplified to be either sinusoidal or a modified sawtooth waveform. Glide F0 was varied about center frequencies of 141, 199, and 282 Hz. Normal listeners labeled the direction of pitch movement. For both types of modulation, performance steadily decreased as center F0 increased. Therefore, pitch information can be derived from temporal envelope cues, but the utility of these cues is limited for higher voice pitch ranges. For all glide center F0s, performance was better with modified sawtooth rather than sinusoidal modulation, suggesting that the salience of temporal pitch cues can be enhanced by temporal sharpening of the modulation envelope. However, initial data from implant users only partially support these conclusions. In tests where modulation frequency was discriminated, performance generally decreased with increasing frequency, but there was little evidence of an effect of modulation waveform shape.

781 Comparison of Forward Masking Pattern for Different Stimulation Modes in Cochlear Implant

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Comparisons were made between monopolar (MP) and bipolar (BP) stimulation modes in a Nucleus® 24 cochlear implant. The BP mode is usually hypothesized to be subject to channel interaction to a less degree than the MP mode, as the BP mode induces a more narrowly tuned neural excitation pattern than the MP stimulation. To test this hypothesis, masked thresholds in the forward masking paradigm by MP and BP stimulation were compared. The masker was 500 ms in duration and presented on electrode 7. The probe with a short duration (50 ms) was presented with a delay of 20 ms after the offset of the masker and the level of probe was adaptively adjusted (2-down, 1-up, 3 alternative forced choice) to determine the masked threshold. The probe stimulation was delivered to a series of electrodes to obtain the masking pattern across the masker-probe proximity. Two levels (soft and medium loud) of the masker were determined and, for each level, the loudness was balanced between the MP and BP mode.

The results with 4 subjects to date show that, at the medium level of masker, the masking patterns for both modes are similar—a clear peak around electrode 7. At the soft level of masker, however, the BP mode in general produces a broad masking pattern, whereas the MP mode produces a clear masking pattern. At both levels, the amount of masking was generally greater with the BP mode than the MP mode. Contrary to our expectation, the BP mode does not create a masking pattern significantly sharper than that of the MP mode when the two modes are loudness-matched. One possible explanation is the effect of high specificity in the BP mode might have been compromised by the high current (or high amount of charge) chosen to match the loudness of the MP stimulation. Based on the present findings, the use of BP is not recommended at least at a soft level to reduce channel interaction, as it appears that MP mode leads to better channel specificity.

782 Further Investigations of a Possible Supra-Threshold Stochastic Resonance Phenomenon in Cochlear Implant Listeners

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Cochlear implant listeners' ability to detect modulation deteriorates with decreasing carrier level and increasing modulation frequency. We have previously found that at low carrier levels, modulation detection in cochlear implant listeners can be enhanced with the introduction of an optimal amount of external noise into the carrier envelope, an effect suggestive of stochastic resonance (JARO 2(2): 159-171). The effect was stronger at higher modulation frequencies. Originally, we had used a multiplicative form of noise (numbers drawn from a uniform random distribution scale the amplitudes of successive pulses in the carrier). We have since found that multiplicative and additive noise (noise linearly added to or subtracted from carrier pulse amplitudes) have identical effects on modulation detection. We have also found that we can "induce" the noise-related enhancement in modulation sensitivity at a higher carrier level by concurrently introducing an off-channel masker. Such a masker can degrade modulation detection even at a higher carrier level. The introduction of noise into the signal carrier envelope in this situation can improve modulation detection thresholds significantly. Taken together, our results suggest that a low-level signal carrier is not necessary to produce the stochastic resonance effect. The only necessary condition is that the "modulation signal" be weakened -- by reducing carrier level, by increasing modulation frequency, or by introducing a masker on another channel. We hypothesize that the effect occurs at a relatively central level of processing. A model incorporating an expansive nonlinearity, a lowpass envelope extraction stage, an internal noise source, and a bistable modulation processor is being implemented to further our understanding of the processes involved.

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783 Relative Contributions of Spectral and Temporal Cues for Phoneme Perception as Revealed by Acoustic Simulations of Cochlear Implants

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Cochlear implants provide users with limited spectral and temporal information. In order to study the relative contribution of spectral and temporal cues on phoneme perception, we systematically varied the amount of information presented through acoustic simulations of cochlear implants. The spectral information was controlled by varying the number of frequency bands between 1 and 16 (i.e., 1, 2, 3, 4, 6, 8, 12, and 16). The temporal information was controlled by varying the lowpass cutoff frequencies (LPFs) of the envelope extractors from 1 to 1024 Hz in octave steps (i.e., 1, 2, 4, … , and 1024 Hz). Twenty consonants in a “Ca” context and 12 vowels in an “hVd” context were processed using these 88 (8 x 11) spectral and temporal conditions. The processed speech materials were then presented to 11 normal-hearing subjects for phoneme identification. The results showed that for vowel identification, the performance improved as a function of number of channels up to 12. There were no significant changes in performance when the LPFs were >4 Hz. For consonant identification, the performance also improved as a function of number of channels up to

12. There were no significant changes in performance when the LPFs were >32 Hz. In the range of LPFs between 1 and 32 Hz and number of channels between 1 and 12, there was a trade-off between the LPF and number of channels. That is, in those ranges, the spectral information could compensate for the diminished temporal information, and vice versa. These data indicate that phoneme recognition is dominated by the contribution of the spectral cues. Minimal temporal cues are required for vowel (LPF <4 Hz) and consonant (LPF <32 Hz) recognition. These results are in contrast to our report of contribution of temporal and spectral cues for tone recognition in which we found that, over a fairly large range of conditions, both temporal cues and spectral cues contribute to tone recognition.

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784 Frequency Modulation Detection in Cochlear Implants Listeners

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Although amplitude modulation detection has been extensively studied in both acoustic and electric hearing, frequency modulation detection has been rarely studied in electric hearing. Here we systematically studied the cochlear implant listeners' ability to detect three types of frequency modulations including, upward sweep, downward sweep, and sinusoidally frequency modulated stimuli. Difference limens (i.e., 70.7% correct response in a 3IFC, 2-down and 1-up procedure) were measured as a function of baseline frequency (from 75 to 1,000 Hz). Factors studied included electrode position (apical vs. basal), stimulation level (soft vs. comfortable), and modulation frequency (from 5 to 320 Hz dependent on the baseline frequency). Three postlingually deafened adults using Nucleus-22 cochlear implant participated in the experiment. For comparison, similar data were also collected in normal hearing listeners. Preliminary data showed an insignificant effect of electrode position and stimulation level but a significant effect of baseline frequency and modulation type on frequency modulation detection. Consistent with previous data in simple rate discrimination, difference limens in detecting all three types of frequency modulation increased monotonically as a function of the baseline frequency. Despite of large individual variability, difference limens for the sinusoidally frequency modulation were about half of that for the upward and downward frequency sweeps. The present data suggest that cochlear implant listeners may be more sensitive to dynamic frequency changes than steady-state frequency changes. We hope to explore this difference to dynamically encode the temporal fine structure in speech and music sounds for cochlear implant users.

785 Autoimmune Mouse Antibodies Recognize Multiple Antigens Proposed in Human Hearing Loss

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Serum antibodies of hearing loss patients recognize several proteins that are proposed as possible antigenic targets in the ear. This often leads to a clinical diagnosis of autoimmune inner ear disease, although it is not clear how these antibodies impact the ear. Therefore, to better understand the relationship of autoantibodies and ear disease, an examination was made of serum autoantibodies in the MRL/MpJ-Faslpr (MRL) autoimmune mouse with hearing loss. It was hypothesized that autoimmune disease mice with hearing loss will have autoantibodies against the various cochlear antigens proposed in clinical autoimmune inner ear disease. Similar antibody patterns in the mouse would provide an animal model in which to investigate potential autoimmune mechanisms of this hearing disorder.

Sera from MRL autoimmune mice and normal C3H mice were tested by the ELISA technique for reactivity against various reported cochlear antigens. These included heat shock protein 70 (bovine, human, bacterial), laminin, heparan sulfate proteoglycan, cardiolipin, and collagen types II and IV. The autoimmune mouse sera showed significantly greater antibody reactivity against all of the antigens when compared to normal mouse sera.

Serum antibodies from autoimmune mice recognized several putative autoantigens reported for patients with hearing loss, suggesting comparable antigen-antibody mechanisms may be operating. However, the recognition of multiple antigens did not identify any one as being the specific target in autoimmune hearing loss. Nevertheless, the parallel between antibodies in the MRL autoimmune mouse and human studies demonstrates the value of this mouse model for investigations of potential cochlear antigens in autoimmune inner ear disease.

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786 The Patulous Eustachian Tube - New Aspects of the Tubal Mechanics

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Objectives: The role of the paratubal muscles, especially in cases of a patulous Eustachian tube, still is unclear. The aim of this study was to develop a new dynamic model of the muscular compliance of the tube.

Methods: High-resolution cross-sectional T1-MRI data of the first author`s paratubal structures were used creating a new functional 3D-model of the Eustachian tube and its related structures visualized by the Hamburg VOXEL-MAN digital image system. This 3D-model was compared to the MRI-findings of patients with the clinical presentation of a patulous tube.

Results: Functional 3D-reconstructions of the paratubal structures reveal that the medial pterygoid muscle is acting as a movable hypomochlion of the tensor veli palatini muscle. Contraction of the medial pterygoid muscle increases, relaxation decreases the opening pressure of the distal part of the tube. MRI-observations in patients with a clinically patulous Eustachian tube underline the role of an atrophic pterygoid muscle as one cause of this syndrome.

Conclusion: The influence of the medial pterygoid muscle upon the opening pressure of the Eustachian tube gives a new aspect on the understanding of tubal pathophysiology.

Key words: Eustachian tube - muscular compliance - medial pterygoid muscle – tensor veli palatini muscle – movable hypomochlion

787 Detection of Labyrinthine Fistula in Human Temporal Bone By Threshold Variation on Three Dimensional CT Scan

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Detection of small labyrinthine fistula is difficult by imaging, and may require surgical exploration. The aim of this study was to evaluate the sensitivity of the three dimensional (3D) CT scan with threshold variation in the detection of labyrinthine fistula.

Five human temporal bones were studied. Temporal bones were labelled. In 4 specimens, after a radical mastoidectomy, the bony labyrinth was drilled in the postrior, superior, and lateral semicircular canals, and the basal turn of the cochlea. In these locations, calibrated holes with diameters ranging from 0.8 to 0.3 mm were created. On the last temporal bone serving as control, a radical mastoidectomy without experimental fistula was performed. A CT scan (General Electric, Light ultra speed) was performed on each temporal bone before and after

dissection in a simple blind manner by the radiologist. Axial sections of 0.5 mm thickness with a spiral acquisition were obtained. Data were processed by 2 in-build protocols: virtual endoscopy, and surface rendering by threshold variation.

With these 2 processing protocols, holes of 0.4 mm could be detected. No false positive images were observed in these anatomical locations. This method yields a significant improvement in detection of labyrinthine fistulas, and can be used in a routine manner.

788 Superior Semicircular Canal Dehiscence Increases Sensitivity to Bone Conduction in Chinchillas

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Superior Semicircular Canal Dehiscence (SSCD) Syndrome is a recently defined clinical disorder primarily associated with vertigo induced by loud sounds, but also including heightened sensitivity to bone conduction stimuli and reduced sensitivity to air conduction. Developing a better understanding of the mechanisms leading to increased sensitivity to bone conduction stimuli will lead to better understanding of the syndrome as well as potentially aid in the creation of better diagnostics of SSCDs and provide insight into the third window hypotheses of Ranke and Bekesy.

The effect of SSCDs on the response to bone conduction stimuli was evaluated in seven chinchillas. Induction of a SSCD produced an increase in cochlear sensitivity to bone conduction (as measured by cochlear potential). Attempts were then made to seal the dehiscence and restore the initial pre-SSCD condition. Only partial reversal of the SSCD induced increases in CP were seen upon plugging the dehiscence with metal rods and jeltrate. As a supplement to the physiological data, a mathematical model of bone conduction and the potential effects of SSCD was also created. This model, based on the acousticomechanical properties of the ear provides insight into the phenomena of bone conduction and the effect of SSCD.

789 Hydroxyapatite Cement Use in Ossicular Reconstruction

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Objective: To examine the audiometric results and complications when using hydroxyapatite cement for ossicular reconstruction in patients where traditional prostheses failed to accomodate the middle ear anatomy.

Methods and materials: A retrospective chart review of four patients undergoing ossicular reconstruction with Mimix (Walter Lorenz Surgical) hydroxyapatite cement between the years 1999-2002 was completed. Ossicular reconstruction came in two forms: partial reconstruction from the stapes superstructure to the tympanic membrane and an incus strut graft extending from the stapes superstructure to the malleus. A middle ear risk index (MERI) score was calculated for each patient.

Results: A total of four patients underwent hydroxyapatite cement ossicular reconstruction. The average follow-up was 10 months. The average MERI score was 2. The average pre-op air-bone gap was 18dB. The average post-op air-bone gap was 6dB. There were no extrusions. All ears remained dry throughout the follow-up period.

Conclusion: Hydroxyapatite cement can be used to mold an ossicular prosthesis in patients undergoing reconstruction when traditional prostheses fail to accomodate the middle ear anatomy.

790 Phenotypic Presentation of Children With Non-Syndromic Sensorineural Hearing Loss and Incidence of Connexin 26 Homozygosity

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This report describes the presentation of children with nonsyndromic sensorineural hearing loss (SNHL) to a multidisciplinary clinic. The patient population consisted of 33 boys and 29 girls from one to 16-years of age. All patients underwent a comprehensive evaluation including examinations of prenatal/antenatal risk factors, family history, history of present illness, physical exam, metabolic testing, high-resolution temporal bone imaging, physchoacoustic audiograms, tympanograms and DPOAEs. In addition, each patient received a genetics evaluation and testing for Connexin 26 and A1555G mitochondrial mutations. Eight-percent of these 62 patients tested homozygous positive for Connexin 26. All of these homozygous patients presented with bilateral hearing loss, compared to a rate of 32% unilateral hearing loss in the non-homozygous group. In regard to stability, 80% of the homozygous population presented with stable hearing loss and 20% demonstrated a progressive form. Similarly, 74% of the non-homozygous Connexin 26 patients presented with stable hearing loss, 16% exhibited progressive forms and 10% had fluctuating hearing loss. Assessment of severity found 60% of the patients in Connexin 26 population possessed moderate SNHL and 40% had profound SNHL. By comparison, 26.32% of the remaining population presented with mild SNHL, 24.56% with moderate loss, 24.56% with severe loss and 24.56% with profound loss. None of the homozygous Connexin 26 patients presented with inner ear anomalies while high resolution CT scan demonstrated inner ear malformations in 16% of the remaining population. In conclusion, homozygous Connexin 26 patients possessed greater rates of bilateral hearing loss, a similar incidence of stable hearing loss, more often increased severity and decreased rate of inner ear malformations in comparison to non-homozygous Connexin 26 patients.

791 Designing Outcomes Measures for Tinnitus: Influence of Questionnaire Wording

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It is well known that the specific wording of questionnaires can affect respondents' answers. Data from 1094 patients with severe tinnitus are presented to illustrate such differences on tinnitus questionnaires. Two different question formats were used: Format 1 developed for the Tinnitus Severity Index (TSI) of the Oregon Hearing Research Center, and Format 2, similar to that of the Chronic Illness Problem Inventory *(Kames et al 1984)*. For Format 1, response options were: 1=Never, 2=Rarely, 3= Sometimes, 4=Usually, 5=Always. For Format 2, response options were: 1=Not at all, 2=A little, 3=Moderate, 4=Quite a bit, and 5=Very much. Patient responses to the Format 1 item "Have hearing difficulties or tinnitus made it difficult to concentrate?" were: 1=4%, 2=10%, 3=36%, 4=27%, 5=23% for a total of 14% in the two lowest categories. Patient responses to Format 2 item "I have difficulty concentrating" were: 1=25%, 2=30%, 3=20%, 4=14%, 5=11% for a total of 55% in the two lowest categories. These differences were highly significant and similar differences were also found for items concerning difficulties relaxing, and feeling irritable or nervous. We hypothesize that the question structure of Format 2 shows less severity of tinnitus impact mainly because it implies that the difficulties are global or fixed personality characteristics rather than reactive states induced by a specific health problem. These observations appear to support the suggestion that global or generic health scales do not function as

efficiently as disease-specific scales for evaluating the severity of impact of chronic health conditions or for demonstrating treatment effects *(Guyatt, G.H. et al, J. Clin. Epidemiol. 52:187-192, 1999).*

792 "Typewriter" Tinnitus: A Treatable Unilateral Tinnitus Syndrome of the Elderly

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Anecdotes abound about medications abolishing tinnitus but well-designed studies have failed to show any oral medication that will abolish tinnitus. One consideration that may account for this discrepancy is that these studies make no distinction between the characteristics of the tinnitus and/or the subjects being studied; typically all subjects with any type of tinnitus are studied. We have identified two subjects with similar tinnitus and subject characteristics whose tinnitus was abolished with the same medication.

The subjects were 77 and 87 year old women who had the abrupt onset of tinnitus in one ear. Both described a staccato quality to their tinnitus ("like a typewriter in the background"). There were no other hearing or vestibular complaints; their audiograms were symmetric and consistent with their ages.

Both subjects completely suppressed their tinnitus with carbamazepine (tegretol).

These tinnitus/subject characteristics bear similarities to other cranial nerve mononeuropathies, namely trigeminal neuralgia, hemifacial spasm, and glossopharyngeal neuralgia. All are unilateral, occur in the elderly, have a staccato quality, respond to carbamazepine, and have no demonstrable deficit in the function of the associated cranial nerve. These other cranial nerve mononeuropathies have been associated with compression of the root entry zone of the cranial nerve by an aberrant blood vessel and, when refractory to medications, often can be relieved by surgical decompression of the root entry zone. As such the similarities suggest that this tinnitus syndrome may also be associated with compression of the root entry zone of the auditory nerve by an aberrant blood vessel. If other cases can be identified following this same pattern, then a well-defined treatable tinnitus syndrome would be established.

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793 Tinnitus Suppression with Subthreshold Sounds

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Suppression of peripheral tinnitus with subthreshold sounds was present in 64.7% of patients in a preliminary study (n=34) by Burkhard Franz. This technique is based upon the electromodel and the concept that the IHC are electroreceptors that are controlled by potentials from the OHC. When some OHC are lost, there is less natural suppression of the IHC and they become hypersensitive. In this excitable state they may respond to stray electrical potentials that results in tinnitus. The IHC are sensitive to cathodal stimuli and positive potentials such as the +SP from the OHC suppress that sensitivity. After a cochlear injury, some OHC usually remain but their diminished number is not sufficient to control the IHC sensitivity. By applying subthreshold sounds it is apparently possible to evoke sufficient +SP from the remaining OHC to suppress the IHC and relieve the tinnitus. This concept of the electromodel of the auditory system opens new tinnitus treatment strategies. This concept can help explain: 1] COCB stimulation leading to higher CM but lower CAP. (Wiederhold and Peake, JASA, 1965); and 2] noise presentation or hypoxia that causes lower CM but higher CAP. (Pierson and Møller, Hear Res, 1982a,b)

794 Some Statistical Issues Affecting Audiometric Outcome Measures in Otolaryngologic Research: Interdependence of Variables and Appropriate Models Of Variance.

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Objective: Research in Otology often takes the form of treatment efficacy trials, with audiometric measures used as outcomes. The use of standard outcome measures, specifically Pure Tone Average (PTA) and Speech Intelligibility, carry with them significant a priori concerns regarding the implications of variable interdependence and the appropriate models of variance. The present study was undertaken to characterize these effects and consider alternative means of comparing audiometric outcomes.

Methods: Herein we used a real data set (N=334) of pre- and post-treatment audiograms from idiopathic sudden sensorineural hearing loss (SSNHL) patients to illustrate the effects of the interdependence of audiometric outcome with severity at initial diagnosis, and also expectations for using the several variants of PTA. For studies where PTA is not useful, combination-vector and area-under-the-curve approaches for audiometric analysis are described. Speech Intelligibility, as measured by monosyllabic word recognition, was also examined, and the use of the binomial distribution tables for significant differences explained. Our SSNHL data set has been used to illustrate the expected effects on sensitivity and specificity of choosing any single percent-correct value as a limit of significance.

Conclusions: Appreciation of the strengths and limits of audiometric outcome measures is essential to avoid or minimize statistical pitfalls. This information can be used to guide choices in otologic research design that will strengthen validity and clinical relevance of group comparisons, allowing better insight into the medical or surgical treatment of hearing loss.

795 Cochleo-Toxicity of Povidone-Iodine Solution and Scrub in Young and Old Guinea Pigs.

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Introduction: The purpose of this study is to evaluate cochleo-toxicity of Povidone-Iodine solution, which is widely used as pre-operative disinfectant in the surgery of the ears. Cochleo-toxicity of Povidone-Iodine Scrub, which is used as a disinfectant of the hands are also evaluated. A small number of reports are available which allude no toxic effects. Only one report indicates toxic effects of the Povidone-Iodine solution in chinchillas.

Materials and methods: Guinea pigs (N=81) were used. The test solution of various concentrations was filled in one side of the middle ear cavity, and saline solution was filled in other side of the middle ear cavity as a control. Compound action potentials (CAP) was measured from the round window membrane at 24 hours, one week and one month after instillation of the solution. The responses were averaged 200 times. Also, the growth of the animals was taken into considerations in this series of experiment as well as the effect of dilution of the test solutions. Animals were grouped into three according to the body weight; 100grams, 250grams, and 350 grams.

Results: The ear which had Povidone-Iodine Scrub invariably became totally deaf.

A preliminary report from our laboratory indicated that the Povidone-Iodine solution without dilution did not show appreciable deterioration

of the CAP at 24 hours, it showed reduction in CAP at 7th day, and severe loss at 28days.

In this study, the youngest animal group showed a significant reduction in CAP at 24 hours. At 4 weeks, a group of less than 250 grams showed more reduction of the CAP than the group with more than 350 grams.

Conclusion: In the previous studies of oto-toxicity of the drugs applied in the middle ear cavity, no report ever addressed the relationship between cochleo-toxicity and the degree of growth of the experimental animals. Apparently, the difference in permeability of the round window membrane exists in the young and old animals of the same species.

796 Experimental Investigation on Factors Influencing Tremor in Microsurgical Manipulations

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The aim of this study was to measure hand tremor under simulated microsurgical conditions and to estimate the influence of different kinds of physical strain (e.g., physical exertion and hand exercise), as well as food abstinence and coffee consumption. Furthermore, the effect of one- or two-handed manipulation and microsurgical experience was investigated.

For this purpose the hand movements of 16 adult subjects were assessed during a defined manual manipulation using a stapes model to simulate microsurgical procedures. A laserinterferometric-based displacement technique was developed to measure tremor amplitude and frequency, as well as maximum displacement, to evaluate the subjects' fine motor skills.

Results: The mean tremor frequency was 8.1 Hz and was not dependent on different kinds of physical strain. Two-handed manipulations showed significantly lower tremor amplitudes than one-handed performances. Tremor amplitude and maximum displacement did not change after hand exercise, food abstinence and coffee consumption. But a significant increase in the tremor amplitude was found after physical exertion. Microsurgically experienced surgeons showed smaller tremor amplitudes for one-handed runs.

The results recommend to avoid physical exertion before microsurgery. Two-handed manipulations are preferable for increased hand steadiness. Hand steadiness might be improved by microsurgical training.

797 A Y-linked Inheritance Pattern with the Nonsyndromic Hearing Loss in a Large Chinese Family

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We found a large seven-generation Chinese family with progressive sensorineural hearing loss only occurred in patrilineal males among the generations. The family involved in this study was ascertained through the Chinese PLA General Hospital Department of Otolaryngology-Head & Neck Surgery. The family history was collected through questionnaires, personal interviews and the study of the family¡¯s genealogy of 1000 years. Auditory function was performed in 50 members of the family, including 20 affected and 30 normal individuals. The earliest recorded hearing-impaired family member was born on Apirl 13th, 1847 and he died on Apirl 10th 1898. In his following two generations, there was one male offspring in each generation. The family¡¯s record indicates that these two males were also hearing impaired. Among the fourth generation, there were four affected males and one unaffected female. The four affected males produced 25 patrilineal offspring, of which 23 are alive at the time of our study. Audiograms were obtained from 19 of the 23 patrilineal males and all diagnosed sensorineural hearing loss. Hearing loss in the family may start at as early as six years of age or in the second decade of life, allowing for normal speech development. As seen from the family analysis that showed the ancestor at the base of the genealogy was a carrier of a mutant gene. The gene appears to have been passed on in a Mendelian Y-linked pattern, as there is no cross-gender transmission of hearing impairment in this family. Genetically, the hearing loss appears to be transmitted as a single dominant Y-linked mutation. History study, physical examination and CT scan of the temporal bone have revealed no associated abnormalities in the family and these results support the conclusion of a Y-linked inheritance pattern attributed to the family.

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798 Neural Progenitor cells

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The early-formed neural tube consists of proliferating, morphologically homogeneous cells termed neuroepithelial (NEP) stem cells (Kalyani et al., 1997). Here we show that neuroepithelial (NEP) cells express a characteristic spectrum of markers and that both positive and negative selection criteria can be used to isolate stem cells. Sox-2, Fz-9 and FGFR4 are expressed by stem cells and are downregulated upon differentiation while other markers such as nestin, musashi, Brn-1, etc are expressed by both stem cells and other progenitors (Cai et al., 2002). NEP cells generate more differentiated cells via the generation of more resticted progenitor cells that include GRP's (glial restricted precursors) and NRP's (neuron restricted precursors). GRP cells express a characteristic spectrum of markers and generate oligodendrocytes and astrocytes in vitro and in vivo. Differentiation is regulated by multiple pathways that include Nkx2.2, ngn-3, BMP and HES1 and 5. GRP cells generate astrocytes via the generation of an astrocyte precursor cell that can be distinguished from other cells by the expression of CD44 and the absence of GFAP expression and its inability to generate oligodendrocytes. Each of these cell types can be isolated from ES cell cultures and data from human ES cells will be presented. Each of these cell types can be transplanted for therapy and the relative advantage or disadvantage of transplanting NEP, GRP, APC will be discussed.

799 Studies of the Neuronal Stem Cell of the Olfactory Receptor Neuron Lineage

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Neurogenesis proceeds throughout life in the vertebrate olfactory epithelium (OE), indicating that a neuronal stem cell exists in this tissue. Our studies of mouse OE have shown that olfactory receptor neurons (ORNs) are the products of a lineage that contains at least three distinct stages of proliferating neuronal progenitors: the neuronal stem cell; its progeny, a transit-amplifying progenitor that expresses the proneural gene homologue MASH1 (a bHLH transcription factor); and the Immediate Neuronal Precursor (INP), a second transit amplifying progenitor that is the progeny of the MASH1-expressing progenitor and gives rise directly to ORNs. Using tissue culture, genetic, and in vivo surgical approaches, our experiments have revealed several important principles about the regulation of neurogenesis in this system: (1) overall ORN production appears to be regulated by feedback inhibitory signal(s) that regulate(s) progenitor cell proliferation; (2) development of cells at each stage in the ORN lineage is regulated by both extrinsic and intrinsic factors; (3) negative signaling plays at least as important a role as positive signaling in regulating olfactory neurogenesis; (4) expression of proneural genes appears to be crucial in determining the

fate of neuronal progenitors. Experimental findings supporting each of these principles will be discussed.

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800 Blood Stem Cells: Paradigms for Stem Cell Biology

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In the stem cell biology field, the hematopoietic stem cell (HSC) is the best-defined example of an undifferentiated cell that can expand dramatically and be induced to differentiate into a wide range of specialized cell types. Bone marrow transplantation (BMT) serves as a benchmark for clinical applications of stem cell therapies. The clinical success of BMT as a form of stem cell therapy is most likely due to the robust nature of the normal process of hematopoiesis, since the short life-span of most blood cells results in a requirement for ongoing production of these cells throughout life. Only epithelial tissues rival hematopoiesis in the ongoing process of tissue replacement. Unlike epithelium, however, the HSC must provide progenitor cells for a wide variety of differentiated cells including erythrocytes, platelets, and the myeloid and lymphoid lineages of leukocytes. During the ontogeny of hematopoiesis, the primary site of blood development changes several times. There are two distinct genetic programs which are initiated in multiple locations by the hemangioblast, a cell with potential to form both endothelial and hematopoietic progeny. Bone marrow contains more than one type of stem cell, and recent studies have suggested some degree of plasticity in the ability of stem cells to differentiate into widely divergent lineages. Since stem cells provide a self-renewing source of normal differentiated cells, the potential for therapeutic application is great. Gene correction therapy in the autologous bone marrow transplant setting has proven feasible, although insertional mutagenesis has been shown to occur. Future directions for the field include expansion of functional stem cells ex vivo, and the optimization of allogeneic transplant approaches as an alternative approach to gene therapy.

801 The Characterization of Mesenchymal Progenitor Cells and their Application to Cartilage Repair.

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A bank of conditionally immortalized clones from mouse bone marrow have been characterized for their ability to differentiate into mesenchymal phenotypes. These marrow-derived clones of the adherent subset of marrow cells were assessed for their ability to differentiate into adipocytes, chondrocytes, osteoblasts and stromal support cells. The results indicate that mesenchymal progenitor cells (MPCs) of marrow show differentiation potentials that range from mono-potential to quadri-potential. Efforts to fit these results into a classical model of lineage transitions have been unsuccessful. It is hypothesized that the lineage of adult-derived MPCs is subject to extensive phenotypic plasticity and contains a range of intermediary progenitors. The Stochastic Repression /Induction model has been proposed to account for these differentiation features of MPCs. The regulation of the lineage transitions of MPCs are discussed in the context of know inductive and repressive regulatory factors for mesenchymal phenotypes.

Recent studies in laboratory have focused on the use of MPCs and other progenitor cells for the repair of cartilage. A method has been developed to target progenitor cells to the cartilage matrix repair site by coating cells with antibodies to extracellular matrix molecules. Chondro-progenitor cells were incubated a vital fluorescent dye (Vybrant, Molecular Probes), and then coated with palmitated protein G followed by antibodies to type II collagen, keratan sulfate and chondroitin sulfate and incubated with cartilage explants. Cell binding to the explants was examined by fluorescence microscopy of histologic sections and by confocal microscopy on whole mount preparations. Coating of chondro-progenitor cells with antibodies is shown to be an effective method for targeting progenitor cells to cartilage extracellular matrix.

802 Therapeutic Potential of Stem Cells in the Damaged Mouse Cochlea

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Hearing impairment has a profound effect on the American population, with almost thirty million people exhibiting some level of deafness or hearing loss. This disability impairs more people in terms of both numbers and economic impact than epilepsy, multiple sclerosis, spinal injury, stroke, Huntington's and Parkinson's diseases combined. The goal of our research is to investigate the therapeutic potential of stem cells transplanted into the auditory system. To this end, we will describe the experiments we have undertaken to assess the ability of stem cells to integrate into the cochlear sensory epithelium and replace damaged hair cells. Our first series of experiments was designed to exploit the developmental potential of stem cells by defining the in vitro conditions in which neural stem cells transdifferentiate into cochlear cell types. In the second series of experiments, we have examined stem cell integration into organotypic cultures of the organ of Corti. The third series of experiments investigated the regenerative capacities of stem cells by transplanting them into the otic capsule of a noise-damaged mouse model. Our hypothesis is that the transplanted stem cells will integrate into the damaged sensory epithelium, respond to local environmental cues and differentiate into cochlear cell types.

This work has been supported by a grant from the National Organization for Hearing Research Foundation.

803 Transplantation of Hair Cells and Stem Cells in the Inner Ear

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We have developed a molecular marker for committed hair cell (HC) precursors and HCs, using a promoter fragment from the pou4f3 gene to drive expression of green fluorescent protein (GFP) in transgenic mice. GFP was used to select donor cells for transplantation and to identify donor HCs that were transplanted into GFP-negative recipients. A neural stem cell line derived from hippocampus and stably expressing GFP was used for additional transplantation studies. Controls consisted of free GFP, killed GFP+ cells or irrelevant GFP-expressing cell types.

Using an in vitro utricular macula preparation, we observed that immature HCs from fetal macula can be successfully transplanted into a postnatal sensory epithelium that was previously exposed to ototoxins to destroy most resident HCs. The transplanted HCs showed integration into the macular epithelium, and formation of stereociliary bundles. In contrast, fully differentiated HCs were not successfully transplanted. The neural stem cells tested did not show similar integration or transdifferentiation into HCs in vitro.

Immature cochlear HCs transplanted into the adult cochlea in vivo survived for weeks, but showed no evidence of migration toward or integration into the organ of Corti. Neural stem cells appeared to cluster and migrate after transplantation in vivo. They also integrated into cochlear tissues, apparently in response to physical damage. However, no morphological signs of transdifferentiation were observed.

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804 Avian Sensory Progenitor Cells: Purification and Transplantation into Injured Auditory Epithelial In Vitro.

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Our laboratory studies mechanisms used by birds to regenerate hair cells. We are attempting to purify cells from the mature avian auditory epithelium (AE) that can divide and form hair cells over several passages in culture. We hope to use these post-embryonic sensory progenitor cells (SPCs) in future studies to characterize their molecular features, to make comparisons between avian and mammalian supporting cells, to identify molecules that promote SPC proliferation and hair cell differentiation, and to determine lineage relationships in the avian AE. We have used a standard cell culture method (serial passaging) to amplify and purify avian SPCs. Auditory epithelial cells (AECs) were isolated from control chickens and cultured in EBSS/BME/5% FBS for 2 days with streptomycin to kill all hair cells, then in control media until confluence (7-11 days). Cells were dissociated, split, cultured in the same media until confluence, and fixed or passaged again. In this manner, AECs have been serially passaged 3 times. When subcultured at 10,000 cells/cm2, AECs take 7-9 days to reach confluence. Five days after the first passage, 95% of AECs in secondary cultures incorporate BrdU, most cells express supporting cell antigens, and some AECs express hair cell antigens by 7 days post-plating. These findings show that SPCs are maintained and amplified after subculturing. We are currently testing whether various basal media, supplementary mitogens (insulin, insulin-like growth factor-1, transforming growth factor alpha, forskolin), and supplementary substrates (collagen I, laminin) can improve the rate of SPC growth. Also, we have injected DiI and BrdU-labeled cells from secondary cultures into the intact AE of cultured cochlear ducts from control and drug-exposed chickens. Transplanted cells incorporate into the host AE and survive for as long as 1 week. We are currently testing the ability of purified SPCs to divide and form new hair cells once transplanted.

805 In Vitro Growth and Differentiation of Sensory Hair Cell Progenitors from the Embryonic Mouse Inner Ear

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To better understand the molecular control of progenitor cell fates within the developing mammalian inner ear, we have established an in vitro system that supports the growth and differentiation of hair cells and supporting cells from mitotic progenitors taken from mouse vestibular and cochlear epithelium. Differentiation of new hair cells from dissociated otic epithelium occurs in distinctive epithelial structures and requires the presence of an unidentified mesenchymal factor, either for the formation of these structures or the differentiation of hair cells and supporting cells. Based on the distinct stereocilia morphologies of in vitro generated hair cells derived from cochlear and vestibular tissue, we suggest that the E13.5 mouse inner ear may contain separate progenitors for at least these two main types of hair cell. Efforts to further characterize the growth and differentiation requirements for hair cells and supporting cells in culture will be discussed.

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806 Generation of Hair Cell-Like Cells by Directing the Differentiation of Embryonic Stem Cells

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We used growth factors whose receptors are expressed by the cells of the developing inner ear to create a cell culture environment that promotes inner ear sensory cell formation. Using a step-by-step strategy we were able to create from embryonic stem cells derivatives that displayed characteristic features of neural-like progenitor cells. These progenitor cells expressed a combination of marker genes, including the stem cell marker nestin and early inner ear markers such as Pax2, BMP4, and BMP7. Expression of the embryonic stem cell marker Otx2, which is also a marker for the developing inner ear was maintained in the progenitor cell population. In addition, we found expression of marker genes for the developing sensory epithelia, including Math1, delta1, jagged1 and jagged2.

We induced in vitro differentiation by removal of all mitogenic stimulators from the culture medium and we found differentiated cells that expressed immunological markers indicative of hair cells, for example myosin VIIA, parvalbumin 3, and espin.

807 Sub-millisecond Voltage-Dependent Bundle Movement in Frog Vestibular Hair Cells

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In response to a voltage step, a hair cell′s stereocilia bundle moves in three distinct phases. Two phases, attributed to a myosin-1c motor and to channel closure after Ca^{2+} binding, occur in tens of milliseconds or milliseconds respectively. The fastest and least understood phase is a movement towards the kinocilium with hyperpolarization that occurs in < 1ms, which we will call the ″flick″. To move the bundle, we employed a gradient force light trap (″optical tweezers″) which uses a Nd:YAG laser refracted by a 2μm polystyrene bead attached to the kinocilliary bulb of a hair cell to exert a force of several hundred pN. The movement of the bead is separately detected by a HeNe laser and a quadrant photodiode. We measured deflections with a time constant of 0.2 ms of up to 6 nm for a 160 mV depolarization. Flick amplitude is roughly linear with voltage between -160 and +60 mV, but saturates at larger depolarizations. Neither changing calcium influx through transduction channels by reducing external calcium from 4 to 0.1 mM, nor blocking transduction with 200 μM gentamicin significantly affected the flick. However eliminating transduction with external BAPTA (which cuts the tip links of the transducer unit) abolished the flick. A positive bias of the bundle with constant force increased the flick, whereas it was eliminated by large negative bias. These data suggest that the flick is associated with the transduction channel but does not result from calcium entry through the channel, and may reflect a voltage-dependent conformational change.

808 Frequency Selectivity of Free-Standing Hair Bundles

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Hydrodynamic models suggest that mechanical resonance of free-standing hair bundles can account for the frequency selectivity of hair cells in the alligator lizard cochlea (Freeman and Weiss, 1990a-d; Shatz, 2000). Previous measurements (Frishkopf and DeRosier, 1983; Holton and Hudspeth, 1983) show that the motion of these hair bundles is frequency dependent, but do not characterize the nature of this frequency dependence. To further test the models, we have measured the frequency dependence of motion of free-standing hair bundles in an isolated preparation of the alligator lizard cochlea. Measured hair bundle rotations were normalized by the velocity of the reticular lamina to obtain a transfer function H(f). At low frequencies, H(f) magnitude was constant or rose with frequency with a phase at or above zero. At high frequencies, H(f) magnitude fell with frequency with a phase

approaching -90 degrees. For about 1/3 of the hair bundles, the transition between these two regimes presumably occurred below the lowest frequency measured. For the remaining hair bundles, the transition occurred at frequencies that varied systematically with bundle height. To quantify this variation, we fit the Freeman/Weiss model to H(f) for each bundle. The peak frequency of the fit varied inversely with bundle height to the 3/2 power. These frequencies were consistently about one octave lower than those estimated from neural data (Holton and Weiss, 1983). The sharpness of tuning of H(f) was consistent with that of hair cell receptor potentials in response to high-level tones. Fitting the measured H(f) with a low-pass filter gave qualitatively similar results. These results show that the tuning seen at the level of the auditory nerve is already present in the mechanical responses of hair bundles. However, the mechanism responsible for increasing the sharpness of tuning at low levels remains unclear.

809 Loss of Striated-Sheet Matrix, Aberrant Collagen Fibril Organisation, and Changes in the Tectorial Membrane's Frequency and Level-Dependent Properties in Beta-Tectorin Null Mutant Mice

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The mammalian tectorial membrane is composed of three collagens and three non-collagenous glycoproteins, alpha-tectorin, beta-tectorin and otogelin. The collagens form fibril bundles that run radially across the tectorial membrane and are imbedded in a tectorin-based, striated-sheet matrix. To determine the role played by beta-tectorin in matrix formation, and to further understanding of tectorial membrane function, transgenic mice were produced in which the first 4 coding exons of beta-tectorin were deleted. In mice homozygous for this deletion, beta-tectorin cannot be detected in the tectorial membrane, the striated-sheet matrix is completely absent, and the collagen fibrils are atypically organised, being packed densely along the upper edge and projecting vertically downwards towards the surface of the organ of Corti. The tectorial membrane is attached to the spiral limbus, has a covernet and a marginal band, but lacks Hensen's stripe. With low-level primaries, DPOAEs appear above the noise floor only at high frequencies (>40 kHz). They appear at progressively lower frequencies as the intensity is increased (>30 dB). At 75 dB SPL, DPOAEs have a similar magnitude and frequency range as those in wild types. Round window CAP recorded over the 5-30 kHz range is ~30 dB less sensitive in null mutants than in wild-types. CM recorded from the high frequency region of the cochlea in wild type and null mutant mice is similar in amplitude. These results show that beta-tectorin is required for striated-sheet matrix formation, and that this non-collagenous matrix is required for correct collagen fibril organisation. They also suggest that the frequency and level-dependent mechanical properties of the tectorial membrane are altered in beta-tectorin null mutant mice.

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810 Thermodynamic Analysis of Nanotube Formation in Prestin-Containing Membranes: Unraveling the Area Motor/Bending Motor Controversy

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Recent evidence indicates that prestin is the motor protein responsible for outer hair cell electromotility. However, the mechanism of action of prestin is not understood. At least two models of electromotility have been proposed. One proposes the conformational change of the motor protein induces in-plane strain in the membrane, often specifically considered to be a change in membrane area. The other postulates that a conformational change in the motor protein produces curvature strain in the membrane. Presently, experimental evidence can not distinguish between the two models. However, the recent application of tether formation experiments to outer hair cells provides an experimental scenario in which nanoscale curvature changes can be distinguished from in-plane area changes. Current thermodynamic models of tether formation predict that the length of a tether depends upon the pulling force (F) and the bending stiffness of the membrane. We have further developed these models to include the free energies associated with active area and curvature changes. This extended thermodynamic model predicts that the equilibrium tether length (L_t) and radius (R_t) will depend upon the applied voltage and the mode of electromotility. Specifically, voltage-induced changes in curvature are postulated to translate the F vs. R_t curve, while changes in membrane area will alter the shape of the curve. The application of the model to experiments in which tethers are formed from outer hair cells and HEK cells transfected with prestin under controlled voltage could potentially resolve whether prestin acts by changing membrane area or by changing membrane curvature.

811 How Does the Cochlear Amplifier Stimulate the Inner Hair Cells?

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We have recently proposed that the cochlear amplifier is a fluid pump driven by outer hair cell (OHC) somatic motility [Hubbard et al. (2002) ARO Abstracts 25:236]. According to this hypothesis, acoustic energy propagates down the cochlea via the classical (transverse) traveling wave until it approaches the best place. At this point the OHCs begin pumping fluid into the tunnel of Corti (TOC) creating a second type of traveling wave which we call the organ of Corti (OC) wave. It is the OC-wave and not the classical traveling wave that is amplified by the OHCs according to the fluid-pump hypothesis. The question remains, however, how does the motion of the OC-wave get coupled to the inner hair cell (IHC) stereocilia?

To address this question, a simple model of OHC motility was developed that included a 3-state Boltzmann model for the apical tension-gated conductance and a 2-state Boltzmann model for the voltage-dependent shape changes of the prestin molecules in the basolateral membrane. The rate of fluid transfer from the spaces of Nuel around the OHCs to the TOC was assumed to be directly proportional to the rate of OHC contraction. The TOC was modeled as an elastic tube and the pressure changes produced in the TOC by OHC contractions were computed. For frequencies above the cutoff frequency of the OHC membrane, the pressure in the TOC was proportional to OHC stereocilia displacement. For lower frequencies, at high stimulus levels, the pressure waveform in the TOC was distorted in a manner consistent with the distortion observed experimentally in IHC receptor potentials [Mountain and Cody (1999) Hear. Res. 132: 1-14]. These results are consistent with the hypothesis that the IHC hair bundles are stimulated by pressure changes in the TOC created by the OHCs. This stimulation may be the result of displacement and/or rotation of the IHC cell body.

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812 Computation of the Fluid-Structure Interactions and Vibrational Modes in the Basal Region of the Cochlea.

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At the base of the cochlea, the basilar membrane (BM) can be accessed directly via the scala tympani, but the organ of Corti (OC) and the tectorial membrane (TM) are inaccessible in vivo. Thus a reliable computational model will be very useful to analyze the detailed micromechanical movements of an intact cochlea in this region, where the mechanical events are expected to differ from those at the apical turn. Because of the difficulty of visualization within the cochlear duct, the interactions of the cochlear fluid and cellular structures as well as the micromechanical vibrational modes of the cochlear partition (CP) remain unclear. In an earlier work we developed a finite element model (Abstract of ARO Midwinter Meeting, pp. 236, 2002) for the radial vibrational modes in the apical region of the cochlea. In the present

study we apply our model to calculate the fluid-structure interactions and the high frequency vibrational modes of the CP in the basal region of the cochlea, where calculation meshes are relatively more difficult to generate due to the extremely small CP size compared to the fluid compartments. Axial elastic coupling is now included via a BM modeled as an orthotropic clamped plate, and solid damping is included in the model by assuming a Voigt solid and using a complex Young's modulus for BM, OC and TM solid domains. Simulation of passive radial modes in the basal region of a guinea pig cochlea for frequencies between 17-20 kHz indicates monophasic vibration of the BM but multiphasic modes of deformation of the TM and the reticular lamina. The calculated axial wavelength at 18 kHz is less than 1 mm. Differences exist between basal and apical mode shapes, e.g. the rotation angle of the three rows of the OHC increases from inner row to outer row in basal turn simulations.

813 Responses from a Nonlinear, Multi-compartment Model of the Cochlea

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Using realistic parameters, a linear, multi-compartment model of the cochlea can couple outer hair cell (OHC) force generation into a slow-traveling wave inside the organ of Corti. We have now included a saturating nonlinearity in the hair-cell force-generation mechanism to make the model even more realistic. Impulse ("click") stimuli at increasing sound levels yield basilar membrane (BM) responses that grow in a compressive, nonlinear fashion that is qualitatively similar to data obtained from the BM. Model reticular lamina (RL) responses are like BM responses, but they are not identical. The model's BM instantaneous frequency (IF) "glides" are also similar to experimentally measured glides. Model RL glides are like BM glides, but they are not identical. The model's steady-state BM responses to tones of increasing sound level increase linearly at low SPLs, saturate, and then begin to grow again, as a second, near-linear regime is entered. The BM responses can show dips or notches that are not observed experimentally.

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814 Does the Cochlea Exhibit an Andronov-Hopf Bifurcation?

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Two groups have argued that the cochlea contains a set of oscillators, each operating near an Andronov-Hopf bifurcation (Camalet, S., et al. (2000) *Proc. Natl. Acad. Sci.* **97**:3183; Eguíluz, V.M., et al. (2000) *Phys. Rev. Lett.* **84**:5232). They claim that nonlinear cochlear responses to tones of varying amplitude directly reflect the nonlinear responses of these oscillators, and associate the oscillators with the outer hair cells.

Andronov-Hopf bifurcations are explained and their relevance to cochlear mechanics examined. Both theory and experiment rule out any simple connection between measurements of basilar membrane motion, and bifurcation characteristics of outer hair cells.

815 Computer Simulation of Three-Dimensional Cochlear Macro Mechanics

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We present the results of a series of large scale computer simulation experiments with a realistic and detailed model of the human cochlea. Our model uses a three-dimensional, curved description of the cochlear anatomy, with the material properties of the curved elastic surfaces within the cochlea (such as the basilar membrane) described using the partial differential equations of shell theory. The viscous fluid is a non-linear system whose behavior is described using the Navier-Stokes equations. The fluid-structure interactions are computed using the immersed boundary method. Our simulation code is numerically compute intensive, so our experiments require the use of a partitioned supercomputer, the 32 processor Hewlett Packard "SuperDome" installed at Caltech's Center for Advanced Computing Research. In the series of experiments presented, we have applied a pure tone input to the cochlea. The simulation results are in good general agreement with the accepted macro-mechanical behavior of the cochlea: a traveling wave on the Basilar Membrane is observed, which rises to a broad maximum excursion, and is followed by a sharp descent. Moreover, our experimental results reveal a wave pattern that has additional unexpected features that prompt further investigation. In particular we find that while the wave pattern is continuous and smooth, its envelope is not smooth: there are locations along the basilar membrane where the envelope of the wave rises or falls very sharply. These non smooth locations depend on the input frequency.

816 Perturbative Analysis of the Effects of Roughness on the Helmholtz-equation Formulation of 2- and 3-d Cochlear Mechanics

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The perturbative theory of apical cochlear-wave reflection via distributed inhomogeneities (roughness) or nonlinearity in one-dimensional cochlear models (Shera and Zweig, 1993; Zweig and Shera, 1995; Talmadge et al., 1998, 2000) is generalized to the case of two- and three-dimensional cochlear models. Key elements of this approach are the scala fluid pressures integrated over the scala cross sections (Duifhuis, 1988). When applied to a "smooth" cochlea, the basic formalism may be used to determine approximate WKB-type wave-solutions. These solutions differ from previously determined ones based on energy-flow considerations (e.g., Whitham, 1974; Lighthill, 1978,1981; Steele and Taber, 1979; de Boer and Viergever, 1984).

817 Spatial Patterns of Basilar Membrane Vibration in the Sensitive Cochlea

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The volume velocity of the transverse vibration of the basilar membrane (BM) in response to a single tone, and the longitudinal patterns of the BM responses to two tones were measured using a scanning laser interferometer in this study. The bullas of young gerbils with normal hearing were opened and the round window was surgically enlarged to exposed approximately 1 mm of the BM. Magnitude and phase of the BM response to a single tone were measured as functions of the longitudinal and radial locations and the volume velocity of BM vibration centered at the characteristic frequency location was calculated. For a two-tone experiment, magnitude and phase of BM vibration were measured as a function of the longitudinal location at frequencies of primaries and of the cubic distortion product. The observed vibration demonstrates compressive nonlinear growth and a shorter wavelength and slower propagation velocity along the cochlear length than previously reported. For 16 kHz tones, the phase delay is up to 6 π over the observed cochlear length (<1,000 μm), and the detectable basilar membrane response to a low-level tone occurs over a very restricted (~600 μm) range. The volume velocity gain of BM vibration is ~40 dB lower than that of point velocity. There is no detectable backward traveling wave along the cochlear partition although a normal forward traveling wave is seen. The data provide new insights for the study of the cochlear amplifier and the generation of otoacoustic emissions.

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818 Chlorpromazine Increases CAP & DPOAE Thresholds and Inhibits the Contralateral Medial Olivocochlear Reflex in Guinea Pigs

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The cochlear amplifier is powered by a membrane-based motor mechanism located in the outer hair cell lateral wall plasma membrane. Chlorpromazine has been shown to alter membrane mechanics and shift the electromotility transfer function in isolated outer hair cells. This study was designed to determine whether cochlear function is also affected by performing in vivo perilymphatic perfusion of the guinea pig cochlea. Perfusion with chlorpromazine resulted in an increase in both the compound action potential threshold (35 dB) and the distortion product otoacoustic emission threshold (9 dB). Additionally, the contralateral medial olivocochlear reflex was blocked. These effects were completely reversible within two minutes by perfusion with artificial perilymph, suggesting that no intracellular effects were induced by chlorpromazine. As a comparison, perilymphatic perfusion of salicylate was performed. Similar to chlorpromazine, salicylate modulates OHC membrane tension; however, it also inhibits electromotility. This study revealed that salicylate increased the compound action potential threshold (41 dB), increased the distortion product otoacoustic emission threshold (17 dB), and blocked the contralateral medial olivocochlear reflex. However, these effects were only partially reversible within the same two-minute time frame. These findings suggest that a proper lipid environment within the outer hair cell plasma membrane is important for normal hearing and provides additional information about the manner in which the membrane protein prestin interacts with the plasma membrane to produce electromotility. Amphipathic drugs may produce membrane disorganization that interferes with the summation of electromotile forces from individual prestin proteins.

819 Couplin, a Novel 27 kDa Protein, Links Prestin to the Actin-Spectrin Cytoskeleton in Auditory Outer Hair Cells

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The outer hair cells (OHCs) of the mammalian cochlea are specialized sensory-motor cells that rapidly contract and elongate in response to voltage, a process known as electromotility. Prestin, a recently cloned integral membrane protein, has been identified as the 'motor' protein that drives OHC electromotility. Prestin is densely packed throughout the lateral plasma membrane of OHCs, and voltage-induced conformational changes in prestin are likely transduced to an underlying and highly organized actin-spectrin network, the 'cortical lattice'. Cross-linking 'pillar' structures whose molecular nature is unknown connect the plasma membrane to the cortical lattice. To identify binding proteins to prestin, we screened a mouse organ of Corti yeast two-hybrid library with the cytoplasmic carboxyl-terminus of mouse prestin. One clone, which binds to prestin in yeast under high-stringency conditions, encodes a novel 27 kDa protein with similarity to the calponin homology (CH) domain. Northern and Western blot analysis demonstrate expression of this clone in several tissues including the cochlea. Confocal and immunogold electron microscopy with affinity purified antibody specific to this protein colocalize with prestin at the cross-linking 'pillar' structures. Interestingly, the CH domain predicts binding to F-actin, and we also colocalize this protein with actin filaments in cultured cell lines. These results suggest that this novel protein may link prestin to the underlying actin-spectrin network of the cortical lattice. Accordingly, we propose the name "couplin" for this novel clone and suggest that this protein may be a structural element of the pillar structures that transfer mechanical force from the membrane-based prestin motors to the cortical cytoskeleton to engage electromotility of OHCs.

820 Imaging of the Cortical Cytoskeleton of Guinea Pig Outer Hair Cells Using Atomic Force Microscopy

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The lateral walls of the mammalian outer hair cell (OHC) consist of three layers. In the outermost plasma membrane, there are protein motors whose conformation is probably changed according to the cell's membrane potential. As a result of their conformational change, change in the area of the plasma membrane occurs. The cortical lattice beneath the plasma membrane is thought to convert this area change in the plasma membrane into length change in the axial direction of the cell. Therefore, the cortical lattice seems to play an important role in the motility of the OHC. Some electromicroscopic studies have been performed to investigate the cytoskeleton of OHCs. However, details of the ultrastructure of the cytoskeleton have not been clarified. In this study, the ultrastructure of the cytoskeleton of a fixed OHC extracted with Triton X-100 was investigated using an atomic force microscope (AFM). The cortical cytoskeleton which is formed by discrete oriented domains was imaged, and circumferential filaments and cross-links were observed within the domain. Morphological change of the cytoskeleton of the OHC induced by diamide treatment was then examined using the AFM, and reduction of cross-links was observed. Findings indicate that the cortical cytoskeleton consists of circumferential actin filaments and spectrin cross-links.

821 Calcium-Induced Calcium Release in Cochlear Outer Hair Cells

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Several lines of evidence suggest that the motor output of cochlear outer hair cells (OHCs) is modulated by changes of intracellular Ca^{2+} and that this Ca^{2+}-dependent regulation is a part of the efferent feedback, controlling the organ of Corti operation. It was hypothesized that Ca^{2+}-regulation in OHCs involves Ca^{2+}-induced Ca^{2+} release from intracellular Ca^{2+}-stores, which are presumably located in these cells within *subsurface* and *synaptic cisternae*. However, Ca^{2+}-release from these organelles hasn't been documented yet. Here we have used DM-nitrophen, a caged Ca^{2+} compound, to produce a rapid rise of intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) and stimulate Ca^{2+}-induced Ca^{2+} release in isolated OHCs. Non-ratiometric Ca^{2+} imaging in the whole-cell patch-clamp conditions showed that an initial fast and uniformly distributed spike of $[Ca^{2+}]_i$ induced by UV-photolysis was followed by a slowly developing increase of $[Ca^{2+}]_i$, first in the vicinity of lateral wall and later below the cuticular plate. The increase of $[Ca^{2+}]_i$ in the lateral wall region suggests the release of Ca^{2+} from the expected Ca^{2+}-store in the *subsurface cisternae*. The location of the second delayed Ca^{2+}-release below the cuticular plate coincides with an IP_3-sensitive Ca^{2+}-store, which has been shown to be activated by extracellular ATP (Mammano et al., J. Neurosci., 19: 6918-6929, 1999). It is also not surprising, since the operation of IP_3-receptors could be effectively modulated by $[Ca^{2+}]_i$. In a separate set of experiments, the commonly used modulators of intracellular Ca^{2+}-release receptors, ryanodine and caffeine, produced no or very little Ca^{2+}-release from *subsurface cisternae*, suggesting that these Ca^{2+}-stores may have an unusual pharmacology.

822 Investigation of Outer Hair and HEK Cell Membrane Mechanics Using Optical Tweezers

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The lateral wall of the cochlear outer hair cell (OHC) is a trilaminar structure composed of the plasma membrane (PM), the cortical lattice (CL), and the subsurface cisternae (SSC). This composite organization is believed to strengthen the PM, which undergoes constant cycles of stretching and relaxation during normal hearing. In this study we used optical tweezers to investigate some of the characteristics of the PM from: 1) the OHC lateral wall; 2) the OHC basal end; and 3) Human Embryonic Kidney (HEK) cells.

A polystyrene bead was optically trapped and brought into contact with the PM of the test cell. The cell was then moved away using a piezoelectric stage to form a membrane tether between the trapped bead and the cell. We measured the tether formation force (TFF) and the instantaneous tethering force (ITF) of the PM using a quadrant photodetector during membrane pulling.

The TFF values from HEK cells and the OHC basal end were considerably less than that from the OHC lateral wall. The tethering force profiles were similar for the PM tethers formed from both HEK cells and the OHC, but a lower steady state value for ITF was found in HEK cells and the OHC basal end, when compared with the values from the OHC lateral wall.

The differences in TFF indicate the presence of a stronger structural coupling between the PM and cytoskeleton of the OHC lateral wall when compared to that found in HEK cells and the basal end of the OHC. The higher steady state values of ITF for the OHC lateral wall suggest that the membrane composition in the OHC lateral wall may be different from that in the HEK cells and the OHC basal end.

823 Carboxy-terminal Truncations and Mutations of Prestin; Affects on NLC.

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The integral plasma membrane (PM) protein, prestin, the outer hair cell (OHC) lateral membrane motor (Zheng et al,Nature 405,2000; Santos-Sacchi et al,J Physiol 531,2001), likely underlies our auditory system's acute sensitivity. One of the possible mechanisms whereby the gain of the cochlear amplifier could be tweaked is by modulation of the operating voltage range of prestin. For example, we have previously shown (Santos-Sacchi and Rybalchenko, ARO 2002) that intracellular chloride shifts the motor's voltage dependence.

The electrical signature of prestin is its displacement current or nonlinear capacitance. Here we use this measure to evaluate the effects of site-directed mutations of Prestin. We tested truncations of prestin's C-terminus, and found that nonlinear capacitance was abolished when stop-codons were placed at or N-terminal to residue 705. We are not yet sure whether this is due to abnormal protein structure or trafficking problems. However, preliminary data indicate that truncation at residue 724 is not deleterious. Charge substitutions within the C-terminus at specific residues or groups were also tested (D/E to K at 516,518, 522, 524, 527, 528, and 531; R/K to D 571, 572, 573, 576, 577, and 580; R to D at 571; E/D to K at 608, 609, 610, 611,612, and 613). All substitutions showed nonlinear capacitance . All but one of the substitutions that we tested so far (E/D to K at 608, 609, 610, 611,612, and 613) showed shifts in the C-V function along the voltage axis in the depolarizing direction, ranging from 15 to 40 mV. These data indicate that the C-terminus may be important in controlling the operating voltage range of the cochlea amplifier, possibly playing a role in the protein's allosteric modulation by anions.

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824 Prestin Activity May Support Outer Hair Cell Lateral Membrane Chloride Flux

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The outer hair cell (OHC) lateral membrane motor, prestin (Zheng et al, Nature 405,2000; Santos-Sacchi et al, J Physiol 531,2001), is thought to underlie the mammalian cochlea amplifier. Intracellular chloride is known to influence prestin activity (Oliver et al., Science, 292, 2340, 2001). Recently, we discovered a stretch/voltage sensitive conductance (G_{metL}) that non-selectively passes small cations and anions through the OHC lateral membrane (Rybalchenko and Santos-Sacchi, ARO, 2002). Furthermore, we showed that the flux of chloride through this conductance strongly influences prestin activity, the binding of which increases the probability of prestin being in the compact state.

G_{metL}, which exhibits a V-shaped G-V function, is partially blocked by a few stretch-channel blockers, including gadolinium, tamoxifen and quinine, but is resistant to a host of other channel and transporter blockers. Although the molecular identity of G_{metL} remains unknown, it is clear that G_{metL} and prestin share some characteristics, including restriction to the lateral membrane, and sensitivity to chloride channel blockers (e.g., niflumic acid). One possibility is that G_{metL} arises from conformational changes within prestin or mechanical interactions among prestin molecules. If this hypothesis is correct, G_{metL} might express additional characteristics of prestin, for example, prestin's substantial temperature sensitivity (~ 1-2 mV/ ^{o}C shift in V_{pkcm}; Meltzer and Santos-Sacchi, Neuroscience Letters 313,141, 2001). We report here that, indeed, the operating voltage range of G_{metL} and prestin-associated nonlinear capacitance (NLC) co-vary with temperature. Heating OHCs shifts both functions to depolarized levels, while cooling shifts them to hyperpolarized levels. The average shifts (n=3; +/-sem) were 1.28.+/- 0.24 mV/^{o}C for G_{metL} and 1.19 +/- 0.10 mV/^{o}C for NLC. Interestingly, the magnitude of G_{metL} is also substantially temperature sensitive. These data support the hypothesis that prestin and G_{metL} arise from the same structures.

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825 Voltage-Dependent Somatic Stiffness of Cochlear Outer Hair Cells.

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Outer hair cell lateral wall is a unique trilaminate structure consisting of the plasma membrane, a subplasmalemmal cytoskeleton, termed the cortical lattice, and a membraneous organelle called the subsurface cisternae. Morphological studies have shown that large protein particles (~10 nm in diameter) with packing densities possibly exceeding 5,500 per square micron, cover as much as 75% of the plasma membrane. It is now known that the membrane-potential-driven length changes of OHCs are the result of the conformational changes of the voltage-sensitive protein named prestin (Zheng et al., 2000, Nature 405:149-155). During length change intracellular anions act as intrinsic voltage sensors (Oliver et al., 2001, Science 292:2340-2343). We demonstrated that OHCs also changed their axial stiffness when their membrane-potential was altered, and that the electrically evoked stiffness changes and motility had very similar characteristics and might arise in a common process (He and Dallos, 1999, PNAS 96:8223-8228). In this study, we attempted to address three issues. 1) To confirm that the

voltage-dependent stiffness changes and motility indeed arise from a common process, the motor protein. 2) To determine the contribution of stiffness generated by the motor proteins to the global axial stiffness of the cell. 3) To examine whether ACh, the neurotransmitter of efferent fibers, could modulate the stiffness of motor protein and/or the stiffness of other membrane structures. The experiments were done on isolated guinea pig OHCs in the whole-cell voltage-clamp mode. Motions were measured by a photodiode-based measurement system. Axial stiffness was determined by loading the cell with a fiber of known stiffness at the apical surface of the cells. Our results show that voltage-dependent stiffness and motility disappear, and the static axial stiffness of the cells drops ~60% on average after removal of intracellular Cl^-. The results suggest that stiffness of the motor protein is the major contributor of the global axial stiffness of OHCs.

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826 Modulation of The Outer Hair Cell Motor by Sulfonate-Containing Anions

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Chemical modulation of the outer hair cell (OHC) membrane motor protein, prestin, probably contributes to high-frequency cochlea amplification.

In patch-clamped isolated guinea pig OHCs we confirmed that intracellular Cl^- maintains maximal values of prestin's total charge transfer (Q_{max}) and "apparent" valance (z) of its voltage sensor. Our main observation was that Cl^- is important, but not indispensable in supporting prestin's voltage sensitivity. We replaced Cl^- in the patch pipette by several anions not thought to substitute as prestin's hypothetical extrinsic voltage sensor. Q_{max} remained substantial and depended on the nature of the substitute anion. Significant charge transfer was sustained by sulfonate (SO3) containing anions ($HEPES^-$, SO_4^{2-}, pentane-sulfonate$^-$) compared to those lacking sulfonate (e.g., maleate^{2-}). Pentane-sulfonate shifted prestin's operating range to extreme hyperpolarizing potentials, whereas $HEPES^-$ and SO_4^{2-} to moderate and extreme depolarizing potentials. With intracellular SO_4^{2-} solutions containing low Cl^- concentrations, charge transfer remained as robust as obtained with pure chloride solutions; nevertheless, the voltage operating range for prestin was significantly shifted to depolarized levels.

Our data suggest that Cl^- functions as a strong allosteric modulator of prestin rather than its voltage sensor. Cl^- binding increases Q_{max} and shifts prestin's voltage operating range to negative physiological membrane potentials. SO3 containing agents are potentially active allosteric modulators of prestin, as well, influencing primarily prestin's voltage operating range. SO_4^{2-} anions present in native OHCs may counter-balance the effect of Cl^-, shifting prestin's operating range to more positive membrane potentials.

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827 The Strain Ratio of the Outer Hair Cell Motility Motor

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The isolated cochlea outer hair cell (OHC) contracts axially and dilates in diameter when depolarized, consistent with an increase in internal pressure under the constraint of constant volume. It behaves similarly when its internal pressure is increased. In contrast, when an isolated OHC in the microchamber is electrically stimulated, one segment contracts and the other extends. Further, the segment diameters dilate and contract respectively, which suggests antiphasic segmental pressure changes, an untenable proposition. An alternative explanation is that the motility motor generates negative axial strain and positive radial strain on depolarization. We can define the motor strain ration as the ratio of the intrinsic radial and axial strains of the motor protein. To determine the relationship between the strains, concurrent length and diameter change measurements were made in isolated OHCs in the microchamber, for cells of a range of lengths and at varying extrusion factors. The radial and axial strains in each segment were determined and their ratio calculated as an estimate of the motor strain ratio. The results suggest that the motor strain ratio is –0.5. A model of OHC mechanics implies that this ratio is close to ideal for maintaining constant volume in each segment, independent of events in the other segment. Thus the OHC may undergo no significant pressure change during contraction and extension cycles. This result is reasonably independent of the passive membrane properties of OHCs for a range of values around previous estimates.

828 High-Frequency Electrical Interrogation of Isolated Outer Hair Cells

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Micro-electric-impedance (microEI) methods were applied to study the effective dielectric properties of outer hair cell (OHC) membranes using interrogation signals ranging from acoustic to radio frequencies (100 Hz to 15 MHz). Hair cells were isolated from guinea pig cochleas and positioned within the interrogation chamber of micro-machined channels to fit tightly between opposing pairs of metal electrodes. Voltage and current were recorded simultaneously between the electrode pairs using pure sinusoidal stimuli and/or two-tone distortion product stimuli. These data were used to determine the effective electric impedance of the membrane structures as well as nonlinear distortion products. Results exhibited significant differences when the electrodes were apical to the nucleus over the subsurface cisterna (SSC) vs. basal to the nucleus or over the nucleus itself. The electric impedance data were compared to other cell types and to predictions of a two-dimensional simulation of Maxwell's equations. The computational model incorporated the approximate geometry and dielectric properties of the plasma membrane, SSC, nuclear membrane and major intracellular fluid compartments. A finite-difference frequency-domain method was applied to solve for the complex-valued electric field and impedance.

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829 Perceptual Consequences of the Loss of Cochlear Compression

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The normal cochlea supports important auditory functions in terms of the exquisite sensitivity and frequency tuning. When these normal cochlear functions are bypassed in electric stimulation of the auditory nerve, they need to be replaced by a front-end processor in the cochlear implant. More importantly, the direct stimulation of the auditory nerve also provides an opportunity to observe the perceptual consequences of the loss of cochlear functions. Processings in intensity, time, and frequency by cochlear implant users will be contrasted to that by normal listeners in order to delineate the roles of cochlear processing in auditory functions.

830 Intensity Coding for Speech Processors

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Loudness is usually controlled in cochlear implants via a fixed relationship between the output level of each acoustic filter and the level of electric stimulation at the corresponding electrode position.

However, this method ignores the effects of temporal and spatial loudness summation in the output of the sound processor. The aim of the work described here is to develop an advanced algorithm for coding loudness that takes into consideration loudness summation effects and thus will improve perception by implantees. Data from loudness summation experiments were used to develop a model of loudness for electrical stimuli and a practical method that can be used to predict the perceived loudness for arbitrary electrical stimulus patterns. A new sound processing strategy, called SpeL, was devised and implemented in a research sound processor. SpeL uses both an acoustic model and our new electric model to control loudness in real time. The processor analyses the incoming signal to extract the specific loudness (loudness contribution per cochlear place or ERB) and then calculates the electrical stimulus pattern which results in a similar specific loudness pattern. Initial evaluations of SpeL included the assessment of loudness perception. Results showed that the perceptual loudness range with SpeL was less compressed than that using SPEAK or ACE strategies and that the loudness growth of low-level broadband sounds was closer to that with normal hearing with SpeL. Speech perception with the new strategy is currently undergoing evaluation.

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831 Concurrent Sound Segregation by Cochlear Implant Users

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Normally hearing listeners use a number of cues to perceptually separate the voices of competing talkers. We have been studying how cochlear implant users might perform a similar task. To do so, we used both complex patterns of electrical stimulation applied to one or more channels of an implant, and acoustic simulations of electric pulse trains played to normal listeners. These simulations consist of pulse trains that have been bandpass filtered into high frequency regions, such that variations in the temporal pattern of stimulation do not result in "place of excitation" cues. The results show that, when two inharmonically related pulse trains are mixed into the same channel, neither group of listeners can extract the two underlying periodicities. Instead, listeners hear a single pitch corresponding to the higher pulse rate. In addition, a modification of Shannon's "vocoded speech" simulation, in which the envelopes from two voices modulate pulse trains of either the same or different rates, suggests that this phenomenon can affect speech perception: Using different pulse rates for the target and interfering speech only helps when the target is played on the higher rate. Finally, we have concurrently stimulated four channels of the Nucleus CI24 implant at a rate of 100 pps per channel, and studied which manipulations, applied to a single "target" channel, allow users to "hear out" the target from the mixture. Stimulation was in BP+1 mode with a pulse duration of 100μsec/phase. Results show that increasing the current applied to the target channel, or turning it on later than the others, provide effective cues. In contrast, altering the timing of individual pulses such that those on the target channel are about 5 ms apart from those on the other channels does not provide a consistent advantage.

832 Physiological Assessment of Spatial Tuning and Channel Interaction in Cochlear Implants

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The neural recruitment pattern and, in particular, the spread of neural activity across fibers in a longitudinal direction along the cochlea may have a significant effect on the ability of a multi-channel implant to encode stimuli with multiple frequency components. The resulting overlap in neural excitation, or channel interaction, may be affected by a number of factors such as the pattern of nerve survival and electrode placement. With the advent of reverse telemetry systems in cochlear implants, the electrically evoked compound action potential of the auditory nerve (ECAP) can be routinely measured in cochlear implant users. This presentation will outline physiological measures, using the ECAP, to assess spatial response patterns as well as methods that more directly assess channel interactions. Spatial response patterns are evaluated using different recording sites along the electrode array in response to stimulation at a fixed site. Channel interaction measures use simultaneous or non-simultaneous (forward masking) stimulation of two channels in order to assess the effect of stimulation on one channel on the response to another channel. Response interactions are evaluated as the masker stimulation site is varied with a fixed probe. Results to date (in 17 cochlear implant users, using a non-simultaneous assessment of channel interaction) demonstrate significant correlation with speech perception scores. Subjects with broader channel interaction functions demonstrate poorer speech perception scores.

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833 Psychophysics of Channel Interaction

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Channel interactions have been suggested as a possible reason for the variability in the level of speech recognition for cochlear implant subjects, and several studies have shown some relationship between speech recognition and various measures of channel interactions (e.g. Shannon, 1983, Henry et al., 2000; Throckmorton and Collins, 1999). Since the physiological properties that result in channel interactions are difficult to assess or modify, one method for improving speech recognition may be to tune each individual's speech processor based on measurements of channel interactions. Such an approach was demonstrated successfully with implanted subjects in one study (Zwolan et al., 1997), although other studies have had limited success. However, focusing on the measure of channel interactions that is most detrimental to speech recognition may be more reasonable, in terms of cost versus benefit, than investigating possible tuning methods for all measures. Acoustic models can provide an independent comparison between the effect that each measure has on an otherwise unimpaired system, thus providing remediation research focus.

834 An Information-Theoretic Assessment of the Benefits of Noise for Analogue Cochlear Implant Coding

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We are investigating a rigorous information-theoretic design methodology that may circumvent many of the *ad hoc* aspects of current coding strategies. We propose that a cochlear implant strategy for a patient should be designed to maximize the information conveyed

by their cochlear nerve. Our methodology is based on the initial assumption that the neural encoding of acoustic stimuli in the normal ear is almost optimal in terms of the information transmitted to the brain and that increasing the amount of information conveyed by a cochlear implant will increase speech comprehension.

In initial experiments, we have quantitatively measured the amount of information conveyed by an array of nerve fibres from the sciatic nerve of the toad *Xenopus laevis*, which we used as a physiological model of the human cochlear nerve. We extracellularly stimulated the nerve with a Gaussian signal 6 dB above threshold (1 s duration, correlation time 1.6 ms) and recorded the response of single fibres using a microelectrode. The signal, which was identical for each presentation, was presented in background noise with pseudo-random levels up to 20 dB above an arbitrary baseline. Ten presentations were made at each noise level, which is equivalent to simultaneously recording the responses of ten identical fibres to a single stimulus. We measured the transinformation between the input signal and a reconstructed signal, which was formed from the spike times of fibres in the virtual array. The optimal addition of noise doubled the information transmitted, compared with transmission of the signal alone. This is the first demonstration of suprathreshold stochastic resonance in a physiological system.

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835 Perception of High-Rate Stimuli with Cochlear Implants

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The stimulation rate for pulsatile cochlear implant speech processors has been steadily increasing over the past fifteen years. A number of theoretical and electrophysiological studies suggest several ways in which high-rate stimulation may be beneficial. Speech perception data with high-rate processors show mixed results in different studies with some subjects showing an improvement in speech perception and others a decline. We have performed a series of psychophysical experiments in implant patients using high-rate stimulation that closely parallel studies performed with computational simulations and animal models.

These simulations and models suggest that increased dynamic range, more gradual loudness growth, a shallower psychometric function and better temporal resolution for low frequency sinusoids are possible if a high-rate (eg. 5000 pps) "conditioning" or "desynchronizing" pulse train is added to the sinusoid. They also suggest that it is possible to exploit stochastic resonance using such stimuli. Using a DSP-based interface to the Clarion CII cochlear implant, we have tested these predictions in over thirty human subjects. The results confirm that increased dynamic range and more gradual loudness growth indeed occur in virtually all subjects tested. Some subjects show findings consistent with stochastic resonance. The results also suggest that loudness growth in electrical hearing is significantly related to the growth of spike rate. Studies are ongoing to determine if the other model predictions are correct and if improved speech and music perception will occur as implied by the theoretical predictions and the experimental findings thus far.

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836 Investigating the Functional Connections of the Human Inferior Frontal Gyrus: Electrical Tract Tracing Studies

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The inferior frontal gyrus (IFG) of humans is known to play a critical role in speech. The IFG is highly convoluted, forming three sub-gyri, which may represent an evolutionary departure from other primates. It is reasonable to speculate that during speaking the IFG, or some portion of it, influences via cortico-cortical connections the oro-facial representational area of primary motor cortex

To test this hypothesis, electrical-stimulation tract tracing experiments were performed intra-operatively on nine human subjects undergoing surgical treatment of medically intractable epilepsy. Bipolar electrical stimulation (single, charge balance, 0.2ms duration, square wave) was applied to sites on the IFG, while the resulting evoked potentials were recorded from other portions of the IFG and oro-facial motor cortex, using a multi-channel recording array. Protocols were approved by the University of Iowa Institutional Review Board.

Stimulation of the IFG evoked polyphasic waveforms on both language-dominant and non-dominant hemispheres. The most prominent evoked potentials (EPs) were recorded from other sites on the IFG. The EPs had an initial large negative component (onset latency 2-11 msec, peak latency 15-30 msec) followed by a large positive component (peak latency 50-85 msec). A smaller and later (peak latency 140-250 msec) negative component was sometimes seen. Stimulation of anterior superior temporal gyrus also evoked large EPs on the IFG. Surprisingly, less prominent EPs were recorded from motor cortex.

These data suggest that the human IFG may be a cortical complex having functionally interconnected subdivisions. They also show that this IFG complex receives widespread input from both the temporal and frontal lobes and has functional connections with motor cortex.

837 Emergent Cortical Activation Induced By Audiovisual Speech Processing

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Under natural conditions, speech processing is a combined analysis of the vocal tract acoustics and the associated face/lip movements. Neurophysiologically, initial processing of auditory and visual information occurs in distinct areas of cortex. As shown previously (e.g., McGurk and MacDonald, 1976), perception of the acoustic speech signal may be enhanced or altered by the visual speech signal. In this study, we investigated when and where in the brain auditory and visual speech information is processed. Source reconstruction analysis was applied to event-related potentials generated by speech signals presented in auditory alone (AO), visual alone (VO), and two auditory-visual (AV) stimulus conditions. The AV conditions followed the McGurk Effect in which the auditory and visual speech signals were either congruent (AVc) or incongruent (AVi). Current density reconstruction provided evidence of emergent activity in the left posterior temporal and parietal cortex, encompassing Wernicke's cortex, for both the AVc and AVi conditions. This activity emerged 150 to 170 ms after stimulus onset and persisted up to 50 ms. Further analyses of cortical activity centered around 200 ms showed activation strength was greater in the AVc than the AVi condition. For both AV conditions, enhanced activity (relative to AO and VO) was found in superior temporal and in occipital cortex. Evidence was also found for early (100 ms) enhanced activation, but only in superior temporal cortex. This early enhancement did not show a differential sensitivity between the AV conditions. These results suggest that audiovisual speech perception is more than the sum of unimodal perceptual processes and provides evidence of an emergent area of cortical activation in the left hemisphere that occurs only when auditory and speech signals are presented concurrently.

838 Temporal Similarity in Auditory Nerve Fiber Representations of Naturally Produced and Sinewave Synthesized Word Initial Stop Consonants.

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Sinewave speech is a form of synthetic speech that replaces the naturally occurring formant structure with just three, time-varying sinusoids. Perceptual studies with sinewave speech demonstrated that these spectrally reduced stimuli are still recognized accurately (Remez et al., 1981). More recent studies found that other spectrally reduced versions of speech elicit a linguistic percept based on their temporal characteristics (Shannon et al., 1995; VanTasell et al., 1992). This study compared the representations of the temporal structures for natural and sinewave speech tokens in the auditory periphery.

Individual auditory nerve fiber responses to natural and sinewave tokens of the words "ball" and "dirty" presented at 3 intensity levels, were recorded in ketamine anesthetized chinchillas, and used to compute global average peri-stimulus time histograms (GAPSTs) for each token. Comparisons of GAPSTs for the word initial /b/ revealed a pattern of 3 peaks, consisting of a large peak at consonant onset (P1), a smaller second peak (P2), and a large peak at vowel onset (P3). The time between P1 and P3 was 18 msec for the natural /b/, and 15 msec for the sinewave /b/. GAPSTs for the word initial /d/ showed a 2 peak pattern for consonant onset and vowel onset, with the time between P1 and P2 being 41 and 43 msec for the natural and sinewave /d/'s respectively. Chi-squared tests revealed that these intervals did not differ when comparing the natural /b/ to the sinewave /b/, nor when comparing the natural to the sinewave /d/, but did differ significantly for all comparisons of /d/ to /b/. The results demonstrate that the neural representations of sinewave and natural speech tokens have similar temporal structures, which may allow sinewave speech to be recognized accurately despite being reduced spectrally.

839 Effects of Stimulus Repetition Rate and Background Noise on the Representation of Speech Sounds in the Inferior Colliculus, Medial Geniculate Body, and Auditory Cortex of Guinea Pig

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Two previous studies from these authors have investigated effects of stimulus repetition and background noise on the representation of speech sounds, as reflected by brainstem (*ARO Midwinter Meeting*, 2002) and cortical (*Clinical Neurophysiology*, 2002) far-field evoked potentials, in normal and learning-impaired children. In both studies, abnormal, asynchronous neural encoding of speech was related to poorer performance on measures of speech and language skills. The current study intends to describe, and further anatomically localize, effects of repetition and noise in the mammalian central auditory system by recording near-field multiunit activity from within the auditory pathway of the guinea pig.

Microelectrodes (1 MΩ) were placed in the central nucleus of the inferior colliculus, the ventral division of the medial geniculate body of the thalamus, and primary auditory cortex. A 10 kΩ epidural electrode was also placed over primary auditory cortex. A synthesized speech sound /da/ (40 ms, 80 dB SPL) was presented in trains of four, separated within a train by 12, 24, 50, 150 or 350 ms. Stimuli were presented in quiet and in background noise (65 and 55 dB). Response peak and RMS amplitudes, spectral power, and response correlations were measured.

This study incorporated multiple repetition rates and signal-to-noise ratios in an effort to parametrically describe the separate and combined influences of these factors on stimulus representation. Of particular interest is comparison of the representation of harmonic activity, such as vowel sounds, versus the encoding of transient elements, such as stimulus onset, as well as potential interactions of these factors with anatomic level within the pathway.

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840 Effects of Training on Lateral Representation of Speech Sounds in Learning Impaired Children

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Many children with learning problems exhibit deficits in both the perception and neural encoding of speech sounds. In particular, learning impaired children are likely to exhibit atypical patterns of speech sound lateralization. Auditory perceptual training programs have led to distinct alterations of neurophysiologic responses in the left and right hemispheres of normal adults (K. Tremblay and Kraus, JSHLR, 2002) and learning impaired children (TL Richards et al., Ajnr, 2000; E. Temple et al., PNAS, 2000). Specific changes exhibited in patterns of lateralization have not been consistent across these studies and require further investigation.

The effects of training on the lateral representation of speech sounds in learning impaired children were assessed with cortical potentials. Children with reading deficits (n=24) were evaluated before and after an 8-week curriculum using commercial auditory processing training software. Control children (n=21) did not participate in remedial programs during this time period and were evaluated over similar intervals. Relative hemispheric contributions (RMS and peak amplitudes) of the P1-N2 cortical response were investigated.

Trained children demonstrated improved auditory processing abilities compared to controls but failed to show alterations in the lateral cortical representation of speech sounds. However, asymmetry patterns at pretest were associated with improvement in syllable and word perception. These findings suggest that improvements in auditory processing in learning-impaired children can occur despite the relatively hard-wired lateral representation of sound and that patterns of cortical asymmetry appear to mark children who are good candidates for specific types of auditory training.

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841 Encoding of Speech Sounds in Quiet and Background Noise in the Brainstem: Normal and Learning Impaired Children

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The electrical activity of neuronal ensembles encodes acoustic features of speech in a variety of different structures along the path from the periphery to the cortex. At the level of the brainstem, previous studies showed neuronal timing deficits for learning problem (LP) children listening to syllables in quiet conditions. The observed delays were exacerbated by the addition of background noise (1,2).

These studies set precedents for the performance of an encyclopedic study of encoding mechanisms in the brainstem and the establishment of normative values for a large normal (NL) population. Our goal is to catalogue the properties of NL and LP responses in quiet and noisy conditions. To do this, we scrutinized onset responses that operate at transient intervals of tenths of milliseconds (e.g. peak latency), as well as the sustained, periodic frequency-following responses (FFR) that occur over tens of milliseconds (e.g. Fourier transforms, correlations).

Group differences in quiet were observed. Background noise caused different patterns of response degradation in NL and LP groups. Relationships between encoding deficits and type of learning problem

were also evident in subsets of the LP population. Our findings indicate that transient and sustained brainstem response properties reflect discrete encoding mechanisms. Furthermore, the pattern of these responses provides insight into the nature of auditory encoding deficits in LP children.

This study brings us closer to understanding the link between the structure of a speech stimulus, the neural mechanisms that encode it and the behaviors that operate on them.

1. King et al., Neurosci Lett 2001; 27: 111-115

2. Cunningham et al., Clin Neurophysiol 2001; 112: 758-767

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842 Speech Encoding During Binaural and Dichotic Stimulus Application

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Spoken-word recognition depends upon encoding of information bearing elements of the acoustic speech signal. To further delineate the underlying neurophysiological mechanisms brain activation was studied during binaural and dichotic application of speech sounds using fMRI.

Local blood-oxygenation level dependent images were acquired using a 1.5 Tesla MR scanner (EPI, 15 slices/5mm, FOV 220mm, matrix 128x128). A home-made noise attenuating sound system delivered auditory stimuli to 11 healthy volunteers under two experimental conditions: Firstly, standardized sentences consisting of 5 words were presented binaurally. Secondly, similar stimuli were splitted into two unintelligible complementary elements using a toggle-switch gate at a frequency of 3.6 Hz and delivered dichotically. Dichotic application of the unintelligible but complementary stimuli allowed to fully understand the semantic information. Images were analysed by statistical parametric mapping (SPM96).

Binaural stimulation revealed a bilateral activation of the prim. and sec. auditory cortices (BA 41/42) and a left-lateralized activation of the sup. temp. gyrus (STG) including BA 22/21. Furthermore, perisylvian activation was observed in the right hemisphere. Dichotic stimulation revealed consistently a bilateral activation of BA 41/42. In contrast to binaural stimulation, dichotic stimulation showed a bilaterally enhanced activation of the STG (BA 22/21) and the perisylvian cortex, which was clearly pronounced in the right hemisphere.

The results suggest a reconsideration of the hypothesis that auditory speech comprehension is restricted to the left hemisphere. They further indicate that the right STG is essentially involved in paralinguistic aspects of speech perception.

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843 Facts and Artifacts in Auditory Chimaeras

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Smith, Delgutte and Oxenham (Nature, 416:87–90, 2002) produced "auditory chimaeras" by systematically mixing one sound's temporal envelope with another sound's fine temporal structure as a function of frequency bands (1-64). They found that "the envelope is most important for speech reception, and the fine structure is most important for pitch perception and sound localization." Here we identified two technical problems that one should be aware of when interpreting results derived from auditory chimaeras. First, one should be aware of the ear's natural ability to recover the narrow-band envelope with the broad-band processing for a small number of frequency bands (e.g., 1 and 2). Second, one should be concerned about filter artifacts with the narrow-band processing for a large number of bands (e.g., 32, 48, and 64). In addition, we conducted two experiments to challenge Smith et al.'s assertion regarding the envelope and fine structure as the acoustic basis for the "what" and "where" mechanisms. In one experiment, we used Smith et al.'s program to chimaerize two sentences that had either a 15-dB interaural level difference or realistic interaural differences through HRTF filters. Under these conditions, we found that it was the envelope, rather than the fine structure, that determines sound localization. In another experiment, we performed classic filtering manipulation on the chimaerized sounds with 16 bands and a 700-ms delayed envelope or fine structure. With a low-pass filter having an 800-Hz cutoff frequency, we found that one could lateralize the sound to the side with leading fine structure but could not recognize speech. Conversely, with a high-pass filter having an 800-Hz cutoff frequency, one could easily recognize speech but could not lateralize the sound. This result suggests that the dichotomy revealed by the auditory chimaeras is an epiphenomenon of classic duplex perception between low- and high-frequency pathways.

844 Independent Contributions of Amplitude and Frequency Modulations to Auditory Perception. I. Consonant, Vowel, and Sentence Recognition

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Previous studies have demonstrated that one can understand speech with primarily either temporal or spectral cues. However, it is not clear why both cues are present in natural sounds and how they are processed in the auditory system. Here we developed a signal processing strategy that independently extracted slowly-varying amplitude and frequency modulations within a frequency band with the number of bands as an independent variable. Normal-hearing listeners were presented with original speech sounds and processed sounds including amplitude modulation only and both amplitude and frequency modulations. The speech materials were vowels, consonants, and IEEE sentences, presented in quiet, or speech-shaped noise, or a single competing talker. The addition of frequency modulation significantly increased speech recognition with amplitude modulation only, particularly with less frequency bands and in challenging noise conditions. For example, the average vowel recognition score with amplitude modulation only and four frequency bands was 62%, 38%, and 32% for quiet, 0, and –5 dB signal-to-noise ratio conditions, respectively. With the addition of frequency modulation, the corresponding score improved to 75%, 63%, and 52%, respectively. Similarly, the addition of frequency modulation improved sentence recognition by about 50 percentage points from 20% in the presence of a competing talker. These results suggest that, while amplitude modulation provides essential information for speech recognition in quiet, frequency modulation can enhance speech recognition by allowing the listener to extract signal from noise. Further results in tonal language, music and speaker identification will be presented in a companion paper. These results are relevant to design of cochlear implants and audio coding strategies.

845 Independent Contributions of Amplitude and Frequency Modulations to Auditory Perception. II. Melody, Tone, and Speaker Identification.

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In a companion paper, we showed that amplitude modulation provides sufficient information for speech recognition in quiet, but additional frequency modulation is needed in noise. Here we evaluated relative contributions of amplitude and frequency modulations to melody, tone, and speaker identification. Twelve familiar melodies were generated with or without tempo information. Twenty-five Mandarin syllables, each having 4 tonal variations, were produced by a male and a female talker. Six vowel tokens (3 used for training and 3 used for testing) produced by 3 males, 3 females, 2 boys, and 2 girls were used for speaker identification. Stimuli were processed to extract slowly varying amplitude and frequency modulations from a number of frequency

bands (1-64 bands). Melody and speaker identifications were conducted in both normal-hearing and cochlear-implant listeners, whereas tone identification was conducted in normal listeners only. Results showed that amplitude modulation only (i.e., 1 band) produced about 80% correct performance for melody identification with tempo and also for tone identification in quiet. However, for melody identification without tempo and for tone identification in noise (0 dB S/N), the performance dropped to about 40% even with 8 frequency bands. Similarly, listeners could recognize most of the vowels but could not identify the speakers. When frequency modulation was added, performance was restored to a level similar to the unprocessed stimuli. These results suggest that amplitude and frequency modulations independently contribute to auditory perception, with amplitude modulation contributing gross temporal information and frequency modulation contributing detailed spectral information for accurate pitch perception and signal-and-noise separation.

846 Clear Speech Perception in Normal-Hearing and Cochlear-Implant Listeners

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Previous studies have demonstrated that when instructed to speak clearly to people with hearing loss, talkers can produce “clear” speech, which has significantly higher intelligibility in noise than “conversational” speech. Here we measured clear and conversational speech perception at various signal to noise ratios covering a range over which intelligibility increased from about 0% to 100%. Stimuli consisted of ten sets of BKB sentences produced by a male and a female talker in clear and conversational speech. Speech-spectrum-shaped noise was used to produce the different signal-to-noise ratios. Real cochlear implant users and cochlear implant simulations were also tested to measure the contribution of temporal envelope cues to the clear speech advantage. A sigmoid function was used to fit the measured data, producing 2 parameters indicative of the speech reception threshold (i.e., the signal-to-noise ratio at which 50% intelligibility was achieved) and the slope of the psychometric function. We found that the speech reception threshold was –9.1 dB for clear speech and –6.3 dB for conversational speech in normal listeners, and was correspondingly –4.6 dB and 1.0 dB in implant listeners. The differences in speech reception threshold translated into about 15 percentage points in improved intelligibility scores for normal listeners and about 20 percentage points for implant listeners. Cochlear implant simulation produced similar results to that obtained in real implant listeners. The present results confirmed and extended previous findings in normal listeners. In addition, the implant and its simulation data suggest a direct contribution of temporal envelop cues to the intelligibility advantage of clear speech over conversational speech. Further analysis of temporal envelope cues in clear speech should yield results that are not only important for understanding mechanisms of speech perception but also for developing novel processing algorithms in auditory prostheses.

847 Effects of High Presentation Levels on Recognition of Low- and High-Frequency Speech

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For listeners with normal hearing, speech recognition scores tend to decrease as presentation levels are raised above moderate levels. This “rollover” effect generally has been treated as a broadband phenomenon: the negative consequences of high signal levels are assumed to be similar across frequency. For example, the Speech Intelligibility Index (ANSI, 1997) includes a level distortion factor (LDF) which systematically decreases predicted speech scores when the overall level of the speech increases above 73 dB SPL. The LDF is applied uniformly across the entire frequency range.

To examine rollover for high- and low-frequency speech cues independently, normally-hearing listeners were tested for sentence keyword recognition using lowpass- and highpass-filtered sentences presented at a range of levels. In a preliminary task, adaptive tracking was used to determine the lowpass and highpass bandwidths required by each listener for approximately 80% correct keyword recognition at a moderate presentation level (overall levels determined by filter bandwidth but reflecting a broadband level of 75 dB SPL). The resulting high- and low-frequency speech bands were then tested further with sentences presented at 75 dB SPL (broadband level) and also 10, 20, and 30 dB above this level. The low-frequency band showed some evidence of rollover with about a 10% decrease in keyword recognition as levels increased. The high-frequency band showed greater rollover with scores decreasing by about 30% with the 30 dB increase in input level. The greater influence of presentation level on processing of high-frequency speech cues is consistent with both physiological and psychoacoustic results suggesting that cochlear processing may be more nearly linear (less level-dependent) in apical, low-frequency regions than in basal regions tuned to higher frequencies.

848 Speech Intelligibility and Spatial Release From Masking in Children and Adults for Various Types of Interfering Sounds

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It is well known that speech recognition improves when a "target" speech signal is spatially separated from an interfering sound. We recently demonstrated this effect, known as "spatial release from masking" (SRM) in young children. However, the effect is smaller in children, and in general they require higher signal-to-noise ratios in order to demonstrate SRM. In our previous work, the interfering sounds were also speech, adding an "informational masking" component. The present work was aimed at comparing SRM in young children for various interfering sounds, to try and account for the differences between adult and children listeners. These differences might be due to developmentally-dependent effects of interferers that contain energetic, informational and/or linguistic components, as well as temporal modulations.

To begin teasing apart the various factors that might contribute to SRM, in the present study interfering sounds consisted of speech, reversed speech and modulated speech-shaped noise. Children ages 5-6 as well as adults were tested in an IAC sound booth with speakers positioned at a distance of 5 feet. Speech Recognition Thresholds (SRTs) were measured under three conditions: QUIET (no interferer), FRONT (interferer and target originating from the front), and RIGHT (interferer at 90° to the right and target in front). Results showed that, in general, children's performance is worse than adults' (higher SRTs and smaller SRMs). In addition, SRT varies with the type of interfering sound, and this variation interacts with age.

849 Auditory Processing Skills And Literacy Development

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Phonological awareness skills during the preschool years are predictors of later developing reading development. Auditory processing training program outcomes offer strong support to the hypotheses that children with delayed phonological skills or disabled readers have delayed/impaired auditory processing, and that these delays/impairments can improve with appropriate training. The facts that auditory processing skills are essential to phonological awareness, and prone to improve with appropriate intervention, may open new avenues for supporting reading disabled students. They may also provide useful insights in enhancing general literacy instruction methods. This study investigated changes in auditory processing, phonological awareness, and literacy skills in two groups of normally developing first graders. The first group underwent a few months of intense phonological awareness training while the second group,

consisting of age and intelligence matched peers, attended language stimulation sessions of similar duration. The study further examined specific auditory processing skills affected by and related to development of phonological awareness and reading in young children.

850 Effects of Early Auditory Deprivation on Vocal Production in the Common Marmoset

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As a highly vocal New World monkey with a well-developed auditory system the common marmoset (Callithrix jacchus) may serve as a valuable model for the analysis of mechanisms related to human vocal communication. Young marmosets produce vocalizations that differ significantly from those of adults. As the monkeys mature these calls gradually take on adult forms and the use of typically "baby-like" vocalizations eventually disappears. Such changes may result in part from physical maturation; however, the highly social nature of primate vocal communication suggests that auditory experience may also play a role in determining final parameters of the adult vocal repertoire. We have studied the development of communication sounds in infant twin pairs born in a captive colony. One member of each pair was deafened shortly after birth by a series of neomycin injections, and the vocalizations of each animal were tracked for a period of up to one year when the vocalizations of normally developing infants are no longer discernable from those of adults. Spectral and temporal characteristics of these vocalizations were quantitatively analyzed and compared with those of age-matched controls collected earlier from the same colony. Call types typical of immature monkeys, such as "baby-cry" and "babbling," were commonly observed in the deafened animals even after they matured to adulthood, and some call types failed to fully acquire adult form. A subset of spectral and temporal parameters of vocalizations in deafened animals showed abnormal development from infancy to adulthood. Our observations suggest that vocal development in young marmosets is influenced by auditory experience. These findings provide a foundation for further studies to explore the underlying neural and anatomical basis of such vocal plasticity.

851 The Relationship Between Different Levels of Sound-Sequence Analysis and Reading Ability

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Competent reading skills are related to good phonological awareness. There is a growing body of evidence to suggest that the prosodic features of speech play a critical role in the development of efficient phonological skills. In particular, the prosodic cues that signal stress play an important role in infancy in segmenting the speech stream into words, and this is thought to form a platform for the development of phonological abilities. Here we assessed the perception of patterns of pitch change, the most effective cause of perceived stress in speech, and we investigated the relationship to measures of phonological skills and reading ability. We employed a paradigm based on detection of differences between 6 note pitch sequences based on an octave scale of 7 equal steps. This enabled distinction between the perception of actual pitches in a sequence ('local' structure) and the perception of changes in pitch direction ('global' structure or contour). In a sample of 30 normal adults it was demonstrated that only the perception of global structure pitch contour is statistically predictive of performance on measures of phonological skill and reading ability. This auditory measure uniquely accounted for 17% of the variation on a test of phonological decoding skill, beyond that attributable to measures of verbal and nonverbal intelligence. In contrast, this measure was not a unique predictor of orthographic skill, a non-phonological reading skill, suggesting that the perception of pitch contour is an important constraint on the phonological skills involved in reading. The results demonstrate that the perception of meaningful patterns in pitch is related to phonological ability, and supports the role of speech prosody and word boundary cues in literacy acquisition.

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852 The Mobile Phone for Hearing-Impaired People (The First Report)

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Abstract

The speed of development of mobile phones is miraculous. Most of people have one mobile phone a person. Mobile phones have the speaker and microphone system. In this study, we compared mobile phones with HAs from the point of view of improvement of the hearing-impaired persons□f speech discrimination and the degrees of their satisfaction.

Methods

We used the second-generation mobile phone with sound recording system. The speech test as the speech discrimination test and APHB/ HAPI as the evaluation of the hearing-impaired persons□f satisfaction were applied.

Results

The improvement of the speech discrimination and the degrees of the satisfaction with mobile phones was good as with HAs. The wireless communication made both of them worse. The high cost, bulky, useless outside the network, and difficult operation were in questionnaires.

Conclusion.

Several tests to evaluate HAs were done for this study to compare HAs and mobile phones for hearing-impaired people. For this point, this is the first report of our best knowledge. We considered the development of mobile phones would solve problems of results.

853 Deficits In Low-Level Auditory Processing In Reading-Disabled Adults

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The exact nature of perceptual deficits associated with reading disability is still unclear. The dominant current theory, commonly referred to as the 'fast temporal processing deficit hypothesis', states that processing of phoneme-rate stimuli is impaired in some disabled readers. This study critically assessed this hypothesis in a population of disabled readers and their age and education matched controls. We administered an extensive battery of psychoacoustic tasks designed to probe temporal processing on a broad range of time constants, from sub-milliseconds to several seconds. About a third of the disabled readers in our sample were found to have difficulties in most of the administered tasks: detection of frequency differences at widely varying inter-tone intervals, detection of tones in narrowband noise, detection of amplitude modulation, and perception of the lateralized position of tones based on their interaural phase differences. On the other hand, comodulation masking release and binaural unmasking were normal in this subgroup. Disabled readers with poor auditory processing did not differ from other disabled readers on any reading measure, but they scored lower on some tests of general cognitive ability. We conclude that disabled readers do not have a specific deficit in processing brief or rapidly presented stimuli, as suggested by the fast temporal processing deficit hypothesis. Alternatively, we suggest that this subgroup may have reduced signal-to-noise ratio, possibly due to increased internal noise at the output of the auditory channels. Since the majority of disabled readers do not

have perceptual deficits, these deficits are not necessary for reading disability. They may, however, be sufficient to impair reading, as none of the good readers in our sample had consistently poor psychoacoustic performance.

854 Auditory Temporal Processing in Dyslexia - A New Approach

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Developmental dyslexia is a specific reading disability, which is also associated with impairments in basic auditory perception. We previously suggested a psychoacoustic based subtyping of dyslexia (Banai & Ahissar, 2000). Individuals with comprehensive auditory deficits have a reading disability (RD) with additional learning difficulties, whereas those with mild auditory deficits or none have a more specific reading disability (SRD). We now asked whether SRDs also have more pronounced auditory processing deficits, which are revealed when the temporal structure of stimulus presentation is manipulated.

We assessed 2-tone frequency and intensity discrimination ("which tone is higher/louder?") for 50ms pure tones, with a fixed reference (1000Hz, 60dB, respectively), under various ISIs (0.05-5s). Thirty adult dyslexics (D) and 30 age and intelligence-matched controls (C) participated in this study. Under typical ISIs (0.7 – 2s), each D subgroup had a deficit with respect to its intelligence-matched C subgroup, consistent with previous findings. Yet, the thresholds of SRDs were only mildly impaired and not worse than those of controls with lower IQ scores. Under shorter ISIs (0.1-0.5s), SRDs' frequency discrimination became increasingly poor whereas performance of the other subgroups remained the same. Thus, for short ISIs, frequency JNDs of D subgroups were significantly poorer than those of C subgroups.

Intensity discrimination was not impaired among either D subgroup compared with controls when measured with longer intervals (1-2s), consistent with previous findings. Under shorter ISIs (0.2-0.5s), SRD's performance was significantly impaired with respect to their C subgroup.

These findings show that SRDs, whose general psychoacoustic abilities are only mildly impaired when measured with longer ISIs, have enhanced difficulties with sequential auditory processing when stimuli are retained for subsequent comparisons across intervals of hundreds of ms.

855 Auditory Temporal Processing Deficits Among Individuals with a Family History of Specific Language Impairment

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Children with specific language impairment (SLI) exhibit deficits on some measures of auditory temporal processing (Tallal & Piercy, 1973; Wright et al., 1997). Evidence suggests that SLI is heritable (Bishop et al., 1995) and may segregate as mendelian traits in some families (Bartlett et al., 2002). The purpose of this study was to investigate the hypothesis that family members of children diagnosed with SLI exhibit deficits on auditory temporal processing measures. Normative reference data were collected from volunteers (age 15 years to 76 years) with no history of SLI, learning problems, or hearing impairment. Affected families were selected on the basis of one child receiving special services for SLI and a history of at least one other family member with language or learning problems. Members of four affected families participated in the study. A range of auditory temporal processing measures were presented, including gap detection, backward masking at 0 and 50 ms, duration discrimination, rhythm discrimination, temporal order discrimination, and temporal order identification. A battery of standardized and experimental language measures was also presented. Results showed that at least one first-degree relative of each affected family member exhibited abnormal performance on one or more measures of auditory temporal processing. The auditory temporal processing measures that revealed the largest deficits among affected family members were backward masking, duration discrimination, and temporal order identification. These findings support the hypothesis that deficits in auditory temporal processing are associated with a positive history of familial language impairment.

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856 Effects of Age and Hearing Loss on Gap Detection and Echo Thresholds

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Older listeners and listeners with impaired hearing often demonstrate poorer than normal performance on tasks of speech understanding and auditory localization. Underlying these deficits may be a deficit in temporal processing as timing cues are inherent in both tasks. For speech understanding, temporal cues occur for individual speech sounds and perception of such cues is studied using a gap detection paradigm in which a listener must report the interval in which a silent gap was detected (two sounds versus one sound). This paradigm is similar to one used to measure echo thresholds in a precedence effect task. The precedence effect is an auditory phenomenon during which early reflections perceptually fuse with a direct sound. The perceived spatial location of the fused image is dominated by the direct sound, aiding localization. Echo thresholds are measured by varying the onset-to-onset (OTO) interval between a leading sound and a lagging sound. The listener reports if their perception is more of one fused image or two separate auditory events. It has been suggested that resolution of a silent gap between similar signals presented monotically or diotically requires within-channel processing (within a single perceptual channel), and across-channel processing (across separate perceptual channels) is required for signals presented dichotically. The purpose of this investigation was to determine if within channel (measured with monotic and diotic gap detection) or across-channel (measured with dichotic gap detection) temporal resolution has an influence on echo thresholds. Gap detection thresholds (GDTs) and echo thresholds were measured for three groups of listeners: (1) young normal hearing, (2) old normal hearing, and (3) old with impaired hearing. Preliminary results indicate age and hearing loss differentially affect echo thresholds and GDTs.

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857 Onset Asynchrony as a Cue to Sound Segregation by Hearing-impaired Listeners

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The ability of hearing-impaired listeners to use onset asynchrony as a cue to sound segregation was evaluated in a concurrent vowel experiment. Listeners were presented with two simultaneous steady-state vowels. The "target" vowel had a duration of 250 ms, and the "distractor" vowel varied in duration between 350 ms and 550 ms. The vowels had simultaneous offsets, and therefore the onset asynchrony between the two vowels ranged between 100 ms and 300 ms. Two fundamental frequencies were tested, 120 and 151 Hz, with the target and distractor vowels either having the same fundamental or different fundamentals. The percentage of trials in which the listeners correctly identified the second vowel of the two-vowel series was measured as a function of onset asynchrony. While hearing-impaired listeners performed more poorly than normal-hearing listeners, both groups of

listeners showed similar increases in performance with increasing onset asynchrony. Both groups of listeners also performed best in conditions where the fundamental frequencies of the two vowels differed. However, at the smallest onset asynchronies, hearing-impaired listeners did not benefit from the fundamental frequency difference to the same degree as the normal-hearing listeners. When errors were made, all listeners tended to respond with the distractor vowel label at the shortest onset asynchronies. As onset asynchrony increased, these order errors were reduced. The changes in error rate also were similar for both groups of listeners. In summary, these data indicate that onset asynchrony remains a useful to cue to sound segregation by hearing-impaired listeners who may be unable to use differences in fundamental frequency to segregate sounds as well as normal-hearing listeners.

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858 Modulation Discrimination Interference At Low Modulation Rates: Evidence For Two Mechanisms For The Discrimination Of Frequency Modulation Depth.

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Moore and Sek (1995) have proposed that amplitude and frequency modulation (AM, FM) may be detected using a common mechanism when the modulation rates are 10 Hz or more and suggested a model based on the non-optimal use of the excitation pattern. At lower rates they proposed the existence of a second mechanism for the detection of FM, based on phase-locking information. In a modulation discrimination interference (MDI) task, the signal is a change in modulation depth of a sinusoidal carrier, in the presence of other sinusoidal interferers that are remote in frequency. The discrimination of both AM and FM depth is impaired in the presence of AM or FM interferers, but not in the presence of unmodulated ones. Interference has been observed for modulation frequencies of 10 Hz or more, where Moore and Sek (1995) propose AM and FM are coded on a single mechanism. The present work investigated the interference effects of AM and FM on the discrimination of AM or FM depth using a range of target modulation rates between 2 and 10 Hz. If AM and FM are coded by different mechanisms at a 2-Hz rate, then there should be less interference between them. Results replicated previous findings for the 10-Hz target rate. Presence of both AM and FM produced MDI and the effect showed tuning for modulation rate. With the 5-Hz rate, the effect was somewhat smaller. With the 2-Hz rate MDI occurred only when both the target and the interferers were AM and modulated at the same rate. When either the target or interferers were FM, there was no MDI. Further studies investigated whether the absence of MDI was due to possible modulator phase effects. In all cases there was little or no effect of relative modulator phase. These results support the existence of an additional mechanism for the detection of FM at modulation rates as low as 2 Hz, possibly based on phase-locking information.

859 Modulation Masking Produced by 2nd-order Modulation Using Audible and Inaudible Carrier Modulations

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Recent psychoacoustic studies have demonstrated that listeners can detect "2nd-order amplitude modulation" (AM) (sinusoidal AM - of rate fm' - applied to the depth of a 1st-order sinusoidal AM of rate fm), even though the physical modulation spectrum does not contain a component at fm'. According to the modulation filterbank model, the detection cue should appear in two distinct regions of the internal modulation spectrum evoked by 2nd-order AM: (1) as a distortion product of rate fm', and (2) as a "beat" of rate fm' appearing at the output of the modulation filter tuned to or near fm. The goal of the present study was to characterize the distortion product evoked by 2nd-order AM using a range of values of fm, including values that fall outside the range of audible modulations. Initially, 1st-order TMTFs and 2nd-order TMTFs with fm = 64, 180 or 2000 Hz were obtained using four normal-hearing listeners. Then, masked modulation thresholds were measured for a 5-Hz probe modulator in the presence of a 5-Hz 2nd-order AM masker (for each fm) as a function of relative phase between the probe and the masker. Finally, masked modulation thresholds were measured for a 5-Hz probe modulator in the presence a 5-Hz 2nd-order AM masker (with fm = 2000 Hz) as a function of probe modulation depth (the relative phase between the probe and the masker was fixed at a value leading to maximum amount of masking, i.e., 180°). Overall the results are compatible with the existence of a weak distortion product (in the range of 5-10%) in the internal modulation spectrum evoked by 2nd-order AM. However, the relative phase of this distortion product depends on fm.

860 Processing of Complex Amplitude Modulation in Normal-Hearing and Hearing-Impaired Listeners

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Natural sounds like speech and music contain a number of simultaneous amplitude modulations. The temporal properties of such complex sounds are not entirely described by the linear envelope of the stimulus. Recently, the envelope of the envelope, referred to as the "venelope", has been suggested to reflect the perceptual properties of sounds modulated by multi-component modulators [Ewert et al., 2002, J. Acoust. Soc. Am., 112, in press]. It is still questionable how the auditory system extracts the venelope and whether envelope and venelope are processed independently by the auditory system. Masking experiments were performed in order to investigate the interaction between envelope and venelope fluctuations. The data demonstrate that venelope and envelope are not processed independently. Similar masking effects between venelope and envelope were found in normal-hearing and in cochlear hearing-impaired listeners. The results strongly suggest that cochlear compression is not responsible for the perceptual salience of the venelope. The empirical findings are compared to model predictions based on a model that extracts the venelope of the stimuli.

861 External and Internal Limitations in Amplitude-modulation Processing

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The present study explores the relative role of "external" signal variability and "internal" resolution limitations of the auditory system in the detection and discrimination of amplitude modulations (AM). In the first experiment, AM-depth discrimination performance was determined using sinusoidally amplitude modulated broadband-noise and pure-tone carriers. AM-depth discrimination thresholds were a constant fraction (Weber fraction) of the AM depth of the standard for a high standard modulation depth. For smaller standards, AM-depth discrimination thresholds were constant on an linear scale. In the second experiment, AM-detection thresholds were obtained for different signal-modulation frequencies (4 to 256 Hz), applied to either a band-limited random-noise carrier or a deterministic (frozen) noise carrier, as a function of carrier bandwidth (4 to 2048 Hz). In general, detection thresholds were higher for the random- than for the frozen-noise carriers. For both carrier types, the threshold followed the pattern expected from frequency-selective processing of the stimulus envelope. The third experiment investigated AM masking at 4, 16 and 64 Hz in the presence of a narrowband masker modulation. The variability of the masker was changed from entirely frozen to entirely random, while the long-term average envelope power spectrum was held constant. The experiment

examined the validity of a long-term average quantity as the observation variable, and the role of memory in experiments with frozen-noise maskers. The empirical results were compared to predictions obtained with two modulation-filterbank models. The predictions revealed that AM-depth discrimination and AM detection are limited by a combination of the external signal variability and an internal "Weber-fraction" noise process.

862 Are There Separate Mechanisms for the Detection of Amplitude and Frequency Modulation?

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The question of whether changes in the amplitude and frequency of sounds are detected by the same mechanism (e.g., Zwicker, 1956; Maiwald, 1967) or independent mechanisms (e.g., Feth, 1972; Coninx, 1978) is important for auditory research. The aim of this study was to address this question by investigating the effect of narrowband noise modulation maskers on the detection of amplitude modulation (AM) and frequency modulation (FM) at a low modulation frequency (2 Hz).

The modulation maskers were narrow bands of noise, centered on 2 Hz, which were then used to either amplitude-modulate or frequency-modulate a carrier. The carriers were either 1-kHz or 5-kHz pure tones. Detection thresholds for 2-Hz sinusoidal AM and 2-Hz sinusoidal FM applied to both carriers were measured in the presence of the noise modulation maskers. The results show that an amplitude-modulation mask increased detection thresholds for 2-Hz AM imposed on a 5-kHz carrier and for 2-Hz FM imposed on both carriers, but had a greater effect on 2-Hz AM imposed on a 1-kHz carrier. The frequency-modulation mask increased detection thresholds for 2-Hz FM imposed on a 5-kHz carrier and for 2-Hz AM imposed on both carriers, but had a larger effect on 2-Hz FM imposed on a 1-kHz carrier. These results are inconsistent with the idea of completely independent mechanisms for the detection of AM and FM at low modulation frequencies.

863 Behavioral Limits of Auditory Temporal Resolution by the Rat: Amplitude Modulation and Duration Discrimination

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A conditioned avoidance procedure was used to determine thresholds for discrimination of temporal features of sounds under two stimulus conditions: detection of amplitude modulation and detection of an increase in the duration of a sound. For detection of amplitude modulation, rats were trained to respond (make a withdraw response from a water spout to avoid a shock) to the introduction of a sinusoidal amplitude modulation in a white noise carrier. The depth of modulation was gradually decreased until a limit for detecting the presence of the modulation was reached. Psychophysical curves were generated and thresholds for modulation depth were obtained at different modulation frequencies between 5 and 2000 Hz. The thresholds increased with modulation frequency over a range of 100 to 2000 Hz and the modulation transfer functions were similar to those previously reported for humans and chinchillas. For duration discriminations, the rats were trained to respond to an increase in duration relative to a series of regularly repeated background noise bursts. Psychophysical curves were obtained with background noise bursts set at different values between 10 and 1000 ms. The size of thresholds was proportional to the duration of the background noise over a range from 250 to 1000 ms. Thresholds for shorter durations (100 ms or less) were invariant with changes in the background stimulus. The sound pressure level of the stimulus was held constant for all noise bursts used in duration discrimination tasks including the shorter stimuli. The data for rats are compared with those previously published for other species including man.

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864 A Physiological Model for Neural Responses to Amplitude-Modulated Stimuli and for Psychophysical Modulation Tuning

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Neural cues carrying information about a signal's temporal envelope change form as they ascend the auditory pathway. A physiologically-based processing model was developed to understand the transition from a temporal envelope code based on peripheral and lower brainstem responses to a rate-based code as observed at higher auditory processing centers. A computational model of the auditory nerve (AN) [Bruce et al., JASA, in press] was used to generate excitatory and inhibitory inputs for postsynaptic model cells. One stage of interactions enhances temporal modulation tuning and increases synchrony to the stimulus envelope, consistent with physiological studies of the cochlear nucleus. A second layer of convergence results in output cells which are rate-tuned to specific modulation frequencies, as seen in inferior colliculus recordings. The key features of modulation tuning in the model cells are the temporal interactions taking place between fast excitatory inputs and relatively sluggish inhibitory inputs, as suggested by visual cortex modeling studies of responses to modulated stimuli [Krukowski and Miller, 2001, Nat Neuro 4:424-430]. Important parameters describing the dynamic interactions vary across the two stages; excitation is stronger than inhibition in the first layer, while the relative strength of inhibition is greater in the second stage. Delays between respective inputs also shape the final model output by influencing the stimulus modulation rate to which a given model cell responds most strongly. Responses of the second-stage model cells were used to predict psychophysical responses in a modulation masking paradigm. A decision variable based on the rate of a single cell can predict both the shape and bandwidth of modulation domain tuning curves that are consistent with behavioral measurements. Time-domain responses of the model cell can be directly compared to the output of modulation filters used previously to explain the masking results [Ewert and Dau, 2000, JASA 108:1181-1196].

865 Effects of Reduced Temporal Uncertainty on Infants' Pure-Tone Sensitivity: Effect of Cue-Signal Delay

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It is well established that adults' sensitivity in sound detection is improved when the listener knows the time that the sound will occur. In previous work we have shown that infants' detection performance does not improve when temporal uncertainty is reduced by introducing an auditory cue to trial onset and may actually deteriorate when the cue precedes the trial by a long interval. In the present study, we asked whether decreasing the duration of the cue-signal delay to 200 ms would lead to an improvement in infant performance in the cued condition. The subjects were 7-9-month-old infants and 18-30-year-old adults, all expected to have normal hearing. An observer-based conditioning procedure was used. Stimuli were presented monaurally via Etymotic ER1 insert phone. The listener's task was to detect a 200-ms, 1-kHz tone in a continuous background of 20 dB spectrum level noise, low-pass filtered at 2.5 kHz. The level of the tone was fixed at 50 dB SPL for infants and 42 dB SPL for adults. These levels had previously been found to be detectable about 70% of the time (d' = 1) for the two age groups, respectively. Trials were 4 s in duration. The 1-kHz signal was presented at trial onset on 50% of trials. On no-signal trials the noise background continued and no tone was presented. The cue was a 10-dB increase in the level of the background noise, 200 ms

in duration. In the cued condition, the offset of the cue preceded the onset of the trial by 200 ms. In the uncued condition, trials were presented without warning. The results showed that while a temporal cue improved sensitivity for adults, it led to a decrease in sensitivity for infants. The results suggest that an auditory cue preceding the trial may actually distract the infant from listening for the signal under most conditions.

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866 Temporal Gap Evoked Cortical Potentials in Normals and Auditory Neuropathy

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Auditory cortical potentials to temporal gaps in noise (60 dBnHL) were recorded (Fz, Cz, Pz, C3, and C4 scalp sites) from normal and auditory neuropathy (AN) subjects. Gaps of 2, 5, 10, 20, and 50 ms were presented in a random sequence once every 1.75 sec with a total of 50 trials for each gap. The entire sequence required about 7 min. Subjects were tested in two conditions: (1) while detecting gaps in a reaction time task (RT); and (2) while passively listening. Continuous EEG records (bandpass: DC to 100 Hz) were stored and processed offline for drift and ocular artifact correction. Individual trials to each of the gaps were averaged (epoch: -.1 sec to + .8 sec) and measures (peak latency, peak amplitude) of the evoked potential components were made. RESULTS: Accuracy was 100% for the 10, 20, and 50 ms gaps but dropped to 81% for 5 ms gaps and 20% for the 2 ms gaps. In normals, RTs averaged 285 ms to 10, 20, and 50 ms gaps, 350 ms for the 5 ms gap, and 425 ms for the 2 ms gap. Evoked potential components (N100, P200, and P300 in the RT condition, and N100 and P200 in the passive condition) were identified in all normal subjects to gaps between 5 and 50 ms. The amplitude and latency of N100 and P200 were significantly affected when gap durations were 5 and 2 ms. In AN, cortical potentials were only present to gap durations the subjects could identify. CONCLUSION: Cortical potentials (N100, P200) correlated with behavioral gap detection in both normal and AN subjects.

867 The Temporal Effect in Listeners With Cochlear Hearing Impairment

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Previous studies have shown that a higher signal-to-masker ratio is needed to detect a short-duration signal presented at the onset of a longer-duration masker than is necessary if the signal is delayed from the onset of the masker. This will be referred to as the temporal effect. This effect is interesting because it seems to be related to active processing in the cochlea. The temporal effect decreases or disappears with temporary or permanent cochlear impairment, and in these cases listeners with impaired cochleae can detect the signal at a lower signal-to-noise ratio than those with normal cochleae when the signal is at masker onset. This suggests that active processing is high at masker onset, and decreases during the course of the masker, possibly due to efferent feedback to the cochlea.

The present study examined the role of active processing in the temporal effect in more detail. Several studies have shown that the pattern of threshold change with signal level in the short-delay condition may reflect the nonlinear input-output function of the basilar membrane. In the present study, the temporal effect was measured for listeners with cochlear hearing impairment, whose thresholds for long-duration tones in quiet ranged from near normal to 50 dB above normal. The signal was a 10-ms tone. It was presented either near the onset of a 200-ms broadband masker (short-delay condition), or in the middle of a 400-ms broadband masker (long-delay condition). Signal frequency depended on the frequency of the hearing loss. Signal levels were set from just above threshold to 95 dB SPL. The pattern of threshold change with signal level in the short-delay condition was consistent with a decrease in active processing in the cochlea. As expected, the pattern of results in the long-delay condition was the same as for listeners with normal hearing, supporting the hypothesis that active processing plays less of a role in this condition.

868 Effects of Cochlear Damage on 2nd-order Modulation Detection

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Dynamic information in acoustic stimuli not only is carried by variations in amplitude (the 1st-order characteristics of the sound, such as onsets, offsets, or amplitude modulation (AM)) but also can be carried by differences in 2nd-order acoustic characteristics (such as AM depth). Recently, auditory sensitivity to 2nd-order temporal envelope cues has been examined by measuring 2nd-order temporal modulation transfer functions (TMTFs) which relate detection thresholds for 2nd-order modulation (that is, sinusoidal modulation applied to the modulation depth of a sinusoidally amplitude-modulated tone or noise carrier) to fm', the rate of 2nd-order modulation. Here, the modulated tone or noise acts as a carrier stimulus of rate fm. Second-order AM is a complex envelope with 3 AM-components of rates fm, fm-fm' and fm+fm', and shows a salient envelope beat of rate fm'. Previous studies suggest that 2nd-order AM detection is partly based on the detection of a distortion product at rate fm'. The present study aims at examining the role of cochlear nonlinearities in 2nd-order AM detection by comparing 2nd-order TMTFs measured in normal-hearing listeners, listeners with moderately severe cochlear damage and listeners wearing a cochlear implant. Overall, 2nd-order TMTFs are similar and lowpass in shape in each group. In cochlear implant patients, applying strong amplitude compression via the speech processor of the implant does not affect 2nd-order AM detection thresholds. Taken together, these data indicate that cochlear nonlinearities such as compression, transduction, or short-term adaptation do not contribute to the generation of the envelope distortion product involved in 2nd-order AM detection, and reveal a possible role of central auditory nonlinearities.

869 Masking by Schroeder-Phase Harmonic Complexes in Gerbils: Behavioral Data

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Recent behavioral data demonstrated that harmonic complexes with Schroeder phases are generally more effective maskers in several bird species than in humans, and the difference in masking between the positive and negative Schroeder stimuli is much smaller and opposite in direction in birds. Physiological data from gerbils suggests that Schroeder stimuli might also be more effective maskers in gerbils than in humans, and the masking difference between positive and negative Schroeder stimuli in gerbils appeared small. Here we report behavioral data on masking of a 2.85 kHz tone by Schroeder-phase complexes in gerbils for comparison with behavioral data from birds and humans. Schroeder stimuli were the same as those previously used in the behavioral experiments testing birds (Dooling et al., 2001, Hear. Res. 152, 159-172), with fundamental frequencies of 50, 100, and 200 Hz. Six humans were also tested with these stimuli at a fundamental frequency of 50 Hz.

Consistent with published data, the positive Schroeder masker was about 10 dB less effective in our human subjects than the negative Schroeder masker. In contrast the positive Schroeder tended to be a slightly more effective masker in gerbils at a fundamental frequency of 50 Hz. In gerbils tested at 100 Hz there was no significant difference in masking between the positive and negative Schroeder, and at 200 Hz, the positive Schroeder stimulus was slightly less effective as a masker.

In addition, the Schroeder maskers with a 50 Hz fundamental frequency were on average over 10 dB more effective in gerbils than in humans. In summary, the difference in masking between these positive and negative Schroeder stimuli was much smaller in the gerbil than in humans, reminiscent of findings in birds. This could be related to different phase characteristics of the traveling wave in the inner ear of both species, resulting from differences in lengths of the basilar membrane/papilla and group delays.

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870 Temporal Integration of the Pressure Envelope of Sounds Underlies Neuronal and Perceptual Thresholds

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The auditory system has to integrate sound over time for signal detection and identification but confusion exists over how this is achieved. At the single neuron level, thresholds are commonly specified in terms of pressure only, implying that they are independent of time. At the perceptual level, however, threshold amplitudes decrease with increasing stimulus duration, which has led to the common, but never tested, view that the system integrates sound intensity over time and thus has an energy threshold. The deviations of observed thresholds from constant energy have been interpreted to be due to leaky integration of intensity. However, long time constants, unknown to physiology and at variance with the high temporal resolution of the auditory system ("resolution-integration paradox"), are required to fit the data.

Here we show, by exploiting first-spike timing as an indicator of when threshold was reached, that cat auditory-nerve (AN) fibers and cortical (AI) neurons reach threshold by integrating a sound´s pressure envelope and not its intensity. We show further, by re-analysis of detection thresholds of cats (Solecki & Gerken, 1990) and humans (Gerken et al., 1990) that the same applies to perceptual thresholds. The functions relating pressure envelope integration thresholds and time for AN fibers, AI neurons and perception in one species are remarkably similar and can be described by a simple power law which resolves the resolution-integration paradox. Strikingly similar perceptual threshold functions in many vertebrates indicate a conserved mechanism for the temporal integrator. We show that this must be located in the first synapse in the auditory pathway and argue that it involves the molecular processes of vesicle fusion and transmitter release.

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871 Asymmetrical Generalization of Learning Between Sound Onset and Sound Offset in an Asynchrony Detection Task

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The human ability to perceive whether different frequency components of a complex sound begin synchronously or asynchronously aids in the identification of speech sounds and the grouping together of frequencies that arise from the same sound source. We are interested in the plasticity and organization of the mechanisms that underlie such asynchrony detection in humans. We previously observed that listeners trained to detect asynchrony at sound onset improved significantly on the trained condition, but did not generalize that learning to sound offset [1]. To determine whether learning was actually possible at sound offset, we performed the converse experiment. We trained 9 listeners one hour per day for 8 days to determine whether two tones at 0.25 and 4 kHz ended synchronously or asynchronously. Before and after training we tested these listeners and 15 to 25 untrained controls on the trained offset condition and on the previously trained [1] onset condition. The trained listeners improved significantly on the trained condition during the training period, and learned markedly more than controls on both the trained offset condition and the untrained onset condition. Their improvement on the onset condition actually matched that of listeners trained on that condition. Thus, the mechanism(s) underlying asynchrony detection at both sound onset and sound offset are malleable in human listeners. Further, the fact that training on sound offset yielded improvements on sound onset, but not vice versa, indicates that there is an asymmetric relationship between the two mechanisms.

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872 Generalization to Untrained Carriers After Training on Sinusoidal-Amplitude-Modulation Rate Discrimination

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The perception of amplitude modulation is vital for understanding many everyday sounds, including speech. As a first step toward determining whether training might help listeners who have difficulty in perceiving amplitude modulation, we have been examining learning patterns in normal-hearing listeners trained to discriminate sinusoidal-amplitude-modulation (SAM) rate. These patterns have the additional benefit of providing information about the organization of the site affected by learning. Previously we observed that learning of SAM-rate discrimination with a 0-5 kHz carrier modulated at 150 Hz failed to generalize to untrained rates with the trained carrier. Here, we instead asked whether learning of SAM-rate discrimination generalized to the trained rate with untrained carriers. We trained 9 listeners 1 hr/day for 6 days on SAM-rate discrimination using a standard 3-4 kHz carrier modulated at 80 Hz. Before and after training, we tested these listeners and 8 untrained controls on the trained and 5 untrained conditions in which the SAM rate was always 80 Hz, but the carrier bandwidth or center frequency varied. Overall, both the trained and control listeners showed significant improvements across the tested conditions. However, the proportion improvements of the 5 trained listeners who learned significantly during the training phase were significantly larger than those of controls across the tested conditions, suggesting generalization of learning induced by multiple-hour training to untrained carriers of the same rate. Combined with our previous result, these data suggest that SAM-rate-discrimination training (1) should encompass several modulation rates but not necessarily several carriers, and (2) affects a site that processes different rates, but not different carriers, separately.

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873 Gap Duration Discrimination in Listeners of Different Ages: Effects of Gap Duration, Marker Frequency, and Presentation Complexity

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Temporal processing is generally accepted to be poorer in elderly listeners than in young adults. However, the degree to which a decline in temporal processing is evident in the pre-senescent (i.e., middle-aged) auditory system is less clear. Some sparse evidence indicates that a deterioration in the detection and discrimination of temporal gaps can be observed in middle-aged listeners. The purpose of this study was to test the hypothesis that temporal processing of silent intervals deteriorates early in the aging process, particularly for intervals bounded by spectrally-disparate markers and under complex listening conditions. Gap duration discrimination (GDD) was measured for

standard gaps of 0, 35, and 250 ms marked by 20-ms tonebursts. The brief markers had either similar (432 & 458 Hz), or disparate (432 & 2188 Hz), frequencies. For the 35-ms gap, the pair of gap markers was either presented in isolation or formed part of a 3-toneburst train. Both young listeners (< 25 years) and middle-aged listeners (40-55 years) participated. Preliminary results suggest that middle-aged listeners exhibit poorer performance for some gaps bounded by spectrally-disparate markers, as well as gaps that are embedded within a train of tonebursts.

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874 Softness Imperception: Evidence for Elevated Loudness at Threshold in Cochlear Hearing Loss

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Listeners with hearing losses of primarily cochlear origin have a normal rate of loudness growth near their elevated thresholds [Buus and Florentine, J. Assoc. Res. Otolaryngol. 3, 120-139 (2001)]. This implies that loudness at threshold is greater than normal when thresholds are elevated by cochlear hearing losses. In other words, listeners with cochlear hearing losses do not show recruitment in the sense of an abnormally rapid growth of loudness near threshold. Rather, they cannot hear some low loudnesses that are audible to normal listeners: when a sound is just audible it will be louder for a listener with a cochlear hearing loss than for a normal listener. We call this phenomenon *softness imperception* [Florentine and Buus. In: Tranebjærg, L et al. (eds) Genetics and the Function of the Auditory System. Tåstrup, Denmark, GN ReSound, 411-426 (2002)]. To test for softness imperception, measurements of reaction times (RTs) to 200-ms tones presented at and above threshold were measured at two frequencies in listeners with sloping high-frequency hearing losses: one frequency had normal or near-normal threshold, the other had elevated threshold. Results from six cochlearly impaired listeners clearly indicate that RTs are faster at and near elevated thresholds than they are near normal thresholds, whereas results from three normal listeners show no consistent effect of frequency. These findings support the presence of softness imperception in cochlear hearing loss. They indicate that listeners with cochlear hearing losses have reduced dynamic ranges both in terms of dB SPL and loudness. Although the present findings contradict the classical notion of recruitment, they provide a coherent view of loudness perception in cochlear hearing losses. They also agree with modern findings in auditory physiology, clinical observations, and many aspects of current clinical practice.

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875 Adaptive Recalibration of Chronic Auditory Gain

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Results will be reported for an exploratory study of an adaptive chronic "gain" (amplification of supra-threshold information) process within the auditory system. In theory, this gain process is plastic and can be systematically modified and recalibrated. The idea of an adaptive auditory gain mechanism is a fundamental concept in the treatment of both tinnitus and hyperacusis. This notion, however, has gone virtually untested. The hypothesis of this research is that judgements of loudness provide a functional index of chronic auditory gain. Further, chronic auditory gain can be manipulated either upward of downward in a controlled way by prolonged reduction or enhancement in the levels of background sound to which a listener is exposed. To evaluate these assertions, 10 normal-hearing volunteers have been randomly assigned to continuous (23 hours/day), chronic (four-week) external sound treatments. Subjects are exposed either to low-level sound produced by bilateral in-the-ear noise instruments or are fitted bilaterally with sound-attenuating earplugs in a sequential crossover design. Both treatments produced elevated audibility thresholds mainly above 1000 Hz. The effects of each treatment type on loudness judgements will be described. Predictions are that: (1) the earplugs will enhance the magnitude of perceived loudness and the resulting loudness growth functions will become steeper as a consequence of chronic sound attenuation (consistent with enhanced system gain in response to the diminished sound input from the periphery); whereas, (2) the noise instruments will reduce the magnitude of perceived loudness and the resulting loudness growth functions will become shallower (consistent with diminished system gain in response to the elevated background input). Preliminary data are consistent with the above predictions, providing support for a (1) plastic, chronic auditory gain process and (2) the use of sound therapy in TRT.

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876 Estimates of Basilar-Membrane Compression in Listeners with Normal-Hearing Derived from Growth-of-Masking Functions and Temporal Masking Curves

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A loss of cochlear compression may underlie many of the difficulties experienced by hearing-impaired listeners. An accurate and efficient behavioral estimate of basilar-membrane (BM) input-output functions may therefore be of clinical value. The magnitude of cochlear compression in human ears can be estimated using a forward-masking paradigm. This paradigm assumes that the response of the BM to tones around the characteristic frequency (CF) of the site of measurement is nonlinear, whereas the response to tones an octave or more below the CF is linear. Two procedures that use the forward-masking paradigm to derive BM input-output functions are growth-of-masking (GOM) [e.g., Oxenham et al., J. Acoust. Soc. Am. 101: 3666-75, 1997] and temporal masking (TM) [e.g., Nelson et al., J. Acoust. Soc. Am. 110: 2045-64, 2001]. With the GOM procedure, masker levels necessary to just mask a signal are measured at several fixed levels. A high-pass noise is used to limit off-frequency listening. With the TM procedure, masker levels necessary to just mask a fixed low-level signal are measured as a function of the time delay between the masker and the signal. The use of a fixed low-level signal assures that the place along the BM of maximal signal excitation remains constant.

This work was undertaken to determine the following: 1) Whether the two measures described above produce within-subject results that are consistent across a range of CFs and 2) which measure provides more efficient estimates of compression. GOM functions and TM curves were measured at signal frequencies of 1, 2, and 4 kHz in a group of normal-hearing listeners. Results will be discussed in terms of inter-test consistency and test efficiency.

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877 Effect of Masker Variability On Forward Masking and Increment Detection.

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Performance of four subjects with normal hearing was measured in forward masking and increment detection tasks in a two-interval forced choice (2IFC) paradigm. The masker level was either constant from interval to interval or was selected at random from a rectangular distribution with a range of 7, 14, 21, or 28 dB. The signal was a 4-kHz, 10-ms tone. In the forward-masking task, the masker was a 2.4-kHz or a 4-kHz, 200-ms tone presented at 70 dB SPL with 0-ms delay between masker offset and signal onset. In the increment-detection

task, only the 4-kHz masker was used and signal onset began 95 ms after masker onset. In each task, the signal level was set for each subject to yield performance in the range of d' = 1 to 1.5 in the absence of masker variability. Signal level then remained constant while various amounts of masker variability were introduced. Eight 50-trial blocks were run for each condition. There was little difference between the results obtained in the on- and off-frequency forward masking conditions or between the forward-masking and increment-detection conditions. Analyses of decisions on individual trials indicated that subjects did not base their decisions on a comparison of stimulus levels in the two intervals, as the standard model of the 2IFC paradigm assumes. Performance was governed by the average level of the masker in the interval containing the signal, but was not strongly influenced by the degree of variability in that level. The small effect of masker variability in both tasks suggests a large effect of internal noise. These results differ markedly from those obtained in a separate study of intensity discrimination.

Work supported by NIDCD

878 The Role of the Envelope in Intensity Increment Detection.

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Tonal signals added in-phase to an ongoing tonal pedestal produce changes in the energy at the frequency of the tone as well as the shape of the stimulus envelope. Comparison between one-dimensional models reflective of these two aspects of the stimulus were investigated by: 1) changing both the duration and the rise/fall times of a signal defined by a single tone, and 2) by presenting signals consisting of a series of two, four, eight or sixteen brief tones. Of primary interest were improvements in performance produced by increasing either the duration of the single tone or the number of tones in a series. The results are not fully described by an energy-detection model (Green and Swets,1966, *Signal Detection Theory and Psychophysics*) since performance did not grow as rapidly as predicted. Nor are they fully described by an envelope-extraction model based on temporal integration of the signal's level (Oxenham, 1997, *J. Acoust. Soc. Amer.*, p. 1779) which does not predict the similar performance found with asymmetrical (ramped or damped) rise/fall times.

879 Intensity Difference Limens as a Function of Frequency and Level in Hearing-Impaired and Normal-Hearing Canaries

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In humans with cochlear damage, intensity difference limens (IDLs) are often smaller than in normal-hearing humans tested at equal sensation levels. At equal SPL levels, however, IDLs are more similar between hearing-impaired and normal-hearing listeners. The purpose of the present study was to investigate intensity discrimination in normal-hearing canaries and a strain of canary with a hereditary hearing loss involving damaged and missing hair cells, the Belgian Waterslager canary. This strain has been used in neurobiological and behavioral studies of song learning, as well as in hair cell regeneration studies, and has a permanent high-frequency hearing loss associated with hair cell abnormalities in the basilar papilla. IDLs were obtained from birds trained using standard operant conditioning procedures and the Method of Constant Stimuli. Birds were trained to respond to small increases in the intensity of repeating pure tones. Hearing-impaired birds had smaller IDLs at low frequencies than normal-hearing birds when tested at 75 dB SPL, but not at 60 dB SPL. Hearing-impaired birds also generally had smaller intensity difference limens at lower sensation levels than normal-hearing birds. Normal-hearing birds generally showed more change in threshold with increasing sensation level than hearing-impaired birds. In sum, these results in Belgian Waterslager canaries with abnormal basilar papillae are similar to those reported for humans with cochlear damage.

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880 Improved Representations of Individual Stimuli Induced by a Two-Interval Discrimination Procedure

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An influential model of auditory processing [1] suggests that two different procedures used in auditory experiments can induce distinct processing modes: some two-interval procedures induce "trace" mode, in which listeners compare sounds by subtracting the stimulus in one interval from that in the other, while one-interval procedures induce "context-coding" mode, in which listeners compare each stimulus presentation with internal representations of previously heard sounds. One interpretation of this model is that performance in trace mode depends on representations of the stimulus subtraction, not of the individual stimuli, whereas performance in context-coding mode depends on representations of the individual stimuli. If true, training in trace mode should improve listeners' capacity to subtract the stimuli, but not their capacity to represent the individual stimuli. Therefore, training in trace mode should result in improved accuracy when measured in trace mode, but not when measured in context-coding mode. To test this prediction, we trained seven listeners one hr/day for eight days on a non-speech contrast using a two-interval discrimination procedure designed to bias them towards trace mode. The trained contrast was between two stimulus categories: tones that ended either at the same time or at different times. In contrast to the prediction, listeners trained in trace mode were significantly more accurate on the trained contrast than four untrained controls, regardless of whether we measured accuracy in trace or context-coding mode. These results suggest that training in trace mode, at least on a two-category non-speech contrast, can induce the creation or refinement of individual stimulus representations.

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881 Level Effects in Psychophysical Suppression

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Two-tone suppression has been observed at different levels along the auditory pathway. It has been shown at the level of the auditory nerve and in the mechanical response of the basilar membrane (BM). Geisler et al. (1990) postulated that suppression originates from the saturation of outer hair cell receptor currents, which in turn results in saturation of a positive feedback, and thus causes a reduction in gain. As a consequence, responses on the basilar membrane are less compressive in the presence of a fixed-level suppressor. Such a linearization should result in a decreasing amount of suppression with increasing level of a suppressed tone. The present study measures how suppression of the response to a tone depends on the level of that tone when the level of a suppressor is kept constant. A forward-masking paradigm was used in which a short 4-kHz probe presented at a fixed level was masked by a longer duration 4-kHz masker. In the suppression condition, the same probe was masked by the 4-kHz masker presented together with a fixed-level 4.8-kHz suppressing tone. In both cases, the level of the masker necessary to mask the probe was estimated using a 3IFC tracking procedure. The task was repeated for different levels of the probe, which allowed for estimating suppression at different levels of the suppressed tone (masker). The results are consistent with a linearization of the BM response in the presence of a fixed-level suppressing stimulus. Different levels of a suppressor led to different slopes of the masking-growth functions. Implications of this result will be discussed.

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882 The Effects of a High-Frequency Suppressor on Derived Basilar Membrane Response Functions

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Psychological and physiological studies have shown that the normal auditory system processes sound non-linearly. One such non-linearity is compression, which enables the mapping of a large range of sound levels onto a smaller range of basilar membrane (BM) vibration. A consequence of this is suppression, which is the reduction in the BM response to one stimulus as a result of the presence of another stimulus, usually at a different frequency and level. Physiological studies have revealed that suppression affects BM compression, possibly by reducing the gain of the "active mechanism". The present study was undertaken to investigate this effect psychophysically. Forward-masked psychophysical tuning curves were obtained using a fixed, low-level signal at a frequency of 4 kHz, masker frequencies of 2 to 5.5 kHz, and masker-signal gaps of 10 to 100 ms. An adaptive 2I-2AFC procedure was used to obtain the masker level at threshold. This procedure was repeated with the addition of a 4.75-kHz suppressor at 50 or 60 dB SPL, gated with the masker. Estimates of equivalent rectangular bandwidths were increased, and estimates of compression from the derived input/output (I/O) functions were decreased, in the presence of a suppressor as compared to the no-suppressor condition. The results are consistent with physiological results, which show that suppression leads to a partial linearization of the BM I/O function.

883 Psychophysical Estimates Of Basilar Membrane Compression At 4 kHz In Normal Hearing and Hearing-Impaired Listeners.

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Basilar membrane response functions were measured psychophysically at a characteristic frequency of 4000 Hz in sixteen normal hearing listeners and eight listeners with age-related, sensorineural hearing loss. A 2IFC forward-masking experiment was run using a brief 4000-Hz tonal signal (8 ms) fixed at 10 dB SL. Signals were preceded by a tonal masker (104 ms, at 2200 Hz or 4000 Hz), with silent masker-signal intervals ranging from 0-120 ms. Masker level was varied adaptively to estimate the level required to mask the signal. Temporal masking curves (TMCs), showing masker threshold as a function of masker-signal interval, were measured for each masker frequency. A comparison of each listener's off-frequency (2200 Hz) TMC with their on-frequency (4000 Hz) TMC provided estimates of basilar membrane compression and gain. Normal hearing listeners showed compression exponents in the range 0.1-0.4, consistent with previous studies. Hearing-impaired listeners also showed evidence of compression, with exponents lying in the range 0.15-0.5 in listeners with 30-50 dB of hearing loss. Sensorineural loss may be associated with a reduction in the overall gain of the cochlear response, rather than a change in the slope of the response function.

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884 Psychophysical Evidence for Auditory Compression at Low Characteristic Frequencies

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Some recent psychophysical estimates of compression suggest that the human auditory system is less compressive at low characteristic frequencies (CFs) than at high CFs (consistent with physiological measurements on other mammals). However, these estimates have been based on the assumption that the response to a tone well below CF is linear. Physiological data suggest that this assumption may not be valid at low frequencies. Three alternative measures of compression are reported here that do not depend on this assumption. In the first experiment, the growth of forward masking with masker level was measured. For low-level signals, the slope of the function relating signal threshold to masker level is an estimate of the compression applied to the masker. In the second experiment, the effect of masker-signal interval on the masker level at threshold, for a fixed low-level signal, was measured. The slope of this function, when compared to a linear reference (the same function for a high-frequency signal and lower-frequency masker), is also an estimate of compression. Finally, the effects on signal threshold of combining two equally effective forward maskers were measured. Any excess masking (above the 3-dB increase compared to the single-masker condition expected on the basis of linear energy integration) can be attributed to compression of the signal. The results of all three experiments suggest that auditory compression is just as great at 250 Hz as at 4 kHz, with compression exponents in the range 0.2-0.3. However, unlike at 4 kHz, compression at 250 Hz does not seem to vary substantially with frequency relative to CF.

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885 Duration and Frequency Effects in Loudness Enhancement and Reduction

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Loudness enhancement, a perceived elevation in loudness, of a standard tone may occur when the tone is preceded or followed by a more intense "inducer" tone. In these experiments, the standard and inducer were 1-kHz pure tones, and the inducer level was 90 dB SPL. In the first experiment, the loudness of a 10-ms standard was enhanced whether preceded, followed, or flanked (preceded and followed) by a 10-ms inducer. The amount of enhancement was greatest (about 25 dB) with preceding inducers and with mid-level standards between 30 and 50 dB SPL. With following inducers, there was a smaller peak (about 15 dB) in the amount of enhancement, whereas with flanking inducers there was no clear dependence of amount of enhancement on standard level. When a 10-ms or 200-ms standard was accompanied by a 200-ms inducer the enhancement effect disappeared, and loudness *reduction* was observed at high standard levels. A second experiment employed a 10-ms, 50-dB standard, and varied the duration of the inducer between 10 and 200 ms. The perceived loudness of the standard decreased with increasing inducer duration, switching from enhancement to reduction at 100-ms inducer duration. A third experiment investigated the effect of using a variable-frequency comparison tone. Loudness matches for a 10-ms, 40-dB SPL standard tone to a 10-ms comparison tone, with frequencies between 250 and 4000 Hz, were made with and without a 10-ms inducer. Greatest enhancement was again observed with preceding inducers, relative to following or flanking inducers. In contrast to the predictions of the loudness recalibration hypothesis of Scharf et al. (2002), the amount of enhancement did not vary substantially as a function of the frequency of the comparison tone.

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886 Informational Masking for Constant SL and SPL Maskers in Normal-Hearing and Hearing-Impaired Listeners

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Thresholds for a 2.0-kHz signal in the presence of simultaneous multitone maskers were measured in both normal-hearing and hearing-impaired listeners. The maskers consisted of fixed-frequency tones that occurred independently and at random with a probability p of either 0.5 or 1.0 on each trial. The fixed frequencies ranged from 522-8346 Hz at

1/3-octave intervals excluding the 2/3-octave interval on either side of the signal. In different conditions the levels of the individual masker tones were set to be constant in SPL or were adjusted for each listener to be constant in SL=10 dB. The difference between thresholds for the p=1.0 and p=0.5 conditions was taken as a measure of the amount of informational masking. For the constant SPL condition the results showed significant amounts of informational masking in normal-hearing listeners, but only small amounts of informational masking in the hearing-impaired listeners. For the constant SL condition small amounts of informational masking were observed in both group of listeners. Also, normal-hearing listeners showed small amounts of informational masking when masker tones were adjusted to be at the average SL of tones for the hearing-impaired listeners in the constant SPL condition. The results suggest that reduced sensitivity accompanying a hearing loss effectively reduces masker uncertainty, and so the amount of informational masking, by reducing the audible level variance associated with individual masker components

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887 Cueing Effects in Simultaneous Multi-Tone Informational Masking

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The ability to detect a tone added to a randomly drawn multi-tone masker is poor even when the maskers have little energy in the frequency region of the signal. Pre-trial cues are used in this experiment to reduce the frequency uncertainty inherent in such a task. Observers detected a tone added to a randomly drawn 6-tone masker in a single-interval paradigm. The frequency of the signal tone was randomly chosen from 5 possible frequencies. Three conditions were run. In one condition there was no cue (No Cue condition). In the Signal Cue condition a preview of the signal preceded each trial and in the Masker Cue condition a preview of the masker preceded each trial. For the Signal and Masker Cue conditions several interstimulus intervals (ISIs), the time between the cue offset and trial onset, were tested. ISIs ranged from 5 to 500 ms. Thresholds are approximately equal for the Signal and Masker Cue conditions, 15 dB lower than thresholds in the No Cue condition. The sole exception to this rule is for the Signal Cue condition when the ISI is 5 ms; in that condition thresholds were only slightly lower than in the No Cue condition. Trial-by-trial analyses revealed that in the No Cue and Masker Cue conditions observers tend to concentrate their attention to the higher signal frequencies. In the Signal Cue condition observers¡¯ attend to the frequency region of the imminent signal.

888 Informational Masking: Psychometric Functions for "Frozen" and "Mixed" Maskers

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To better understand the effects of masker uncertainty in informational masking, experiments were conducted to examine how psychometric functions (not merely detection thresholds) were altered when such uncertainty was present. In these experiments, five listeners attempted to detect a 1000 Hz tonal target in the presence of multitone maskers using a one-interval Yes-No paradigm with feedback. The maskers were 8-tone complexes having frequencies that were chosen randomly from the range 200-5000 Hz, with the subrange 800-1250 Hz excluded (the "protected region"). Psychometric functions were measured individually for each of 10 maskers held constant across trials (the "frozen" condition), as well as when the masker was chosen randomly on each trial from the set of 10 maskers (the "mixed" condition). In addition, synthetic psychometric functions were constructed by mixing the results from the frozen conditions and sorting the results from the mixed condition. In this presentation, all of these psychometric functions are compared. Also reported are the results of attempts to interpret these data in terms of the energy detector model combined with various assumptions about listener strategy.

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889 Spatial Release from Informational Masking of Speech in Normal-Hearing and Hearing-Impaired Listeners

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Improvements in speech recognition due to spatial separation between signal and masker can be substantially larger when the masker is primarily informational than when it is energetic [e.g. Arbogast et al. (2002) J. Acoust. Soc. Am., in press]. This result may be due to the perceptual effect of spatial separation, which is an effective cue for segregation when the masking is primarily informational. Psychometric functions for speech recognition (CRM sentences) were measured in listeners with normal hearing and sensorineural hearing loss in the presence of each of three maskers. Sentences were processed into 15 log-spaced frequency bands (pure-tone carriers modulated by the corresponding narrow-band speech envelopes). The signal and informational masker were both intelligible and contained random, mutually exclusive bands on each trial. The signal and control masker also contained random, mutually exclusive bands, but the masker was not intelligible as speech. The energetic masker was comprised of bands identical to the signal, but was not intelligible as speech. Two horizontal plane spatial conditions were tested: 0 deg and 90 deg separation between signal and masker. Results in hearing-impaired listeners were quite variable and will be discussed in terms of perceptual segregation and in relation to other factors such as age and audiologic measures.

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890 Psychophysics of Auditory Grouping and CMR: a Songbird as a Model

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The European starling (Sturnus vulgaris) is a bird species that shows perceptual effects of auditory grouping and scene analysis that are very similar to those observed in humans. Common modulation of signal envelopes as well as common onsets and offsets provide cues exploited by the auditory system for grouping. In this study, we present data from CMR experiments comparable to those pioneered by Grose & Hall (1993, JASA 93: 2896-2902) with multiple narrow-band noise maskers at two different sound pressure levels. These behavioral data provide the reference for a study of responses of auditory forebrain neurons in the same species (Klump & Hofer, this volume)

Four European starlings were trained in a Go/NoGo paradigm to report the detection of a 2 kHz tone (400 ms duration) temporally centered in gated noise maskers. These consisted of 25 Hz wide bands of noise that were presented either synchronously (all seven masker bands 600 ms duration) or asynchronously (on-frequency band [OFB] centered at 2 kHz, starting 100 ms before and ending 100 ms after the flanking bands [FB] of 600 ms duration) with the masker envelopes being either uncorrelated or coherently modulated. Signal detection theory was applied to determine the birds' detection thresholds; threshold criterion was a d' of 1.8.

Masking release for coherently modulated maskers versus uncorrelated maskers was on average about 18 dB for signal detection in synchronous maskers at 20 dB/Hz and 28 dB at 50 dB/Hz masker level. Masking release was significantly reduced by about 5 dB for asynchronous maskers of either spectrum level. FB separation had a small effect on masking release. In comparison to humans (Grose & Hall 1993) starlings show more masking release and are affected less by an asynchronous onset of masker elements.

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891 Benefits of Streaming in Psychophysical Tasks

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Most psychophysical tasks require the listener to regard the stimulus as a single sound source, and make judgments based on changes in the sound as a whole. In contrast, in everyday listening sounds emanate from different sources, and the listener separates the sources into streams and tracks changes in one or more streams. Does streaming allow for better resolution of changes in one of the streams? Stimuli were constructed by combining two sound sources consisting of consecutive harmonics of two different fundamental frequencies. Listeners' thresholds for changes in acoustic parameters of one of the sources, such as AM depth, temporal offset of a central component, and frequency excursion, were obtained under two conditions: *a)* the two sources were presented concurrently and perceived as a single sound; and *b)* the two sources were presented with a brief temporal offset and perceived as separate streams. In most cases, thresholds were lower in the streaming condition, demonstrating that streaming does enable listeners to obtain more information from the signal. However, in conditions where combining the information from the two streams yielded cues that were not available from the individual sources, listeners were able to take advantage of such cues.

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892 Regularities in Real-World Sounds That Could Facilitate Perceptual Grouping

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Often, the sound arriving at the ears is a mixture from more than one source, but we usually only interested in one. If we have *a priori* knowledge of the acoustic characteristics of the source we wish to listen to, then we might be able to selectively attend and devote more processing to sounds like these. However, we might not know the exact acoustic characteristics of the source we wish to listen to, or there might be more than one source with characteristics like the target. Fortunately, there is another form of information that the auditory system can exploit. There are regularities in sounds produced by common sources that allow their parts to be perceptually grouped over frequency and time. By identifying a range of simple acoustic features and then performing experiments using these, several cues that affect perceptual grouping have been identified, such as common onset, harmonicity, and continuity of pitch over time. However, the choice of acoustic features tested is somewhat arbitrary and not made with reference to the properties of common real-world sounds. In the current study, we analyse three sets of common sounds (speech, music and environmental sounds) to identify the regularities in them that might be most useful for perceptual grouping. A good general agreement was found between the strongest regularities and those found to be important in previous studies, while those cues not found to be important do not correspond to strong regularities. We evaluate the degree to which simple first order correlations and higher levels of regularity such as those extracted using Independent Component Analysis might be important. The implications of these findings for the mechanisms of auditory perceptual grouping and their development are discussed.

893 Mechanisms for Matching Spectral Templates with Noise and Harmonic Carriers

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This work assessed the ability of listeners to discriminate the spectral shape of harmonic and noise stimuli. This is relevant to the recognition of auditory objects that can have both forms, such as voiced and unvoiced vowels. We hypothesised the existence of central mechanisms for the extraction of spectral envelope regardless of carrier. Harmonic stimuli were based on a 100Hz fundamental and 40 harmonics with Schroeder phase. Noise stimuli had matched amplitude spectra and random phase spectra. Three types of spectral envelope change were applied to a trapezoidal reference envelope, with skirts at 1kHz to 2kHz and 3kHz to 4kHz. 1) In the 'pitch-height' condition the envelope was frequency shifted 2) In the 'M' shape condition increasing attenuation was added at 2.4 kHz 3) In the 'brightness' condition the attenuation at 4kHz was decreased. 2I 2AFC testing required subjects to listen to two pairs of stimuli, and detect the pair that differed in spectral envelope. Subjects heard pairs of harmonic (H) and noise (N) stimuli in four conditions (HH, NN, HN, NH). Full psychometric functions were evaluated based on 6 points and 60 trials/point. 75% detection thresholds and confidence intervals were derived from fitted Weibull functions (maximum likelihood estimation) and a bootstrapping procedure respectively. Thresholds for the pitch-height condition were all less than 5Hz, all 'M' shape condition thresholds were less than 1 dB and thresholds for the brightness condition were between 1 and 2 dB.

Comparison between the four conditions for each subject showed similar thresholds between conditions for the three envelope changes. These data are consistent with the existence of a mechanism for the analysis of spectral envelope that is not concerned with fine spectral structure. We suggest a cortical basis for spectral template comparison.

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894 Generating Acoustic Stimuli with Minimal Perceptual Error for Psychophysical Experiments Involving "Normal Hearing" Subjects

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We present a model-based method for preparing external acoustic stimuli that minimizes the extent of perceptual error (PE) due to peripheral processing. First, we compute the neural activation pattern (NAP) induced by a stimulation paradigm (e.g., a cochlear implant (CI)) using an appropriate model. We measure the PE between this NAP and a candidate NAP, generated under a "normal-hearing" (NH) model, using specially developed perceptual difference metrics (PDMs). Given a PDM and a model of the NH peripheral auditory system, we solve for the best acoustic simulation that minimizes the PE. We call this simulation the "reversed" simulation because it is derived through the inverse of the peripheral auditory system.

In general, psychophysical experiments targeting high-level auditory processing of hearing augmentation strategies often present acoustic simulations of degraded auditory stimuli to NH subjects. For example, noise-bands are presented to NH subjects in experiments targeting the neurological effects of CIs. Although these simulations capture the nature of the signal degradation from the hearing loss and augmentation, they generally do not account for the perceptual effects imposed upon the experiment by the peripheral auditory system.

Using PDMs, we have shown that noise-band simulations of the CI contain less PE when compared with the initial acoustic source than they do with simulated CI NAPs. Therefore, we propose to use reversed acoustic simulations in place of general simulations (e.g., noise-bands) for NH subjects. The reversed simulations will more closely approximate the desired perceptual effect, after the impact of the peripheral auditory system, and can be used in experiments targeting language and auditory processing.

895 Slope of the Human AN Response at the tail

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The characteristics of the auditory neural (AN) response in the tail region (region basal to the Characteristic Place) is important as it

reveals the influence of subtectorial structures on the hair cells in the cochlea and is critical in explaining such phenomena as Two Tone Suppression (2TS) and Upward Spread of Masking (USM) [Allen&Sen 2002, Mechanics of Hearing].

In the absence of in-vivo human AN data, psychophysical tuning and masking data have provided an insight into what the AN response might be. The similarity between USM and 2TS [Allen & Sen, ASA meeting 1998] suggest that psychophysical responses are indeed closely matched to AN response.

Psychophysical data are however scarce for regions basal to the CF. Simultaneous masking data for example [Zwicker, 1980] reveal the triangular functions that are prevalent in most audio compression algorithms. These data however show no "tail" region that is distinguishable from the CF region by a marked reduction in slope. This is in sharp contrast to other mammalian AN data. The cat for example has an extremely "flat" (or place and frequency invariant) tail (about 3 dB/octave) about an octave higher than the CF. We also know that this "flat" tail region is critical in explaining USM and 2TS [Allen & Sen, Biophysics of the Cochlea, 2002] which leads us to conjecture that the human AN and therefore psychophysical response at the tail must also be fairly invariant with place and frequency.

In this presentation, we analyze results from various psychophysical USM studies as well as our own experiments to reveal the characteristics of the AN response at the tail.

896 Vocalizations, Loudness and Critical Bandwidths in the Owl Monkey (*Aotus trivirgatus*)

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An animal's reaction to sound is determined by the biological significance of the sound and by experience. Communication sounds among conspecifics or between predators and prey are examples of biologically significant sounds, but arbitrary sounds may also acquire biological significance and thereby affect auditory behavior. In this presentation, we show exemplary waveforms and spectrograms of communication calls recorded in a colony of owl monkeys and present psychophysical results obtained using simple tones that fell within the frequency-intensity domain of the calls.

Three general categories of calls were observed: Single sounds (Grunt, Squeal); single sounds that were often repeated at ~2-3 Hz (Hoots, Peeps); and sounds characterized by periodic elements (Twang at ~10 Hz, Twitter at ~ 30-40 Hz).

A reaction-time paradigm was used to determine percent detection and response latency to tones (0.25-16.0 kHz) over an 80 dB range of sound pressure levels. Minimum threshold (50% correct responses) was at 1.0 kHz and 4.0 kHz, respectively, in two trained monkeys, and equal loudness contours derived from latency-intensity functions were generally parallel to the threshold functions. Critical masking ratios derived from masked thresholds were used to estimate critical bandwidths (CBs) at 1.0, 4.0, and 8.0 kHz. CBs ranged from < 0.5 octaves at 1.0 kHz to < 0.2 octaves at 8.0 kHz. From these estimates we assume that two signals with non-overlapping CBs (e.g., 1.0 kHz and 2.0 kHz) are processed in separate channels by the auditory system. The psychophysical results will be compared to data obtained from cats, monkeys and humans published by other investigators.

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897 A Neural Model for Detection of Tones in Wideband noise: Cross-frequency Coincidence Detection with Inhibitory/Excitatory Interactions

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Our recent model for detection of tones in noise used cross-frequency coincidence detection to extract temporal information from the responses of low-CF auditory-nerve (AN) fibers. This model is limited to low frequencies; however, performance in tone-detection tasks is generally similar for low and high frequencies. Also, the general anatomical and physiological properties of low- and high-CF cells in the cochlear nucleus (CN) are similar. Here we explore an extension to our previous model that maintains the desired features of cross-frequency coincidence detection at low-frequencies, but that also uses rate and timing cues present in high-CF AN responses. The model presented here, which applies to all CFs, combines inhibition with coincidence detection across excitatory AN inputs. The inhibition is presumed to be from cells that are driven by the same AN fibers that provide the excitatory inputs. Convergence of matched-CF excitatory and inhibitory inputs in the CN is consistent with anatomical and physiological reports.

In response to wideband noise, high-CF fibers phaselock to the envelope of the narrowband-filtered version of the stimulus, which is highly modulated. Interactions between fast excitatory inputs and relatively sluggish inhibitory inputs result in an enhanced representation of temporal modulations. The discharge rates of these model cells are reduced when a tone is added to a noise masker because the degree of modulation in the stimulus envelope is reduced. The decision variable used to predict psychophysical performance based on these model cells is thus a reduction in rate for the stimulus interval with a tone. At low frequencies, where temporal responses to the fine-structure dominate the responses, the reduction in rate is due to phase-opponency. At high frequencies, where envelope cues dominate temporal responses, the reduction in rate is due to the interactions between fast excitation and slow inhibition.

898 Effects of Carboplatin-Induced Inner Hair Cell Loss on Chinchilla Behavioral Measures of Hearing

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The perceptual consequences of inner hair cell (IHC) loss are poorly understood due to the paucity of suitable animal models. Here, we describe some of the basic changes in hearing that occur in chinchillas treated with carboplatin, an antineoplastic agent that selectively destroys the IHC and type I ganglion neurons relatively uniformly along the length of the cochlea. A group of five adult chinchillas were trained to respond across a variety of acoustic conditions to stimuli presented in quiet or in the presence of masking noise. Using a shock avoidance conditioning technique, behavioral thresholds were obtained for pure tones (250-11,000 Hz) in quiet and in the presence of broadband noise (50 dB SPL). Temporal resolution was assessed by obtaining gap detection thresholds presented in broadband noise. Tone-in- narrow band noise (70 dB SPL) masking patterns were measured to assess frequency resolution in narrow bands of noise centered at 500, 2000, and 4000 Hz. All behavioral measurements were measured before and after administration of 75 mg/kg of carboplatin. In addition, ABR and cochlear potentials were measured with clicks and tone bursts.

Carboplatin induced large IHC lesions (>80%) but caused little or no outer hair cell (OHC). Despite large IHC lesions, subjects were still able to respond to all stimuli. Minor changes were observed for pure tone thresholds in quiet. However, pure tone thresholds were elevated significantly in the presence of broadband noise. Tone thresholds in narrow noise were also elevated, but the off-frequency thresholds showed greater elevation than thresholds near the center frequency of the noise. Gap detection thresholds, a measure of temporal resolution, were also impaired. These results help to define the constellation of hearing deficits that can be ascribed to selective loss of IHCs and type I spiral ganglion neurons.

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899 Using Behavioral Neuroanatomy to Define 'Age' in a Mouse Model of Age-Related Hearing Loss

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Attempts to correlate the functional and anatomic changes associated with presbycusis have been frustrated by the variability in rates of progression between individual subjects. We present a behavioral neuroanatomical approach, which relates the behavioral stages of auditory dysfunction in C57BL/6J mice, an animal model of presbycusis, with structural alterations in the cochlea. Our study derived a general system of functional classification for the hearing deficits in C57 mice by measuring tone detection thresholds at high versus low frequencies, in quiet and in the presence of background noise. Long-term assessments of the same mice under different listening conditions revealed presbycusis-like patterns of high-to-low frequency hearing loss and increased sensitivity to masking noise. Task-specific performance deficits followed an orderly sequence despite age variations in the onset of major degenerative events between subjects. These results suggest that insights into the mechanisms of presbycusis may be gained by classifying C57 mice in terms of this functional continuum. Since this behavioral characterization is meant to correct the ambiguities of chronological age assessments, it is designated the ‘functional age” of hearing loss. Cochlear analysis of the animals was conducted according to the functional age of the mice. Hair cell counts conducted from 3-D computerized reconstructions of serial sections revealed an orderly base-to-apex progression of cochlear degeneration. All mice followed the same progression of OHC loss, although subjects showed considerable variation in the rate at which they advanced through this uniform sequence of structural changes. The histological analysis revealed that the “leading edge” of OHC loss correlated with frequency-dependent changes in hearing thresholds. These data suggest that behavioral neuroanatomy has great potential for precisely identifying structural correlates of hearing and deafness.

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900 N1m Evoked by Bone-conducted Ultrasound in Man: Effect of Stimulus Duration

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Ultrasound can be heard by bone conduction in man. However, there has been no consensus about the perception mechanism of bone-conducted ultrasound. In the current study, to clarify the central auditory system of bone-conducted ultrasound, the effect of stimulus duration on the brain was investigated using magnetoencephalography.

Method: The frequency of bone-conducted ultrasound was set at 30 kHz. The N1m amplitudes evoked by bone-conducted ultrasound were measured, varying the stimulus duration at 10, 15, 20, 30, 40 and 60 ms randomly. They were compared with those by an air-conducted 1 kHz sound.

Results: Basically, the longer the duration, the larger the N1m amplitudes for both sounds, and the growth of N1m amplitude for both sounds saturated at the duration of 40 ms. However, below the saturation points, the N1m amplitudes for bone-conducted ultrasound were smaller than those of the air-conducted sound. Significant differences were observed between both sounds at the durations of 10, 15 and 20 ms.

Discussion: According to our results, since the saturation point in the growth of N1m amplitude for bone-conducted ultrasound was the same as audible sound, the temporal integration system of bone-conducted ultrasound is similar to that of audible sound. However, significant differences in the growth were observed between both sounds. The results indicated a possibility that there are some differences in the central auditory system between bone-conducted ultrasound and audible sound.

901 Forward Masking at the Level of N1m: Adaptation or Integration?

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The auditory threshold is elevated by a stimulus preceding it in time, and this phenomenon is called forward masking. According to previous studies, forward masking has been demonstrated in a neural adaptation model and a temporal window (integration) model. To address forward masking in the central auditory system, the effect of signal delay in forward masking was investigated using magnetoencephalography.

Method: The N1m amplitude was measured in the presence of forward masking. The frequencies of the masker and signal were set at 1 kHz, and the intensity was set at 85 dB HL. The signal delay was randomly varied at 0, 10, 20, 40, 80, 160 and 320 ms.

Results: For signal delays above 40 ms, the N1m deflection increased as the signal delay increased. On the other hand, for signal delays below 40 ms, the N1m deflection decreased as the signal delay increased. The N1m deflection was lowest at a signal delay of 40 ms.

Discussion: In the adaptation model, the masking increases as the signal delay decreases. However, in our results, the minimum N1m amplitude was observed at a signal delay of 40 ms. As the signal delay decreased from 40 ms, the N1m amplitude increased although the masking increased. Our results suggest that the growth of the N1m amplitude largely depends on temporal integration at signal delays below 40 ms.

902 Click Train Regularity and Object-Background Decomposition in Human Auditory Cortex

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Auditory objects like the vowels of speech and the notes of music are often periodic, whereas background sounds are often noise. We studied object-background separation using continuous click trains (average inter-click interval = 12 ms) which changed from regular to irregular every 720ms. The neural activity in auditory cortex was studied in 12 listeners using magnetoencephalography. Spatio-temporal dipole source analysis was applied and source locations were corregistered on individual MRI. The continuous stimulation paradigm avoids cortical responses to intensity changes and sets the sustained field evoked by the background sound to baseline (Gutschalk et al. 2002). This technique

allows separation of the regularity-specific anterior sustained field (SF) in lateral Heschl's gyrus by eliminating the intensity specific activity. Experiment 1 involved a 100% regular signal and an irregular background in which stimulus regularity was 75, 50, 25, or 0% (jitter ± 1.5-6 ms). Experiment 2 involved an irregular background (0% regular) and a signal with 25, 50, 75 or 100% regularity. In experiment 1, the transient N1m and the SF decreased with increasing background regularity; that is, when the object and background became more similar. In experiment 2, the N1m was only observed for 75 and 100% regular objects. The SF amplitude decreased slowly with regularity and was still observed with 50% regularity. The results show that the N1m is only observed when there is a clear cut border to the auditory background, while the SF seems to encode the amount of regularity over a longer time interval.

Gutschalk A, Patterson RD, Rupp A, Uppenkamp S, Scherg M (2002) Sustained magnetic fields reveal separate sites for sound level and temporal regularity in human auditory cortex. NeuroImage 15:207-216.

903 Time-Domain Deconvolution of Overlapped Waveforms by "q-Sequences"

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At the previous ARO meeting we presented a frequency-domain method of deconvolving evoked-response waveforms that overlap because of a rapid stimulus repetition rate. At the prior poster, we presented the method using frequency-domain calculations on the data. We now show an improved variant of the method that analyzes time-domain data in the time-domain.

The essence of the method are "q-sequences", which are pseudo-periodic stimulation patterns with a small amount of "jitter". This contrasts with pseudo-random sequences (such as maximum-length-sequences) that have much larger jitter. The constraints on q-sequences occur in both the time-domain and frequency-domain.

QSD (q-sequence deconvolution) is generally applicable to any overlapped waveforms, as long as the overlap is a superposition (a linear summation). Superposition occurs in evoked responses when there is temporal overlap of the electrical activity from two separate neural generators.

The QSD method provides the opportunity to investigate a stimulus-rate regime in brain activity that has previously been un-available to experimenters. Thus, brain responses to supra-fusion stimulus rates can now be determined.

Our previous poster called the method WAAD (Wrap-Around Average Deconvolution). We now formally abandon this acronym because it is misleading. The average is not "wrapped around" by a cyclic-averaging process with a 100% duty-cycle. It is the *response* that is wrapped around! We now re-name the algorithm "QSD", thus concentrating on the critical element of the method, rather than on the characteristics of the waveform analyzed. We apologize for any confusion that may arise due to this acronym-lability.

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904 Characterizing Phase-Locked versus Non-Phase-Locked EEG Activity in Passive and Active Oddball Paradigms

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Auditory evoked potentials are typically analyzed using a strategy whereby time-averaged waveforms are extracted from epochs of EEG by stimulus-triggered events. This approach assumes that data contained within individual trials is composed of a linear combination of phase-locked activity plus background noise. However, linear averaging does not capture rhythmic EEG, which is reactive to the stimulus, but not phase locked to an event.

To examine this issue, we computing single-trial spectra of auditory ERPs in which the ensemble average was subtracted or regressed with varying degrees of lag (0, +20, 40 or 80 ms) from individual data trials. The first approach, where the average ERP was subtracted from each individual trial follows methods proposed by Kalcher and Pfurtcheller (1995). With the second approach, regression of the average waveform was computed for each individual trial and the weighted average was subtracted from individual trials. When time lags were varied, a function was computed for the average waveform regressed with each individual trial and the weighted average was subtracted at the time point that produced the largest regression coefficient. We focused on the 3 Hz band as this represents the predominant stimulus-evoked energy concentration within the oddball paradigm for long latency ERPs (Cacace and McFarland, in press).

The results indicate that subtraction of the average from individual trials removes some stimulus-evoked energy from within the 3 Hz band. The regression procedures were also successful but did not remove substantially more energy in the 3 Hz band compared with the simple subtraction. This analysis shows that a substantial portion of event related activity is not phase-locked to the stimulus. Therefore, it is unlikely that such activity will be detected using standard time domain averaging.

905 ERP Indicators of Cognitive Processing of Temporally Compressed Sentences

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Some listeners have difficulty processing speech that has been compressed across time. Several factors affect performance, including age, rate of compression, and type of speech material. There is scant electrophysiological evidence of the underlying neural correlates that are involved when listeners process speech that has been temporally altered. In this study, two rates of compression were applied to sentences that differed in the predictability of the final word in order to characterize the cortical event-related potential across normal and compressed speech. Young adult listeners were presented with sentences from the Revised Speech in Noise Test (SPIN; Bilger, et al. 1984) spoken at the normal rate and at temporally compressed rates of 40% and 60%. Sentences were presented in three blocks of 400 so that each ear received 100 presentations of sentences ending with either high or low predictability words. All presentations were randomized across each block. An event-related potential triggered to the onset of the final word of each sentence was recorded from 32 scalp electrodes (Neuroscan Synamp and QuickCap). Across all rates, final words failed to produce a negative peak in the early components of the response, regardless of predictability. Responses to standard rate sentences were highly variable across subjects and no stable peaks could be reliably defined. Average latencies and amplitudes were similar for the two ears and for the two types of final words, but not for rate. A peak positivity occurred following presentations of the compressed sentences, at approximately 900 to 1000 milliseconds for the 40% compressed sentences and at approximately 600 to 700 milliseconds for the 60% compressed sentences. The average amplitude of the response at electrode Cz increased with higher rates of compression. Topographic brain maps revealed alterations in peak amplitude distribution across the scalp in different conditions.

906 Neuromagnetic Responses Differ for Positive and Negative Autocorrelated Regular Sounds

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Iterated Rippled Noise (IRN) is generated by delaying a copy of Gaussian noise and by adding it *n* times to the original noise. The perceived pitch is proportional to the inverse of the delay (*d*). If gain *g* = -1 (delay-and-subtract), pitch differs from *g* = +1. Yost et al (J Acoust Soc Am 2349:2361, 1998) reported that for *n* > 4 in the delay-and-subtract condition the pitch shifts an octave below (1/2*d*) the corresponding delay-and-add process. However, they also found if the sound is produced with less than four cascaded delay-and-subtract iterations, the perceived pitch is approximately 1/(1.1*d*) and 1/(0.9*d*) compared to the delay-and-add process.

To investigate the physiological response of the auditory cortex we employed continuous sounds with alternating gain (+1/-1) and fixed *d* and *n*. According to the psychoacoustic findings of Yost the number of iterations *n* was either set to 2, 8 or 4096. Furthermore, we varied *d* (2ms, 16ms) in different runs.

A whole head magnetoencephalograph (MEG) was used to derive the responses of the auditory cortex. For each condition 350 sweeps were averaged. A spatio-temporal source model with one equivalent dipole in each hemisphere was used to analyze the auditory evoked fields.

In each condition we found a prominent late N100m that differed in latency and amplitude. The most distinctive peak was observed in the *d* = 2ms condition. For the positive versus negative condition we revealed significant differences in latency. In all conditions the latency of the N100m increased with a decreasing number of iterations while the amplitude attenuated.

In summary the neurophysiological generator of the N100m reflects the perceived pitch in amplitude. Furthermore, the delayed and enhanced N100m response of the *g* = -1 condition indicates a different pitch encoding process compared to *g* = +1.

907 Rapid Loudness Growth and Auditory Evoked Magnetic Fields in Humans

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Introduction: An abnormally rapid rate of loudness growth for given increments in stimulus intensity is seen both in patients with inner-ear hearing loss and in normal listeners under noise masking conditions. To elucidate whether there is difference or not between the cortical representations of these psychoacoustically similar phenomena, we measured AEFs in 10 inner-ear hearing loss patients with loudness recruitment, and 8 normal hearing subjects under two different conditions, with and without continuous masking noise.

Methods: The sound stimuli used was a 1 kHz pure tone, which was presented monaurally. The sounds with four different intensities (40, 50, 60 or 70 dB SPL) were presented randomly and equiprobably within a single sequence to each ear. In masking noise session, continuous 55 dB SPL white noise was presented binaurally. AEF was recorded with a 122-channel whole-head magnetometer (Neuromag Ltd.).

Results: The latency of N100m decreased as a function of sound intensity in both patients and normal subjects regardless of masking noise. The equivalent current dipole (ECD) moment for N100m increased as a function of sound intensity in both groups. Overall, the moment was significantly larger in patients and significantly smaller in normal subjects with masking noise session than normal subjects without noise session.

Discussion: The results of under noise conditions differed from those in inner-ear hearing loss patients at the point of moment. The cortical activation associated with the two similar rapid loudness growth phenomena was different, and the mechanisms underlying those may be quite different.

908 Mapping the 40 Hz Auditory Steady State Response (aSSR)

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We used PET and electrophysiological source localization techniques in 9 normal subjects. Pure tones activated sites bilaterally in auditory cortex, the left thalamus, left pulvinar, right middle frontal gyrus, left medial frontal lobe, and left anterior insula. PET identified three regions specifically responsive to the 40 Hz aSSR: right Heschl's gyrus, left insula bordering Heschl's gyrus, and right cingulate. A variety of source localization techniques were employed to identify the temporal sequence of activations involved in generating the aSSR. LORETA analyses, independent of the PET results, identified peaks in current density in right temporal lobe, right brainstem-cerebellum, left temporal lobe, and right frontal lobe. Dipole-based analyses were influenced by the modeling assumptions. Using the PET results to inform LORETA did not substantively change the cortical sources, but eliminated the subcortical source. PET-seeded dipole analyses were influenced by the inclusion of a possible source to account for activity outside the field of view of the PET camera. Thus, even with PET constraints, dipole methods were dependent on subjectively-determined modeling parameters. Convergence of PET and electrophysiologic data indicate that the aSSR is generated by a widely-distributed network of cortical sites including regions of the temporal and frontal lobes. Other potential contributors include regions of the parietal lobe and subcortical sites. The presence of these sources and temporal sequence of activations were influenced by modeling parameters. Depending on the model chosen, the aSSR could be viewed as the result of a reverberating network, a heirarchical network, or activity synchronized by a neural clock over widely-spaced areas of the brain.

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909 Hemispheric Activation in Human Auditory Cortex of Normal Hearing and Hearing Loss Subjects

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Hemispheric differences are evident in the normal processing of speech sounds (Phillips & Farmer, 1990) and have been reported using electrophysiologic (King et al., 1999; Kraus et al., 2000) and neuroimaging methods (Zatorre et al., 1992). Some studies have shown that a lack of asymmetric hemispheric activation may have functional implications. In this study, we use functional magnetic resonance imaging (fMRI) to examine hemispheric differences in the activation of the auditory cortex presented with controlled speech stimuli. The speech stimuli are synthesized and natural syllables that vary in frequency, but are similar in overall bandwidth, RMS amplitude and duration. Calibrated stimuli were presented monaurally to the right and left ear of adult subjects with either normal hearing (n = 20) or moderate degree of sensorineural hearing loss (n = 6). High resolution 3D anatomical images were acquired to superimpose the functional anatomy of each subject. The total number of activated voxels ($p \leq 0.01$) was calculated by hemisphere. Preliminary data suggest that normal hearing subjects show different hemispheric response asymmetry patterns than subjects with hearing loss. An overall reduction in the number of activated voxels was observed for hearing impaired subjects. While the location of cortical activation was

distributed similarly in TTG and PT by hemisphere for normal hearing subjects, subjects with hearing loss showed substantially less activation in PT. The findings suggest that moderate hearing loss affects the location of hemispheric activation and the degree of asymmetry in the presence of monaural speech stimuli.

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910 Central Auditory System Development in Children with Hearing Impairment.

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The aim of this study was to examine the effect of hearing impairment on the development of the central auditory pathways. Our measure of maturation of central auditory pathways was the latency of the P1 cortical auditory evoked response. We recorded P1 responses to a speech sound /ba/ in hearing-impaired children who ranged in age from 6 months to 15 years. Recordings were made in sound field with the children wearing their hearing aids. Preliminary results revealed a significant positive correlation between degree of hearing loss and development of the P1 cortical response latencies. That is, children with mild and moderate losses showed age-appropriate P1 latencies while children with more severe losses demonstrated abnormal cortical response latencies. In a subset of children in whom behavioral measures could be obtained, we found a significant negative correlation between development of P1 response latencies and speech perception scores. That is,– as P1 latency decreased, speech perception scores tended to increase. Our findings suggest that the latency of the P1 cortical auditory evoked potential may be a clinically useful marker of the development of the central auditory pathway – and therefore the potential for speech and language development– in infants and children with hearing loss who have been fit with hearing aids.

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911 The T-Complex in Patients With Aphasia

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The t-complex was first noted in the auditory late response (ALR) by Wolpaw and Penry (1). The t-complex is measured in the ALR recorded over the temporal lobes. Its waveform peaks, Ta and Tb, occur at approximately 100-110 ms and 150-160 ms in normal subjects. Tonnquist-Uhlen (2) has shown that the t-complex is absent in approximately 40% of children with severe language impairment and 5% of children with normal language development. The presence of the t-complex or its latency and amplitude may be related to language-impairment in other individuals such as patients with aphasia.

Results for 10 patients with aphasia are presented. ALRs were elicited by synthetic /ga/ syllables. Results were compared to language ability, measured with standardized tests of aphasia. The t-complex was present over both hemispheres in 8 subjects. Tb could not be identified over the left hemisphere in 1 subject and no response could be identified over either hemisphere in 1 subject. Peak amplitude and latency of the responses were compared to responses of an age-matched group with normal language. Results show a significant difference in response amplitude for severely aphasic patients compared to moderately aphasic patients and the normal-language participants. No differences were seen between response amplitudes of moderately aphasic patients and the group with normal language. Significant correlations were found between response amplitude over the right temporal lobe and language ability measured by the aphasia tests.

1. Wolpaw and Penry (1975). A temporal component of the auditory evoked response. Electroencephalography and Clinical Neurophysiology, 39, 609-620.

2. Tonnquist-Uhlen, I.(1994). Topography of auditory evoked long-latency potentials in children with severe language impairment: The T complex. Acta Otolaryngol (Stockh), 116, 680-689.

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912 A New fMRI task for Pre-Surgical Planning: A Comparison of Auditory and Visual Sentence Completion

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There is current interest in functional MRI (fMRI) as a tool in pre-neurosurgical planning. Tasks are currently being sought for use by neurosurgeons that can determine language areas in relation to tumor or other lesion sites so that benefit/risk decisions concerning the surgery can be more easily made. Because patient time within the scanner is an issue especially if multiple tasks are run, tasks that combine more than one cortical function (activating more than one site at once) are desirable. In terms of a language task, a single task combining receptive and expressive areas has been a major goal of our laboratory. The target cortical areas for activation were Wernicke's and Broca's regions. Whereas a naming task using visual stimuli has been used by others to activate Broca's area, we have found that an auditory version of the task (auditory sentence completion) activates both receptive (Wernicke's) and expressive (Broca's) areas in a single task. This study compares the effectiveness of visual versus auditory versions of the sentence completion task in terms of degree and extent of activation. Results show that both versions activate Broca's and Wernicke's areas; however, the auditory version is more robust, activating Wernicke's area to a greater extent. An example is given of a surgical case (tumor) in which language areas are successfully activated by the auditory version of the task. The case, showing an unusual right hemisphere dominance for language, illustrates the utility of the task as an aid to pre-surgical decision making.

913 How Do Neuronal Populations Encode Complex Communication Sounds?

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The mustached bat, *Pteronotus parnellii*, uses complex sounds or "calls", for social communication. Neural mechanisms for the perception of these calls in bats and other animals are largely unknown. To examine population responses to complex sounds, we recorded event-related local field potentials (ERPs) in the posterior region of the primary auditory cortex during presentation of social calls to awake bats. Extracellular recordings were obtained from cortical layers III-V and filtered between 1-1000 Hz. Spectral and temporal properties of the ERPs were studied at 140 recording sites in 5 bats using fourteen syllables and their statistical variants presented at various intensity levels. The ERP specificity for calls was largely determined by the magnitude/frequency of the spectral peak in the gamma range (30-100 Hz) along with temporal parameters e.g., time of the first peak and total ERP duration. Increase in magnitude of the gamma peak (up to 10-fold) was inversely correlated with time of the first peak (9-25 ms). The ERP duration varied for different calls of the same duration by 30-80% (132-258 ms). Our data suggest that fast oscillatory components (in the gamma range) of the ERP reflect perceptual call-specific neural dynamics within cortical circuits. Therefore, not only the generic neuronal connectivity in a particular cortical region, but also the acoustic structure of complex sounds, determines the spectral and

temporal parameters of the ERP. Accordingly, a combination of these parameters may constitute an effective code for the discrimination and possibly identification of calls.

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914 A Mouse Model for the Cortical Processing of Species-specific Vocalizations

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The availability of a genetically-manipulable animal model for studying the cortical processing of species-specific vocalizations would enhance our ability to dissect the mechanisms behind the auditory processing of communication sounds. Towards this goal, we are developing a framework to study ultrasound communication processing in the mouse auditory cortex.

Mice display robust ultrasound communication behaviors. Isolated mouse pups emit ultrasounds that reliably elicit retrievals from adults, and adult males often emit ultrasounds in proximity to adult females. We recorded the vocalizations from CBA/CaJ mice, and analyzed their spectral and temporal variability. Pup isolation calls narrow with age in their distribution of frequency and duration, and shorten in their repetition period. Pup and adult calls fall into two distinct spectral and temporal categories, providing an ideal receiver a means to acoustically distinguish between them, and potentially categorically perceive them along those dimensions.

To investigate the cortical representation of these vocalizations, we played pup and adult calls to anesthetized adult females, and monitored multiunit responses in the left auditory cortex. Many units showed peak temporal modulation tuning to, and stimulus-locked oscillations with, periods near the natural inter-call period of approximately 200 ms. Establishing the neurophysiology underlying the processing of these calls in the mouse is the first step in building a framework for studying cortical sensory coding that combines neuroethological and genetic methods.

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915 Representation of the Voice Onset Time (VOT) Speech Parameter by Population Responses in Primary Auditory Cortex (A1) of the Awake Monkey.

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Neural representation of stop consonant-vowel syllables with variable VOT is examined in A1 of awake monkeys to test the hypothesis that categorical-like temporal response patterns reflecting onsets of consonant release and voicing are maintained in large scale population activity. Measures of population activity include multiunit activity, current source density, and cell cluster responses. Low best frequency (BF) areas respond with categorical-like features; syllables with VOTs of 0 and 20 ms evoke a peak of activity time-locked to consonant release, while those with VOTs of 40 and 60 ms VOT elicit an additional peak time-locked to voicing onset. Aspiration noise is represented by sustained increases of neural activity in higher BF areas. Similar response patterns are found in the activity of thalamocortical afferents. However, there is an accentuation of categorical-like activity induced by intracortical mechanisms. Supragranular responses, indicative of intracortical, polysynaptic activity, maintain response patterns observed in middle cortical laminae. Physiological response patterns are compatible with the perceptual boundary of 20 to 40 ms that occurs in many languages, and support the hypothesis that this interval represents a natural psychoacoustical boundary utilized for VOT perception. Responses represent a straightforward way in which stop consonants can be rapidly encoded, using the well described propensity of A1 for responding to low frequency acoustic transients. Population responses are poised to provide potent inputs to secondary auditory fields involved in further phonetic encoding. These fields likely integrate the activity patterns occurring in the disparate tonotopic regions of A1, uniting responses representing the various acoustic cues used for the differential perception of stop consonants.

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916 High Order Processing of Auditory Information by the Songbird Brain.

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Songbirds, like humans, learn to vocalize by integrating auditory feedback with vocal motor control. This learning paradigm implicitly requires neurons to be highly tuned to auditory input that matches the desired vocal output. In songbirds, the nucleus HVc is at the apex of an auditory-vocal pathway essential to song production and learning. A remarkable auditory property of HVc neurons that distinguish them from their auditory afferents is an extremely selective response to playback of the bird's own song (BOS), but not to playback of temporally altered BOS or other bird's songs. This response is characterized by generation of exquisitely timed and highly phasic action potential bursts during playback of BOS, but not to other song types. We sought to examine the process by which this high order selectivity arises in HVc. To this end, we made *in vivo* extracellular recordings from a putative HVc auditory afferent, nucleus interfacialis (NIf), paired with simultaneous intracellular recordings from identified HVc projection neurons. This technique allowed us to correlate the output of NIf with the input of HVc neurons, and monitor the transformation of auditory stimuli across cell types, an analysis not possible using only extracellular recordings.

We found that NIf shows sustained BOS-evoked responses and broad responsiveness to playback of all song types, with a bias to BOS. Dual recordings show that the output of NIf is highly correlated with the subthreshold (i.e. input) response of single HVc neurons to BOS playback. Spike-triggered averaging showed that NIf action potentials precede EPSPs in HVc neurons by 2-5 msec, consistent with a monosynaptic connection. These recordings suggest a single synapse transformation of a broadly tuned input to an output that is exclusively selective to the BOS, likely through a thresholding process.

917 Physiology of Auditory Grouping and CMR: a Songbird as a Model

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The European starling (Sturnus vulgaris) is a proven animal model for studying CMR and auditory grouping. Here we present data from CMR experiments comparable to those by Grose & Hall (1993, JASA 93: 2896-2902). We recorded neurons in the auditory forebrain of awake unrestrained starlings. The neurons' masked thresholds for 400 ms tones at the characteristic frequency were determined in various masker configurations: (1) a 25 Hz-wide band of noise of 600 ms duration centered on the tone frequency and starting 100 ms before tone onset (on-frequency band, OFB); (2) OFB noise plus six 25 Hz-wide bands of noise (flanking bands, FB) of 600 ms duration, three being presented above and below the OFB within the limits of the excitatory frequency-tuning curve (FTC) and gated on synchronously with the OFB; (3) the same as in condition 2, but with the maskers being presented in the suppressive side-bands of the FTC; (4) the same as in condition 2, but with the OFB starting asynchronously 100 ms before and ending 100 ms after the FB; (5) the same as in condition 3, but with an asynchronous OFB as in condition 4. The envelopes of the masker bands were either uncorrelated or coherently modulated. Tone thresholds were significantly lower in coherently modulated maskers with synchronous onsets compared to synchronous uncorrelated maskers that were presented within the limits of the neurons' excitatory

tuning curve indicating CMR. A reduced CMR could also be found for maskers with synchronous FBs that were presented in the suppression areas of the tuning curve. Asynchronous onset of the OFB (cond. 4,5) abolished the CMR effect. CMR observed in the neuron population will be compared to the CMR observed in the behavioral study using similar stimuli (see Langemann et al., this volume).

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918 Neural Correlates of Auditory Stream Segregation in the Avian Forebrain

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Auditory stream segregation refers to the process by which the auditory system groups sensory input to form coherent perceptual representations of various sound sources. One well-studied stream segregation phenomenon is the "streaming effect," in which sequences of alternating tones (e.g., ABABABAB) are segregated into perceptual representations, or "auditory streams," corresponding to separate tone sequences (e.g., A–A–A–A– and –B–B–B–B). In humans, the streaming effect is more pronounced at faster tone repetition rates and with larger spectral differences between sequential tones. We investigated the neural basis of the streaming effect in the European starling (*Sturnus vulgaris*), a songbird with auditory stream segregation capabilities similar to those of humans (MacDougall-Shackleton et al., JASA, 103:3581-3587, 1998). We recorded multi-unit activity in the auditory forebrain of awake and freely behaving birds. We presented birds with repeated tone sequences (ABA–ABA–) in which the frequency of the "A tone" was specified as the recording site's characteristic frequency. Within a stimulus sequence, the frequency of the "B tone" differed from that of the A tone by 2, 4, 6, 8, 10, or 12 semitones. Tones within a sequence were presented at one of three durations (25, 40, and 100 ms) and four repetition rates (tone period = 100, 200, 400, and 800% of tone duration). Responses to the B tone exhibited marked suppression at frequency differences above 4 to 8 semitones, depending on the tuning characteristics of the neurons. Thus, differential responses to the A and B tones could be observed at a frequency separation that was below the 9 semitone difference used by MacDougall-Shackleton et al. to elicit the streaming effect in behavioral tests in the same species.

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919 The Neural Basis of Stream Segregation in the Auditory Cortex

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We investigated the hypothesis that perceptual auditory stream segregation is reflected in the auditory cortex by the differential suppression of neural responses to off-best-frequency (BF) tones by preceding on-BF tones. We recorded the responses of single units in the primary auditory cortex (A1) of an awake monkey to 10-s ABA_ sequences of tones - where A and B represent 125-ms tone pips of different frequencies, and _ stands for a 125-ms silent gap. The A-tone frequency was always close to the unit's BF, whilst the B-tone frequency was set to 1, 3, 6, or 9 semitones (STs) above A. The amplitude of the neural response to the B-tones decreased over part or all of the sequence duration for A-B separations of 3, 6, and 9 ST, but not 1 ST. In contrast, A-tone responses either remained constant or increased slightly over time. As a result, the ratio of B-tone to A-tone responses increased over time. No decrease in B-tone response amplitude was observed when the A tones were removed, indicating that the effect was not simply due to long-term adaptation of off-BF responses. These electrophysiological results suggest that the differential suppression of responses to off-BF tones by preceding on-BF tones, which has been proposed as the neural basis of stream segregation at the cortical level (Fishman et al, 2000), is a dynamic phenomenon that builds up over the first 5-10 s of stimulation. This is consistent with psychophysical data in humans, which indicate that the percentage of "two stream" responses increases over the same period. [Funded in part by an Engineering and Physical Sciences Research Council Life Sciences Network grant GR/M90146 to Prof. C.J. Darwin, Exptl. Psychol.,University of Sussex]

920 Neural Correlates of Auditory Stream Segregation in Primary Auditory Cortex (A1) of Awake Monkeys: Effects of Frequency Separation, Presentation Rate, and Tone Duration.

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Perceptual organization of sequential sound components, or "auditory stream segregation" (SS) is an important aspect of auditory scene analysis. SS can be experimentally demonstrated by listening to a sequence of tones in an alternating pattern, ABAB. When frequency separation (ΔF) between the tones is small (<10%) or presentation rate (PR) is slow (<10 Hz), a single alternating sequence is perceived. At large ΔFs or rapid PRs the sequence segregates into two perceptual streams, one composed of 'A' tones and the other of 'B' tones, each occurring at half the PR. Increasing tone duration (TD) enhances SS. We previously demonstrated neural correlates of SS in A1. Here, we examined whether neural correlates of SS are enhanced by increases in ΔF, PR, and TD. Physiological measures included multiunit activity and current source density recorded from the thalamorecipient zone. Alternating tone patterns were presented with ΔF, PR, and TD independently varied. 'A' tones were fixed at the best frequency (BF) of the recording site, while 'B' tones were situated away from the BF by an amount ΔF. For ΔFs > 10% and PRs > 10 Hz, 'B' tone responses were suppressed to a greater degree than 'A' tone responses. Increasing TD further enhanced differential suppression of 'B' tone responses. These results parallel psychoacoustic data on SS and support the relevance of synchronized neural ensemble responses in A1 for the perceptual organization of sequential auditory patterns. Results can be partly explained by differential forward masking, wherein there is a decrease in forward masking of 'A' tone responses by 'B' tones with increasing ΔF. Effects of prolonged TD may be due to decreased inter-tone interval, as suggested by psychoacoustic studies (Bregman et al., 2000).

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921 Neurodynamics for Auditory Stream Segregation: A Role for Target-Tracking in Mustached Bats?

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In humans, a sequence of alternating tones differing marginally in frequency (Δf≤50%; typically <1 kHz) can lead to "streaming" when presented at a high rate (>10 Hz). Mustached bats emit echolocation pulses at high repetition rates (>10 Hz) that contain a constant frequency component (PCF_1) in addition to other harmonics. These bats therefore hear a sequence of alternating PCF_1 and its harmonics in the echo ($ECF_{2,3,4}$) that differ widely in frequency (Δf>100%; >30 kHz). We wondered whether combinations of PCF_1 and ECF_x (x=2, 3 or 4) could lead to streaming, so that mustached bats can track targets of interest in a cluttered environment. First, using a simple and realistic neural network, we discovered that recurrent inhibition together with combination-sensitivity in the frequency domain is necessary to enable a node in the network to generate streaming. Next, we recorded single unit activity and event-related local field potentials (ERPs) extracellularly from the primary auditory cortex of an awake bat. We presented the ECF_2 tone either repeatedly (as control) or alternating with the PCF_1 as 25 ms tone bursts. At high presentation rates (>10 Hz),

the ERPs "latched on" to the ECF_2 and disregarded the PCF_1 even though the ERPs followed both PCF_1 and ECF_2 presented singly at the same rate. The effect was not apparent, however, in the activity of single cells. The ERPs in bats correspond to the output of our model and extend the earlier neurophysiological data in monkeys on streaming for tones contained within a critical band (Fishman et al. 2001). Furthermore, our data suggest that auditory streaming is widespread across mammalian species, and in bats could be used to track an ECF_x for insect pursuit and capture.

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922 The Role Of Spectral Contrast In Stimulus Encoding By Auditory Cortex Neurons

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Auditory cortex neurons are generally tuned to the center frequency of a narrowband acoustic stimulus (e.g., tones or bandpass noise) and usually show characteristic monotonic or nonmonotonic response curves as a function of sound level. Frequency response area (FRA) maps—constructed from the discharge rates elicited by a narrowband stimulus delivered at various frequency and level combinations—are commonly interpreted as characterizing the input/output functions of these neurons. We explored the hypothesis that neurons in auditory cortex respond to spectral contrast in a fashion not predictable from their FRAs. Spectral contrast represents a stimulus dimension independent from frequency and level that can only be probed using wideband acoustic stimuli. We used parametric wideband stimuli (PWS) to sample the space of all possible static-spectrum sounds and thereby to explore the shapes of static rate functions along the two dimensions of level and contrast. To do so we first constructed linear estimates of frequency tuning from neuronal responses to a randomized set of PWS. We then systematically varied the sound level and spectral contrast of a single PWS matched in spectral shape to the frequency tuning of the neuron under study. Most surprising, we found neurons unresponsive to high-contrast stimuli at any sound level but that responded vigorously to the identical stimuli under low-contrast conditions. Conversely, other neurons responded well to high-contrast stimuli and poorly to low-contrast stimuli. A continuum of response characteristics was found to exist between these two extremes. These results suggest that spectral contrast represents an important property of sounds to which auditory cortex neurons can be highly selective. Furthermore, parametric wideband stimuli represent essential tools for thoroughly exploring the characteristics of rate functions in auditory cortex.

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923 Influence of Auditory Cortex Lesions on the Ability to Discriminate between Rising and Falling Frequency-Modulated Tones in Rats

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Categorical discrimination of the direction of frequency-modulated (FM) tones was found to be disturbed in gerbils with lesions of the auditory cortex on the right side, whereas lesions on the left side did not result in such a disturbance (Wetzel et al., Neurosci. Lett. 1998). In our experiments young female rats, strain Long Evans, were trained with a conditioned suppression/avoidance procedure to recognize rising and falling FM tones. Thirsty rats were trained to break contact with a spout delivering water after the presentation of one type of stimulus by following the sound with a mild shock delivered through a metal grid (falling FM tone as a warning stimulus). The animals learned to maintain contact with the spout when a rising FM tone (a safe stimulus) was presented. Stimuli (frequencies of tones were between 3 and 6 kHz) were delivered in a quasi-random manner with a ratio between the number of safe stimuli and warning stimuli of 1.8:1. The index of performance was calculated as a hit rate - (hit rate x false alarm rate) (Heffner and Heffner, J. Comp. Psychol., 1988). Five groups of animals were tested: i/ with auditory cortex lesions on the right side, ii/ with lesions on the left side, iii/ with bilateral lesions, iv/ sham operated, v/ controls. Control and sham operated animals learned to discriminate between rising and falling FM-tones in ten sessions; their perfomance index was above 0.6. In animals with cortical lesions the learning curve was less steep and their index of performance was lower. The index of performance in animals with a right cortex lesion or with a bilateral lesion was slightly worse than in animals with a left cortex lesion. Further control experiments are ongoing to explain the way in which animals (even without both auditory cortices) are able to discriminate between both stimuli.

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924 Intensity Discrimination Following Cortical Lesions in Macaques: Detection of Increments, Decrements, and Amplitude Modulation.

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With the exception of several early studies of cats, few ablation-behavior studies have investigated the effects of auditory cortex lesions on discriminations of sound intensity. In the present study, macaques (*Macaca fuscata*) were trained to detect absolute increments and decrements in the intensity of a 625-Hz tone at 65 dB (SPL), as well as to detect sinusoidal amplitude modulation (SAM) in a carrier with the same frequency and similar level. Of the five animals tested, one was normal, one had a bilateral lesion, and three had unilateral lesions. Unless otherwise stated, testing was conducted in the free-field.

For the detection of increments, normal and unilateral animals had thresholds between 1.8 and 2.7 dB, and the bilateral animal had a threshold of 3.5 dB. For the detection of decrements, normal and unilateral animals had thresholds between 2.6 and 3.8 dB, and the bilateral animal had a threshold of 9.5 dB. One of the unilateral animals was tested with earphones and was unable to detect a 16-dB decrement in the ear opposite its lesion despite a normal threshold in the ear opposite its intact cortex. These results suggest that the ability to detect increments and decrements of intensity can be differentially effected by cortical lesions.

For the detection of SAM, normal and unilateral animals had modulation-depth thresholds between 1.9 and 2.1 dB. Tested with earphones, the one unilateral animal had a threshold of 4 dB in the ear opposite its lesion (its free-field threshold was 2.1 dB). The bilateral animal had much lower asymptotic performance than the other animals and a threshold of ~10 dB. One possible explanation for these results is that bilateral auditory cortex lesions result in a diminished, though not absent, ability to detect amplitude modulation—a cue that is very salient for normal animals.

925 Early And Late Electrocortiogram Patterns Of Stimulus-Related Activity In Primary Auditory Cortex Of Trained Animals

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Epidural electrocorticograms were measured using 18-channel (3×6) electrode arrays implanted over the right auditory cortex (field AI) in four animals (Mongolian gerbil) trained to discriminate between a rising and a falling frequency modulated tone (frequency range 2-4 kHz, duration 250 ms). Using a previously introduced classification procedure transient patterns of cortical activity suitable to discriminate between the rising and the falling modulation were identified. *Early* (locked to stimulus onset) and *late* (emerging at variable times after the stimulus) patterns could be differentiated. Deletion of increasing numbers of randomly selected electrodes were used to determine a critical density of recording channels required to capture the

discriminative power of the early and late patterns. Statistical analysis of the classification revealed a sigmoid dependence of the discriminative power from the number of remaining electrodes with an inflection point at 12 electrodes. The analysis of the minima of the classification statistic revealed that in the early patterns discriminative information was focal on regions corresponding to the tonotopic representation of the stimuli whereas in late patterns this information seemed to be distributed non-focally across larger cortical regions. This analysis supports the previous notion of the coexistence of topographically organized activity states related to the physical stimulus features and non-topographically organized states largely determined by intrinsic factors (Ohl et al., *Nature*, 2001, **412**: 733-736).

926 Feature Detector or Novelty Detector? Processing of Stimulus Probability by Auditory Cortex Neurons.

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The ability to detect rare auditory events may be critical for survival. Furthermore, stimulus statistics strongly influences the nature of the optimal neural code. We presented pure tones at two frequencies f1, f2 and manipulated the interval df between them (df=37%, 10% and 4%), and the relative abundance of the two tones (p=90/10%, 70/30% and 50/50%). We recorded single-neuron activity in primary auditory cortex (A1) and in the auditory thalamus (MGB) of cats under halothane anesthesia.

We found that neurons in A1, but not in the MGB, responded more strongly to a sound when it appeared rarely in a sequence than to the same sound when it was common. The difference in the responses to the common and rare sounds was a result of a stimulus-specific decline in the responses to the common sound (stimulus-specific adaptation, SSA), or a facilitation of the responses to the rare sound. This differnce in responses was positively correlated with df, and negatively correlated with the abundance of the rare tone. Thus, A1 neurons are sensitive to global stimulus statistics.

Significant differences between the responses to the common and the rare sounds were found in many neurons even for a frequency interval of 4%. These neurons exhibited therefore hyperacuity, a frequency resolution that is an order of magnitude better than receptive field width in either A1 or the auditory periphery, and that is better for rare than for equiprobable sounds.

We hypothesize that this form of SSA is a neural correlate of mismatch negativity, an important auditory event-related potential, implicated in sensory memory. Our results suggest that auditory cortex neurons, in addition to processing the acoustic features of sounds, may play a role in sensory memory and in novelty detection.

927 Neuronal Activity and Local Field Potentials in Rostral Superior Temporal Cortex of the Monkey During Performance of Auditory Delayed Matching-to-Sample

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Ablation-behavior experiments in monkeys have shown that the rostral part of the superior temporal gyrus (rSTG) is important for performance on auditory short-term memory tasks. However, the neuronal mechanisms underlying this behavior are unknown. In the current study, neuronal activity and local field potentials (LFPs) were recorded from rSTG while the monkey performed an auditory delayed matching-to-sample task. The stimuli consisted of both simple and complex sounds, and the monkey was required to release a touch-bar when the test stimulus matched the sample, which could occur on either the first, second, or third stimulus presentation after the sample. We found that: (1) the LFPs evoked by stimulus presentations usually have two positive components, one at about 80 ms (P80) and the other at about 150 ms (P150), their amplitudes differed depending on whether the stimulus presented was a sample or a test. That is, the sample stimuli evoked a relatively small P80 and a larger P150, whereas the test stimuli evoked an enhanced P80 and a suppressed P150. No significant differences were observed between the amplitudes of P80 or P150 elicited by match and nonmatch stimuli. (2) Single rSTG cells could be activated by both the simple sounds (such as pure tones and bandpass noise) and the more complex ones (ripples and monkeys calls). As with the LFP amplitudes, the strength of the neuronal discharge to a stimulus depended on its position in the trial, for single neurons test stimuli often elicited smaller discharges than those elicited by the samples. However, (3) a small population of neurons showed match enhancement, i.e. an enhanced response when the test stimulus matched the sample, although intervening nonmatch stimuli usually reduced this enhancement. (4) Some rSTG neurons showed sustained activity during the delay period after the sample stimuli. This delay activity, however, was disrupted or reset to a lower level after intervening nonmatch stimuli.

928 Activation of Human Auditory Cortex Territories During Reference and Working Memory Tasks: A fMRI Study

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This study compared fMRI-activation of the human auditory cortex produced by two different types of memory driven tasks using the same set of stimuli. Six linearly frequency modulated tones (FM) with 500 ms duration were used in both cases. The two tasks were the retrieval of a previously encoded FM target in the set (reference memory - RM) and the sequential two back identification of matching FM pairs (working memory - WM). A previously pilot study by one of the authors [K.Y.] showed in nine healthy normal hearing subjects that activation by the two types of memory are different in a particular area (territory T3) of auditory cortex. The working memory task produced significantly more activation in a subarea of the left planum temporale. Since it was also found that task performance was higher for RM than for the naturally more difficult WM task - the focus of the present study was to test the influence of task difficulty on these fMRI activation of the auditory cortex during these types of memory driven tasks.

16 right-handed subjects with normal hearing participated in this study. The working memory task was again more difficult and produced significantly more activation in the left territory T3. This opens up two testable possibilities to explain the higher left T3 activation during WM. It could be a WM-specific effect of stimulus processing or a result of task difficulty. We ranked subjects according to difference of sensitivity index between RM and WM tasks. Then we divided the group into two subgroups of same size i.e. with small differences (sdiff) and large differences (ldiff) of sensitivity index d'. Using a one-sided t-test separately we found a significant activation difference in left T3 for the two tasks only in the sdiff group. This largely excludes that task difficulty was the main reason for the increased left T3 effect during WM.

929 Nonverbal Sounds Abolish Frontal Cortex Activity Associated with Arithmetic Task Processing

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The neurobiological basis of the effect of nonverbal sounds on cognitive processing is not well understood. In two separate fMRI studies, T2-weighted gradient-echo EPI images were obtained from right-handed male subjects using a 1.5T MRI scanner. For one experiment, BOLD contrast changes related to processing nonverbal sounds were measured in 7 subjects relative to silence. Results of this experiment revealed bilateral albeit asymmetric activity favoring the right side when attending to nonverbal sounds [left to right voxel ratio of ~6:8]. This activation pattern is similar to that obtained for music [left to right voxel

ratio of ~6:7] (Kanwal et al,2000). Bilaterally activated regions included the insula, pre- and postcentral gyri, and the transverse, middle, and superior temporal gyri. The cuneus, inferior parietal lobule, and supramaginal gyrus were activated in the left hemisphere whereas the medial and inferior frontal gyri, caudate, and putamen were activated in the right hemisphere. For a second experiment, BOLD contrast changes were measured in 11 subjects when performance of an arithmetic task was coupled with simultaneous presentation of nonverbal sounds to the left ear. Normally, arithmetic task processing activates the left inferior frontal gyrus (Dehaene et al,1999; Kazui et al.,2000). In the presence of nonverbal sounds, arithmetic task processing produced activity in the cuneus and precuneus ($p<0.001$;z-max=5.26); significant activation in the frontal lobe, however, was not obtained. Abolition of frontal lobe activity with nonverbal sounds is in contrast to the shift in activity to the left anterior cingulate reported when the same subjects performed the arithmetic task coupled with simultaneous presentation of music to the left ear (Washington and Kanwal,2002).

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930 Acoustic Experience is Essential for the Development of Normal ITD Processing

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Interaural time differences (ITD) are a major cue for sound localization. In mammals, ITDs are encoded in the medial superior olive (MSO) by a complex temporal interaction of binaural excitatory and inhibitory inputs. Interestingly, glycinergic inhibition from the medial nucleus of the trapezoid body (MNTB) seems to be important for tuning ITD sensitivity to the physiological relevant range of ITDs (Brand et al., Nature 417:543, 2002). In normally developing animals this glycinergic input undergoes a specific structural refinement during the first days after hearing onset. This refinement depends on auditory experience and fails to develop in animals, which are reared in omnidirectional white noise designed to mask most spatial acoustic cues (Kapfer et al., Nat. Neurosci 5:247, 2002).

Here we show that ITD tuning in animals that have been exposed to omnidirectional noise around hearing onset from P10 to P25 fails to adjust to the physiological range of ITDs. Their ITD sensitivity resembles that of MSO neurons during blockade of glycine. Animals that have been exposed to white noise as adults do not show such an impaired ITD tuning.

Our data show that experience of spatial acoustic cues is essential for developing proper low frequency sound localization capabilities. Moreover, it seems likely that the structural effects of rearing animals in omnidirectional noise are related to the functional differences in ITD tuning in these animals. In other words, the specific elimination of glycinergic inputs resulting in the observed spatial refinement of glycinergic inputs on MSO neurons appears to be crucial for correct ITD tuning.

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931 In Vivo Neuromodulation of LSO Neurons

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The lateral superior olive (LSO) is one of relatively few auditory nuclei to which a particular function is widely ascribed. This function, the azimuthal localization of high-frequency sound by processing binaural differences in the intensity of sound, is based on the interaction of ipsilateral glutamatergic excitation and contralateral glycinergic inhibition. Previous in vitro studies suggest that excitatory-inhibitory (EI) balance can be altered by neuromodulators present in the LSO. We investigated this issue in the LSO of intact gerbil by manipulating serotonin and GABAB receptors during the binaural presentation of tones that varied independently in intensity at each ear. Serotonin, GABA, the GABAB agonist baclofen, and the GABAB antagonist CGP 46381 were iontophoresed before, during, and after binaural stimulus presentation. Activating either the GABAB or serotonergic pathways caused changes in response magnitude, often without altering the overall shape of the EI function. Serotonin either increased or decreased the spike count. Baclofen almost always decreased the response, while in the same neurons CGP 46381 increased the response. The fact that a GABAB antagonist could by itself alter the response level suggests an ongoing modulation of GABAB receptors in vivo. In a subset of neurons we also observed a shift in the balance of excitation and inhibition. Finally, the effects of manipulating either serotonin or GABAB receptors were often most pronounced on the sustained part of the neural response, with relatively little effect on the onset portion. These results suggest important roles for serotonin and GABA in regulating binaural interactions in the LSO.

932 Do Neurons of the Medial Superior Olive Cross-correlate their Inputs?

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Interaural temporal disparities (ITDs) are a major cue for sound localization along the azimuth. Neurons of the medial superior olive (MSO) are binaural comparators that take monaural inputs and create a sensitivity to ITDs. Psychophysical modeling assumes that a neuron in the MSO cross correlates its inputs, but tests of this assumption are few.

Here, we reexamined the relationship between monaural and interaural synchronization coefficients (SCs) to tones from earlier studies of MSO neurons in the cat (Yin & Chan, 1990) and the rabbit (Batra et al., 1997). For ideal cross-correlation, which is mathematically similar to convolution, the interaural SC equals the product of the SC of the two monaural inputs. SCs of the inputs were estimated in two ways: from the SCs of the MSO neuron to monaural tones and from the SCs to the tones at either ear during a binaural-beat stimulus, which were obtained by analysis of each frequency component of the response. SCs to monaural tones overestimated the interaural SC, but those derived from responses to binaural stimuli were smaller, and more closely matched the interaural SC.

Modeling of the response and varying the parameters involved indicated that the SC to a monaural tone typically matched the SC of the input. This, coupled with the observation that these SCs overestimate the interaural SC, implies that neurons of the MSO do not cross-correlate their inputs, but discharge instead over a wider range of ITDs than anticipated. The modeling also indicated that the weaker-than-expected interaural SC was a result of the neuron discharging when the input from only one side was activated. This effect also explains why monaural SCs derived from responses to binaural-beat stimuli more closely match the interaural SC.

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933 Conditioned Enhancement and Suppression in the Lateral Superior Olive

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Binaural neurons that are excited (E) by stimulation of one ear and inhibited (I) by stimulation of the other ear (EI or IE) are sensitive to

interaural intensity differences (IIDs). The lateral superior olive (LSO) is the first location where EI neurons are found, and populations of these cells are present at each subsequent synaptic level from brainstem to cortex. In the inferior colliculus (IC) of the gerbil midbrain, Sanes, et al (1998 J Neurosci18 794-803) found that a dynamic change in IID of an ongoing stimulus often resulted in an enhanced or suppressed spike-count (depending on the direction of change) compared to that generated by a static stimulus with the same IID. This type of conditioned enhancement and suppression is thought to contribute to motion sensitivity. In the present study, we used extracellular, single unit recording techniques to determine if the same phenomenon was present in the gerbil LSO. Although our stimulus paradigm differed somewhat from that of Sanes et al, we also observed both conditioned enhancement and suppression in most LSO neurons. This result suggests that sensitivity to motion may begin at the first level of binaural processing. Preliminary results with iontophoresis of the GABA-B receptor antagonist CGP 46381 suggest that GABA contributes to the phenomenon.

934 Interaural Time Coding: Binaural Inhibition in the Frog Dorsolateral Nucleus .

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The frog dorsolateral nucleus (DLN) is is the first auditory nucleus in the CNS. In contrast to its analogue, the monaural mammalian cochlear nucleus the frog DLN receives prominent contralateral innervation. We have investigated the responses of neurons to combinations of binaural interaural time differences (ITD) and interaural intensity differences (IID) of pure tone stimuli.

We recorded from single cells in the DLN of adult grass frogs (*Rana temporaria*). The frogs were placed in a closed-field setup and stimulated with brief tone bursts emitted dichotically using calibrated headphones. The medulla was exposed via a dorsal approach, and recordings (glass microelectrodes, impedances 30-50 MΩ) were made at electrode depths between 10 and 300 μm relative to the surface. Approximately 70% of the cells in DLN responded only to monaural stimulation, some (<10%) only to contralateral stimulation. Among the binaural cells the responses depended on a combination of ITD and IID. Selectivity for a certain ITD was always seen as inhibition and always dependent on the IID. Typically the response was weaker if the contralateral stimulation was 0.5 to 1 ms ahead of the ipsilateral (responses measured as spikes/stimulation). In some cells a very distinct combination of ITD and IID completely inhibited the response of the cell.

In conclusion, detection of ITD in the frog DLN seems to be based on inhibition, and the responses show notable similarity to those recently reported in the mammalian MSO (Brand et al., Nature 417:543-547, 2002). We have not found evidence of any excitatory coincidence detection, nor of any segregation of time and intensity pathways.

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935 Spatial Unmasking of Chirp Trains in a Simulated Anechoic Environment: Behavioral Results and Model Predictions

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Gilkey and Good (Human Factors 37:835-843) hypothesized that improvements in detection with spatial separation of a target (T) and masker (M) come about due to low-frequency binaural effects and/or high-frequency changes in the target-to-masker ratio. The current study examines the relative importance of low and high frequency (binaural and energetic) cues for broadband stimuli.

Detection thresholds were measured for a broadband periodic 40-Hz chirp train (T) in the presence of a broadband noise M for various T and M spatial configurations (using procedures and stimuli similar to Lane, Delgutte, and Colburn, 2003 ARO abstract). Results are compared to model predictions to test whether thresholds are determined by the "best" single frequency channel or if information is integrated across channels. Various T and M spatial configurations were simulated using non-individualized head-related transfer functions. Measurements were made for both broadband and low-pass-filtered stimuli; monaural, high-pass, and narrowband conditions were measured for a subset of conditions.

Results suggest that broadband thresholds depend primarily on high-frequency monaural cues. Low-frequency information and binaural processing do not contribute significantly to broadband performance. For low-pass stimuli, both energetic and binaural factors are important. While low-pass performance is worse than broadband, the improvement in detection with spatial separation of T and M (compared to when T and M are in the same direction) is similar for low-pass and broadband stimuli for angular separations of up to 45°. At larger angular separations, spatial unmasking is greater for broadband stimuli than for low-pass stimuli, presumably due to additional increases in high-frequency head-shadow effects. For both broadband and low-pass conditions, "best channel" predictions underestimate performance, suggesting that listeners integrate information across frequency channels.

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936 Psychophysical Measures and Peripherally Based Models of the Role of Non-Simultaneous Masking in the Precedence Effect

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This study addresses the effects of a binaural masker click on the discrimination of interaural time delay in a binaural target click. The relative level and time of occurrence of each click is varied in both the experiment and the analysis. The working hypothesis of the study is that two factors, non-simultaneous masking (NSM) and the precedence effect (PE), contribute to the observed effects of the masker click. The psychophysical experiment measures "discrimination masking," the effect of the masker click on the level of the target necessary to discriminate a given change in the target's interaural time difference. When the target follows the 40 dB SL masker, for inter-stimulus-intervals (ISIs) up to 8 ms, over 20 dB of discrimination masking is measured. When the target leads the masker, only small levels of discrimination masking are measured for even the shortest ISIs. The analysis of the experiment is based upon the integration of existing peripherally based models. Peripherally based models of NSM, such as Oxenham [J Acoust Soc Am 109, 732 (2001)], are monaural and therefore do not account for localization phenomena. The Hartung and Trahiotis [J Acoust Soc Am 110, 1505 (2001)] peripherally based PE model, unlike its NSM counterparts, includes binaural processing in the form of a cross-correlation stage. The two models begin with peripheral filtering and end with temporal integration. One of the biggest differences between these models, apart from the obvious monaural/binaural difference, is the nature of the temporal integrator. Predictions from a peripherally based PE model, with a temporal integrator time constant that gives accurate predictions of NSM, will be discussed.

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937 Ideal Population Coding of Interaural Phase Difference

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Interaural time or phase differences (ITDs and IPDs) are used as a cue for the lateral position of the sound source. Neurons in the auditory brainstem show a bell-shaped tuning curve for IPDs of a pure tone, peaking at a best IPD. Various schemes have been proposed to account for how populations of neurons encode the range of IPDs encountered under natural listening conditions, eg: a homogenous place code (Takahashi and Konishi, 1986). Due to evolutionary constraints, it is likely that the actual population coding regime in the brain is close to the ideal. We used point estimation theory to determine the ideal distribution of best IPDs for a population of neurons to encode most accurately the ecologically possible range of IPDs. Three optimum distributions were observed, depending on head size and tone frequency. The first ideal distribution positioned best IPDs in two channels, one either side of zero IPD, with best IPDs outside the ecological range. This distribution was ideal for small head sizes and/or low frequencies, eg: guinea pigs below 1000Hz, consistent with physiological observations (McAlpine et al. 2001), or humans below 200Hz. The second ideal distribution distributed best IPDs homogenously across the ecological range of IPDs. This distribution was ideal for large head sizes and/or high frequencies, eg: barn owls above 3000Hz, or humans in the range 700-1300Hz. This is similar to the model for barn owl IPD coding that has been proposed from the physiological evidence. A third, complex, ideal distribution pattern was found for medium head sizes and/or middle frequencies, eg: 200-700Hz tones in the human. Thus, according to ideal coding models, different coding strategies may be used depending on tone frequency. Psychophysical predictions were made of the relative distribution of IPD estimation errors for different tone frequencies.

McAlpine D, Jiang D, Palmer AR (2001) Nat Neurosci 4: 396-401.

Takahashi T, Konishi M (1986) J Neurosci 6: 3413-22.

938 Phase-locking to Low Frequency Sounds in the Auditory Nerve of the Rabbit

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The substrate for sensitivity to interaural time differences (ITDs) is phase-locking, transmitted from the auditory nerve to the central auditory system. One question is whether phase-locking at the periphery can account for the tuning to ITDs at the site of binaural comparison, or whether some intervening sharpening occurs. Another is whether phase-locking in the nerve is constant below the corner frequency.

Rabbits were used because data are available on ITD sensitivity in the superior olivary complex (SOC) and higher levels. While anesthetized with urethane, the bulla was vented and the auditory nerve exposed. Recordings were made with micropipettes (20-40 MOhms) filled with 1M NaCl. Stimuli were suprathreshold tones (typically 70-80 dB SPL).

Fibers synchronized from ~0.2-3 kHz. On average there was an abrupt decline in synchronization index below 300 and above 1500 Hz. Within this range, the maximum indices were typically between 0.70-0.85. In the SOC, the index to interaural phase should be the product of the monaural indices (Batra et al., J.Neurophysiol. 78:1237-47, 1997). In the rabbit SOC, the maximum synchrony for most neurons is between 0.45 and 0.85. The lower end of this range, which includes most neurons, is consistent with the product of typical synchronies in the nerve. The higher synchrony in some SOC neurons suggests sharpening does occur.

If synchrony is constant with frequency, the time jitter of the spikes will decrease by 1/frequency (1/f). The width of tuning to ITDs in most central neurons decreases by less than 1/f, indicating a bias for encoding time rather than phase. In the nerve, the widths usually declined by nearly 1/f. However, some declined by slightly less than 1/f, and this small difference would be magnified when the inputs from the two sides are compared. Consequently, at least some of the bias for encoding time rather than phase occurs in the periphery.

939 Sensitivity to Changes in Interaural Time Difference of Tones and Noise and to Changes in Interaural Correlation in the Inferior Colliculus of Guinea Pigs.

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Previously we showed that, in inferior colliculus (IC), the just noticeable difference (jnd) for the interaural time difference (ITD) of short tones is comparable with human jnds, while the jnd for interaural correlation of short noises is much worse. To determine if this is due to stimulus differences, or in the relative processing of interaural correlation and ITD, we measured tone ITD jnds, interaural correlation jnds and noise ITD jnds for the same 10 noise tokens (1ERB wide, centred on BF), in 30 neurons in the IC of urethane anaesthetised guinea pigs. We estimated the contributions of intrinsic neural and stimulus induced variability to the responses. ITD curves from peak to trough spanning zero ITD in 0.02 cycle steps were obtained with 50-ms, 20 dB suprathreshold best frequency (BF) tones and noise. Interaural correlation curves were obtained between ±1 in 0.1 steps, measured at the estimated characteristic delay. Fifty repetitions were obtained with 10-repeat blocks of the three measures interleaved.

ITD jnds were similar for both tone and noise, and comparable with previous reports. Different noise tokens yielded different mean firing rates, accounting for 18% or 27% of the variance at a given ITD or correlation value, respectively. Pooling across all tokens gave noise ITD jnds and correlation jnds that were higher than the average jnd across individual tokens, but the pooled jnd was usually within the range for individual tokens. Thus, even for the best noise token, correlation jnds were much worse than human jnds. Stimulus variability does not explain this discrepancy. Although individual neurons can contribute to ITD discrimination without obligatory pooling, the same neurons cannot when involved in correlation discrimination. Discrimination of ITD and interaural correlation must therefore depend on different processing strategies.

940 A Parametric Analysis of Binaural Interactions in Ferret Auditory Cortex.

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Traditionally, binaural interactions of neurons have been described in a largely qualitative manner, and assigned to classes depending on overt excitatory or inhibitory influences from each ear. Here we describe a new, quantitative, approach based on the statistical analysis of neural responses using generalized linear modelling techniques.

We recorded responses to 100 binaural noise intensity combinations, varying randomly between 0 and 90 dB SPL in each ear. The evoked spike counts were then fitted by multiple Poisson regression. To test for significant non-linear trends in the data, as well as for non-linear interactions between the ears, we allowed second-order terms and interaction terms in the regression model. We used the following maximal model:

$$\log(\hat{y}) = a L + b L^2 + c R + d R^2 + e L R + f L^2 R + g L R^2 + h,$$

where $\hat{y}$ is the predicted mean spike count, L and R are the sound levels in the left and right ear and a-h are the parameters of the model. Starting from the null model we introduced additional parameters in an exhaustive search until adding further parameters no longer produced a significant reduction in residual deviance at the 5% level. The parameters of the final model can then be used to derive an objective classification of the modelled neuron. For example, a positive parameter c can be taken as evidence for an overt excitatory influence of the right

ear, a non-zero *d* indicates a non-monotonic level dependence, while a positive or negative value for *e* would be indicative of non-linear facilitation or occlusion of the binaural responses respectively.

In a preliminary analysis of 34 auditory cortex neurons using this method we found that the final model captured the underlying trend of the data in all cases, and could account for 20 - 75% of the total deviance of the data.

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941 Role of the Auditory Cortex in Adaptation to Altered Binaural Cues in Adult Ferrets

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Auditory localization relies on the detection and interpretation of binaural and monaural spatial cues. Plasticity during development allows the neural pathways involved in spatial hearing to be calibrated by experience of the acoustic cue values available to individual listeners. Less is known about the capacity for adaptation in adults or what the driving forces and brain areas involved in any changes might be. We have investigated this issue by plugging one ear in adult ferrets and measuring the accuracy with which they approached the source of a broadband sound as well as that of the initial head-orienting response. Plugging one ear led to an immediate impairment in both measures of auditory localization, demonstrating the importance of binaural processing in these tasks. However, the performance of the animals improved substantially after periods of weeks or even days of monaural occlusion, both for sound durations of 1000 ms and 40 ms (which are too short for any benefit to be gained from head movements). The recovery in localization accuracy did not require response feedback and was also apparent after plugging one ear in visually-deprived ferrets. Moreover, the time course of adaptation appeared to be related to the frequency of testing sessions, rather than the duration of ear-plugging. In contrast, adaptive changes were not observed, even for stimuli of 1000 ms in duration, in either the accuracy of approach-to-target or head-orienting responses by adult ferrets that had previously received a bilateral lesion of the auditory cortex. This suggests a specific role for cortical processing in calibrating these responses and in the capacity of the mature auditory system to adapt to abnormal localization cues.

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942 Binaural Interactions in Ferret Auditory Cortex.

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We recorded neural responses in ferret auditory cortex to binaural broadband noise stimuli. 100 binaural noise intensity combinations were randomly presented from a square matrix covering 0 - 90 dB SPL in each ear. We effectively presented interaural level differences (ILDs) at a range of average binaural levels (ABLs). Our approach provided more information on binaural interactions than the conventional method of classifying units according to the ILD function at a single ABL

Like Semple & Kitzes (1993, J. Neurophysiol. 69:449-462), who used a similar approach, we analysed our data as a function of the sound level at the right and left ears. However, we quantified binaural interactions by fitting evoked spike counts using multiple Poisson regression (see abstract 684).

Analysis of 34 units showed that there was an overt excitatory influence from the contralateral ear in all cases. In 26 cases we found evidence for response saturation non-linearities or significant non-monotonicities. The ipsilateral ear provided overt excitatory input in 20 cases, and overt inhibition in 3 cases. Although 10 units showed no overt effects from the ipsilateral ear, all 34 units appeared to be influenced by the left ear through first or second-order multiplicative interactions between the ears. The first and second order interactions, with squared input from the ipsilateral ear, were almost all negative. This agrees with our qualitative observation that high ipsilateral sound intensities tended to inhibit activity for many units. Seven units were highly non-monotonic and responded in a focal manner to a specific range of interaural intensities. These focal responses usually favoured the contralateral ear.

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943 Gender Effects on High-Frequency Distortion Product Otoacoustic Emissions in Humans

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Many characteristics related to evoking distortion-product otoacoustic emissions (DPOAEs) with higher frequency stimuli (>8 kHz) have been reported (Dreisbach & Siegel, 2001), but if high frequency DPOAEs are to be used clinically, subject characteristics also need to be defined. Gender has been reported to affect DPOAE latency and amplitude when elicited with lower frequency stimuli (<8 kHz) (e.g., Bowman et al., 2000; Shehata-Dieler, et al., 1999). In the current study, custom equipment was used to determine the effect of gender on DPOAEs at higher frequencies.

DPOAEs were measured in 37 subjects (20 female and 17 male). Behavioral thresholds were measured through 16 kHz using Békèsy tracking procedures. Ratio sweeps (fixed f2, varied f1) were used to calculate latency and frequency sweeps to measure amplitude. Ratio sweeps were obtained at f2 frequencies of 1, 2, 4, 8, 10, 12, 14, and 16 kHz, with L1=60 and L2=45 dB SPL, with f2/f1 varied from 1.0 to 1.3. Frequency sweeps were measured with L1=60 and L2=45 dB SPL and an f2/f1 of 1.2 at discrete f2 frequencies between 1 and 16 kHz. The general shape of the average behavioral thresholds for each gender group showed a steep rise in threshold above 8 kHz, with a slight improvement in threshold at 14 kHz, rising again at 16 kHz. Latency values decreased as frequency increased for both groups and the greatest differences between the groups occurred at 1 and 2 kHz. The mean emission amplitude obtained from the frequency sweeps were averaged for each gender group and the greatest differences between the groups were found for frequencies greater than 8 kHz. Data were subjected to a repeated-measures ANOVA. The frequency by gender interaction and the main effect of gender were not significant for the behavioral results, in agreement with previous reports. However, significant frequency by gender interactions were found for latency, for data from 1-8 kHz, and amplitude, for data from 9-15 kHz.

944 Optimal Primary Tone Levels For DPOAE Measurements In Guinea Pigs.

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For investigating loss of sensitivity and loss of compression of outer hair cell amplifier during noise exposure, drug treatment etc. by means of DPOAE a stimulus paradigm need to be applied which is able to reflect cochlear nonlinear compressive sound processing known from direct basilar membran measurements (e.g. Ruggereo et al. 1997). In humans, such a paradigm has already been established, which accounts for the different compression of the primary tones at the DPOAE generation site L1 = 0.4L2 + 39 dB SPL with f2/f1 = 1.2 refered to as scissor paradigm (Janssen et al. 1998). The purpose of the present study was to elaborate an equivalent parameter setting for guinea pigs. 96 different L1-L2 combinations were presented to 24 ears in 18 normally hearing guinea pigs at 7 frequencies (f2 = 2 - 16 kHz, f2/f1 = 1.2). Measurements were performed using a DP2000 system (Starkey) and an ER10C probe (Etymotic) and custom made software. L2 was varied in 5 dB steps from 20 to 60 dB SPL, L1 between 20 and 65 dB SPL (1 - 3 dB steps). An extremal value analysis was performed to achieve maximum DPOAE levels (Ldp) for L1 in subject to L2. A correlation analysis of maximum Ldp was performed for single frequencies and

independent of frequency. Linear regression analysis yielded a guinea pig specific scissor paradigm, which is similar to the human paradigm. As in humans, also in guinea pigs only little variation with f2 could be observed. Optimal primary tone levels that led to maximum Ldp were close to L1 = L2 at high stimulus intensities. With decreasing stimulus intensity down to threshold the difference between L1 and L2 increased (L1 > L2).

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945 Toneburst-Evoked Otoacoustic Emissions at High Stimulus Rates using Maximum Length Sequences

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The application of the maximum length sequence (MLS) technique to recording click-evoked otoacoustic emissions (CEOAEs) allows for a reduction in test time by up to two orders of magnitude. This is because the technique permits the use of extremely high click rates, as inter-click intervals need not be greater than the response duration. The MLS technique also provides novel tools for studying CEOAE and cochlear nonlinearity, as, for example, the amplitude of the response progressively reduces with stimulus rate ("rate suppression"). However, the MLS technique has thus far only been applied to CEOAEs – this study attempts to extend it to toneburst-evoked OAEs (TBOAEs). Our primary aims were to determine whether TBOAEs can be recorded using the MLS technique and whether they demonstrate rate suppression similar to MLS-recorded CEOAEs.

CEOAEs and TBOAEs at 0.5, 1, 2 and 4 kHz were measured in 21 normal adult ears at different stimulus levels and stimulus rates up to 2500/s, using both the conventional and MLS recording techniques. Reliable MLS TBOAEs were obtained for tonebursts at 1 and 2 kHz, while TBOAEs at 0.5 and 4 kHz were generally close to the noise floor. TBOAEs also exhibited rate suppression as stimulus rate increased, in a manner broadly comparable with the corresponding CEOAEs. However, system nonlinear artifacts were more problematic in recording TBOAEs than CEOAEs.

This study demonstrates that the MLS technique can be extended to TBOAEs. Rate suppression occurs in TBOAEs with a magnitude comparable to that in CEOAEs. The findings suggest new possibilities for (a) investigating cochlear nonlinearity, and (b) clinical assessment of cochlear function, using frequency-specific stimuli.

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946 Effects of f2/f1 Ratio and L1-L2 Difference on 2f1-f2 DPOAE Amplitude and Phase

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Objective: Stimulus parameters play an important role in DPOAE production. In this study, 2f1-f2 amplitude input/output functions and phase functions were measured for f2=1001 Hz in the human ear with various f2/f1 ratios and L1-L2 differences to observe their effects on 2f1-f2 DPOAE. Methods: The level of f2 (L2) was increased from 35 to 70 dB SPL at a 1 dB step to generate 2f1-f2 amplitude input/output functions. The level of f1 (L1) was increased together with L2. The frequency and intensity of f1 were determined by f2/f1 ratios ranging from 1.05 to 1.35 and L1-L2 differences ranging from –4 to 10 dB. The magnitude and phase of 2f1-f2 DPOAE were plotted as functions of f2 intensity and f2/f1 ratio or L1-L2 difference. Results: Increasing the intensities of primary tones resulted in systematic increases of 2f1-f2 DPOAE levels with little phase changes. Increasing the f2/f1 ratio produced systematic changes in 2f1-f2 phase. Varying f2/f1 ratio and L1-L2 difference caused complex changes in 2f1-f2 amplitude input/output and phase functions. Notches in the amplitude input/output functions corresponded to sudden changes in phase. Conclusion: These results re-emphasize the complex relationship between DPOAE amplitude, phase and the stimulus parameters that should be considered when interpreting DPOAE measurements. The complex patterns of DPOAE changes in response to parameter variation may reflect non-linear interactions between energies of primary tones along the basilar membrane.

947 A Novel Method for Making Fast Measurements of DPOAE as a Continuous Function of Primary Level.

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Distortion product otoacoustic emission (DPOAE) is often measured as a function of primary stimulus level. Such input-output (I/O) functions are traditionally measured at discrete values of primary level. In humans, DPOAE-I/O functions have been used to estimate behavioral thresholds or to select "optimal" primary levels to elicit the highest possible DPOAE levels in subjects with normal hearing. In non-humans, a DPOAE-I/O function often exhibits a trough in DPOAE level accompanied by a rapid phase change. Such troughs have implications for cochlear models and have been linked to DPOAE adaptation mediated by the medial olivocochlear reflex (Kujawa & Liberman, JARO, 2001; Kim et al., in Biophysics of the Cochlea, 2003). The present study describes a novel method for measuring the DPOAE-I/O function. We used an exponential function for the rise/fall envelope of the two-tone stimulus waveform such that the rate of primary-level change was a constant dB per unit time. A typical stimulus had: rise/fall rate = 15 dB/s, rise/fall duration = 2 s, total duration = 5.5 s. The combination of exponential rise/fall with heterodyne analysis (Kim et al., JARO, 2001) allows the measurement of DPOAE level and phase as a continuous function of primary stimulus level. Recordings of DPOAE-I/O functions in humans reveal that the "optimal" primary levels can differ among normal-hearing ears. Recordings of DPOAE-I/O functions in cats demonstrate the capability of the present method to map efficiently the location of an I/O trough in an L1-L2 space. The continuous nature of the resulting DPOAE-I/O curve allows the position of a trough to be precisely determined in a short period of time. This method should be useful in studies that involve measurements of DPOAE-I/O functions, especially studies in which "optimal conditions" are needed or in which it is important to define the I/O function in fine detail.

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947-B Close Primary DPOAE Macrostructure : Origin and Significance

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Upper and lower DPOAE components obtained with close frequency primary tones (f2/f1<1.02) at 75dBSPL exhibit predominantly 'place fixed' (ie reflection) emission phase characteristics and show a common quasi-periodic DP frequency dependant amplitude modulation. A consistent pattern of rounded intensity peaks and deep notches has been found between 2 and 4kHz in 14 normal young adults, confirming and extending the findings of Knight and Kemp (JASA 2001;109:1513-25). Typically the peak spacing is 400Hz and the peak to peak modulation is 10-20dBSPL.

In so far as very close primary tones approximate to a continuous single tone these DPOAE would be expected to share many characteristics with stimulus frequency OAEs. But periodic modulation of this magnitude is only seen in SFOAEs obtained with near threshold stimuli where multiple reflections between middle ear and the OAE reflector site cause strong resonances. With 60dBSPL stimulation and above, multiple reflection is largely suppressed.

Strong modulation is observed in 2f1-f2 DPOAE with f2/f1~1.2 and stimuli >60dBSL. This is due to interference between comparable amplitude place and wave-fixed sources. Interference can also result from internal multiple reflections of the DP even in the presence of strong primary stimulation because at this wider primary spacing DP frequency specific reflector sites are not within the suppression range of the primaries.

948 Group Delays and Production Mechanisms for Tone-burst Evoked OAEs in the Guinea Pig

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In mammals, acoustically evoked otoacoustic emissions (OAEs) are thought to arise from two different mechanisms: nonlinear wave-related distortion and linear place-fixed reflection. Broader basilar-membrane excitation patterns in the guinea pig (smaller Qs than humans - see Shera et al., 2002) suggest that nonlinear distortion may play a greater role in the production of stimulus-frequency OAEs than it does in humans. Tone-burst evoked OAEs were measured in the guinea pig to determine both their group delays and the relative contribution of reflection- and distortion-sources to the total OAE. Group delays were measured using amplitude modulated tone-burst evoked OAEs by computing the envelopes of the stimulus and emission waveforms with the Hilbert transform, fitting each envelope with a sine function, and determining the time shift between the respective sinusoids. The contribution of each emission mechanism to the total OAE was determined by unmixing the two emission types using Fourier analysis and time-domain windowing. The results suggest:

i. Group delays for OAEs evoked by 18 kHz stimuli are consistent with a round-trip delay based on comparison with basilar-membrane delay measurements.

ii. A more complex interaction between the two emission mechanisms than is observed in humans, in which tone-burst evoked OAEs appear dominated by a linear reflection mechanism.

949 Multi-component Distortion Product Otoacoustic Emissions in the Leopard Frog, *Rana pipiens*

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It is currently thought that basilar membrane (BM) vibrations are boosted by an active feedback mechanism [1]. Although the absolute contribution of this mechanism to basilar membrane vibration increases with increasing stimulus intensity, its relative contribution is highest at low stimulus levels [2]. Consequently, distortion product otoacoustic emission (DPOAE) characteristics at low stimulus levels are mainly determined by the active feedback mechanism, while at high stimulus levels DPOAEs are determined by the passive mechanical properties of the BM. In frogs no cochlea is present. Instead, the inner ear holds two papillae specialised in detecting airborne sound. These papillae are not over a BM. Also, hair-cell length changes have never been shown, and are unlikely to be present in frogs. The absence of both a BM, and of hair-cell length changes as an active feedback mechanism in the frog inner ear might give rise to different DPOAE input-output curves as compared to those found in the cochlea.

Here we report on DPOAE recordings performed in the leopard frog, *Rana pipiens*. DPOAE input-output functions recorded from the amphibian papilla (AP: f_1< 1.4 kHz) as well as the basilar papilla (BP: f_1=1.5-2.5 kHz) consisted of two regions separated by a notch around 70-75 dB SPL stimulus level. As in the cochlea, high-level DPOAEs (above the notch), reflect the passive emission component, while low-level DPOAEs (below the notch) seem to be dominated by an 'active' component. A model, based on the mechanoelectrical properties of hair cells [2], which seems applicable to the 'simple' BP, can not explain the phase behaviour of the DPOAE input-output curves found in this study.

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950 Do DPOAEs Require Low Stimulus Levels or Intact Cochlear Feedback to be Frequency Specific?

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It is widely held that distortion-product otoacoustic emissions at 2f1-f2 (DPOAE) are generated where maximum interaction occurs between stimuli, i.e., likely close to the place tuned to f2, thus exhibiting large frequency specificity. Accordingly, impaired cochleae with well-defined transitions from normal to damaged places often exhibit DPgrams (i.e., plots of DPOAE levels against f2) with clear-cut roll-offs showing good correlations with audiogram roll-offs. It suggests that DPgrams afford interesting mapping capabilities, but actually, it is surprising because DPOAEs are usually derived from rather high-level stimuli, which should show some degree of smearing on the basilar membrane - even more so in a damaged, poorly tuned cochlea. Thus, one would expect that the notch of a pathological DPgram would appear increasingly blurred with increasing stimulus OHC damage, owing to the resulting loss of tuning sharpness. In order to explore this issue, mice and gerbils with high-frequency cochlear dysfunction either due to genetic impairment of basal OHCs or exposure to a loud high-frequency tone, were explored with the help of cochlear potentials and DPOAEs. The levels of DPOAE-eliciting stimuli were increased stepwise from 40 to 80 dB SPL and the limit frequencies separating normal from decreased DPOAE or cochlear potential responses were compared: they were similar regardless of stimulus level. The next step consisted of administering furosemide or inducing ischemia in the already impaired animals so as to decrease their endocochlear potential, thereby turning off the OHC-based cochlear loop ensuring the gain and frequency selectivity of basilar membrane movements. As expected, DPOAEs elicited by stimuli below 60 dB SPL vanished at all frequencies instead of remaining present at low frequencies. Yet, the boundary between the interval with initially impaired OHCs and the lower-frequency one not only reappeared, but also looked sharper with increasing stimulus level from 65 dB SPL on up. However, it could shift downward by about 1 kHz. It suggests that instead of relying upon the existence of an intact cochlear feedback loop, the frequency-specific presence of DPOAEs is ensured by the integrity of a nonlinear element in OHCs, this element showing both tuning and lack of sensitivity to furosemide (stereocilia would be good candidates).

951 Post-mortem Distortion Product Otoacoustic Emission in the Leopard Frog

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Distortion product otoacoustic emission measurements were performed in the leopard frog, *Rana pipiens*. Animals were sacrificed by destruction of the CNS(double-pith procedure) or by cardioectomy. The time course of emission decay was monitored by measuring DPOAE input-output functions for stimulus frequencies in the amphibian papilla frequency range (f1=1011 Hz, f2/f1=1.1), and in the basilar papilla frequency range (f1=2011 Hz, f2/f1=1.1). Input-output curves consisted of two parts, separated by a knee point near 70 dB SPL input level.

Above the knee point, the emission is a passive nonlinear response; below the knee point there is an additional component, which we will tentatively refer to as the active component. For the amphibian papilla, the active component disappears within 6 minutes following cardioectomy, and between 5 and 25 minutes following CNS destruction. For the basilar papilla, the active component disappears between 15 and 64 minutes following cardioectomy or destruction of the CNS. The passive component at high stimulus levels has been observed up to 71 hours post mortem. The results show that low-level otoacoustic emissions in frogs are more vulnerable in the amphibian papilla than in the basilar papilla. The high-level passive emissions are remarkably robust compared to those in other vertebrates.

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952 Interaction of Bone-Conducted and Air-Conducted Sounds

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This study was designed to determine the magnitude and phase relationships between one signal delivered via bone conduction and another delivered via the air conduction pathway. Three measurement techniques are being investigated: DPOAE, adaptive 2AFC behavioral threshold, and E-Coch-G. For DPOAE measurements, F1 was always delivered via air conduction. F2 was delivered in three different ways. First, F2 was delivered via air to produce a "conventional" DPOAE measurement. Second, F2 was delivered via bone vibrator. The level of F2 (bone) was then adjusted to produce equivalent DPOAE levels. Third, F2 (air) plus F2 (bone) were equated for level so that the relative phase could be varied. For the behavioral measurements, air- and bone-conducted signals were equated for level and then systematically varied in phase. The levels of both air- and bone-conducted signals were adjusted in a 2-UP, 1-DOWN adaptive rule to track the 70.7% correct level as a function of the relative phase between air and bone signals. DPOAE results clearly indicate that cancellation within the cochlea occurs at a different air-bone phase than is required to cancel in the ear canal. Behavioral results indicate that cancellation can approach 30 dB. E-Coch-G measurements are still in process. It is anticipated that an E-Coch-G signal could provide the "error" signal for an active noise reduction system designed to reduce bone and body conducted sound within the cochlea. Results for all three measurement techniques on the same subjects will be presented. Individual differences and male vs. female differences are evident in the early results.

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953 Comodulation Masking Release and the Role of Wideband Inhibition in the Dorsal Cochlear Nucleus

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Human psychophysical studies have shown that the detection of a masked signal is improved when the masker is coherently modulated over a wide frequency range. This phenomenon is commonly referred to as comodulation masking release (CMR). Studying responses of single units in the ventral cochlear nucleus of the guinea pig, Pressnitzer et al. [Pressnitzer, D., Meddis, R., Delahaye, R. and I.M. Winter (2001). J. Neurosci. 21: 6377-6386] hypothesized that wideband inhibition of narrowband units underlies CMR. However, the strongest inhibition in the cochlear nucleus is found in the dorsal region (DCN) and therefore this study tests the wideband-inhibition hypothesis by recording from single units in the DCN of the anaesthetized guinea pig. A pure tone signal, usually at the unit's best frequency, was temporally placed in the dips of a 10-Hz sinusoidally amplitude-modulated masker tone. The masker was centred at the same frequency as the signal and is referred to as on-frequency masker (reference condition). Flanking bands were then added which were pure tones, amplitude-modulated either in phase (comodulated condition) or out of phase (codeviant condition) with the on-frequency masker. Whenever possible the flanking bands were placed into inhibitory regions of the unit's response area. For 22 out of 29 units the addition of the comodulated flanking bands reduced the response to the masker and as a result the salience of the signal response was enhanced in comparison to both the reference and codeviant condition. Our results are consistent with the hypothesis that wideband inhibition underlies CMR as observed psychophysically in humans.

954 SAM Stimulus Response of DCN Cells Indicates Coincidence Mechanisms and Complex Neural Circuitry

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Previous researchers documented firing rate modulation and spike entrainment, in response of DCN single units to SAM stimuli. Ghoshal and Kim (1994) supported the hypothesis that these observations (i) could be explained by ISI regularity in simple neuron models, and (ii) are closely related to the intrinsic oscillations in DCN cells. Their simple neuron models possessed refractory and integrate-and-fire components, and had continuously varying inputs. However, Joris et al (1994) have shown that leaky integrate-and-fire neuron models, sensitive to input spike time coincidence, exhibit strong entrainment accompanied by strong rate modulation. Additionally, Yang et al. (1999) proposed coincidence models for auditory brainstem cells that incorporate inhibitory mechanisms. The goal of present research work has been to determine whether SAM response in DCN cells could indicate the neural mechanisms that have not been included in simple spike-generating neuron models proposed for DCN cells.

Mathematical measures that aid in quantifying phase locking properties (Marangos et al. 1998) and spike entrainment (Aggarwal et al. 1999), have been presented previously. Differences in the SAM response properties between coincidence models and simple neuron models, have also been characterized (Aggarwal et al. 2001). In this study, the results of the modeling study (Aggarwal et. al. 2001) are compare with DCN single unit recordings. Such data analysis supports the hypothesis that temporal integration and coincidence detection mechanisms exist in the DCN.

Furthermore, a detailed analysis of the ISI regularity and intrinsic oscillations in DCN cells validates the SAM response analysis results. Intrinsic oscillation analysis indicates that, unlike simple neuron models, DCN cells receive multiple inputs along complex neural pathways. These observation supports the possibility of a complex neural circuitry in the DCN, that influences the temporal response properties of DCN cells.

955 Parameters of the Random Spectrum Stimuli that Determine Nonlinearity of DCN Type IV Neurons

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Random spectrum stimulus (RSS) sets have been used to estimate the spectral weighting function that a neuron applies to sound energy across frequency. These weights can be applied to predict responses to other stimuli, and prediction performance is a measure of the model's validity. In particular, if a neuron is highly nonlinear, a 1st and 2nd order model will not be predictive. Spectral weighting functions have been used with success in modeling the responses of linear neurons in VCN and ICC, but not for nonlinear Type IV units in DCN (Yu and Young, 2000).

The question we ask is: does this apparent nonlinearity change with RSS parameters that were not previously varied, such as bin energy standard deviation, tone density, or bandwidth? To test for effect of standard deviation (E_{sd}), we presented four different RSS sets with E_{sd} of 12, 6, 3, and 1.5 dB, where 12 dB was the value used previously. Preliminary results show that when E_{sd} is decreased, 7/14 Type IV neurons in DCN respond in a more linear way, i.e. model performance improves. Weights also grow larger with improved model fit, and can

change in spectral shape and sign. Sorted RSS responses also show that the stimuli eliciting the maximum or minimum firing rates cannot be predicted by the 1st order weights for 11/14 units. 2nd order weights improve the prediction, so that the "optimal" stimuli are predicted for all but 4/14 highly nonlinear units. As E_{sd} is decreased, however, the models start to predict the "optimal" stimuli for these units as well. These results show that 1) nonlinearity decreases with stimulus standard deviation, which is expected because systems can be linearized for small deviations from an operating point, and 2) shapes and signs of weights can change with E_{sd}, suggesting that Type IV neurons are adaptive in their coding.

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956 Representation of Spoken Whispered Vowels in Cat Cochlear Nucleus

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Although a significant number of studies have dealt with the representation of normally voiced vowels in the auditory nerve and cochlear nucleus of a variety of animal species, few published works have examined the representation of whispered vowels (e.g., Voigt et al., Hear. Res. 8: 49-58, 1982; Stevens and Wickesberg, Assoc. Res. Otolaryngol. Mid-Wint. Meet. Abst., 2002). Unlike voiced vowels, spoken whispered vowels lack a fundamental frequency and well-defined spectral peaks. We studied the temporal representation of three spoken whispered vowels (/i/, /u/ and /a/) in the discharges of auditory nerve fibers (ANFs) and ventral cochlear nucleus (VCN) neurons in the anesthetized cat. Fourier analysis of the responses of ANFs, primary-like and primary-like with notch neurons in the VCN indicates that those neurons provide an accurate representation of the first formant (F1) of the vowels. The representation of the second formant (F2), however, was very weak in the cases in which /i/ or /a/ was used as the stimulus. For chopper neurons, the representation of F1 and F2 in the time structure of their discharges was almost nonexistent.

957 Multi-unit Temporal Pattern Analysis of Duration Coding in the Cochlear Nucleus

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Some single neurons in the midbrain and cortex are "tuned" to stimulus duration, yet there is no evidence for such tuning at lower levels of the system. Alternatively, we have shown that duration may instead be carried in the temporal firing patterns of single neurons (Eddins and Leong, 2001). The present study was designed to explore whether duration coding by temporal firing patterns, as analyzed with an artificial neural network (ANN), improves with the combined activity of multiple neurons recorded simultaneously. Using a multi-electrode recording probe, we studied the activity from two neurons recorded from one electrode or two neurons recorded from two separate electrodes in the chinchilla cochlear nucleus. After the response areas were measured, temporal firing patterns were recorded for tone burst stimuli of varying duration (2-256 ms), presented at a frequency to which both neurons responded, at levels from 30 to 50 dB SPL, in 5-dB steps. The temporal firing patterns served as input to train and test a one-layer ANN to recognize stimulus duration. The firing patterns were segmented into multiple, brief time bins whose temporal positions were fixed at the onset of the stimulus. Network performance was measured as the correlation between the estimated and the actual stimulus duration and was evaluated with patterns from individual and paired neurons. For both individual and paired neurons, network performance improved as the size of the time bin increased (t=1-5 ms) and also as the number of bins increased (n=1-10). Moreover, network performance improved significantly for paired neurons across all test conditions. In general, the performance of paired neurons recorded from the same channel was significantly better than that of paired neurons recorded from separate channels. The results suggest that duration is better represented by the temporal firing patterns of more than one neuron.

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958 Phaselocking Accuracy of Bushy Cells in the Anteroventral Cochlear Nucleus is Influenced by Inhibition

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Recent morphological and physiological findings have revealed that inhibitory inputs influence signal transmission in cells that receive their excitatory input through large calyx synapses. Earlier we have shown that inhibition alters the frequency tuning, level-dependent responses and encoding of the signal onset in spherical bushy cells of the anteroventral cochlear nucleus.

The present study examines the role of inhibition in the encoding of the sinusoid phase of the stimulus, known as the phaselocking ability of spherical bushy cells (SBCs). We used neuropharmacological application of strychnine and bicuculline in an in vivo preparation (Havey and Caspary, 1980) to examine the contribution of GABAergic and glycinergic transmitter systems ('drug-units'). The results complemented data of SBCs in which the presynaptic action potentials of endbulb-terminals and the respective postsynaptic action potentials were recorded ('pre/post-units'; Kopp-Scheinpflug et al., 2002 accepted). An input-output analysis of pre- and postsynaptic activity or alternatively of activity before and during drug application was performed. Results were compared to control experiments in which NaCl was applied and tested for their statistic significance by means of a permutation test (Siegel, S. and Castellan Jr., 1988). For the majority of units, spike rate decreases during the synaptic processing (95% of pre/post-units, 42% of drug units). Rate decrease occurred with either no change of phaselocking accuracy or with an increase of the accuracy (53% of pre/post-units, 57% of drug units). Responses in control experiments were dominated by rate changes in the opposite direction as found in pre/post-units or showed no change.

The data provides evidence that the accuracy of phaselocking of SBCs is improved at the auditory nerve-SBC synapse.

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959 Source of the Commissural Pathway Connecting the Cochlear Nuclei in the Rat

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Some neurons in the cochlear nucleus (CN) project their axons to the opposite CN. This commissural pathway indicates that the CN is the initial site of binaural processing in the central auditory system. In this report, we studied the anatomy of commissural neurons in the ventral cochlear nucleus (VCN) of the rat. Previously, these cells have been described as large, glycinergic multipolar cells with radiating dendrites. These features also describe "radiate cells" of the VCN. Radiate neurons innervate the ipsilateral dorsal cochlear nucleus (DCN) in the rat and our hypothesis was that they also project to the opposite CN. We tested this hypothesis by injecting biotinylated dextran amine into the DCN (labeling radiate cells) and filling the opposite cochlear nucleus with Diamidino Yellow (labeling commissural cells). Several radiate cells contained both tracers thus confirming our hypothesis. However, in the same experiments, we also observed neurons that were not double labeled. The morphology of these non-radiate cells was studied by filling the cochlear nucleus with Fast Blue (FB) in two rats. The size and distribution of labeled commissural cells were analyzed and compared to that of radiate neurons labeled in prior experiments. For the FB-filled commissural cells, 63% (181/287) had small cell bodies ($<250\ \mu m^2$). In contrast, radiate cells are typically large as only 15% (17/111) have cell body size $<250\ \mu m^2$. The small commissural neurons

were frequently found near the granule cell regions of the VCN, whereas radiate cells are predominantly located in the magnocellular core. These results demonstrate that VCN commissural neurons in the rat are comprised of at least two populations: radiate cells and small cells. Radiate cells inhibit their targets in the contralateral CN. Small cells may represent an excitatory pathway linking the two cochlear nuclei. Perhaps radiate and small cells serve to accentuate differences between binaural patterns of activity.

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960 Temperature-Sensitivity of the Low-Threshold Potassium Current in Octopus Cells

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Octopus cells are specialized to detect the synchronous firing of groups of auditory nerve fibers. At rest two opposing voltage-sensitive conductances, a depolarizing hyperpolarization-activated mixed cation conductance and a hyperpolarizing low-threshold K+ conductance are partially activated (Bal and Oertel, 2000, 2001). These conductances make synaptic responses small and rapid and prevent firing if synaptic responses rise too slowly (Ferragamo and Oertel, 2002). Current-and voltage-clamp recordings were made at three different temperature ranges, 22-23°C, 26-27°C, and 33-34°C. The present results show that the characteristics of the low-threshold K+ current are strongly sensitive to the temperature at which they are assayed.

Measurements of the low-threshold K+ current under voltage-clamp revealed a lower threshold of activation, larger peak amplitudes, and more inactivation at high than at low temperatures. The rates of rise and inactivation also increased with temperature. The proportion of the peak K+ current blocked by DTX-K, a blocker of currents mediated through channels that contain at least one Kv1.1 subunit, at –40 mV also increased with temperature (37% at 22-26°C, 61% at 33-34°C).

The sensitivity to temperature was also observed in current-clamp recordings. Amplitudes of action potentials were significantly smaller and briefer at their bases at high than at lower temperature (11.5 ± 2.2 mV, 0.4 ± 0.05ms at 33-34°C; 15.9 ± 5.6 mV and 0.7 ± 0.02 ms at 26-27°C, 20.3 ± 3.9 mV and 1 ± 0.04 ms at 22-23°C). The resting potential depolarized with increasing temperature. Transiently the differences in resting potential between 22 and 33°C were up to 10 mV. In the steady state resting potentials were on average –62 ± 2 mV at 33°C, -65 ± 3 mV at 26°C, and 67 ± 5 mV at 23°C (5 cells each).

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961 Golgi Staining in the Human Auditory Brain Stem

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Neuronal morphology in the human auditory brain stem has traditionally been studied by Golgi or modified-Golgi silver impregnation techniques. It is through these histological studies that cell descriptions such as bushy, octopus, stellate, and others have been reported. The Golgi technique is capricious and numerous modifications have been made to improve the rate of adequate staining. We describe a simple technique that has demonstrated improvement in staining outcomes and has been applied to human autopsy material for the study of auditory neuronal morphology.

Human adult brain stems were obtained from autopsy after determination by a Neuropathologist that they were free of disease. The brain stems were fixed by immersion for several weeks in formalin and sectioned in the coronal plane into 2-3mm slices. The human cochlear nucleus lies at the level of the pontomedullary junction with a bias to the pontine side. Slices were immersed in a mordant solution (Adams, 1979) under continuous vacuum for 4 days. This procedure promoted penetration of the solution deeper into the tissue than simple immersion. The brain stem slices were then immersed under vacuum in 1% silver nitrate solution for another 4 days. 50-100 micron thick sections were taken using a Vibratome and mounted for light microscopy.

Our results demonstrated more consistent silver staining of neurons in the auditory brain stem and cerebellum with vacuum immersion than with simple incubation. Several Endbulbs of Held were identified and cells consistent with octopus and multipolar cells were found. Detailed protocols will be presented.

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962 Neurotrophin-3 (NT-3) Expression in Neurons and Astrocytes is Developmentally Regulated in the Mouse Postnatal Cochlear Nucleus

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The neurotrophins and their receptors have been implicated in peripheral auditory system development but their role in cochlear nucleus (CN) maturation has received little attention. To begin to address this topic in CN we have started to profile the expression patterns of these proteins in F1 hybrids of C57Bl/6J x CBA/J mice at successive stages of development i.e., postnatal day (P) 3, 8, 14, 30, and 60. Light microscopic observations of the immunostain patterns produced by antibodies highly specific (by Western blot) for NT-3 and a glutamate transporter (GLT-1) in 15-µm serial CN sections suggest that the expression of NT-3 gradually shifts from primarily axonal at P3 to an astrocytic expression in the adult animal (P60). At P3, NT-3 immunostaining revealed many long, continuous tracts of immunopositive punctae consistent with axonal localization. The tract-like projections were most evident and thickest at the ventral aspect of the CN and are most likely cochlear nerve fibers. The observed pattern contrasted with the feathery, web-like profile produced by antibodies labeling GLT-1, a glutamate transporter located primarily in astrocytes. The CN of P8 mice displayed NT-3 staining patterns consistent with both axonal and astrocytic expression, while NT-3 antibody staining in P30 and P60 animals resembled GLT-1 labeling of astrocytes. Neuronal somata became heavily outlined in mature animals, probably due to astrocytic processes wrapping around neuronal perikarya, not as the result of cytoplasmic or cell membrane localization. The observed shift in NT-3 expression from axons to astrocytes suggests that, depending on the stage of postnatal development, NT-3 can act directly through axons in situ or indirectly by way of astrocytic processes.

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963 Single Cell Electroporation in NL Neurons in the Chick Brainstem

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One goal of our laboratory is to understand the mechanisms whereby afferent activity regulates neuronal architecture in the brainstem auditory pathways. To accomplish this goal we needed to establish an efficient, reliable method for labeling individual Nucleus Laminaris (NL) neurons for live imaging studies. Of the methods tested (diolistic transfection, retrograde tracing, and single cell electroporation), we found that single cell electroporation was the most effective. Acute or cultured slices of brainstem containing NL are prepared from embryonic day 17-21chicks. Slices are placed on filter paper soaked with artificial cerebral spinal fluid. A micropipette filled with anionic rhodamine dextran (10% in sterile saline) is placed just above the NL cell body line, and a brief train of square wave pulses is applied between the fluid surrounding the slice and the micropipette (50V, 8 pulses, 50ms duration, 10 Hz), releasing the tracer. NL cells seem to fill instantaneously, however a 15-30min incubation period is given to ensure loading of distal dendrites. With this procedure we are able to

label multiple, spatially separate NL cells on each side of the slice. Electroporated cells appear to fill completely, and display the same morphological features observed in NL cells stained with the Golgi method, e.g. dendritic symmetry and dendritic length gradient (Deitch & Rubel, 1984, J Comp Neurol, 229:66, Smith, 1981, J Comp Neurol, 203:309). Imaging studies also demonstrate that fluorescent label remains stable after multiple imaging sessions and over many hours (0-8hrs). Further studies have shown that single cell electroporation can readily be combined with electrophysiological techniques. This combination will allow us to dynamically image NL cells during the course of afferent stimulation, providing us with an ideal system in which to study the role of neuronal input in the regulation of cell structure.

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964 Distribution Patterns of Catestatin-like Immunoreactivity in the Central Auditory Pathway Within the Human Brainstem and in the Spiral Ganglion of the Human Inner Ear

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In vivo, Catestatin is a potent vasodilator whose actions appear to be partially mediated by a release of histamine and activity at H1 receptors. Catestatin can be displaced from chromaffin cells by Substance P suggesting that Catestatin and Substance P may occupy an identical or overlapping non-competitive site on varying nicotinic receptors. Chromogranin A, a 48 kD acid protein initially found in catecholamine storage vesicles of the adrenal medulla, participates in the inhibition catecholamine release. At present, several Chromogranin A-derived peptides have been identified and shown to exert an affect on the secretory function of parent cells. As a peptide precursor and a peptide hormone, Chromogranin A is suspected of indirectly inducing neuronal apoptosis in the presence of microglial cells. The aim of the present study was to investigate the presence and distribution sites of Catestatin within human auditory central and peripheral pathways. For cytoarchitectual orientation, series of adjacent brainstem sections were stained with cresyl violet and with hematoxylin-eosin for the inner ear. The density of Catestatin-immunostaining was assessed in the spiral ganglion and the central auditory pathways using an image-pro analysis system. Densities of catestatin were graded as low (+), moderate (++) and high (+++) according to the intensity of immunostaining. Results show that Catestain positive staining was demonstrated in cochlear neurons of the spiral ganglion, the nucleus cochlearis, the nucleus vestibularis, the superior olivary complex and the inferior colliculus inferior. Findings and functional implications are discussed.

965 Projections of the Inferior Olive to the Cochlear Nucleus in Rats

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The granule cell domain (GCD) of the cochlear nucleus receives auditory and non-auditory inputs and projects in turn to the dorsal cochlear nucleus (DCN). Some of these inputs are in the form of mossy fiber endings that arise from the cuneate nucleus, nucleus of the spinal trigeminal tract, and the pontine nuclei. In addition, Fast Blue injections in the DCN retrogradely labels some neurons of the inferior olive. Because the structure of the DCN resembles that of a cerebellar folium, we asked whether the inferior olive might project to the GCD in the form of climbing fibers. We used the anterograde tracer, biotinylated dextran amine, to demonstrate a bilateral projection from the inferior olive to the GCD in 23 rats. We used labeled climbing fibers in the cerebellum as a positive control. The projection was modest, but appeared as long relatively straight axons with short side branches, ending with small bouton endings. *En passant* and terminal swellings marked this termination. Collectively, labeled swellings were found throughout GCD, concentrated in the lamina and superficial layer, but found to a lesser extent in the subpeduncular corner, strial corner, and granule cell islands. There were also scattered swellings in the DCN but very few in AVCN. The swellings contained small round vesicles and formed asymmetrical synapses. The structure of these endings was similar to that of climbing fibers. In a few cases, injection of the inferior olive did not yield labeling swellings in the cochlear nucleus but climbing fibers were found in the cerebellum, indicating that not all parts of the inferior olive project to the GCD. This novel projection indicates that the GCD not only processes polysensory information but also integrative information from centers such as the pontine nuclei and inferior olive.

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966 Mossy Fiber Projections from the Spinal Trigeminal Nucleus to the Cochlear Nucleus

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The granule cell domain (GCD) of the cochlear nucleus is known to receive nonauditory inputs from a variety of sources and to contain numerous mossy fiber endings. These mossy endings form synaptic relationships with the resident neuronal elements, but the source of the inputs and their targets are not completely known. Using anterograde neuronal tracers in rats, we have determined that the spinal trigeminal nucleus projects into the granule cell lamina and the deep layers of the dorsal cochlear nucleus (DCN). The labeled endings vary in size and shape. We sought to determine the ultrastructural characteristics of these spinal trigeminal endings and to identify the postsynaptic neurons. Terminals labeled with biotinylated dextran amine (BDA) were examined using an electron microscope. We observed en passant and terminal swellings, many of which resembled mossy fiber endings of the cerebellar cortex. These mossy endings were characterized by irregular shapes, round synaptic vesicles, prominent postsynaptic densities, and contacts with granule cell dendrites. The trigeminal mossy fibers resembled those of the cuneate nucleus (Wright and Ryugo, 1996). The structural similarities between the separate somatic sensory inputs may suggest functional cohesion where proprioceptors converge in the GCD to convey information about head and pinna position.

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967 Somatosensory Pathways from the Spinal Column to the Dorsal Cochlear Nucleus are Plastic

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The principal cells of the dorsal cochlear nucleus (DCN) receive inputs from many auditory and non-auditory sources, including the medullary somatosensory nuclei (MSN). Projections to the MSN that, when electrically stimulated, yield evoked potential (EP) responses in the DCN include those from spinal nerves associated with the cervical vertebrae. In this poster, we investigate the hypothesis that the somatosensory inputs are strengthened or weakened according to their association with auditory activity.

We have found that the EP produced in the DCN by electrical stimulation of individual spinal nerve roots can be strengthened with an

appropriate training paradigm. One such paradigm involves a combination of 100Hz tetanic burst stimulation to the spinal nerve roots with simultaneous suprathreshold acoustic noise stimulation to the ipsilateral ear. Notably, our results show that the potentiation requires both types of stimuli to be present. Neither the electrical stimulation alone nor the acoustic stimulation alone resulted in an increase of the EP response in the DCN.

Under normal conditions, electrical stimulation of the second cervical nerve (C2), which includes projections from the pinna, yields the strongest EP response relative to that induced by stimulation of the other cervical nerve roots. The present results suggest that the strength of the C2 response is a direct result of its innervation of the pinna muscles, in the sense that movements of the pinna are expected to be associated with acoustic stimuli.

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968 Threshold Sensitivities of Neuronal Populations in Cochlear Nucleus (CN) Determined by Sound-Induced Fos Expression: EVIDENCE for Acoustic Stimulation of Granule Cells.

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Threshold sensitivities to sounds were examined among the different subdivisions and cell types of the CN. Unanesthetized, unilaterally tympanectomized rats were exposed to narrow band noise bursts (8 kHz center, 0.5 octave, 100/400ms) in the free field for 2 hrs. Sound levels were varied from –14.5 to 45.5 dB in spectral level (dBSL, overall levels 20-80 dB). Rats were isolated from ambient sounds in a sound-attenuating booth for 4 days prior to exposure. Immunocytochemical localization of the nuclear protein, Fos, was used as our metric for neuronal activity since Fos expression increases with firing rate in neurons. Granule cells measured in the external granular layer in Nissl-stained sections had nuclei 23.3±7.8μm^2. Consequently, all Fos-stained nuclei less than 32μm^2 (roughly one std. dev. above the mean) were defined as granule cells; larger nuclei were defined as non-granule neurons. Control animals not exposed to sound showed little or no Fos expression in the CN. Threshold sensitivities for the three major subdivisions were similar, but differed between granule and non-granule neurons within each subdivision. Threshold for non-granule neurons was approximately 0 dBSL. Increases in intensity (–4.5 to 45.5 dBSL) resulted in a monotonic increase in the number of these neurons expressing Fos. Threshold for granule cells was approximately –10 dBSL, and these showed a rapid increase in numbers (8-10 fold) between -4.5 and +5.5 dBSL. Well-defined, tonotopically appropriate laminae were formed by the non-granule neurons, and the laminae increased in breadth with increasing intensity. Fos expressing granule cells were also tonotopically organized but were more broadly distributed within subdivisions. These data suggest that granule cells have sound-evoked action potentials at lower absolute thresholds than other CN neurons.

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969 Low Levels of Lead Induce Gliosis and Neuronal Changes During Development of the Murine Auditory Brainstem

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Lead remains an important environmental toxin despite its removal from paint and gasoline. Early exposure to lead is a risk factor for reading disability (RD) but it is not known how lead exerts its effects on language and perception. Children with language impairment have demonstrated deficits in backward masking and preliminary studies indicate that children who have measurable lead levels also have deficits in backward masking. We previously reported that chicks exposed to low levels of lead during development (< 10 ug/dL) show a deficit in backward masking that is similar to the deficit in children with RD. These lead exposed birds exhibit increased gliosis and decreased immunoreactivity for neurofilament protein within the primary auditory brainstem nucleus, and these morphological changes are consistent with the observed behavioral deficits.

The present study was undertaken to determine whether lead exposure during development of the mammalian auditory system also results in neural and glial changes within the primary auditory nucleus Anteroventral Cochlear Nucleus (AVCN). Breeding pairs of Balb/c mice were given a low dose (0.1mM), a medium dose (0.6mM) or a high dose (2mM) of lead acetate in their drinking water throughout gestation. The brains of the lead-exposed offspring were then processed for histology and neurofilament immunohistochemistry at postnatal day 21. Increased numbers of glial cells are observed by Hematoxylin & Eosin staining within the AVCN of all lead-exposed mice compared to control mice that received no lead in their drinking water. In addition, decreased immunostaining for neurofilament is found within the AVCN of lead-exposed mice, particularly in the low lead group. These findings confirm that exposure to low levels of lead induce morphological changes within the mammalian auditory brainstem that could underlie the behavioral deficits observed in lead-exposed children.

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970 Scientific Visualization of Pathways in The Middle and The Inner Ear

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The pathways of aeration within the middle ear and the fluid dynamics of the inner ear are three dimensional phenomena that have been traditionally interpreted from two dimensional formats. Understandably this has resulted in contradicting opinions. Even modern technology such as Computed Tomography and Magnetic Resonance Imaging has added little to our knowledge of exactly where these pathway are because of the inability to demonstrate the delicate soft tissue membranes that determine the parameters of the spaces.

In an attempt to more clearly define these pathways we have created 3D models of the middle and inner ear of the right temporal bone of a 40 week gestation, human neonate with no congenital defects, harvested 19 hours postpartum, embedded in celloidin and sectioned at 20 microns. Microscopic slides of all 780 serial sections were scanned into a high resolution photo scanner and anatomical entities were separated out from each image, color coded and 3D images created using commercial graphics software.

This technique allows for: 1) realistic precise visualization of the membranous endolymphatic system and associated spaces and by extrapolation, the possible fluid dynamics of the inner ear; 2) 3D visualization of the relationship of the tympanic membrane, ossicles, mucosal folds, nerves, and bony walls to each other, thus allowing for conceptualization of the spaces of the normal middle ear, specifically, the only aeration route to the mastoid antrum from the Eustachian tube as well as the most common route to the aeration packet behind the pars flaccida (Prussak's space).

With these scientific visualizations our understanding of the dynamics of aeration in the middle ear and fluid in the inner ear should contribute substantially to unraveling at least some of the pathologic processes (ex. - blockage) that affect these structures.

971 Finite Element Modeling of Human Ear with Temporal Bone Histology

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The finite element (FE) method, a general numerical procedure, has its distinct advantages in modeling complex biological system when compared to other computational methods. The accuracy of FE analysis on a structure system, such as the ear, greatly depends on the geometry and boundary conditions of the system. In this paper, we report a newly established FE model of human ear with external ear canal, middle ear ossicles, and middle ear cavity. Two technologies, 3-dimensional reconstruction of human temporal bones and finite element analysis of ear biomechanics, were combined to construct this computational model for describing function of the ear for sound transmission. A set of histological sections of a human temporal bone was prepared, scanned, and digitized for imaging analysis. The aligned and treated images of the middle ear components, external ear canal and middle ear cavity were constructed with attached ligaments, muscles, and tendons. A 3-D CAD model was first created which provided accurate geometric data. Based on the CAD model, the finite element model of human ear was generated with whole middle ear and ear canal. The model-derived eardrum (umbo) and stapes vibrations were then compared the results obtained from human cadaver temporal bone experiments with 90 dB SPL at the eardrum across auditory frequencies. The model will be further used for acoustic analysis of the ear.

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972 Morphometric Study on Facial Recess in Human Temporal Bone

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During middle ear surgery, to successfully open the facial recess between the mastoid portion of the facial canal (FCMa) and the chorda tympani nerve (CTN) is mandatory. However, the fossa incudis is the only useful anatomical landmark available so far for that purpose. For this reason, we have tried to find a more sophisticated method to delineate not only the location but also the size of the facial recess.

Seventeen human temporal bone specimens obtained from 17 individuals whose ages ranged from one week to 82 years and who had no evidence of ear disease. The specimens were processed histologically and prepared for light microscopic study. The image of FCMa, CTN and the incus in histology sections was identified under the light microscope and entered into a personal computer through the CCD camera for three-dimensional measurement. To identify FCMa and CTN and to measure the facial recess, we used the incus as a reference mark. On the horizontal plane, which is the upper part of the facial recess defined by the reference mark (incus), the distance between FCMa and CTN was measured for and found to be 1.7 to 3.2 mm (average 2.4 ± 0.4 mm). There was no significant difference in the length between children and adults.

The result indicates that as far as the upper part of the facial recess is concerned its surgical opening for children would be as easy as that for adults. Further study on the lower part of the facial recess will be performed and reported to finalize clinically important morphometric information on three-dimensional localization, shape and size of the facial recess in both children and adults.

973 Thickness Distribution of a Human Eardrum Measured with Confocal Microscopy

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In literature, only few data of eardrum thickness are available. In most middle ear studies a rough estimate is used, obtained on a few isolated points by means of conventional light microscopy on histological sections. We used a confocal laser scanning microscope (Zeiss, LSM 410 Invert) to obtain highly accurate thickness measurements of the untreated human eardrum along several complete lines. The optical sectioning technique provides an excellent basis for a quantitative study of a fresh tissue without artefacts as shrinking caused by a conventional preparation method.

For this study, the eardrum was dissected out of the middle ear, stained with a water-based dye to improve the fluorescence signal and immersed in a buffer to avoid dehydration. Because of its cone shape, a few incisions were made so that it could be flattened out on a horizontal microscope slide. Using a long working distance objective with water as the immersion medium, optical depth sections perpendicular to the eardrum's surface were imaged. After scanning neighboring images along lines parallel and perpendicular to the manubrium, data of the thickness distribution could be obtained. The measured thickness data had to be corrected for a 'focal shift' error to know exact thickness. A small difference in refractive index between the immersion medium and the imbedding medium causes a broadening and axial shift of the point-spread-function, affecting the recorded images in axial direction. After an accurate theoretical and experimental study of the point-spread-function, its effect on thickness measurements was taken into account.

The resulting thickness data will lead to finer modeling and a better understanding of the middle ear mechanics, its function and pathophysiology.

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974 Pressure Induced Eardrum Deformation at Progressive Stages of Middle Ear Dissection.

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Quasi static pressure differences between the middle ear (ME) and the ambient pressure in the external meatus constantly occur in everyday situations. It is generally known that large static pressures have an effect on hearing thresholds, and it is also accepted by many clinicians that there is a relation between eardrum pathology and ME pressure deregulation. Quantitative experimental data of both static deformations and vibrations under static load are necessary to build realistic computer models of the ME mechanical system. To obtain a correct model for the large static pressure induced middle ear motions, the components which have an influence on quasi-static ME mechanics should be incorporated.

We studied the effect of static pressure on deformation of the eardrum in different stages of dissection of gerbil temporal bones. With our high resolution moiré interferometer we recorded the shape and deformation of the eardrum as a function of pressure along a line perpendicular to the manubrium and through the umbo, at each dissection stage. First the cochlea was removed with the oval window intact, then the tensor tympani was cut, next all remainders of the cochlea together with the stapes were removed, and finally the incudo-mallear joint was exposed and the anterior mallear ligament was cut The mean displacement perpendicular to the plane of the annulus was also determined in the zone surrounding the umbo, at all stages of dissection as a function of pressure.

The measurements showed that eardrum deformation is not influenced by removing the cochlea and the stapes, or by cutting the tensor tympani. Trimming down the bone so that a full medial view is obtained

on the TM can also be done without changing the static eardrum behavior, but our results show that sometimes changes are introduced even if no apparent damage is seen under the microscope. Our results indicate that the influence of the cochlea, tensor tympani and stapes may be disregarded to obtain a good model of large eardrum deformation under static pressure. Exposing the incudo-mallear joint probably damages the posterior incudal ligament: our measurements show major changes in membrane pressure response. The anterior mallear ligament has an important influence on the shape of the eardrum in rest position.

975 Three-dimensional Displacement of the Gerbil Ossicular Chain Under Static Pressure Changes

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The role of the middle ear is to transmit the sound energy from the tympanic membrane to the inner ear by the vibration of the middle ear ossicles. The middle ear has to perform this tranfer under largely varying ambient pressure conditions that can give rise to large transtympanic pressure differences. We all know from personal experience that the middle ear function is strongly diminished under these conditions. From our earlier studies it is well known that the position, shape and curvature of the tympanic membrane is entirely different for over- or underpressures in the middle ear cavity. The configuration of the middle ear ossicles must also change with middle ear pressure because the cochlea has to be protected from damage by too large medial footplate displacements.

We have studied the changes in the configuration of the ossicles with static pressure using a fully 3-D measuring technique, namely computerized scanning x-ray tomography (CT). The scans were done on fresh gerbil temporal bones using a micro-CT scanner (SkyScan 1072), a laboratory scanner for high-resolution measurements of small objects. Careful segmentation of the 3 ossicles in the virtual sections was performed to measure the spatial position of the ossicular chain for pressure differences of 0, 150, 300, -150 and -300 daPa (1daPa ~ 1mmH2O). Position changes of the ossicles within the middle ear cavity, drastic deformation of the tympanic membrane and shift of the ossicles with respect to each other could all be measured in a same preparation.

976 Simulation of Measurement of Ossicular Mobility: Effect of the Direction of Applied Displacement on Mobility of the Ossicles

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It is important to evaluate ossicular mobility in tympanoplasty surgery, because it affects the prognosis of improvement of the hearing level. However, since quantitative evaluation of ossicular mobility has been difficult to date, we have developed a new apparatus for its measurement. With this apparatus, the ossicles are displaced and the reaction force from the ossicles is simultaneously detected. The clinical applicability of this apparatus has been evaluated by measuring the ossicular mobility in humans. In the case of measurements in humans, as the surgical field in tympanoplasty is limited, it is difficult to unify the direction of applied displacement in each patient. This difference in direction may result in difficulty in comparison between patients.

In this study, measurement of ossicular mobility with the newly developed apparatus was simulated by using a finite-element model of the middle ear, and the effect of the direction of the applied displacement and that of the state of the middle ear on the measurement results were estimated. It was found that the result of ossicular mobility differs not only with the condition of the middle ear, i.e., the stiffness of the ligaments and the presence of I-S joint separation, but also with the direction of the applied displacement. However, in the range of the difference in the direction of the applied displacement which may arise in each operation, its effect on the measurement result of ossicular mobility is considered to be small. Therefore, it was concluded that the apparatus can be used to diagnose the degree of ossicular fixation during surgery.

977 On the Coupling Between the Incus and the Stapes in the Cat

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The connection between the long process and the lenticular process of the incus is extremely fine, so much so that some authors have treated the lenticular process as a separate bone. We review descriptions of the lenticular process that have appeared in the literature, and present some new histological observations. We discuss the dimensions and makeup of the lenticular process and of the incudostapedial joint, and present estimates of the material properties for the bone, cartilage and ligament of which they are comprised.

We present a preliminary finite-element model which includes the lenticular plate; the bony pedicle connecting the lenticular plate to the long process; the head of the stapes; and the incudostapedial joint. The model has a much simplified geometry. We present simulation results for ranges of values for the material properties. We then present simulation results for this model when it is incorporated into an overall model of the cat middle ear. For the geometries and material properties used here, the bony pedicle is found to contribute significant flexibility to the coupling between the incus and stapes.

978 Single-channel Properties Underlying Spiral Ganglion Neuron Firing Pattern Diversity

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The diversity of firing patterns, which vary both regionally and tonotopically, is now known to be a key organizational principle of neonatal spiral ganglion neurons. In order to understand the basis for this diversity we have begun to access the fundamental features of voltage-gated ion channels. Our goal is to determine whether these channels consist of discrete populations of relatively uniform channels that differ only in density or whether the individual channels themselves show variation in their fundamental properties. An extensive series of single channel recordings found that specific properties of the large conductance calcium-activated K^+ channel ($K_{(Ca)}$) most likely contributes to the heterogeneous firing patterns of spiral ganglion neurons.

Cell-attached recordings were made from neonatal mouse spiral ganglion neurons (P3-P6) maintained in vitro 3-20 days. Four major categories were identified; a small hyperpolarizing activated cationic current (n=48), a delayed rectifier channels of <30pS (n=28), a 48.7 pS channel type with predominately inactivating kinetics (n=358), and the $K_{(Ca)}$ channels (>100 pS; n=153). The greatest diversity in ion channel characteristics was seen in the $K_{(Ca)}$ channels. Conductances that ranged from 180pS to 300pS displayed an expansive range of voltage dependence ($V_{1/2}$ = −55 to +50mV under identical Ca^{2+} conditions). Furthermore; heterogeneous gating properties were observed (5ms to >15ms mean open time) at similar open probabilities and conductances.

From these studies we speculate that the heterogeneity of ion channel types in spiral ganglion neurons and complexity of $K_{(Ca)}$ properties are key elements that could dictate the precision with which the auditory nerve encodes information.

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979 Firing Properties of Putative Type II Spiral Ganglion Neurons In Vitro

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Spiral ganglion neurons provide the sole link between hair cells in the periphery and neurons in the CNS. Our electrophysiological analysis of postnatal spiral ganglion neurons in vitro has revealed that type I apical and basal neurons show distinctive responses to threshold depolarization such that basal neurons produce briefer action potentials of shorter latency than apical neurons. We now extend our studies to include type II neurons which are only sparsely distributed in the spiral ganglion and as a result are poorly understood. To elucidate the firing features of these cells, we utilized an in vitro method to record from neurons identified with a marker that selectively labels type II neurons.

Whole-cell current clamp recordings were made from mouse postnatal spiral ganglion neurons (P4-P7). Analysis of these recordings showed that firing properties of basal type II neurons (N=6) differed significantly from their type I counterparts (Adamson et al., JCN 2002). The basal type II neurons fired action potentials with longer latencies (62.2 ± 1.7 ms; $P<0.01$) and slower accommodation (5.8 ± 1.9 spikes/240ms; $P<0.01$), but with indistinguishable durations (1.4 ± 0.08 ms). We also found that thresholds of the putative basal type II neurons (-61.9 ± 1.7 mV) were significantly lower than the basal (-55.3 ± 0.9 mV; $P<0.05$) and apical (-56.3 ± 0.8 mV; N=62; $P<0.05$) type I neurons.

These experiments establish that putative type II basal neurons are not specialized to respond rapidly to depolarizing stimuli as are the type I basal neurons. This is consistent with the speculation that speed is not likely to be the forte of type II neurons which summate inputs from multiple presynaptic cells and conduct action potentials via small diameter unmyelinated processes in situ. Their low thresholds, however, could make the type II neurons more sensitive to slight changes in voltage, thus enabling them to respond to low intensity stimuli.

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980 Needles in the Haystack: Membrane Properties of Identified Type II Spiral Ganglion Neurons

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Type II spiral ganglion neurons (sgnII) provide the afferent innervation to outer hair cells (ohc), and represent only around 5% of all primary auditory neurons. The remaining 95%, type I spiral ganglion neurons (sgnI), provide afferent innervation to inner hair cells (ihc). Little is known about sgnII function or their membrane physiology. To approach this problem we have identified sgnII in slices of the P7-P10 rat cochlea, and have investigated their electrophysiology using the whole-cell patch clamp technique.

SgnII were identified using confocal immunocytochemistry to localize peripherin expression, which is seen only in sgnII at these ages. SgnII were most numerous in the apical spiral ganglion regions close to the intra-ganglionic spiral bundle, and had variable soma diameter. In subsequent patch clamp experiments, the pipette was directed to sgn soma in these regions. Patch pipettes were filled with biocytin or Lucifer yellow to allow post-hoc analysis of sgn peripheral neurite targets. SgnII innervated multiple ohc via spiral fibres, whereas sgnI innervated single ihc via radial fibres. SgnII displayed transient inward sodium currents, and inactivating A-type potassium currents. This phenotype was distinct from that of sgnI, which had potassium currents with slower inactivation. SgnII fired phasic action potentials followed by a slowly depolarizing response, consistent with the inactivation of the A-type current. This depolarizing response was absent in sgnI. Glutamate activated inward currents in sgnII and sgnI, but the response in sgnII was more potentiated by cyclothiazide, suggesting differential AMPA receptor expression. ATP activated comparable desensitizing inward currents in sgnII and sgnI. These data demonstrate a distinct functional phenotype for sgnII compatible with the requirements for integration of cochlear amplifier activity.

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981 Differential Distribution of Calcineurin in Type I and Type II Spiral Ganglion Neurons

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Application of ouabain onto the intact round-window membrane of the gerbil cochlea induces apoptosis in most spiral ganglion neurons, leaving a few type II-like neurons intact (Schmiedt et al; JARO, 3:220, 2002). Peripherin, an intermediate filament protein, is considered a reliable marker for type II spiral ganglion neurons. Immunohistochemical staining revealed that the remaining neurons in the ouabain-exposed gerbil cochlea were peripherin-positive. Knowledge of the presence or absence of certain other key neuron-specific proteins in type I and type II neurons may help to define the biochemical mechanisms that lead to either death or survival after exposure to ouabain.

Calcineurin, also known as protein phosphatase 2B, belongs to the family of Ca^{2+}/calmodulin-dependent protein phosphatases. This protein has a wide distribution in the central nervous system and is a candidate enzyme for regulating cell survival in the rat hippocampus after traumatic and ischemic insults (Morioka et al; Progress in Neurobiology, 1999). Here we show that, like peripherin, calcineurin is present only in type II neurons in the normal gerbil spiral ganglion. In addition, all of the neurons remaining in the spiral ganglion after treatment with ouabain where calcineuron-positive.

These results suggest that: 1) calcineurin provides an additional reliable marker for differentiating type II from type I neurons in the spiral ganglion; 2) the presence of calcineurin only in type II neurons provides insight into their function; and 3) calcineurin may play a protective role in enhancing the survival of type II neurons exposed to ouabain.

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982 Delayed Neurotrophin Treatment Following Deafness Rescues Spiral Ganglion Cells from Death and Promotes Regrowth of Auditory Nerve Peripheral Processes

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Recent studies have demonstrated that neurotrophic factors (NTF) reduce spiral ganglion cell (SGC) degeneration and initiate process regrowth following deafness when treatment shortly follows deafness. In clinical application, to improve benefits of cochlear implants, implantation and NTF treatment would likely be delayed. We, therefore, compared the efficacy of brain derived neurotrophic factor (BDNF @ 100 µg/ml) plus acidic fibroblast growth factor (FGF1 @ 50 ng/ml) on SGC survival and peripheral process regrowth, administered immediately vs. 3 and 6 weeks after deafening. Six groups (n=6 ea) were deafened (kanamycin + ethacrynic acid) with a >60dB ABR threshold shift necessary for inclusion in the study. Groups received BDNF + FGF1 or artificial perilymph (AP controls) into the scala tympani for 26 days of treatment. SGC survival and process regrowth (using pan trk immunostaining) was assessed from mid-modiolar plastic sections. A marked significant enhancement of SGC survival was seen

in each of the NTF treated groups compared to its AP control. The degree of enhancement decreased as the delay increased in the 6 wk delay group. Peripheral processes demonstrated a significant (>2:1 NTF:AP) enhancement in the 4 day treated animals, however there was still immunostaining in the AP controls indicating this might reflect "maintenance" rather than regrowth. A large enhancement (>10:1) in the 3 wk delayed treatment group (ample time for degeneration of peripheral processes), diminishing (>2:1) in the 6 wk delayed treatment group were indicative of regrowth. Thus the combination of BDNF + FGF1 was effective even when deafness induced degeneration after 6 wks had reduced the normal SGC population by >30%. NTF treatment may improve benefits of cochlear implants by maintaining a larger excitable population of neurons and inducing regrowth.

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983 Anatomical and Electrophysiological Changes after Auditory Nerve Demyelination in Chinchillas

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The goals of this study were to develop a reliable method of demyelinating the auditory nerve in chinchillas and to study the physiological effects resulting from such demyelination. Further, we wanted to test the hypothesis that auditory nerve demyelination might be the pathological mechanism underlying auditory neuropathy (AN). Chinchillas received a dose of either Adriamycin, a cytotoxic agent, or a sham (vehicle only) injection into the auditory nerve bundle near the cochlear nucleus. The cochlear microphonic (CM), compound action potential (CAP), and inferior colliculus evoked potentials (IC-EVPs) were recorded before and up to 2 months after injection. In addition, DPOAEs and ABRs were also recorded. The control group, receiving the sham injection, showed no significant anatomical or physiological changes over the 2 month test period. In the experimental group, demyelination of the ganglion cells in the modiolus and the auditory nerve fibers in the internal auditory canal began by the first day after the injection and remained for the entire 2-month study period. Virtually no missing IHCs or OHCs were found. The CM and DPOAE amplitudes were larger after drug injection, especially for the CM. The CAP and IC-EVPs demonstrated substantial threshold elevation and amplitude reduction beginning by the first day after drug injection. While there was progressive deterioration in the CAP over time, some recovery occurred for the IC-EVPs. ABR was absent by the first day and remained absent for the entire study period. The results introduce a potential animal model of auditory nerve demyelination with physiological results that mimic those in AN.

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984 Pathology and Physiology of Auditory Neuropathy

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We studied a family with hereditary sensory motor neuropathy and deafness accompanying a missense mutation in the MPZ gene. Pathological examination of the cochlea in one of the family members revealed marked loss of auditory ganglion cells and central and peripheral auditory nerve fibers within the cochlea. The inner hair cells were of normal number with preserved morphology. The outer hair cells were normal in number except a 30% reduction in just the apical turn. Examination of the sural nerve and the auditory nerve adjacent to the brain stem showed marked loss of fibers with evidence of incomplete remyelination of some of the remaining fibers. Studies of auditory function in surviving family members using electrophysiological and psychoacoustic methods provided evidence that the hearing deficits in this form of auditory neuropathy were likely related to a decrease of auditory nerve input accompanying axonal disease. Altered synchrony of discharge of the remaining fibers was a possible additional contributing factor. The role of axonal and demyelination of auditory nerve fibers to the hearing disorder will be discussed.

985 Electrophysiological Studies on Effects of Intracochlear Perfusion of Capsaicin and Capsazepine: In Search of a Role for Vanilloid Receptors in Primary Auditory Neurons

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Ingestion of capsaicin (CAP), the pungent ingredient of hot pepper, causes intense burning oral pain via the vanilloid receptor type 1 or VR1. The VR1 is a nonselective cation channel that is found in small- to medium-diameter primary sensory neurons of the somatosensory system. Tinnitus is known to bear similarities to pain, yet little is known of possible bases, including molecular-level mechanisms in the auditory periphery. Recent observations of VR1 mRNA and immunoreactivity in the spiral ganglion suggest VR1 as a possible mechanism, and CAP application provides an approach to its investigation. The purpose of this study was to probe the potential effects of CAP on auditory function. Several physiological/electrophysiological methods were employed. A micropipette system was used to perfuse the guinea-pig cochlea with CAP (typically 0.1-10 ppm) and its competitive antagonist, capsazepine (CZP, 0.2-20 ppm), as well as washout with artificial perilymph in scala tympani of the first turn {ST-T1} (drainage at the apex). Electrocochleography (ECochG) was undertaken to register synchronized compound neuronal and gross receptor potentials at relatively low and high stimulus levels of 4 kHz in quiet and with white/masking noise (intracochlear tungsten electrode in T1). In addition, asynchronous background whole-nerve or ensemble background activity {EBA} was recorded from ST-T1, also under quiet and noise. Two groups of guinea pigs received different concentrations or either CAP only or CAP plus CZP. Preliminary analyses suggest small dose-dependent increases around 1 kHz of the EBA during CAP perfusion, which appeared to be blocked by CZP. ECochG results were more variable and complicated, perhaps by extraneous factors of perfusion. Study limitations and theoretical implications of the findings are discussed regarding possible roles of VR1 activation in auditory function.

986 Expression of the trk A, trk B and trk C Proto-Oncogene Receptors in the Adult Human Cochlea

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A primary cause of deafness is damage of receptor cells in the inner ear. Clinically, it has been demonstrated that effective functionality can be provided by electrical stimulation of the auditory nerve. However subsequent to the sensory cell loss there is a loss of afferent nerve fibres.

Brain derived neurotrophic factor (BDNF) is mediated via the trk B receptor, Neurotrophin–3 (NT3) trough tkr C receptor and NGF via the trk A. Cryosections of an human modiolus were used to study the presence of neurotrophin receptors in human spiral ganglion.

The inner ear were obtained during petro-clivial meningioma surgery. The inner ear was fixed in 4% freshly prepared paraformaldehyde. With standard immunohistochemical methods we assessed the expression of neurotrophin receptors in the human spiral ganglion.

The distribution pattern of trk A, B and C in the human modiolus and perspectives for improving cochlear implants is discussed.

987 Stem Cell Survival in Scala Tympani of the Cochlea

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Cochlear prostheses function by stimulation of the auditory nerve and there is increasing evidence that their effectiveness is dependent on auditory nerve survival and excitability. It would, therefore, be valuable if new neuronal connections could be established between the cochlea and the cochlear nucleus, particularly in cases of poor nerve survival. Stem cells provide a potential mechanism. In this study, we have assessed survival, differentiation and migration of mouse embryonic stem (ES) cells placed into the cochlea of hearing and deafened guinea pigs. Cell clumps of either DKO (double null for neurofibromatosis 1 gene) (Barald et al, 2002) or wild type D3 ES cells, some transfected with green fluorescent protein (GFP), were placed in the scala tympani or through a defect in the modiolus into the nerve. Immunotherapy (cyclosporine and Doxycycline Hyclate) started the day of surgery and continued until termination, days 13-26. Animals were perfused with fixative, and paramodiolar cryostat sections of the decalcified cochlea were examined with immunocytochemistry. Both populations of stem cell showed good survival in scala tympani, 13 days following their placement. Neurofilament and Nestin immunoreactive (IR) somata and processes were visible among the mass of cells in scala tympani, suggesting differentiation into neurons, though not all cells were so labeled. There were also host (guinea pig) cells present among the mass of cells in scala tympani, including epithelial and reactive cells. Analysis of modiolar implants and longer survival time is ongoing.

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988 Disruption of Lateral Olivocochlear Neurons Depresses Compound Action Potential Amplitude.

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In previous experiments, we injected melittin, a cytotoxin, into the lateral superior olive (LSO) to disrupt lateral olivocochlear (LOC) projections to the cochlea. The functional effect of the LSO lesion was limited to depression of compound action potential (CAP) amplitude (Le Prell et al. 2001). Here we describe a novel approach to selectively lesion LOC efferents without adverse consequences to the afferent pathways originating in or passing through the LSO. We injected a dopaminergic (DA) neurotoxin into the cochlea; CAP amplitude depression was similar to that produced by LSO lesions.

Guinea pigs were treated with intra-cochlear Ringer solution (control) or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrindine (MPTP), the DA neurotoxin. Substances were slowly injected into basal turn scala tympani, or allowed to diffuse across the round window membrane for 30 min. 6-hydroxydopamine (another DA neurotoxin) disrupts tyrosine-hydroxylase-like immunolabeling of LOC neurons (Densert 1975; Eybalin et al. 1993; d'Aldin et al. 1995). Because multiple putative LOC transmitters are colocalized in the LSO (Altschuler et al. 1983; 1984; 1986; Abou-Madi et al. 1987; Safieddine et al. 1997) as well as LOC terminals (Altschuler et al. 1985; Safieddine and Eybalin 1992), we expected a broad loss of LOC neurons after disrupting DA-containing neurons. At the conclusion of the experiment, cochleae were perfused with fixative and immunoreacted with anti-synaptophysin antibodies. LOC immunbolabeling was somewhat reduced 30 minutes post-MPTP and substantially reduced 7-days post-MPTP. Consistent with the results of the brainstem lesions, these results suggest the main effect of disrupting LOC innervation of the cochlea is a depression of CAP amplitude. This technique for selectively lesioning descending LOC efferents provides a new opportunity for examining LOC modulation of afferent activity and behavioral measures of perception.

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989 Serotonin Induced Plasma Extravasation in the Murine Eighth Nerve: Possible mechanism of Migraine Associated Vertigo.

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It has been proposed that neurogenic vasodilation and extravasation of plasma from dural blood vessels could be an etiologic factor in migraine. We hypothesize that plasma extravasation in the eighth nerve or inner ear has the potential to contribute to fluctuating hearing loss, tinnitus, hyperacusis and vertigo. Plasma protein extravasation can also be induced experimentally by intravenous administration of vasoactive substances, which directly alter vascular permeability. This study used intravenously injected horseradish peroxidase (HRP) as a tracer for protein extravasation in mice. HRP (100 mg HRP diluted in 1 ml of 0.9% saline solution, injected 10 ml/ kg) was delivered via a tail vein followed by a 10^{-7} M/l of 5-HT injection (10 ml/kg) (N=5) or 0.9% saline solution (10 ml/kg) (N=5) 15 minutes later. Forty-five minutes later, mice were euthanized and perfused transcardially. Heads were decalcified in 10% formic acid, infiltrated with OCT compound and cryostat sections were mounted on slides. HRP was visualized immunhistochemically. The distribution of HRP in the tissue was scored quantitatively, ranging from no staining (-) in structures such as brain parenchyme to dense staining (++) of dura, external canal skin and tympanic mucosa. Regardless of the experimental groups, the immunoreactivity of stria vascularis was ranged from + to ++. Hair cells, spiral ganglion cells and Scarpa¡¯s ganglion cells were mostly non-reactive in both groups. However, the staining pattern in the eighth nerve differed between two groups. Non-neuronal elements in the peripheral portion of the eighth nerve were densely HRP-positive (+ to ++) in 5-HT injected rats, but showed little reactivity in control mice. The central portion of the nerve was HRP-negative. This results raise the hypothesis that plasma protein extravasation in the eighth nerve may be an etiologic factor of migraine associated vertigo.

990 Simultaneous Electrocochleography And Cochlear Blood Flow Measurements During Cochlear Hypoxia In Rabbits

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The aim of the study was to evaluate the pattern of cochlear blood flow (CBF) and electrocochleography (ECochG) during transient ischemia of the cochlea. A newly designed probe was used for the simultaneous recordings of laser Doppler CBF and ECochG directly from the round window (RW). The probe enabled the recording of high amplitude compound action potentials (CAP) and cochlear microphonics (CM) with few averages. Experiments were conducted on 5 young albino rabbits. Three consecutive 1-minute followed by a 3-minute compressions of the internal auditory artery (IAA) were conducted to generate episodes of cochlear ischemia. A 10 to 15 minute recovery period was provided between compressions. CBF and ECochG were recorded continuously throughout the procedure. Click stimuli of 50 dB nHL rarefaction and condensation at a rate of 19.3/sec were used. Sixteen sweeps were averaged for each epoch. Successful recordings from 7 ears were analyzed. CAP and CM were extracted by addition and subtraction of condensation and rarefaction recordings, respectively. Latencies and amplitudes of the N1 component of the CAP were evaluated.

Both CBF values and CAP amplitudes showed an overshoot following the release of each compression. The compressions caused amplitude, latency and morphological changes in CAP. The CAP waveform changes were observed to follow the changes in the CBF level very rapidly. CAP amplitude measures were found to be more sensitive to hypoxic changes compared to auditory brainstem responses as observed in previous experiments. CAP needed less averaging and less recording time on each epoch, thus increasing the resolution of the recordings. Although the absolute CM amplitude changes were found to be smaller compared to CAP, CM changes provided additional information which may be a valuable monitoring tool.

991 Protective Effect of MK 801 on Hypoxia-induced Hair Cell Loss

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Hypoxia is a pathogenetic factor in various inner ear diseases, and increasing importance is attached to the pharmacological protection of the cochlea from traumatic influences. Electrophysiological and binding studies have shown that MK 801 is able to block the NMDA receptor channel and may protect the cochlea against excitotoxicity.

Glutamate, a major endogenous excitatory amino acid, is involved in the toxicity in the mammalian cochlea under several pathological conditions. Elevated glutamate contributes to tissue damage by activating calcium-permeable NMDA receptor channels. Thus NMDA antagonists may have an otoneuroprotective effect under hypoxic conditions.

The aim of this study was to evaluate if MK 801 has a protective effect on hypoxia-induced hair cell loss using an in vitro model of the new-born rat cochlea. Organ-typical cochlea cultures were exposed to hypoxia (pO2 = 10-20 mm Hg at 37°C) in normal DMEM medium or in DMEM medium containing MK 801 (10 µmol/l) for 36 h. The cultures were phalloidin-labelled and the number of outer and inner hair cells (OHC/IHC) was counted. The hair cell damage was characterised by missing IHC and OHC.

The mean damage in normoxic controls was 1 - 4%. IHCs revealed a significantly higher susceptibility to hypoxia than OHCs. 36-hour exposure to hypoxia caused a mean loss of about 25% OHC and 60% IHC. In the group treated with 10 µm MK 801, the damage was significantly reduced to about 10% in OHC and 35% in IHC (ANOVA: $P<0.001$), indicating a similar pharmacological effect of MK 801 on hypoxia-induced hearing loss in the in vitro model used in this study.

992 Effects of Vasoactive Drugs on Cochlear Blood Flow After Transient Cochlear Ischemia

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The aim of this study was to evaluate the pattern of cochlear blood flow (CBF) during transitional ischemia of the cochlea in non-pretreated ears and in ears pretreated by papaverine, sodium nitroprusside (SN) and mannitol.

Methods: Young rabbits were used for this study. Reversible ischemic episodes within the cochlea were induced by directly compressing the internal auditory artery (IAA). Cochlear blood flow (CBF), cochlear blood volume (CBVol), and cochlear blood velocity (CBVel) were measured using a laser-Doppler (LD) probe positioned at the round window (RW) niche. In test ears, prior to the IAA compressions, saline (control), mannitol, or SN were administered topically at the RW for 20-30 minutes while papaverine was delivered directly to the IAA and cochleovestibular nerve complex. Each ear underwent 1-, 3- or 5-min IAA compressions.

Results: In all ears, it was observed that a rapid reduction ranging from 50 to 75% of baseline in CBF, CBVol, and CBVel followed each IAA compression. In all cases, an overshoot of CBF reaching 10% to 120% over baseline (BL) value after 30 to 90 sec after IAA releasing was observed. CBFVel appeared to contribute most to the overshoot observed in CBF. Overshoot was greater for all three measures with longer durations of IAA compression. In the pretreated ears, all drugs changed the CBF, CBVol, and CBVel recovery patterns. Overshoot was enhanced 30-50% with papaverine, 5-12% with SN, and 10-25% in saline (control) treated ears. In mannitol treated ears, the overshoot was enhanced 90-120% after 5-min compressions, similar to that seen in saline treated ears. Conclusions: Duration of IAA compression was found to be an important factor influencing the recovery pattern and overshoot of CBF, CBVol, and CBVel. SN and papaverine significantly changed the overshoot seen after release of IAA compression. LD-CBF was found to be a sensitive method for monitoring the cochlear blood deficit and its recovery post IAA release.

993 Interaction Between Dopaminergic and Glutamatergic Transmission in Isolated Guinea Pig Cochlea

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The damage of the afferent nerve due to ischemia or acoustic trauma is caused by the extreme glutamate (Glu) release from the IHCs. The lateral olivocochlear efferent (LOC) fibers making synaptic contact with afferent dendrites and releasing dopamine (DA) has a protective role against Glu-excitotoxicity in case of ischemia and acoustic trauma.

We used in vitro microvolume superfusion to measure DA release from isolated guinea pig cochlea. We found that both the resting and the evoked release of DA significantly increased in response to veratridine, which causes pathologically high sodium influx and mimics the cellular effects of ischemia. This effect was insensitive to ionotropic Glu-receptor antagonists suggesting the lack of effect of Glu- on DA-ergic terminals.

To explore the interaction between DA and Glu we set up a new method, combining the in vitro microvolume superfusion with HPLC to simultaneously measure the release of DA and Glu. The release of both transmitters increased in response to electrical field stimulation that could be blocked by tetrodotoxin, proving the neuronal origin of the transmitters. DA receptor antagonists and agonists did not change significantly the release of Glu suggesting the lack of presynaptic regulation of Glu release from IHCs. Interestingly, both agents increased the outflow of DA.

With respect to the well-known ototoxicity of aminoglycosides, we also studied their effect on DA release from LOC fibers. We found that neomycin decreased the electrically evoked release of DA that may represents a putative site of action of aminoglycosides. Gentamicin and kanamycin had no significant effect on the release of DA. In experiments using cochleae were from animals chronically treated with neomycin, neomycin failed to change the release of DA. It is most likely due to an adaptive mechanism.

994 Upregulation of Glial Cell Line-derived Neurotrophic Factor in the Rat Cochlea Following Kainic Acid Excitotoxicity

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Many studies have showed that an excessive amount of glutamate or glutamate analogs are excitotoxic to the type I ganglion neurons in the cochlea. Application of kainic acid (KA) to the cochlea immediately causes a significant decrease of the compound action potentials (CAP).

The interesting thing is that this damage recovers in a week. The recovery phenomenon is attributed to regrowth of damaged postsynaptic dendrites and reformation of the synapse between inner hair cell and dendrites of afferent nerves. There may be neurotrophic actions associated with repairing cochlear damage. The present study was designed to determine if the rat cochlea damaged by KA could recover and, if yes, to examine changes in GDNF mRNA in the rat cochlea following KA excitotoxicity. KA (20mM) applied on the round window membrane in rats caused a rapid reduction in CAP. Seven days after KA treatment, however, CAP had recovered to their normal value. To detect changes in GDNF mRNA in a rat cochlea, we used quantitative RT-PCR analysis. A recombinant RNA-standard was synthesized and used as internal RT-PCR standard. After KA treatment, GDNF mRNA levels were up-regulated. The result suggests that GDNF may be involved in repairing cochlear damage after KA treatment.

995 Threshold Shift Induced by Paraquat-generated Superoxide Radicals

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Reactive oxygen species (ROS) play a significant role in the molecular processes underlying noise-induced hair cell death and hearing loss (NIHL). Included among the ROS thought to be involved in NIHL is superoxide (O_2^-). While O_2^- is not highly reactive on its own, it can be readily converted to the dangerous hydroxyl radical (OH^-). Thus, the question remains, does exposure to superoxide by itself represent a significant threat to cells and tissue? Previous studies (Nicotera et al., 2002) have shown that an O_2^--generator, paraquat, creates significant HC lesions when applied to the round window (Nicotera et al., 2002). The next question concerns what pattern of hearing loss could be expected from paraquat lesions in the cochlea. Specifically, does paraquat exposure lead to any temporary threshold shift (TTS), or is the threshold shift merely an accumulation of permanent threshold shift (PTS)? Also, does paraquat only cause a basal lesion, or is the effect found throughout the cochlea?

To answer these questions, 12 animals were implanted with bilateral inferior colliculus (IC) electrodes. Following testing of hearing from 500-8000 Hz, each ear was exposed, via round window drug application, to one of four concentrations of paraquat: 10 mM, 5 mM, 3 mM, and control PBS solution. Animals were tested at: 90 minutes, 24 hours, 48 hours, 72 hours, 7 days, 14 days, 22 days. TTS and PTS were calculated. Threshold shift peaked between Day 3 and Day 7, depending on the concentration. All of the paraquat groups showed recovery from peak threshold shift, suggesting some form of TTS. Significant threshold shift was found at all five frequencies tested, suggesting that the drug did reach the apical end of the cochlea following round window application. We are currently exploring a number of hypotheses to explain the appearance of TTS following the paraquat exposure.

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996 Paraquat-induced Hair Cell Damage and Protective Effect of M40403

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Previous studies suggest that aminoglycoside-induced hair cell damage results from the generation of reactive oxygen species such as the superoxide radical. Support for this hypothesis comes from studies showing that animals overexpressing superoxide dismutase (SOD) or animals treated with compounds that scavenge superoxide show significantly less hair cell damage from aminoglycoside treatment, and cochlear cultures treated with M40403, a highly specific, nonpeptidyl SOD mimetic, show significant protection from gentamicin. To further characterize the role of superoxide in cochlear damage and the protective effect of compounds that inactivate it, we treated mouse cochlear organotypic cultures with paraquat, an herbicide that produces high levels of superoxide by redox cycling with oxygen and cellular diaphorases. Cultures were prepared from C57BL/10J mice at PND2. One day later, the cultures were treated for 24 h with paraquat alone (0.01-10 mM) or paraquat (0.01-1 mM) in combination with 10 μM M40403. The number of IHCs and OHCs systematically decreased with increasing concentration of paraquat, with complete loss of hair cells at concentrations of 5, 7.5 and 10 mM. Hair cell numbers were significantly higher in M40403-treated cultures than in cultures treated with paraquat alone, but lower compared to control cultures (no paraquat)--i.e., M40403 provided significant, but incomplete, protection. Given that paraquat predominantly generates superoxide and M40403 specifically targets the superoxide radical, we conclude that endogenous superoxide concentration is critical in mediating cytotoxic responses in cochlear hair cells. The incomplete protection afforded by M40403 points to additional factors, such as dose and/or rate-limiting effects of superoxide dismutation, in paraquat cytotoxicity.

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997 ATP Acts As An Intercellular Signalling Molecule After Laser Lesioning Of Cultured Chick Utricular Epithelia

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Little is known about the physiology of vestibular support cells and in particular how these cells respond to hair cell damage. We have shown that laser lesioning of utricular epithelia generates intercellular calcium (Ca_{inter}) waves that spread from the lesion site (Gale & Warchol, 2001 ARO abstract). ATP is known to act as an extracellular signalling molecule involved in the generation of Ca_{inter} waves (Haydon, Nature Neur Rev 2:185, 2001). We laser lesioned chick utricular epithelia and measured *intra*cellular calcium $[Ca^{2+}]_i$ with Fura2. The spread of the Ca_{inter} wave was reduced by apyrase (20U/ml), which rapidly degrades ATP, indicating the involvement of ATP and thus P2 receptors in the $[Ca^{2+}]_i$ increases. The Ca_{inter} wave was almost completely blocked by thapsigargin (1 μM) showing that release of calcium from intracellular stores is required.

Local perfusion of either ATP or UTP to undamaged cultures activated increases in $[Ca^{2+}]_i$ in support cells. Dose response curves showed that the rank order for agonist potency was UTP>ATP>>UDP~ADP. The EC50s for UTP and ATP were 0.5 ± 0.3 and 13.1 ± 2.5 μM respectively (mean ± sd, n >= 3 for each dose). Again, these responses were blocked by 1 μM thapsigargin. Application of UTP and ATP together at submaximal doses did not result in a greater increase in $[Ca^{2+}]_i$ than application of a single nucleotide, suggesting that the nucleotides act via the same receptor(s). The P2 receptor antagonist PPADS (10 μM) reduced the UTP-activated $[Ca^{2+}]_i$ rise by 75%. The receptor profile we describe indicates that that utricular support cells express $P2Y_4$ and/or $P2Y_6$ receptors. The data also suggest that damage-induced release of extracellular nucleotides can convey a localised signal to vestibular support cells.

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998 The Effect of Heptanol on Lateral Wall Fibrocytes in the Gerbil

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Spiral ligament (SL) fibrocytes are extensively coupled via gap junctions, and this network is thought to play an important role in cochlear K^+ homeostasis. Heptanol, an aliphatic alcohol, acts as a blocker of gap junctions. In this study, 100% heptanol was applied acutely to the round window (RW) of the gerbil for 2 min, then washed out. The gerbils were allowed to recover for short periods of 12 or 24 hrs or for longer periods of 1, 2, or 4 weeks. After short recovery periods, endocochlear potentials (EPs) were markedly reduced (7-18 mV), compound action potential (CAP) thresholds were highly elevated, and only small residual otoacoustic emissions (DPOAEs) were present. Conversely, significant recovery of cochlear function, especially of the EP, was found with the longer recovery periods.

Histopathologic studies 24 hrs after treatment showed a significant reduction in the immunostaining intensity for connexins 26 and 30 and the Na-K-Cl cotransporter among type I, II, and IV fibrocytes. Moreover, nuclear fragmentation indicative of apoptosis was observed in most type II and IV fibrocytes after short recovery periods, especially in the upper basal turn. Some loss of outer hair cells and supporting cells was apparent in the lower basal turn and hook regions, but apoptotic bodies were not observed in these cell types. No evidence of apoptosis was seen in other cell types of cochlea. These results suggest that: 1) 100% heptanol applied acutely to RW of the gerbil selectively induces apoptosis in SL fibrocytes; and 2) loss or dysfunction of lateral wall fibrocytes may be associated with dramatic reductions of EP thereby affecting auditory function.

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999 Mild Carbon Monoxide Exposure Impairs the Developing Auditory System of the Rat.

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The objective of the study was to determine if chronic exposure to mild concentrations of carbon monoxide (CO) caused changes in auditory function during synaptogenesis/auditory development. We exposed rat pups chronically to CO concentrations, 0, 12.5, 25, and 50 ppm in air starting at day 8 through 22 days of age. By using gastrostomy-reared animals, we were able to compare mother-reared pups with the gastrostomy-reared pups, with or without CO exposure. The CO exposed animals had reduced amplitudes of the eighth cranial nerve's action potential upon examination of the auditory brain-stem response. We examined the central auditory regions for basal neuronal activity, using c-Fos immunoreactivity as a marker. In the central nucleus of the inferior colliculus (CIC), the basal c-Fos immunoreactive cells were significantly decreased in the CO exposed animals when compared to controls; however, there was no difference in the number of c-Fos expressing cells in the external nucleus of the inferior colliculus in exposed and control animals. The dorsal cortex of the inferior colliculus at 25 and 50 ppm CO exposure, but not at 12.5 ppm, had decreased c-Fos expression.

Analysis of the cochlea from CO exposed animals at 25 ppm revealed neurofilament expression was decreased in the neurons of the spiral ganglion. In addition, histochemical analysis revealed reductions of NADPH diaphorase, cytochrome oxidase and calcium ATPase. Only mild atrophy of the myelin surrounding the central process of these neurons was observed. Analysis with anti-myelin basic protein antibody corroborated these findings. No changes were observed in the morphology of the neurons in the spiral ganglion and in the inner and outer hair cells. Synaptophysin immunoreactivity was also normal in the hair cells. We conclude that the cochlea and CIC are affected by mild CO exposure during development. The deficits in the CIC appear to be permanent as they persist into adulthood.

1000 Deterioration of Outer Hair Cells in Reperfusion Injury of the Cochlea

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Characteristics of electrophysiological and structural changes of the cochlea occurring during the reperfusion stage after ischemia have not been fully understood. In the present study, we demonstrated that outer hair cells (OHCs) are vulnerable to reperfusion injury and that hydroxyl radical contributes to the OHC injury. Transient ischemia of the cochlea was induced in albino guinea pigs by pressing the labyrinthine artery. Effects of cochlear reperfusion on cochlear potentials (endocochlear potential (EP), compound action potential (CAP) and cochlear microphonics (CM)) and structural changes in hair cells were examined. Although EP exhibited an almost steady value after 20 min reperfusion, CM amplitude tended to progressively decrease during the reperfusion period in the animals subjected to 45 or 60 min ischemia. OHCs were swollen and exhibited alterations of the nucleus at the termination of ischemia. Severer structural deterioration of OHCs was induced by 4 h reperfusion than ischemia itself when the ischemic period was 45 or 60 min. Perilymphatic perfusion of dimethylthiourea, a hydroxyl radical scavenger, partially ameliorated the elevation of the CM pseudo-thresholds and the structural changes of OHCs. These results indicate that cochlear reperfusion induces functional and structural deterioration of outer hair cells probably by hydroxyl radical generation.

1001 3D-Reconstruction Of The Vestibular Endorgans In Pediatric Temporal Bones

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Little is known about disorders of the peripheral vestibular end organs in children. Because of its bony protection, direct examination is not yet feasible without permanent damage of the inner ear. Therefore the final diagnosis can only be made by post mortal histological examination.

The temporal bones of a child (age: 17 hours) without peripheral vestibular pathology and two children with known peripheral vestibular pathology were prepared by the celloidin technique and sectioned in 20µm. Every 5th section was digitized with a Nikon slide scanner. The imaging data was layered anatomically correct and exported into AVS-Express 6.0. The reconstructed 3D models were used for measurements.

The angle between the utricular and saccular macula of the normal vestibular endorgans ranged between 52.1-127.0°. The saccular macula had a length of 2.26mm and a width of 1.63mm. The utricular macula had a length of 2.59mm and a width of 2.09mm. Both maculae were curved in longitudinal and transverse axis. The pathological models differed significantly from all measured distances and angles.

The 3D-reconstruction of the normal pediatric temporal bone revealed similar findings as Takagi and Sando [1] and Naganuma et al. [3] reported previously. We found that the angles between the semicircular canals were not perpendicular as Curthoys et al. had suggested [2]. The findings of the abnormal peripheral vestibular endorgans, such as a complete aplasia of the lateral semicircular canal suggest vestibular deficits.

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1002 Mouse Cochlea Database: The Digital Cytocochleogram

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The Mouse Cochlea Database (MCD) continues to be developed and contains four resources that provide a comprehensive collection of information on the anatomy of the mouse cochlea. The purpose of this communication is to report on the development of one of the MCD resources: the digital cytocochleogram. A cytocochleogram is a graphical representation of the anatomical state of the hair cells along the complete width and length of the organ of Corti. It is usually produced from whole-mount, surface-preparations of the organ of Corti and the condition of each hair cell is assessed microscopically. The digital cytocochleogram consists of light microscopic, digital images of the reticular lamina showing the complete length of the organ of Corti in a CBA mouse. Each digital image was calibrated for microscope magnification and reticular lamina structures were mapped to distance along the length of the basilar membrane. This was accomplished using an arc approximation method using the pillar cells as a reference point. Distance along the basilar membrane was calculated from a montage of individual reticular lamina images. Software tools including Javascript and PHP were developed to allow users with web browsers to extract morphometric information from the images. Users can view and measure anatomical structures of the reticular lamina in real-time, and know the location of a structure as a function of distance along the length of the basilar membrane. A graphical representation of the condition of each hair cell of this cochlea is also provided in the MCD. The MCD digital cytocochleogram thus provides a microscopic view of the complete reticular lamina of a normal mouse cochlea that can be morphometrically analyzed in real-time. Now that these tools have been developed, experimentally altered and mutant mouse cochleas can also be posted and analyzed on the MCD website in the future.

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1003 3-D-Reconstruction of the Human Middle and Inner Ear

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For several reasons it is useful to have a 3-D- model of the ear.

We did a reconstruction of the human ear by using already digitized images of the temporal bone. The preparation was sectioned at 25- 50 µm and pictures were taken. For the image segmentation we worked with a software (Amira 2.3) on a common PC. The originally colored pictures were handled as grey level image stack. To better detect relevant structures the images were cropped and segmentation thresholds were frequently changed. Various bio-materials like bone, ligament, muscle and membrane as well as air and fluid (lymph) were chosen to display the topology as desired. To conceive a 3-D-structure, we processed the picked areas with Generalized Marching Cubes and accomplished tetra- and hexahedrons with special tools to further analyze the data thus obtained.

Interest was first directed towards the middle ear containing the tympanic membrane and the ossicular chain with special focus on the stapes footplate, the oval window and its junction to the inner ear, our second point of interest. Until now, these both parts of the ear had mostly been treated separately. In particular, we put emphasis on the fluid filled scalae, semicircular channels, the vestibule, the ductus reuniens, endo- and perilymphaticus just as the saccus endo-lymphaticus. On basis of the pictures we were not able to differentiate between the endo- and perilymphatic space but we can give a certain predication on the allocation of fluid. The exact geometric reconstruction is essential for a finite element analysis in order to better understand the signal processing of the ear (Boehnke et al., ARO 2003).

The gain of these accurate anatomic results is grave to optimize the stapes- protheses and give recommendations to the surgeon in common.

1004 Three-Dimensional Imaging of the Inner Ear, Using Interactive Virtual Reality Techniques.

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In 1999, we presented 3-D images derived from the National Library of Medicine (NLM) Visible Female data set. In 3-D we displayed the skin, skull, brain, cerebellum, eyes, extraocular muscles, and the vestibular portion of the inner ear. Since then, we have been working to define inner ear structures in more detail, including the semicircular canals, vestibule, cochlea, and middle ear structures.

Due to its small size, in order to visualize the detailed middle and inner ear, we scanned intact "fresh" isolated petrous bones with high resolution, Micro-CT, and 3- and 7-Tesla Magnetic Resonance Imagers. The images consisted of 3D arrays of cubic voxels ranging in size from 20 to 300 micrometers on a side. These images were then analyzed using a comprehensive software system called AnalyzeAVW, developed in the Biomedical Imaging Resource at the Mayo Clinic, Rochester, Minnesota. The segmented images were then used to reconstruct realistic 3-D models that facilitate interactive viewing and detailed quantitative analysis of these structures and their relationships. Using virtual reality techniques, endoscopic viewing will be demonstrated inside the various structures imaged. We are able to display debris in a semicircular canal, fly above and below the basilar membrane in the cochlea and view other portions of the inner ear. These techniques may prove useful in developing a virtual temporal bone library, surgical planning, diagnosis, and cochlear implant or other prosthetic device design.

1005 Tectorin Mutant Mice: Three Mutations, Three Phenotypes

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Alpha and beta-tectorin are major components of the non-collagenous, striated-sheet matrix of the tectorial membrane (TM). Alpha-tectorin contains an entactin G1-like domain, vWF type D repeats and a zona pellucida (ZP) domain. Beta-tectorin has a single ZP domain. The two tectorins are thought to interact via their ZP domains to form the striated-sheet matrix of the TM. Transgenic mice with different mutations in the tectorin genes have strikingly different phenotypes. A targeted deletion in the entactin G1-like domain of alpha-tectorin results in a TM that is completely detached from the surface of the cochlear epithelium, lacks all known non-collagenous components, and only contains randomly organized collagen filaments (Legan et al., Neuron 28:273-285, 2000). In mice with a null mutation in beta-tectorin, the TM contains alpha-tectorin and remains attached to the cochlear epithelium, but completely lacks striated-sheet matrix and has aberrantly organized collagen fibril bundles. In mice with a missense mutation (Y1870C) in the ZP domain of alpha-tectorin, the limbal zone of the TM is considerably reduced in thickness, striated sheet matrix is missing from the region of the TM overlying the internal sulcus, and the

collagen fibrils in this region of the TM are disorganised. These results indicate that interactions between alpha and beta-tectorin are essential for formation of the striated sheet matrix of the TM, that beta-tectorin is unlikely to mediate attachment of the TM to the epithelial surface, and that the organisation of collagen fibril bundles within the tectorial membrane depends upon the integrity of the non-collagenous, striated-sheet matrix.

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1006 Visualizing the Distribution of Terminal Sugars on Complex Carbohydrates in the Guinea Pig Cochlea Using Fluorescently-Conjugated Lectins

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The surfaces of all cells are coated with complex carbohydrates, in the form of glycoproteins and glycolipids, whose terminal sugar moieties are important for specificity of cell adhesion and cell communication. Little is known about the distribution of these sugars in the cochlea. Lectins are plant-derived proteins that specifically bind to individual terminal sugars. The epithelia lining the scala media within the guinea pig cochlea are polarized, with an apical surface exposed to the endolymph, while the baso-lateral membranes are exposed to perilymph. Our aim was to assess the distribution of lectin binding in the organ of Corti and stria vascularis, and specifically investigate whether lectins display epithelial polarity on cells enclosing the endolymphatic space of the scala media.

We used fluorescently-conjugated wheat germ agglutinin (WGA), concanavalin A (Con A), dolichos biflorus agglutinin (DBA), and peanut agglutinin (PNA) and confocal microscopy to survey the distribution of four different terminal sugars in fixed, permeabilized organ of Corti and stria vascularis preparations from adult, pigmented guinea pigs. The preparations were co-labeled with phalloidin to visualize the cytoarchitecture of F-actin in lectin-labeled cells.

WGA and DBA bound both the stereocilia and baso-lateral walls of hair cells. Polarity was found in PNA labeling, with PNA bound to the apical region of the lateral wall of outer hair cells and the basal half of Dieters' cell phalanges. Con A only weakly labeled the stereocilia of hair cells.

In the stria vascularis, WGA, DBA, and PNA bound the marginal cell region, with PNA labeling polarized to the endolymphatic surface. WGA and Con A labeled the cell borders of marginal cells in the region of the junctional complexes. The patterns of WGA and Con A are consistent with previously published studies. Knowledge of lectin binding in the cochlea can be used to design methods for targeted drug delivery to cochlear cells and to perform micromechanical experiments.

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1007 Distribution of TRPV2 in Rat Inner Ears

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TRPV2 (a.k.a. VRL-1), a newly cloned vanilloid capsaicin receptor homologue, does not respond to capsaicin and is thought to be responsible for transducing high-threshold heat responses (Caterina et al., 1999). However, a recent study indicated that TRPV2 is associated with chemosensory functions in visceral sensory neurons (Ichikawa & Sugimoto, 2002). Although high temperature heat detection is not an expected need for the cochlea, there could be a role for TRPV2 in a response to cochlear fluid chemistry. In the present study, we investigated the immunocytochemical distribution of TRPV2 in rat inner ear to determine whether TRPV2 has the potential to be involved in cochlear function.

TRPV2 expression was observed in both inner and outer hair cells, as well as out pillar cells, inner pillar cells, Hensen's cells, Claudius cells and spiral ganglion neurons of rat inner ear. Positive immunolabeling was located mainly in nuclei of the above-mentioned cells. However, no labeling could be detected in the stereocilia. In addition, TRPV2 immunoreactivity was present in medium and large diameter neurons within the dorsal root ganglion, and nerve fibers on the basilar and anterior inferior cerebellar arteries.

These immunolabeling patterns in the rat cochleae suggest that TRPV2 may be involved in temperature or fluid chemistry sensitivity in the peripheral auditory system.

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1008 Ultrastructure of Inner Hair Cell Innervation in the C57BL/6J Mouse

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The structure of afferent terminals and their synapses has important implications for mechanisms of normal sensory-neural transmission and hearing dysfunction. Bouton size, mitochondrial content and terminal locations on IHCs correlate with spontaneous activity and threshold of corresponding auditory nerve fibers in the cat (Merchan-Perez and Liberman, JCN, 371:208, 1996). For this report, we studied IHC innervation in the mouse. Serial section electron micrographs were taken through the afferent endings of three IHCs in the 8 and 16kHz regions of a 2-month-old C57BL/6J mouse with normal hearing. During 3-dimensional reconstructions of the electron micrographs, two morphologically distinct classes of afferent swellings were observed. "Simple" swellings were more numerous (19.8±5.9/IHC), smaller (1.5±2.2 μm^3), and tended to terminate near the inferior pole of the cell on the surface facing the cochlear base. "Folded" swellings, characterized by a deep invagination of the outer membrane, were less numerous (10.5±1.4/IHC), larger (3.8±3.3 μm^3), and terminated on the IHC in more apical locations. Innervation at 16kHz differed from that at 8kHz by the presence of more simple swellings, smaller folded swellings and more distinct spatial segregation of these ending types. Preliminary observations also suggest that a subgroup of simple swellings containing few mitochondria were clustered on the basal surface of the IHC, suggesting that additional subclasses of terminals can be defined by mitochondrion density. These data reveal that the IHC innervation pattern in young adult C57BL/6J mice is quite complicated. Furthermore, there is still much to be learned about the functional significance of these findings.

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1009 Localization Patterns of Secretoneurin: A Neuropeptide in the Cochlea and Vestibular Endorgans of the Rat

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As a member of a highly-conserved family of proteins collectively called Chromogranins, secretoneurin is a secretogranin-II (154-186) derived polypeptide generated by proteolytic cleavage. The aim of this study was to investigate the presence of secretoneurin within inner ear neurotransmission and the patterns of distribution expressed by this protein in the cochlea and vestibular endorgans of rat. Employing a modified method of pre-embedding for immunoelectron microscopy, our findings revealed that secretoneurin- like immunoreactivity is present in nerve terminals of rat organ of Corti as well as vestibular endorgans. In the organ of Corti (OC), positive reactivity was found most notably in portions of the inner spiral bundle, the tunnel spiral bundle, outer radial fibers, and nerve endings in synaptic contact with outer hair cells. Under immunoelectron microscopy, we observed both stained and unstained fibers within each of these nerve bundle systems. Cochlear sensory hair cells, supporting cells, and inner radial fibers were devoid of staining. Similarly, secretoneurin-like immunoreactive terminals in vestibular endorgans formed en passant contacts with both stained and unstained fibers. The strongest show of positive reactivity was found in bouton-type nerve terminals and fibers within vestibular sensorineural epithelium. Immunostaining was predominant in vesiculated nerve fibers although portions of non-vesiculated nerve fibers and terminals (in direct contact with type II cells) were also seen. Both type I and type II vestibular hair cells as well as afferent chalices were devoid of secretoneurin-like immunoreactivity. These results demonstrate that secretoneurin is present in the organ of Corti and the vestibular endorgans of rat. Secretoneurin appears to be distributed within the efferent as well as sub-populations of the afferent system. The present study implicates an extensive involvement of secretoneurin in the neurotransmission of the inner ear.

1010 Projections of Intrinsic Lateral Olivocochlear Neurons in the Rat: Evidence of a Frequency Mismatch Between Origins and Terminations

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The lateral superior olivary nucleus (LSO) contains intrinsic olivocochlear (OC) neurons that, individually, terminate in focal arborizations beneath the inner hair cells (IHC). Evidence that this projection is topographically, and possibly tonotopically, organized, has been reported in several species. We attempted to describe the relationship between the location of tracer injections in the LSO and the projections of intrinsic neurons beneath the IHC, as assessed by frequency/place maps derived from the literature. Twenty-three anesthetized Sprague-Dawley rats received iontophoretic injections of biotinylated dextran amine (BDA) in the LSO and after 10-12 days, they were perfused with an aldehyde mixture. Frozen sections of the brainstem and the intact or bisected cochlea were processed for the demonstration of BDA by the ABC method. The cochlea was embedded in resin, dissected into quarter turns and examined with DIC optics. Frozen sections were mounted and counterstained. The locus of the injections along the medial-lateral extent of the LSO and the basal-apical locations of the centers of efferent labeling beneath the IHC, both expressed as a percentage of total length, were measured for each animal and plotted. We found that the LSO intrinsic efferent terminations in the cochlea were, on average, displaced by some 11% towards the apex when compared to the frequency/place map of the corresponding LSO injection site. Such a displacement represents slightly less than one octave along most of the rat's cochlea. Recently, M.C. Brown reported a somewhat similar apical displacement between the characteristic frequency of most medial OC neurons and their center of termination beneath outer hair cells, as sampled in the guinea pig (Brown, MC, ARO Abstr. 25, 81, 2002). Such displacements of both lateral and medial OC terminations are not predicted by current hypotheses concerning efferent function and merit further study.

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1011 Time Course of Olivocochlear Efferent Fiber Degeneration Following a Single High Dose of Gentamicin and Ethacrynic Acid in the Chinchilla

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In the chinchilla, concurrent administration of a large dose of gentamicin (GM; 125 mg/kg IM) and ethacrynic acid (EA; 40 mg/kg IV) results in complete destruction of cochlear hair cells and spiral ganglion neurons (SGNs). Hair cell loss occurs within days after injection, whereas degeneration of SGNs occurs over a time period of several months (Ding et al., ARO Abstr. 140, 2002). The fate of olivocochlear efferent fibers following GM/EA injection is currently unknown. In this study, we counted acetylcholine esterase-positive tunnel-crossing fibers in cochleas of animals sacrificed at 1, 2, 3, or 4 weeks after GM/EA injection, after all cochlear hair cells had been destroyed. The results show a progressive base-to-apex gradient of efferent fiber loss between 1 and 4 weeks post GM/EA. At one week post GM/EA, approximately 50% of the tunnel-crossing fibers were already missing. The loss progressed to 68% at 2 weeks, 84% at 3 weeks, and 94% at 4 weeks. We are currently assessing efferent fiber loss at earlier and later time points to fully characterize the time course of efferent fiber degeneration after cochlear hair cell loss, and to determine the relationship between efferent and afferent fiber degeneration.

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1012 Proliferation of Supporting Cells in Mouse Cochlear Organotypic Cultures

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Tritiated thymidine has been used to trace the birth of hair cells, supporting cells and neurons in the mouse inner ear in vivo. The production of hair cells, pillar cells, Deiter's cells, Hensen cells, Claudius cells reaches a peak at approximately E14, rapidly declines within 2-4 days so that few new cells are born in the organ of Corti beyond E20-P1. Consequently, the loss of hair cells and spiral ganglion neurons after birth results in permanent hearing loss. During the past few years, we have prepared organotypic cultures of the cochlea from P0-P12 mice and have observed a substantial expansion of the outer sulcus region that we originally attributed to an increase in cell size rather than cell division. To evaluate this issue in more detail, we prepared cochlear organotypic cultures that included cells from the spiral ganglion to the Hensen/Claudius cell boundary; care was taken to exclude the spiral ligament and stria vascularis. P0-P12 cochlear tissues were attached to the surface of rat-tail collagen and cultured in serum free medium for 1, 3, 6, or 10 days. To determine if the expansion of

the outer sulcus was due to cell division, bromodeoxyuridine (BrdU) was added to the culture medium for various time periods. Specimens were stained with rhodamine-labeled phalloidin, and DNA replication was evaluated with the bromodeoxyuridine (BrdU) immunohistochemistry. No evidence of cell proliferation was seen inner and outer hair cells, pillar cells, Hensen's cells, Deiter's cells, cells of the inner sulcus and limbus or the spiral ganglion. However, large numbers of BrdU-labeled cells were seen in the outer sulcus region normally occupied by Claudius and Hensen cells. Cell proliferation was maximal 1-3 days in culture and slowly decreased thereafter. Additional immunolabeling studies are underway to clarify the identity of the proliferating cells in the outer sulcus.

1013 Postnatal Maturation of Cochlear Structure in Mustached Bats

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Exceptionally sharp tuning of the mustached bat cochlea to 61 kHz, the dominant constant frequency component of the echolocation call depends on the concerted action of specialized passive cochlear mechanics and active cochlear amplification. Previous studies showed that during the first 4-6 weeks of postnatal development, the dominant echolocation frequency and the corresponding cochlear resonance shift upward from about 48 kHz to 61 kHz and tuning sharpness increases.

The present investigations of cochlear maturation with light and transmission electron microscopical techniques during this critical period show: 1. Morphology of the organ of Corti in neonates closely corresponds to the stage shortly after onset of hearing in other mammals. 2. At birth, the foveal region of the cochlea has established the specialized basoapical gradients in morphology of the basilar membrane and the tectorial membrane as well as the characteristic regional variation in gross innervation patterns. 3. An efferent innervation of the outer hair cells is already found in neonates. 4. The basilar membrane is loaded with a thick tympanic cover layer at birth which is progressively reduced with age. 5. The cytoskeleton of Deiters cells and pillar cells matures during the first two postnatal weeks.

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1014 Members of the Cadherin Family of Cell Adhesion Molecules are Expressed in Restricted Domains of the Developing Inner Ear in the Chicken

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The inner ear, including the vestibular system, develops from the otic vesicle. Although the structure and function of the mature organ is well studied, relatively little is known about the early embryonic mechanisms and genetic factors, which regulate pattern formation in the otic vesicle and morphogenesis of the inner ear. Here, we study the spatiotemporal expression of several classic cadherins (L-CAM, B-cadherin, R-cadherin, N-cadherin, cadherin-6B and cadherin-7) in the developing inner ear of the chicken from embryonic day 2 (E2) to E12 by immunostaining. Cadherins are cell adhesion molecules that regulate morphogenesis in many organs and tissues. Our results show that each cadherin is expressed in restricted regions of the developing inner ear. The expression pattern for each cadherin is distinct, although there is partial overlap between cadherins. In the otic vesicle, some cadherins are expressed in domains that relate to early pattern formation. At intermediate stages of development, the expression of each cadherin is more clearly related to histogenetic fields that give rise to parts of the vestibular organ and organ of Corti (e.g., sensory epithelia and adjacent regions, semicircular canals, tectorial membrane, etc.). These results suggest that cadherins play a role in the regionalization and morphogenesis of the inner ear, as has been shown also for the CNS.

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1015 Variation in Hair Cell Bundle Characteristics in the Saccule and Lagena of Macrouridae Deep-Sea Fishes

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The family Macrouridae (grenadiers and rat-tails) is one of the most successful groups of deep-sea fishes. They live between 1000 and 5000 meters, depths at which there is little or no light, food is scarce, and there are considerable distances between individuals. Thus, it is likely that macrourids may have auditory specializations to find food, avoid predators, and find mates. These species have well developed inner ears with large saccules and complex saccular hair cell bundle orientation patterns. The lagenae in macrourids have a highly dentate-shaped otolith and large epithelial regions only covered by a gelatinous otolith membrane.

We analyzed the characteristics of the sensory epithelia and ciliary bundles in the saccules and lagenae of five species from three macrourid genera. The shape of the saccule varies among these three genera, as well as between species within a single genus. Ciliary bundle types vary little within each saccule. In contrast, the lagenae not only have variations in overall shapes between species, but they also show extraordinary diversity in ciliary bundle types and sizes within each lagenar macula. In addition, there are substantial inter-specific differences in the distribution of different ciliary bundle types in the lagena.

The variation in hair cell bundles shapes and sizes implies that hair cells in different regions of a macula may respond differently to the same acoustic signal, or that they may respond to different signals. In deep-sea fishes, the inter-specific differences, particularly in the lagena, may be adaptations to specific ecological niches in which the different species live.

1016 Are there Structural Variations in the Ears of Two Deep-Sea Eels that Inhabit Different Depths?

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Due to the absence of light, deep sea fishes must have adaptations in non-visual sensory systems to enable them to do normal activities such as finding food and mates. Indeed, it appears that the ears and lateral lines of many species may be adapted for this special environment. There is evidence that some sound-producing deep-sea species have elongated stereocilia and enlarged saccules. However, little is known about how the inner ear may vary with depth. Two species of deep-sea eels, *Synaphobranchus kapuii* and *S. bathybius*, inhabit different depths. While *S. bathybius* has been found between 731 and 4,855 meters, *S. kapuii* is found between 236 and 3,200 meters. This difference in depth range makes these two species ideal for this project.

Light microscopy and SEM were used to compare gross morphology as well as ultrastructural details and determine hair cell orientation of the sensory epithelia of the two species. These data were correlated with estimated age of the specimen, behavior, and depth range of the species to isolate factors that might signify variation due to depth.

The ears of *S. kapuii* and *S. bathybius* are encased in an unusually robust cranium. Initial results indicate that *S. kapuii* has an unusually

delicate lagenar otolith, a characteristic of deep-sea species. The lagena macula itself is shaped like a teardrop with the pointed end curving dorsally while the saccule macula is shaped like an elongated oval with a slight pinch just rostral of the dorsal-ventral midline of the macula. There are some differences between the ears *S. bathybius* and *S. kapuii* and other deep-sea species, but some general trends in deep-sea structure, particularly the intricate lagenar otolith, are observed in these species.

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1017 Tissue Engineering Implications of Collagen Composition in Human Cartilage Subtypes

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There are numerous potential applications for tissue-engineered cartilage in head and neck reconstruction. These applications include laryngotracheal reconstruction, microtia repair, auricular reconstruction, and repair of septal defects. In order to characterize the differentiation of tissue-engineered constructs, a basic understanding of the collagen composition of different human cartilage subtypes is required.

Cartilage tissue can be divided into three general classes or types: hyaline, elastic, and fibrocartilage. In order to more precisely characterize these subtypes, immunohistochemistry with antibodies to elastin and to collagen types I, II, V, VI, X was performed. In addition, routine staining with toluidine blue, Safranin O, and Verhoeff's stain (for elastin) was performed. Cartilage subtypes analyzed include articular, costal, auricular, and nasoseptal cartilage.

Immunohistochemical staining showed a characteristic pattern of antibody staining for each of the cartilage subtypes, which varied by both position and intensity. Observed differences included positive type X collagen immunostaining of cells in costal and auricular cartilage, but absence of cellular immunostaining in nasoseptal and articular cartilage. Pericellular immunostaining was very intense with all cartilages for collagen type V and VI, with the similar immunostaining of all cartilage subtypes. Only two markers (collagen type X and elastin) are necessary to distinguish between these four cartilage types.

This study provides a method by which the unique collagen composition of different cartilage subtypes in humans can be documented. This method can be applied in evaluating tissue-engineered cartilage in vitro or for post-implantation analysis.

1018 Cochlear Epithelial and Neuroblast Cell Lines from the Ventral Otocyst of the Immortomouse.

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Epithelial cells from the ventral region of the mouse otocyst at embryonic day 10.5 (E10.5) differentiate into many cell types. These include non-sensory epithelial cells, supporting cells, hair cells and neurons of the cochlea. To study the differentiation of these cell types in vitro, we established cell lines from the ventral otocyst (VOT) of the Immortomouse at E10.5. Cells derived from this animal are conditionally immortal. They proliferate if cultured at 33^{o} C in the presence of gamma-interferon and resume partial differentiation at 39^{o} C in the absence of gamma-interferon.

The transcription factor GATA3 is expressed at high levels in the ventral otic epithelium at E10.5 and in cochlear but not vestibular neuroblasts. Cells were removed, cloned by limiting dilution and selected for expression of GATA3. Two cell lines, US/VOT36 and US/VOT33 were subsequently selected with reference to their morphology, expression of the epithelial marker cytokeratin and the neural marker tubulin beta-III. US/VOT33 expressed GATA3, neurogenin1, neuroD, Brn3a and tubulin beta-III, all of which are normally expressed by developing cochlear neurons. US/VOT36 expressed GATA3, cytokeratin, myosin VIIa, Pax2 and Math1, all of which are expressed within the cochlear epithelium. The profiles were not exclusive because VOT33 expressed Brn3c and VOT36 expressed neurogenin1. However, the 2 cell lines executed inherently different developmental programs under differentiating conditions. We have shown that they are amenable to gene 'knock-down' experiments with antisense Morpholinos, allowing us to identify the downstream targets of selected transcription factors against different developmental backgrounds.

1019 Ectopic Embryonic Stem Cells Can Contribute to Otic Development in Vivo

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The long-term goal of this research is to devise a means of enhancing inner ear function when either hair (sensory) cells or nerve cells do not develop correctly or are lost to injury, disease or ageing. We also wish to enhance the success of cochlear implants. We have implanted mouse embryonic stem cells (ES cells) into the developing inner ears of embryonic chicks and are beginning similar experiments in embryonic mice in embryo culture, in attempts to develop models of stem cell therapy. It is our particular choice of stem cell that is important in these experiments. We are transplanting otocyst derived cell lines from the Immortomouse (pluripotent cells derived from embryonic day 9) that may serve either a sensory cell or neuronal cell function. In ES cell experiments, we employ a novel ES cell paradigm: mouse ES cells that produce neurons efficiently because the neural apoptosis gene, neurofibromatosis 1 has been knocked out by homologous recombination (Jacks et al, 1991). We have implanted wild type (D3) ES cells, ES cells that have one affected NF1 allele (SKO=single knockout ES cells) and those that have two affected NF1 alleles (DKO, double knockout ES cells). All of these ES cell lines can integrate into the otic epithelium. However, DKO cells are capable of becoming neuron-like in the absence of nerve growth factors and can contribute to the statoacoustic ganglion in the developing chick. The ES cells also respond to a statoacoustic neurite outgrowth factor made by the otocyst or an Immortomouse inner ear cell line by becoming neuron-like in culture.

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1020 Neural Stem Cell Transdifferentiation into Cochlear Cell Types

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There is a growing body of evidence that point to the potential therapeutic applications of stem cells in diseases of the central nervous system, heart disease, and diabetes. The foundation of this work lies in the observation that stem cells are pluripotent and are able to differentiate into a variety of cell types depending on local environmental cues. We set out to determine if stem cells obtained from the brain had the ability to differentiate into cochlear cell types. To test this, we injected C17.2 neural stem cells (which stably express [beta]-galactosidase and GFP) into the round window of adult anesthetized FVB mice. We found that the C17.2 neural stem cells survived within the cochlear capsule for the duration of our experiments (4 weeks) and had migrated extensively throughout the cochlear duct. After 1 week,

the spiral ganglion contained the highest concentration of stem cells suggesting a preference of the C17.2 neural stem cells for neural tissue . Immunohistochemistry revealed the upregulation of several spiral ganglion structural proteins including NF200, MAP-2, and [beta]-III tubulin, and co-localization with NT-3. Some of the transplanted C17.2 stem cells upregulated myosin 7a and parvalbumin as well. Next we asked whether the C17.2 neural stem cells would adopt an expression profile that more closely resembles that of the organ of Corti. To test this, we co-cultured C17.2 neural stem cells with OC-1 and OC-2 cochlear cell lines and used immunohistochemistry to identify the presence of cochlear specific proteins within the stem cells. We found that myosin 7a, connexin 26, and parvalbumin were upregulated in the C17.2 neural stem cells after 7 days in co-culture with the cochlear cell lines. Furthermore, many of the C17.2 neural stem cells adopted the distinctive OC-1 and OC-2 phenotypes. These results indicate that neural stem cells possess the ability to transdifferentiate into cochlear cell types both in vitro and in vivo.

1021 Investigation of Neural Stem Cell-Derived Donor Contribution in the Inner Ear

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The utilization of multipotent somatic stem cells is a possible therapeutical strategy for diseases of the inner ear resulting from lack of regeneration. In the current study stem cell-derived donor contribution in the inner ear was investigated. Fetal stem cells from the cortex of BCL2-transgenic, Lac-Z marked mice were isolated at E 14 and cultivated in neurobasal media (hFGF and hEGF) for four weeks. The neural stem cells of male animals were harvested, injected into blastocysts and transferred of foster mothers. The resulting mice were investigated six months post partum.

ABR was preformed in six animals. The cochleas of four female animals were dissected and the DNA of the entire cochlea was analyzed for donor contribution by donor-specific by YMT-PCR.

All animals had normal ABR-thresholds. The 342 bp bands for YMT were detected in the cochlea of three of the four female animals investigated. In two animals the expression was unilateral, in one animal bilateral. The results indicate that neural stem cells possess the ability to form structures of the functional adult inner ear.

1022 Generation of Hair Cell-Like Cells from Inner Ear Stem Cells

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Using protocols for the isolation of adult neuronal stem cells, we were able to isolate from the utricular sensory epithelia of 10 week-old mice cells that are capable of forming spheres. Sphere formation, a feature of adult stem cells, happens when individual stem cells proliferate in low-density single cell culture. Cells derived from these spheres were capable of self-renewal and we were able to expand primary spheres by mechanical dissociation and replating.

Sphere cells displayed expression of the adult stem cell marker nestin as well as a number of inner ear developmental markers including Pax2, BMP4, and BMP7. Following initiation of differentiation of sphere-derived cells, we found upregulation of mRNA of the hair cell markers myosin VIIA, Brn3.1, and espin. The differentiated cell population contained up to 15% hair cell-like cells with F-actin-rich protrusions that were also labeled with antibodies against the hair bundle protein espin. Cells with these hair bundle-like protrusions were additionally immunopositive for the marker protein myosin VIIA.

Our results suggest that the sensory epithelium of the adult mouse utricle contains potentially multipotent stem cells. We have isolated these cells and found that they have the main features of neural or neural-like stem cells, that is, they form spheres, they proliferate, and they express the marker nestin. In vitro differentiation of these cells resulted in induction of hair cell marker genes and the appearance of cyto-morphological specializations indicative of hair cells.

1023 Transplantation of R28 Retinal Progenitor Cells into Spiral Ganglion and Cochlear Cultures

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Neural progenitor cells could conceivably be used to replace damaged spiral ganglion neurons (SGNs) in the inner ear; however, their ability to differentiate into suitable phenotypes depends on the receptors they express. The aim of the present study was to determine if R28 retinal progenitor cells express glutamatergic and GABAergic receptors, similar to those found on SGNs, and to determine if they would survive and differentiate in mouse cochlear cultures. In Experiment 1, we made whole cell recordings from R28 retinal progenitor cells cultured under proliferating conditions. No action potentials or voltage sensitive currents were detected; however, non-NMDA and NMDA receptor agonists induced inward currents. The mean amplitude of the inward current was –105±81 pA for kainic acid (KA, 200 μM), –70±8 pA for AMPA (200 μM), and –43±31 pA for NMDA (1 mM). KA and AMPA induced currents that were suppressed by CNQX and DNQX. GABA (200 mM), muscimol (500 mM) and baclofen (500 mM) induced inward currents with mean amplitudes of -53±28 pA, -40±17 pA and -23±8 pA, respectively. Immunocytochemistry revealed GluR1, GluR2, and GluR3, NMDA and GABAa receptors on R28 cells similar to those on spiral ganglion neurons. In Experiment 2, DiI labeled R28 progenitor cells were co-cultured with SGNs or cochlear cultures. R28 cells developed a fusiform shape and extended processes that overlapped those from SGNs. In normal cochlear cultures, R28 cells preferentially localized to the SGN region and inner sulcus, but were seldom seen in the hair cell region. However, when the hair cells were damaged with gentamicin, R28 cells were found within the organ of Corti. This suggests that damage enhances the entry of R28 cells into the sensory epithelium. These results suggest that R28 progenitor cells may have the potential to differentiate into spiral ganglion-like neurons in vivo, and possibly other cell types.

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1024 Cultures of Inner Ear Tissue Reveal Potential Stem-Cells Only Within 2 Weeks After Birth in NMRI Mice

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I:In the mature mammalian ear hair cell loss due to sound damage or ototoxic drug insult results in hearing and balance impairment. Undifferentiated precursor cells with the potential to replace lost hair cells are apparently missing. We try to establish protocols for the in vitro culture of undifferentiated stem cells derived from the inner ear of mice and to evaluate a possible time window for the presence of these cells.

M&M:The cultures of vestibular organs and cochlea from NMRI mice (1,6,14,21 days old) were stored over night in 0.1M phosphate buffer containing 30% saccharose. The tissue was harvested, transferred into PPD-solution (0.01% Papain,0.1% dispase II,0.01% DNAse I,12.4 mM MgSO4 in HBSS without Mg++/Ca++), digested at room temperature.

The resulting single-cell solution was collected, resuspended in Neurobasal medium/supplemented with B27,2 mM L-glutamine,0.1 g/L penicillin/streptomycin,2 µg/ml heparin,20 ng/ml bFGF-2,20 ng/ml EGF. The medium was replaced once a week. Cells were cultured for up to 3 months, evaluated systematically, documented by video microscopy.

R:Only in preparations from 1 and 6 day old mice we were able to grow spheres (that have previously been described for neural stem cell cultures). After 1 week cultures these cells showed evidence for differentiation in morphologically distinct phenotypes. Some of the differentiated cells had neuronal characteristics while others exhibited features resembling glial cell types. In contrast to these cultures attempts to culture inner ear tissue of older mice failed. Our findings take effect in cultures derived from vestibular organs and the cochlea.

C:We are able to establish spheroid cultures from inner ear tissue that resemble those described for neural stem cells. In addition, the presence of these potential stem cells is limited to the time before the onset of hearing in NMRI mice.

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1025 Expression Profiles of Neural Stem Cells Compared to Cochlear Cells by Gene Chip Analysis and Immunohistochemistry

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Results from experiments in which neural stem cells were transplanted into a deafened cochlea showed that the transplanted stem cells would migrate throughout the cochlea and adopt several phenotypic characteristics. In order to determine if transplanted neural stem cells had transdifferentiated into a cochlear cell or simply maintained a CNS profile, we set out to identify molecular markers that are selectively expressed in the cochlea and not expressed in neural stem cells. We compared the mRNA expression profiles of 96 genes of interest obtained from gene chip analysis of the whole mouse cochlea (Chen & Corey, JARO 3:140, 2002) to that obtained from C17.2 neural stem cells. We found 26 genes that were selectively expressed on the adult cochlea and 5 genes that were specific to the neural stem cells. We then surveyed the expression of these genes by immunohistochemistry and found 12 gene products (including NF200, connexin 26, MAP-2, parvalbumin, GluR2,3, and b-III tubulin) that are specific to the adult cochlea and one (Math1) that is specific to the C17.2 neural stem cells. These results elucidate adequate molecular markers that may be used to confirm the in vivo transdifferentiation of neural derived stem cells into cochlear cell types.

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1026 Myosin VI is an Early Marker For Hair Cells In Vitro

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The cells of the inner ear epithelia are organized into a precise mosaic such that hair cells do not directly contact one another, but are separated by supporting cells. How this precise mosaic forms is not yet known. To begin to investigate inner ear cell patterning, we developed an in vitro assay. With this assay it was found that dissociated, embryonic inner ear cells (days E13-14) reaggregated into three distinct patterns within the first 24 h in culture. Only one of the aggregates types, the "dome" contained both hair cells and supporting cells. Hair cells were identified in dome aggregates after several days in culture using various hair cell markers. However, due to the time it took for the hair cell markers to be expressed in the cells, it was not possible to determine whether hair cells differentiated then migrated into dome aggregates, or whether undifferentiated hair cells clustered in the aggregates prior to differentiation. In order to address these possibilities, hair cell markers that are expressed earlier in development are needed.

In the present study an antibody to myosin VI was used to test for earlier labeling of hair cells. In contrast to other hair cell markers, this antibody labeled E14 hair cells as early as 3 h after plating. This method will now allow us to monitor hair cell location throughout the aggregation process and help determine whether hair cell migration is an important mechanism for hair cell patterning in vitro.

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1027 Developmental Expression of Nestin in the Mouse Inner Ear: Implications for Repair

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The goal of the present study was to determine the presence of Stem/progenitor cells in the mouse inner ear. We used the nestin promoter-GFP (green fluorescent protein) transgenic mice (1). We examined the inner ear of mice at P0, P3, P5, P7, P10 and 10 weeks of age. To assess the phenotype of GFP expressing cells in the sensory epithelia anti-calmodulin antibodies and phaloidin were used to identify hair cells and their stereocilia. The whole endorgans or cryosections were analyzed under epi-fluorescent or confocal microscope. GFP expressing cells were found from P0 to P7. Hair cells of the macula utricle displayed GFP in their cytoplasm while the hair cells of the crista ampullaris did not show any GFP. Cells within the stromal tissue underneath the sensory epithelia of the crista and utricle were also GFP positive. The cochlea had scattered GFP+ cells within the spiral ganglia, but not the spiral ganglia neurons. In the organ of Corti GFP signal was only in supporting cells that surround the inner hair cells (GFP-negative). However, outer hair cells were mild GFP+. The inner ear of 10 weeks old animals contained moderate GFP staining in the stroma of the crista ampullaris and utricle but not within in the sensory epithelia or the cochlea. The present data indicate that the expression of nestin in the mouse inner ear is developmentally regulated; yet in the adult inner ear there are some nestin expressing cells indicating an intrinsic repair potential although to a more limited extent than during post-natal life.

1. - Yamaguchi et al Neuroreport 11:1991-1996, 2000.

1028 Multipotent Differentiability of Adult Mammalian Cochlear Cells

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Unlike those in lower vertebrates, mammalian cochlear hair cells and supporting cells are incapable of regenerating normally following degeneration or loss. Loss of auditory hair cells represents the major cause of deafness. We previously reported that the cochlear cells dissected from the adult guinea pigs can grow to form specific floating cell spherical colonies, cochsphere, implying that stem progenitor cells may exist in culture. In this experiment, we continuously tested the proliferative and differentiative capacities of the cell ball to ascertain stem progenitor cells. The cochlear cells were micro-dissected from the adult guinea pig's inner ears and cultivated naturally in vitro without genetic immortalization. The formed cell balls were isolated to test the differentiative capacity individually. The new-formed cochsphere was usually lack of specific cell markers, negative to cytokeratin and nestin. The expression of $p27^{Kip1}$, a cyclin-dependent kinase inhibitor for cell cycle progression, was also low and undetectable. However, with supplement of serum and epidermal growth factor (EGF), or in long-term culture, multiple differentiation and asymmetric cell division could be found and visible in the sphere or the dispersed cells. The differentiated cells included the cells expressed specific hair cell markers, Brn3.1 and Myosin VII. A few cells showed a positive reaction to a outer hair cell marker, prestin. The cochlear supporting cell

markers, cytokeratin and Cx26/30, were also found in some differentiated cells. In addition, non-cochlear cell phenotypes, which were positively reactive to neuronal cell markers, Nerofilament, β-III Tubulin and Vimentin, and a glial cell marker, GFAP, could also find in culture. The data demonstrated that stem progenitor cells may exist in the adult mammalian cochlea; the cochlear hair cells may be able to regenerate through the stem cell differentiation.

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1029 Proliferative Generation of Mammalian Auditory Hair Cells in Culture

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Hair cell (HC) and supporting cell (SC) productions are completed during early embryonic development of the mammalian cochlea. However, increasing evidence suggests that new HCs can be generated in mammalian sensory epithelium. Retinoic acid or growth factors were shown to trigger production of supernumerary HCs in embryonic and neonatal sensory epithelia in vitro. To obtain a production of new HCs in a neonatal sensory epithelium, immature cells must be present, eventually proliferate and retain the potentiality to undergo straightforward differentiation into HCs. This study shows that acutely dissociated cells from the newborn rat organ of Corti, developed into so-called otospheres consisting of 98% nestin (+) cells when plated on a non-adherent substratum in the presence of either Epidermal Growth Factor (EGF) or Fibroblast Growth Factor (FGF2). Within cultured otospheres, nestin (+) cells were shown to express EGFR and FGFR2 and rapidly give rise to newly formed myosin VIIA (+) HCs and p27KIP1 (+) SCs. Myosin VIIA (+) HCs had incorporated bromodeoxyuridine (BrdU) demonstrating that they were generated by a mitotic process. Ultrastructural studies confirmed that HCs had differentiated within the otosphere, as defined by the presence of both cuticular plates and stereocilia.

The demonstration that cultured immature nestin (+) cells present in the newborn rat organ of Corti can proliferate and subsequently differentiate into HCs and SCs together with the detection of nestin (+) cells in vivo at the spiral limbus in the P15 mature organ of Corti open new prospects with regard to the regeneration/repair of the injured organ of Corti.

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1030 The Ability for Differentiation of Neural Stem Cells in the Inner Ear of Mice

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Recent studies have demonstrated that neural stem (NS) cells differentiate into several types of neurons and glial cells after transplantation into brain and retina, which have brought great expectation that severe damage of nervous system can be repaired using stem cells. To evaluate the potential of neural stem cell transplantation into the inner ear, we examined the fate of NS cells for survival and differentiation after transplantation into the inner ear. We induced the injury of inner ear epithelia in 36 adult female ICR mice by injecting a neomycin solution into the posterior semicircular canal. NS cell spheres were derived from the embryos of transgenic mice, of which cells express enhanced green fluorescence protein (GFP), and transplanted into the injured inner ear. Grafted NS cells were identified as lining along the membranes of the perilymphatic space of cochlea, the labyrinth and the surface of vestibular epithelia at 7, 14, 25, 42 days after transplantation. Although most of grafted cells differentiated into glial or neural cells, immunohistochemistry for myosinVIIa, a specific marker for inner ear sensory cells, demonstrated myosinVIIa-positive NS-derived cells in vestibular epithelia. These cells were localized in the sensory layer of the vestibular epithelia and some of them had a stereocilia-like form on the luminal surface. This indicated that NS-derived cells are incorporated into sensory epithelia and that some of them become characteristic of inner ear sensory cells. These findings suggest that NS cells can be used for transplantation approaches to inner ear dysfunction caused by the loss of sensory hair cells in the future.

1031 Transplantation of Autologous Mesenchymal Stem Cells into the Cochlea of the Chinchilla

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The purpose of this study was to investigate whether autologous mesenchymal stem cells (MSC), which are easily obtained from one's own bone marrow and can differentiate into neurons, survive in the cochlea and could be used in inner-ear cell therapy. Since the efficacy of adult stem cell engraftment increases in damaged organs, we used gentamicin-treated chinchillas that undergo severe damages in multiple regions in the inner ear. At 4 weeks after gentamicin treatment of the animal, the cultured autologous MSCs were labeled with DiI and injected into the cochlea. Animals were sacrificed for histological examination at 3 weeks after MSC injection. We confirmed survival of the injected cells in the scala vestibuli and tympani, the lateral wall of the cochlea, and the modiolus. The cells in the scala vestibuli appeared pyramidal or polygonal, while those in the spiral ligament appeared elongated and spindle shaped. The labeled cells in the spiral ligament seemed to have migrated to this region from the scala vestibuli. The shape of the transplanted cells appeared to be dependent on the regions in which they settled, which suggests that MSCs transplanted in the cochlea may have been influenced by the micro-environmental conditions to which they are subjected. Some cells that survived in the spiral ganglion and in the cochlear nerve were NF-200 positive, which indicates that some MSCs transplanted in the cochlea may differentiate into neurons. The present results provide rationale for further investigation of MSC transplantation for potential use in cell and gene therapy of various inner ear diseases.

1032 Transplantation Approaches for Inner Ear Diseases

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Our final goal is to establish a novel therapeutic strategy for inner ear diseases. Recently, the efficacy of transplantation approaches has been reported in a variety of diseases that were considered incurable. In this paper, we examined the potential of transplantation approaches for inner ear diseases. The targets for transplantation approaches included sensory hair cells (HCs), spiral ganglions (SGs) and spiral ligaments (SLs), in which degeneration is considered a major cause of inner ear dysfunction. The aim of cell transplantation was protection and regeneration of these cells. Mice and chinchillas were used as experimental animals. Neural stem (NS) cells and mesenchymal stem (MS) cells were used as donors. Cell transplantation into the inner ear was performed through the round window, cochlear lateral wall, or semicircular canal. These donor cells survived in various regions of the inner ear including the modiolus, Rosenthal canal, perilymphatic space,

sensory epithelia and SL. Transplant-derived cells differentiated into various types of cells including glial cells, neuronal cells and HC-marker-positive cells, indicating that cell transplantation may be utilized for regeneration of HCs, SGs and SLs. In addition, most transplanted NS cells exhibited expression of neurotrophic factors, which are known to have the efficacy for protection of HCs and SGs from ototoxic insults. This indicates that transplantation approach can be utilized for protection of HCs and SGs from cell death. Consequently, transplantation approaches have the potential for treatment of inner ear diseases.

1033 A Potential Approach to Establish Mammalian Cochlear Cell Lines that can Produce Cilia

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To date, no ideal mammalian cochlear cell lines that can produce cilia have been established. However, an approach that we used on airway ciliary cells raises great hope that a viable approach may be forthcoming. As it is critical to find an appropriate way to this end, approaches in the literature were screened and one was selected that might transform the cells yet also maintain normal cellular features. Additionally, because of inherent technical difficulties in manipulating the cochlear cells, airway cells were selected to test the efficacy of the selected approach. Similar to cochlear cells, airway cells can produce cilia. Cilia in airway and in cochlea are both highly differentiated structures made by cytoskeleton, though they differ in their architecture. Here, we report how the selected approach was applied on airway cells and show that the cilia and other features appeared in the airway cell line. For this purpose, the E1A gene was induced into the animal airway cells. The cells immortalized in vitro by E1A were separated by G418 and subcloned. Then, the cloned cells were grown in an extracellular matrix material, and a three-dimensional cell aggregate was soon observed. In these aggregates, cilia were readily detected and grew toward the luminal side of the aggregate. Some cells generated multiple cilia. In addition, other structures such as tight junctions were also seen around the apex of these cells. Further, to verify that the clone was immortalized by E1A gene but not by other factors, we performed a series of tests such as immunostaining, southern blotting, and hybridization. These tests confirmed that the gene had been transferred successfully into the cell line. Thus, under experimental conditions we were able to generate an epithelial cell line with phenotypic expression similar to the cell of origin. Success in airway cells and the refined methods of application lead us to the next step: replicating this phenomenon in the cochlear cells.

1034 Survival and Distribution of Adult-Derived Stem Cells Transplanted into the Adult Mouse Inner Ear

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Hearing loss and vestibular dysfunction can result from disease, noise, toxins and aging. In many cases, inner ear dysfunction results from damage to sensory cells, but unfortunately, there is only a limited ability for hair cell regeneration in mammals. Additionally, other cells in the inner ear are often affected, and in some cases may be the primary cause of the resultant pathology. The ability of stem cells to differentiate into various cell types coupled with their propensity to integrate most readily into regions of tissue damage, suggests that stem cells may be uniquely suited to the challenges associated with damage to the inner ear. We are investigating the use of adult bone marrow derived murine stem cells to treat hearing and vestibular disorders. These stem cells, termed multipotent adult progenitor cells (MAPCs), are capable of differentiating into derivatives of all 3 germ layers in vitro and in vivo, similar to ES cells (Jiang et al, 2002). As such, the MAPCs are ideal candidates for stem cell repair of the damaged inner ear. We are undertaking studies of MAPC transplantation into the inner ear. Several questions are being addressed. Do MAPCs survive within the adult inner ear and can they integrate within the sensory epithelia layer? Does the site of transplantation affect MAPC survival and distribution? Finally, do MAPCs differentiate into inner ear specific cell types and can they affect changes in auditory and/or vestibular function? Our first preliminary results show that MAPCs transplanted into adult mice survive for one week and do integrate into inner ear tissues.

1035 Growth Factors Alter the Survival and Differentiation of Neurons Cultured from the Mouse Cochlear Nucleus

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This study was designed to determine the effects of brain derived neurotrophic factor (BDNF), glial derived neurotrophic factor (GDNF) and neurotrophin-3 (NT-3) on the survival and differentiation of cochlear nucleus (CN) neurons grown in primary culture.

CN cultures were prepared from postnatal day 4 mice, and grown in defined, serum free media (Neurobasal plus B27) supplemented with varying concentrations of each growth factor. CN cells were grown for 4 days in vitro before being processed using microtubule-associated protein 2 (MAP2) and glial fibrillary protein (GFAP) immunohistochemistry.

At physiologically relevant concentrations, BDNF and GDNF significantly increased the survival of CN neurons grown in primary culture. There was no apparent effect of NT-3 on overall neuronal survival, although CN neurons were more likely to be found in clusters in NT-3 cultures. This clustering was related to the presence of astrocytes, which were increased in number in the cultures treated with NT-3. There were no obvious differences in the size and overall morphology of CN neurons among the control and growth factor treated cultures. It can be inferred from these data that the trophic effects produced by BDNF and GDNF on CN neurons differ from those of NT-3.

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1036 Receptors to Bioactive Agents Identified on Retinal Progenitor Cells

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Neural progenitor cells provide a promising method to replace damaged neurons and even highly differentiated cells, such as hair cells, in the cochleae of mammals. The potential for a progenitor cell to develop a functional neuronal phenotype is associated with the expression of various neurotransmitter receptors. Our previous study has demonstrated that R28 retinal progenitor cells express glutamatergic and GABAergic receptors. In this experiment, we went on to further evaluate the existence of receptors to four types of bioactive agents: glycine, 5-HT, acetylcholine (ACh), and ATP. The later two receptors are of particular interest because they are expressed in the inner ear. It was found that no action potentials or voltage sensitive Na+ and K+ currents could be induced on any R28 cells. However, a small but significant inward current was induced in a portion of cells by glycine (14 out of 48), ACh (10 out of 38), and ATP (16 out of 50), but not by 5-HT. The mean amplitude of the inward current was –31.9±18 pA for glycine (1 mM) positive cells, –11.2±7.7 pA for ACh (1 mM), and –16±19 pA for ATP (1 mM). Immunocytochemistry has revealed subpopulations of R28 cells immunoreactive for Ach, glycine, serotonin

and dopamine receptors. Additional studies regarding the morphological identification of ATP receptors are underway.

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1037 Neurotrophin Effects on Spiral Ganglion Neurons From Different Regions of the Cochlea

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Neonatal cochlear hair cells express both brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), while spiral ganglion neurons express the neurotrophin receptors trkB and trkC throughout the cochlea (Ylikoski et al.,1993). However, null mutations suggest that there may be subpopulations of neurons that have different Trk signaling requirements for their survival (Minichiello, 1995). In addition, studies suggest there are regional differences in trk activity along the length of the cochlea in promoting spiral ganglia survival into adulthood (Fritzsch et al., 1998). To determine whether the response to neurotrophic factors is similar at different locations along the length of the cochlea during a period of terminal innervation of the organ of Corti, we cultured neonatal, rat spiral ganglion explants from the apical, middle and basal turns with 10 ng/ml BDNF or NT-3. Neurite number and length were evaluated. Results were compared to explants cultured in media without neurotrophic factor support. BDNF had a minimal effect on the length of SG neurites, but produced a dramatic increase in neurite number compared to control cultures. The increase in neurite number appeared to represent increased survival of SG neurons rather than branching. There was no difference in the responses of neurons from difference cochlear turns. NT-3 exhibited a modest effect on both neurite length and number. The results suggest that BDNF is a more potent regulator of neonatal SG neuron survival than NT-3, in all cochlear turns. Moreover, the signaling that links neurotrophin/receptor interaction to cellular responses is similar in different cochlear regions.

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1038 Interaction of Neurotrophin-3 (NT-3) and Fibroblast Growth Factor (FGF-2) in the Cochlea and Cochlear Nucleus (CN) of the Mouse Embryo.

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Various studies have demonstrated NT-3 expression in the inner ear and hindbrain of postnatal animals. Here we show the expression of NT-3 in the embryonic mouse cochlea and CN from E11, E13, E15, E19 and P1. We used a highly specific antibody for NT-3 (by Western blot). Expression of NT-3 in E11 and E12 embryos was sparse in the otic epithelium (OE) and not detected in the rhombic lip and adjacent CN anlage (CNA). By E13, OE and migratory neuroblasts were stained; in the ganglion and CNA fibers and some cells stained. At E15 in the basal turn immature hair cells and ganglion cells, and CN cells, were well stained. By E19, OE and ganglion staining in all turns was faint but little remained in CN. In sum, NT-3 expression was transitory in the OE, spiral ganglion, and CN early in their formation but declined by birth.

To test the hypothesis that the transitory expression of NT-3 could affect development, we used cocultures of OE and CNA from E11 mouse embryos, treated with FGF-2 , NT-3, or a combination. Control cultures were fed with defined medium. Migration and neurite outgrowth were measured on days 2 (2DIV) and 4 by time-lapse imaging and were immunostained for NT-3 at 7DIV. FGF-2 enhanced OE migration more than NT-3. NT-3 had a stronger effect on neurite outgrowth in the CNA than in the ganglion. NT-3+FGF-2 produced the most robust effects, including apparent contacts between ganglionic and CNA neurons. NT-3 expression was greatly upregulated by NT-3 treatment. Neuronal development in the cultures compared with E15/E18 in situ and the period of transitory NT-3 expression in the same cell populations.

The results support the hypothesis that NT-3 plays an important role in differentiation and neurite outgrowth of both cochlea and CN and may interact with FGF-2.

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1039 Differential Gene Expression Between the Sensory Epithelia of the Chick Cochlea and Utricle

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The avian utricle produces hair cells on an ongoing basis, but hair cell production in the avian cochlea only occurs as a consequence of damage. We used two custom cDNA microarrays to profile gene expression differences between these sensory organs. The first array was comprised of ~400 genes that are known to be expressed in the inner ear. The second array included all known (~1600) human transcription factor (TF) genes. We employed multiple comparative hybridizations and statistical tools to derive a robust set of observations. Twelve genes from the first cDNA array were differentially expressed in the utricle and cochlea. Genes for BMP4, GATA3, MMP18, MYC, EPHA2, & GELSOLIN were expressed at higher levels in the cochlea, while SMAD2, KIT, MMP14, SPARC, MEF2C, & β-AMYLOID were present at higher levels in the utricle. More than 80 TFs were differentially expressed in the utricle and cochlea. Of these, LOC51637, HMG20B, and CRIP2 were upregulated in the utricle, while GATA3, ARNTL, FOXF1, and PRDM7 were higher in the cochlea. Several of the TF changes appear to fall into the TGFβ/activin signaling pathway, while others may be part of the c-KIT pathway. Most TF changes did not fall into known networks. Interestingly, 28 TFs map to chromosomal intervals that harbor uncloned deafness loci. We independently validated many changes by quantitative PCR on additional utricle and cochlea samples. In all cases these agreed with the changes detected by arrays. We also conducted mRNA in situ hybridizations to determine spatial expression pattern for many of these genes. Differential patterns of expression were observed for BMP4, GATA3, GELSOLIN and KIT, as well as for several new TFs.

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1040 Using Microarrays to Identify Target Genes of the Pou4f3 Transcription Factor in the Embryonic Mouse Inner Ear

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The POU4F3 transcription factor is a hair cell specific protein. Previous results have suggested that expression is first detected at mouse embryonic day E12.5 and is confined to post-mitotic hair cells. POU4F3 is required for normal hearing, as demonstrated by studies of a family with progressive hearing loss (DFNA15) associated with a dominant mutation of Pou4f3, and the dreidel and Pou4f3 knock out mice. Analysis of the development of the inner ear of knock out mice demonstrated that Pou4f3 is not required for the determination of hair cell fate. However, in the absence of Pou4f3, all hair cells initiate an apoptotic pathway that ultimately results in a complete loss of hair cells in all inner ear sensory patches in the adult mouse.

To study the role of Pou4f3 in hair cell development, we sought to identify its downstream target genes by comparing gene expression using total RNA extracted from inner ears of E16.5 dreidel homozygotes and wild type mice. RNA from each genotype was processed and hybridized to Affymetrix Murine Genome U74Av2 Arrays. Microarray sensitivity and reliability was evaluated by comparing our data to inner ear expression databases available online. Microarray results were studied using a 'present-absent' breakdown, as well as a combined T-test and fold-of-change analysis. To validate the results obtained by microarray, developmental quantitative expression profiles of Pou4f3, myosin VI, myosin VIIa and some putative Pou4f3 target genes were performed on cochlear sensory epithelium RNA.

1041 Characterization of Homeobox Genes in the Developing Cochlea of Rats

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The homeobox genes play an important role in the organogenesis and pattern formation from *Drosophila* to man during embryogenesis. Very little is known about the expression and role of the homeobox genes in the inner ear, especially in the cochlear hair cells. In this study, we first sought to profile the expression of the homeobox genes in the developing ear of rats from embryonic day 12 (E12) to postnatal day 1 (P1) using Affymetrix microarrays in combination with reverse transcription-polymerase chain reaction (RT-PCR). We then studied the role of some representative homeobox genes in the proliferation and differentiation of a progenitor hair cell line (OC1) that is derived from the organ of Corti of postnatal rats at P5. The cochlear tissue from E12, E14, E16, E18 and P1 was dissected under an operating microscope. Total RNA was isolated and Affymetrix microarrays were performed according to the manufacturer's manual, in triplicate. It was found that at least Hox 1.11, rHox, pem, msx-1, msx-2, Dlx 3, Nkx 2.5 and homeo A were highly expressed as judged by Affymetrix microarrays and their expression in OC1 was confirmed by RT-PCR. The expression of some homeobox genes (Hox1.11, rHox, Msx1, Msx2, Dlx3, Pem, Nkx) in other tissue of the body (brain, liver, lung heart, kidney, thymus, intestine, muscle, skin, spleen) was noted whereas the expression of Homeo A was limited to the cochlear tissue or progenitor hair cell line. Next, we investigated the role of representative Hox1.11 and rHox homeobox genes in OC1. OC1 was starved for 24 hours, incubated with short interfering RNA (siRNA) and antisense oligos for another 24 hours, and radiolabeled with ^{3}H-thymidine for additional 24 hours. Cells were then harvested for cell counts and quantitation of ^{3}H-thymidine incorporation by a scintillation counter and presented as cpm per 10^{4} cells. Cells with empty vector transduction or sense oligos served as controls. It was found that inhibition of either Hox1.11 or rHox genes with antisense oligos or siRNA significantly reduced DNA synthesis and cell counts. The data suggest that both Hox1.11 and rHox play a role in the proliferation of cochlear hair cells.

1042 Analysis of Receptor-Like Protein Tyrosine Phosphatase Expression in the Mouse Inner Ear

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The receptor-like tyrosine phosphatase (RPTP) family comprises six different classes of transmembrane receptors with protein phosphotyrosyl dephosphorylating activity. The supporting-cell antigen (SCA) is a Type III RPTP that is expressed on the apical surface of supporting cells in the avian inner ear and is down-regulated in response to hair-cell loss (Kruger et al., J Neurosci 19:4815-27, 1999). It is not yet known whether mammalian supporting cells express a similar receptor, nor how such a receptor, if expressed, responds to hair-cell loss. The closest mammalian homologue (50% identity at the amino acid level) is Density Enhanced Phosphatase (DEP, also known as RPTPeta, Byp, CD148, PTPRJ), a Type III RPTP that is up-regulated prior to the density-dependent inhibition of cell proliferation in vitro. A total of 17 RPTPs have been identified thus far in the human genome (Schindelholz et al., Development 128:4371-82, 2001) and we have identified the same number in the current mouse genome database. Microarray studies (Chen and Corey, JARO 3:140-8, 2002) have identified 10 RPTPs that are expressed in the mouse inner ear. RT-PCR using specific primer pairs, or a specific primer paired with a degenerate primer designed to the conserved RPTP catalytic site, detects the expression of all 17 known RPTPs in the cochlea and utricle of the mouse inner ear at P3. One of the three Type III RPTPs expressed in these organs is PTPRQ (the hair-cell antigen, HCA), a protein expressed specifically on the apical surface of hair cells. The remaining two Type III RPTPs, DEP (PTPRJ) and glomerular epithelial protein phosphatase (GLEPP, PTPRO), are therefore the most likely candidates for a mammalian homologue of the SCA.

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1043 Wnt Expression in the Developing Embryonic Mouse Inner Ear

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Epithelial-mesenchymal tissue interactions are vital to chondrogenesis in a number of mammalian organ systems, including the limb and inner ear. Intercellular signaling molecules such as transforming growth factor β1 (TGFβ-1), fibroblast growth factor (FGF), and sonic hedgehog (Shh) play significant roles in mediating these epithelial-mesenchymal interactions in the developing cartilaginous capsule of the inner ear. Wnt, a family of secreted glycoprotein signaling molecules, has been shown to regulate limb chondrogenesis in developing mouse and chick embryos. Consequently, we sought to determine if the pattern of Wnt 5a and 10b expression was consistent with a similar role in the regulation of chondrogenesis in the developing mouse inner ear. Sections of embryonic mouse inner ear at day E10.5, E12, E13, and E14 were immunostained using antibodies to Wnt 5a and Wnt 10b to define the pattern of Wnt 5a and 10b protein expression. Additionally, RT-PCR was used to confirm the presence of Wnt 5a and 10b gene expression. Our immunohistochemical results demonstrate that, in the E13 inner ear, Wnt 5a is preferentially expressed in otic epithelial tissue, whereas Wnt 10b is localized to the periotic mesenchyme. Results of RT-PCR indicate that Wnt 5a and 10b gene expression occur in both the epithelial and mesenchymal tissue of the embryonic mouse inner ear. Our findings support a role for Wnt signaling in mediation of otic epithelial-periotic mesenchymal interactions in the developing mouse inner ear.

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1044 Expression of Rab Proteins in the Developing Inner Ear

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The Rab family of Ras-related GTPases plays an essential role in the regulation of intracellular membrane traffic. Although a total of 32 Rab proteins has been identified thus far, specific functions of individual Rab proteins are yet to be determined. We have begun elucidating key molecules involved in lysosomal membrane transport/fusion, since we found that aminoglycoside antibiotics are transported into the lysosome of the hair cell after endocytotic uptake. To identify Rab proteins that are involved in the intracellular transport of aminoglycosides in the hair cell, we evaluated the expression of Rab3a, Rab5, Rab7, Rab11 and Rab27 proteins in the inner ear using western blot analysis and compared their expression levels to those in other tissues. We also sought to elucidate developmental changes in their expression level, because immature hair cells are resistant to aminoglycoside toxicity due

to a lack of cellular accumulation of aminoglycosides. All of the five Rab proteins examined were present in the inner ear as well as other tissues, including the brain, eye, intestine and kidney. Rab3a was predominantly expressed in the brain, whereas Rab3a levels were much lower in the ear and kidney. The same trend was observed for Rab5a. Rab7 was enriched in the brain, ear and kidney in contrast to low levels of expression in the eye and intestine. The expression of Rab11 was approximately at the same level among all tissues examined. These results indicate that the expression of individual Rab proteins vary among different tissues and suggest a unique and complementary role of each Rab in cellular functions. The high level expression of Rab7 in the inner ear and kidney is interesting, given that Rab7 is implicated specifically in lysosomal membrane traffic. This, along with the susceptibility of the inner ear and kidney to aminoglycoside toxicity, raises the possibility that Rab7 is involved in lysosomal targeting of aminoglycosides in these organs.

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1045 Thyroid Hormone & Ion Channels

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Retardation of the expression of the fast-activating potassium channel IK,f or BK-channel was recently documented to be related with deafness in thyroid hormone receptor beta mutant mice (Ruesch et al., PNAS, 1998). In the developing cochlea various ion channels are expressed in outer and inner hair cells as well as spiral ganglia neurons far beyond, shortly before or during the onset of hearing, dependent on the differentiation stage of the cell. We analysed the expression of different ion channels in hair cells and ganglia neurons and studied a presumptive thyroid hormone dependency in animals in which TH is retracted by goitrogen, by TSH receptor mutation or TR mutations. Data may hint to a new principle by which TH affects phenotypically important genes in the inner ear.

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1046 Cloning and Developmental Expression of Nonmuscle Myosin IIA (MYH9) in the Mammalian Inner Ear

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Mutant alleles of *MYH9* encoding a nonmuscle myosin heavy chain have been linked to nonsyndromic or syndromic forms of autosomal dominant hereditary hearing impairment (HHI). These naturally occurring mutations have identified a critical biological role of this motor protein in the auditory organ as well as other target organs and cells including platelets affected by its dysfunction. Myh9 expression has been described in an adult mouse but critical parameters pertaining to its developmental expression remains to be characterized. The current study describes cloning of the mouse Myh9 cDNA and the temporal onset and spatial distribution of Myh9 expression in the developing and the mature inner ear of the fetus, neonate and the adult mouse. The cloned Myh9 cDNA contained two single base pair differences from the genomic sequence, one of which yields an altered codon. Immunoblot of embryonic (E15.5) and adult tissues from several organs including the cochlea identified a single anti-Myh9-immunoreactive band, mw 250 kDa. In situ expression analysis identified Myh9 expression within the epithelia surrounding the cochlear duct at E13.5 and 15.5. Within the neonate and the adult cochlea, M9 expression is observed predominantly within the cells of the connective tissue of the spiral ligament and in the spiral limbus.. Amongst the embryonic organs that are positive for Myh9 expression, the skin displayed the highest intensity of anti-Myh9 immunoreactivity. However, mutant alleles of MYH9 are not associated with skin disorders. In summary, cloning of Myh9 and assessing its developmental expression in the cochlea represent fundamental initial steps towards assessing the role of Myh9 in hearing and its dysfunction.

1047 Localization and Developmental Expression of the Protein Encoded for by the Usher Syndrome Type III Gene.

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Usher syndrome type III (USH3), a relatively rare and unique clinical subtype of the Usher syndromes, is characterized by late onset progressive sensorineural hearing loss and retinitis pigmentosa, with variable loss of vestibular function. The gene for USH3 was recently identified by our group and others, and shown to encode a novel 232 amino acid protein.

To gain insight into the possible function of this protein, we produced antibodies against a synthetic peptide comprising an antigenic portion of the protein. Reactive antibodies were affinity purified and qualified by western blot of cochlear and retinal extracts. The antibodies were found to react with a protein of the correct molecular size. The reagent was then used to immunolocalize the USH3 gene product in murine cochlear cryosections at various developmental stages. In adult murine cochlea, the USH3 protein localized to the matrix of the limbus, the external sulcus and the region of the spiral ligament corresponding to the location of tension fibroblasts. Addition of the peptide immunogen to the primary antibody inhibited this immunostaining, showing that the antibody was reacting specifically with the peptide epitopes in the tissue sections.

Interestingly, developmental activation in the limbus occurred very early (before P0), whereas expression in the external sulcus was later (around P10), and in the spiral ligament even later (young adulthood). The suggested role for tension fibroblasts is to maintain tension on the basilar membrane, which would be more critical in high frequency sound perception. Given that the sensorineural hearing loss is late onset, high frequency specific, and progressive, the functional role of this protein in the region of the spiral ligament where tension fibroblasts are found warrants a closer look.

1048 Expression of the Hair Cell Markers Espin and Parvalbumin 3 During Mouse Inner Ear Development

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Espin and parvalbumin 3 are two proteins that are relatively specific to hair cells. Espin is localized in hair cell stereocilia and mutations in the espin gene appear to cause hair bundle instability followed by hair cell degeneration as seen in the deaf Jerker mouse. Parvalbumin 3 acts as a mobile Ca2+ buffer present in high concentrations in the cytosol of hair cells and appears to be expressed at an early stage of hair cell development in birds.

Antibodies raised against espin and parvalbumin 3 reveal strong immunoreactivity and this, together with their specificity, make them effective tools in the study of hair cell maturation. However the timing of expression of espin and parvalbumin 3 during mouse inner ear development has not been studied.

To be able to utilize these markers together with already established hair cell markers we have determined their initial occurrence in mouse vestibular and cochlear hair cells. In addition, we compared the temporal expression pattern of espin and parvalbumin 3 in hair cells with other marker genes.

We present a comparative immunohistochemical analysis of serial sections of mice inner ears from the thirteenth day of gestation to

adulthood. The sections were stained with antibodies raised against espin, parvalbumin 3 and other hair cell markers.

1049 Potassium Channel KCNQ4

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Cochlear outer hair cells are responsible for the frequency-resolving capacity of the mammalian inner ear. Electrical stimulation during the hearing process induces rapid length changes of these cells, transduced by the novel motor protein prestin (Zheng et al., 2000, Nature 405). Recently Karkovets et al. 2000, PNAS 97 reported the expression pattern of a novel potassium channel, KCNQ4, in hair cells and neurons of the auditory and vestibular systems, the expression of which is linked to nonsyndromic dominant deafness, DFNA2. In outer hair cells and Type I vestibular hair cells this channel is presumed to be similar to the unusual potassium selective `leak` current, termed IK,N, which is responsible for the repolarisation of the outer hair cells. We noted an alteration of the subcellular distribution of the outer hair cell motor protein prestin coincident to an alteration of the subcellular distribution of KCNQ4 prior to the onset of hearing. As the prestin expression itself as well as its subcellular distribution revealed as being under control of thyroid hormone (TH) (Weber et al Knipper, 2002, PNAS 99), we analysed the effect of TH on KCNQ4 expression and focused also on a particular molecular link between TH and the subcellular redistribution of prestin and KCNQ4. Data towards this aim, indicating a role of TH on KCNQ4 will be presented.

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1050 Postnatal Development of the Murine Utricular Macula

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The purpose of the present study was to quantify the extent of the postnatal changes in the utricular macula of the mouse by implementing stereological methods. The total number of all cell types (hair cell type I, hair cell type II and supporting cells) in the utricular macula was estimated in mice from the following age groups: 1, 2, 4, 8, 16, 32, 64, 128, 256 and 512 days post partum (dpp). The total number of the different cell types was estimated from systematically sampled sections using a physical disector.

The results show a total of approximately 6100 cells in the mouse utricular macula at 1 dpp and this number does not change with age. The composition of the cell population changes as type I and type II hair cells differentiate but is constant from postnatal day 16 and onward. The number of supporting cells is 4000 at 1 dpp, decreasing to about 2600 at 8 dpp and remaining at this level in the following age groups. The number of type II hair cells is 1600 at 1 dpp, increasing to a maximum (2200 cells) at 4 dpp. It then decreases to 1300 cells at 16 dpp and remains at this level. There are approximately 200 type I hair cells at 1 dpp which increases to a total of 2200 type I hair cells at 16 dpp.

In conclusion, the sensory epithelium of the utricular macula does not attain a mature composition of cell types until 16 dpp. The results support the idea that cells recognised as supporting cells differentiate into type I hair cells passing through a stage where they resemble type II hair cells. Finally, there is no change in the total number of cells and no decline in the number of hair cells with normal aging.

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1051 Contribution of Innate Immune Response to Cochlear Inflammation

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Immune responses comprise a rapid, innate response and a slow, adaptive response. The cochlear innate response involves IL-1b expression by spiral ligament fibrocytes. We examined the contribution of systemic stimulation of the innate response to the adaptive response. The cochlea was challenged with the antigen, KLH, in systemically sensitized Swiss mice (n=25) with or without injection of lipopolysaccharide (24 mg LPS, i.p.), a stimulator of innate immunity. To avoid cochlear trauma that also stimulates innate immunity, KLH was injected intrathecally. For comparison KLH was injected intracochlearly through the capsule or through the round window. Identical surgical groups were compared to avoid KLH concentration variation. Controls (n=4) received LPS and no KLH. Animals were sacrificed after 7 days. Cochleas were decalcified, cryosectioned and stained with H&E or immunolabeled with anti-IL-1b. Inflammatory cells and immunolabeled fibrocytes/section were counted.

Mice, systemically sensitized to KLH, react to cochlear KLH challenge with infiltration of inflammatory cells. LPS increased the number of cells in each surgical group ($p<0.001$). The mean number of infiltrated cells following intrathecal antigen injection was 40 +/- 24 increasing to 142 +/- 114 with LPS. When KLH was injected through the capsule the mean number of infiltrated cells was 184 +/- 26 increasing to 834 +/- 381 with LPS. With round window injection the number of infiltrated cells was 110 +/- 27 increasing to 684 +/- 57 with LPS. The percent of fibrocytes expressing IL-1b was also greater in LPS mice.

Innate responses contribute to cochlear immunity. Non-specific stimulation of the systemic innate immune system by LPS augments the cochlear response to specific antigen.

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1052 Inflammatory Signals Increase the Expression of MHC Class II Molecules in the Inner Ear

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There is growing evidence that immune reactions occur in the cochlea where they can function either for protection of delicate sensory structures or as a source of inflammation. Since immune responses are initiating by antigen presentation to T-cells, antigen presenting cells expressing major histocompatibility complex (MHC) class II molecules are mandatory.

Under resting conditions, cells from the cochlea express no MHC class II. However it is demonstrated in this study that exposure to interferon-y (IFN-y) induces an increase in MHC expression in neonatal cochlear cells of mice, in vitro. In addition, MHC class II molecules were localized in the inner ear of adult mice after induction of sterile labyrinthitis. This induction of MHC class II molecules either by IFN-y of inflammation may render cochlear cells competent to initiate and participate in immune reaction and may therefore contribute to both immunoprotective and immunopathological responses of the inner ear.

1053 Effect of TNF on Recruitment of Cochlear Inflammatory Cells

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Cochlear inflammation damages the delicate structures that mediate hearing. Cells participating in the immune response reach the inner ear via the spiral modiolar vein and its collecting venules (SMV). TNF is thought to be important in recruitment and extravasation of leukocytes into tissues; it is expressed early in the cochlear inflammatory response. Recent experiments demonstrate that Etanercept (a TNF receptor blocker) reduces the inflammation as well as the hearing loss associated with experimentally induced labyrinthitis. In order to test its direct effect, recombinant mouse TNF (1.6ug/ml, R&D Systems, Inc.) was infused into the inner ear of 9 female Hartley albino guinea pigs through a cochleostomy for 2 or 4 days using an Alzet mini-osmotic pump at 1ul/hr. Auditory evoked brainstem response thresholds were measured before and after treatment. Control ears received PBS via an osmotic pump (n=4) or had no surgery performed (n=17). Paraffin, H&E stained sections were used to count the number of inflammatory cells/cross-section of the SMV perivascular space in the basal turn (5-17 sections/cochlea). Hearing loss was negligible in both treated and untreated ears. After 2 days, the perivascular space of the SMV in experimental cochleas had a mean (S.D.) of 17 (22) inflammatory cells. After 4 days, there was a mean of 47 (51) ($p<0.001$). Control cochleas contained no infiltrated inflammatory cells in the perivascular space.

In the absence of antigen or infection, TNF in the perilymph has the ability to directly recruit inflammatory cells into the cochlea. As a result, it is likely that the effectiveness of Etanercept is due to its ability to block TNF receptors.

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1054 Intratympanic Administration for Disturbances of Cochlear Blood Flow Due to Acute Otitis Media

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In our previous study to elucidate the influence of otitis media on cochlear blood flow (CBF), CBF of treated ears decreased significantly first day after LPS-inoculation, and this decrease was recovered gradually at the seventh and 14th days. In the present study, we evaluated the effects of intratympanic administration of drugs for disturbances of CBF following otitis media. Those animals inoculated with LPS were divided into three groups, and intratympanic administrations were performed 30 minutes after LPS-inoculation. The first group received dexamethasone, the second group received NOS-inhibitor, and the third group received PBS as a control. Twenty-four hours after LPS-inoculation, CBF of each ear of rats was measured using a probe from the laser-Doppler flowmeter placed over the basal turn of the cochlea. The ratio (R) of the value of CBF in the right ear to that in the left ear was calculated and evaluated. Following these examination, Prostaglandin E1 (PGE1) was topically applied to the RWM of the right ear of each inoculated rat and change (C) of CBF was calculated. Measured cochleas were prepared for histological examination. Average for R in rats received dexamethasone or NOS-inhibitor was significantly higher than that in rats treated with PBS. The average for C after application of PGE1 was higher in rats received dexamethasone or NOS-inhibitor compared to that in rat with PBS. Electron microscopic examination revealed that vacuolous changes in the stria vascularis were less severe in rats treated with dexamethasone or NOS-inhibitor. The results show effects of intratympanic administration of steroid or NOS-inhibitor for disturbances of cochlear blood flow caused by an inflammatory condition of the middle ear.

1055 Pathology of Stereocilia in Aging and Otitis Media

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Changes in inner ear ion homeostasis may result in ultrastructural changes of hair-cell stereocilia, especially stereocilia links. Stereocilia links are directly involved in mechanoelectrical transduction of sound; and integrity of the links is highly susceptible to electrolyte concentrations in the inner ear fluids. Damage or loss of the tip links and side links as well as fusion of OHC stereocilia have been observed after disruption of the ion homeostasis of the inner ear fluids by administration of a high concentration of potassium solution in perilymph or by rupture of Reissner's membrane to produce intermixing of cochlear fluids.

It has been reported that biochemical and structural changes in the cochlear lateral wall, composed of the stria vascularis and the spiral ligament, in aging precede the disturbance of inner ear fluid and ion homeostasis and damage of the sensory epithelium. We observed damage to stereocilia links and fusion of stereocilia in aged animals and animals with otitis media induced by Eustachian tube obstruction. These changes in stereocilia were similar to those changes observed after perilymphatic perfusion of potassium ions or after intermixing of cochlear fluids. Fusion of stereocilia and injury to their links can distort signal transduction in the auditory system and result in hearing loss. We hypothesize that both aging and otitis media may be involved in the alteration of ion homeostasis in the inner ear. These changes of the biochemical environment in the cochlear compartments in turn appear to produce morphological effects associated with damage (fusion or disarray of stereocilia) and/or loss of hair-cell stereocilia links that eventually lead to hearing loss.

1056 Protease Inhibitors Alpha 1-Antitrypsin and Ilomastat Are Not Ototoxic In The Chinchilla

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Objectives: Proteases of both the serine and metalloprotease families have been shown to play a role in the pathogenesis of otitis media. Inhibitors of proteases from each of these families have been shown to beneficially impact disease progression in a number of related chronic inflammatory conditions. The purpose of this study was to assess the safety of protease inhibitors when instilled into the middle ear, with a view to their potential use in the treatment of human otitis media.

Study Design: Prospective, randomized, controlled trial in the chinchilla model.

Methods: After completing baseline auditory testing and bilateral transpalatal obstruction of the Eustachian tube (ETO), chinchillas received weekly transbullar injections of protease inhibitor (alpha 1-antitrypsin, ilomastat, or both), vehicle, or saline. After one month, hearing was tested and the animals were sacrificed. Temporal bone histopathology was performed.

Results: All treatment groups demonstrated a statistically insignificant average loss in long-term hearing (0 db) for all measures using clicks and tones ($p > 0.15$ for all conditions). All treatment groups were statistically insignificantly different from one another ($p=0.5625$). Histopathology revealed no significant inner ear changes.

Conclusions: Protease inhibitors that are currently under study in human clinical trials for inflammatory conditions have no significant toxic effect on the inner ear of chinchillas. These findings support the safety of further clinical trials using these inhibitors to treat middle ear inflammation.

1057 Location and Timing of Initial Osteoid Deposition in Meningitic Labyrinthitis Ossificans Determined by Multiple Fluorescent Labels

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Variable amounts of fibrosis and neo-ossification fill the cochlea following bacterial meningitis. This study delineates the timing and location of initial ossification following pneumococcal meningitis. Fluorochromes are compounds which specifically incorporate into ossifying bone. Sequential addition of different colored fluorochromes during osteoneogenesis can define the timing and location of osteoid deposition and mineralization. Mongolian gerbils were infected by intrathecal injection of *Streptococcus pneumoniae* type 3 and control gerbils received saline. Both groups were injected with calcein post-op day 3 followed by xylenol orange, oxytetracycline and alizarin red on days 7, 14 and 28 respectively. Ten experimental gerbils were sacrificed 24 hours after each label, the temporal bones and tibias harvested and embedded in plastic. Wafers (200□ thick) were mounted in sequence and examined. Twelve of 50 experimental animals (24%) were positive for fluorescent labeled osteoid. Fluorescent labeled osteoid was present at all sampling times. Label extended from the endosteal wall into the lumen of the vestibule and scalae tympani (ST). Sites of fluorescence varied with each specimen. They included the ST of the basal turn, the opening of the cochlear aqueduct, and were associated with the organ of Corti, stria vascularis and spiral ligament in all turns from base to apex . These results indicate that osteoid is deposited and begins mineralization by at least post infection day 3 and continues through the first 28 days post infection.

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1058 Screening Mutant Mice for Hearing Impairment at The Jackson Laboratory

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We previously have reported on our screening program at The Jackson Laboratory (TJL) to identify inbred strains of mice with hearing impairment. Here we report our screening results for strains that carry spontaneous mutations. This is the first systematic assessment of hearing ability in the many mutant strains of mice available at TJL. For each strain tested, ABR thresholds for broad-band click and pure tone 8 kHz, 16 kHz, and 32 kHz stimuli were obtained from at least 4 mutant and 4 non-mutant control mice. When possible, known heterozygous mice also were tested. Of the 100 mutant strains tested so far, 18 were deaf and 17 were hearing impaired. Most of the deaf mutants exhibited head bobbing and circling behavior. Mice homozygous for quivering (qv); a mutation of the beta-spectrin 4 gene, (Spnb4), quaking (qk), shiverer (shi); a mutation of the myelin basic protein gene, (Mbp) and slow-wave epilepsy (swe); a mutation of the sodium/hydrogen exchanger gene, (Slc9a1) exhibited abnormal ABR wave-forms. We detected significantly elevated ABR thresholds in mouse strains carrying 13 mutations that have not been previously evaluated for hearing: abnormal feet and tail (Aft), progressive ankylosis (ank), belly spot and tail (Bst), coloboma (Cm), congenital goiter (cog); a mutation of the thyroglobulin gene, (Tgn), Ames dwarf (df); a mutation of the paired-like homeodomain factor 1 gene, (Prop1), Snells dwarf (dw); a mutation of the pituitary specific transcription factor 1 gene, (Pit1), dwarf grey (dwg), flaky skin (fsn), hydrocephaly with hop gait (hyh), mesenchymal dysplasia (mes); a mutation of the patched homolog gene, (Ptch), mocha (mh); a mutation of the adaptor-related protein complex AP-3 delta gene, (Ap3d), and short ear (se); a mutation of the bone morphogenetic protein 5 gene, (Bmp5). These mutant strains now provide new mouse models for different forms of syndromic hearing impairment.

1059 A Zebrafish Mutation Causing Hair Cell Degeneration in the Ear and Lateral-line Organ

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We have conducted a genetic screen in the zebrafish, *Danio rerio*, identifying 19 families defective in the development or function of the inner ear and lateral-line organ. One of the mutant families (848) is unresponsive to vibratory stimuli. The mutant larvae can swim in response to touch; however, they are unable to maintain a consistent orientation with respect to gravity. The overall appearance of the five-day-old larvae is normal, apart from the lack of an inflated swim bladder and abnormal pigmentation in the eyes.

We were unable to measure in mutant inner ears the extracellular microphonic potentials apparent in wild-type fish. When five-day-old larvae were immersed in water containing the cationic fluorophore 4-Di-2-ASP, which is normally taken up by wild-type hair cells in the lateral-line organ, almost no hair cells were visible. Furthermore, when this dye was injected into the inner ear, scant fluorescent labeling confirmed the presence of only a few hair cells. Antibody staining and semi-thin sectioning demonstrated that the mutant fish had approximately 10% as many hair cells as wild-type siblings. Signs of hair cell degeneration were already visible at 24 and 48 hours post-fertilization. The hair cells that remained in five-day-old larvae appeared morphologically abnormal and showed signs of membrane blebbing. In the semicircular canals, the surviving kinocilia were significantly shorter than those of wild-type hair cells.

Using simple sequence length polymorphisms (SSLPs) and single nucleotide polymorphisms (SNPs), we have mapped this mutation to a 200-kb region in linkage group 21. Cloning of the gene mutated in 848 will provide insight into the molecular mechanisms of hair cell development and survival.

1060 The Endocochlear Potential (EP) in C57BL/6 Mice is Resistant to Noise Exposure

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C57BL/6 mice carry the Ahl mutation, which has been associated with progressive degeneration of spiral ganglion, organ of Corti and cochlear lateral wall. Despite lateral wall degeneration in these mice, we and others (Ohlemiller et al., ARO 2002; Lang et al., HR 2002) have shown that the EP remains normal for ages up to 2 years, by which time little hearing remains. This may reflect the compensatory ability of the remaining stria vascularis, or possibly reduced current loading of the stria due to hair cell loss in older animals. We examined the latter possibility by exposing C57BL/6J mice at two different ages (3 and 8-10 mos) to broadband noise at 110 dB SPL for 2 hrs, followed by EP recordings in the cochlear base and apex 1-3 hrs later (5-7 mice/group). Under similar conditions, CBA/CaJ mice show EP reductions in both base and apex (Hirose and Liberman, ARO 2002). We therefore included CBA/J and CBA/CaJ, as well as B6.CAST Ahl+ mice in our study.

Non-exposed mice showed similar EPs in base (109±3 mV) and apex (99±4 mV), independent of strain and age. Noise exposure eliminated compound action potential responses in most animals at 10-40 kHz at the time of EP recording. Noise exposure led to significant reduction of the EP only in CBA/J and CBA/CaJ mice (1-way ANOVA, $p < 0.001$). Post-exposure EPs in C57BL/6J and B6.CAST Ahl+ mice averaged 110±4 mV (base) and 97±7 mV (apex), while those in the CBA strains averaged 69±9 mV (base) and 68±8 mV (apex). Strain effects did not depend on age.

The resilience of the EP in C57BL/6 mice derives from the genetic background, independent of Ahl-related cochlear degeneration. Pilot experiments in albino mice (ICR), and inducible NOS and GPx1

knockout mice (C57BL/6 background) indicate that neither strial pigment nor these protective enzymes mediate differences observed between C57BL/6 and CBA-related strains.

1061 Disrupted Endolymphatic Calcium Homeostasis In Pendrin Knockout Mice

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Pendred Syndrome is associated with deafness and caused by a mutation of pendrin that is expressed in the cochlea and the vestibular labyrinth (Everett et al., 1999; White et al., ARO 2003). The endocochlear potential of pendrin knockout (Pds -/-) mice, which are deaf, is near 0 mV. Endolymph is acidic in these mice but the K concentration is normal (Wu et al., ARO 2002; White et al., ARO 2003). In normal mice, the endolymphatic Ca concentration, which is critical for sensory transduction, is maintained above equilibrium, suggesting the presence of Ca secretory pathways such as the plasma membrane Ca-ATPase in Reissner's membrane. If Ca efflux from endolymph would be driven largely by the endocochlear potential, it would be expected that Ca concentrations rise in animals that lack a positive endocochlear potential. The goal of this study was to evaluate endolymphatic Ca levels in normal mice and mice that lack an endocochlear potential such as PDS -/- mice and KCNE1 -/- mice. Endolymphatic Ca levels were measured with double-barreled ion selective electrodes. Further, the presence of crystallizations in endolymph was taken as indirect evidence for elevated Ca levels. The endolymphatic Ca concentration in the cochlea and utricle of Pds -/- mice (both ~2 mM) was markedly higher compared to Pds +/+ mice (0.04 and 0.24 mM) although the perilymphatic Ca concentrations were similar (1-2 mM). Further, Ca levels in endolymph of KCNE1 -/- mice were apparently high, based on the finding of crystals attached to Reissner's membrane. These results support the concept that Ca efflux in the cochlea is driven by the endocochlear potential and suggest that part of the etiology of Pendred Syndrome is a dramatic disturbance of endolymphatic Ca homeostasis.

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1062 Towards a Molecular Understanding of Usher Type I Syndrome: The Quest of Myosin VIIa, Harmonin, and Cadherin 23 Ligands.

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Deaf-blindness in three genetic forms of Usher type I syndrome (USH1) is caused by defects in myosin VIIa, harmonin (multivalent PDZ domain protein), and cadherin 23. Despite being critical for hearing, the functions of these proteins in the inner ear remain elusive. We generated antibodies against cadherin 23, and harmonin to analyse their distribution, along with that of myosin VIIa, in the inner ear hair cells. To get insights into the cellular function of these molecules and to understand the pathogenesis of Usher type I syndrome, we also sought their interacting partners, in the yeast two hybrid system. A particular attention was paid to ligands whose function would provide explanations of the abnormalities observed in the Usher I patients and/or the myosin VIIa or cadherin 23 defective mouse mutants. For instance, we have recently shown that a molecular complex composed of Rab27A, MyRIP and myosin VIIa bridges retinal melanosomes to the actin cytoskeleton, and thereby mediates the local trafficking of these organelles. The defect of this molecular complex is likely to account for the perinuclear mislocalisation of the melanosomes observed in the retinal pigment epithelium cells of myosin VIIa defective mice. In the inner ear, myosin VIIa also interacts with vezatin at the level of the hair bundle; this interaction is likely to ensure the growing of the stereocilia as a coherent unit. New partners should soon be incorporated to this developmental pathway.

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1063 Cochlear Function in Beethoven Mouse Mutants

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Beethoven (*Bth*) is a new mouse mutant with semi-dominant inheritance. The mutation is a methionine to lysine mutation in the *Tmc1* gene (Vreugde *et al*. Nat. Genet. 30:257-258, 2002). *Tmc1* encodes a protein predicted to contain at least six transmembrane domains. Mutations in the human *TMC1* gene are associated with non-syndromic deafness (Kurima *et al*. Nat. Genet. 30:277-284, 2002).

We studied *Bth/Bth*, *Bth/+* and wild type littermates at postnatal day (P)15, P30 and P60. *Bth/+* mutants show deteriorating CAP thresholds at 12kHz and higher from P15 to P60, but near normal thresholds at 3 and 6kHz. Middle turn IHCs degenerate first, progressing to extensive IHC loss by P60, while OHC loss occurs later and starts in the basal turn. *Bth/Bth* mutants showed a similar pattern of hair cell loss, but occurring more rapidly. Homozygotes showed no CAPs at any age studied, although we could record SPs at high intensities suggesting their hair cells can depolarise. Endocochlear potentials are at least as large in mutants as in controls. Although raised thresholds in *Bth/+* are correlated with (and could be caused by) IHC loss, observations from *Bth/Bth* mutants suggest their hair cells never function normally even when they appear normal.

Single hair cell function was measured by whole-cell patch-clamp in organ of Corti dissected from P6 to P22. Resting potentials and size of outward K+ currents were similar in all three genotypes at immature stages, and furthermore all three genotypes had large transducer currents. In contrast, IHCs of *Bth/Bth* and, to a lesser extent, *Bth/+* mutants show a significant reduction in IK,f, the fast activating BK-type outward K+ current characteristic of mature cells. These results suggest that Tmc1 protein may be required for normal developmental expression of the BK channels in IHCs.

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1064 Deafness and Pigmentation Defects in *Varitint-Waddler* (*Va*) Mice Caused by Mutations in Mucolipin-3 (*Mcoln3*), a New Member of the TRP-family of Ion Channels

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Deafness in spontaneously occurring mouse mutants is often associated with defects in cochlea sensory hair cells opening an avenue to systematically identify genes critical for hair cell structure and function. The classical semi-dominant mouse mutant, *varitint-waddler* (*Va*), exhibits early-onset hearing loss, vestibular defects, pigmentation abnormalities and perinatal lethality. A second allele, Va^J, which arose in a cross segregating for *Va*, shows a less severe phenotype. Using a positional cloning strategy, we identify two new members of the mucolipin gene familiy (*Mcoln2* and *Mcoln3*) in the 350kb Va^J minimal interval and provide evidence for *Mcoln3* as the gene mutated in *varitint-waddler*. *Mcoln3* encodes a putative six-transmembrane protein with sequence and motif similarities to the family of non-selective transient-receptor-potential (TRP) ion channels. In the *Va* allele an Ala419Pro substitution occurs in the fifth transmembrane domain of

Mcoln3 and in *Va*^{J} a second sequence alteration (Ile362Thr) occurring in *cis* partially rescues the *Va* allele. Mcoln3 localizes to cytoplasmic compartments of hair cells and plasmamembrane of stereocilia. Hair cell defects are apparent by embryonic day 17.5 assigning *Mcoln3* an essential role during early hair cell maturation. Our data suggest that Mcoln3 is involved in ion homeostasis and acts cell-autonomously. Hence, we identify a new molecular link between hair cell physiology and melanocyte function. Not the least, *Mcoln2* and *Mcoln3* are candidate genes for hereditary and/or sporadic forms of neurosensory disorders.

1065 Molecular Pathogenic Mechanism of Mitochondrial DNA Mutations Associated with Non-syndromic Deafness

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Mitochonmdrial DNA (mtDNA) mutations are likely to be a significant cause of childhood sensorineural hearing loss, especially with maternally transmitted pattern. These mtDNA mutations, including both base substitutions and rearrangement mutations, have been found to be associated with both syndromicand non-syndromic forms of sensorineural hearing loss. However, the biochemical and molecular pathogenic mechanisms underlying the maternally inherited deafness remain poorly understood. Recently, an African-American family with maternally inherited nonsyndromic hearing loss have been associated with the mitochondrial T7511C mutation in the tRNASer(UCN) gene, which is commonly related to deafness. In addition, homoplasmic mutations T3308C in the ND1 gene and T5655C in the tRNAAla gene have been found in all members of this pedigree and also in some controls. To understand the pathogenic mechanisms of these mtDNA mutaitons, a biochemical analysis has been carried out of transmitochondrial cell lines, constructed by transferring mitochondria from lymphoblastoid cell lines derived from deaf individuals with mtDNA mutations or from controls lacking mutations, into human mtDNA-less (ro) cells. A significant decrease in the amount of tRNASer(UCN) and rate of mitochondrial protein synthesis and oxygen consumption was observed in the mutant cell lines, when compared with control cell lines. These observations suggest that the T7511C mutation in the tRNASer(UCN) gene is a primary mutation responsible for deafness phenotype and that T3308C and T5655C mutations play synergistic roles in the biochemical defect leading to deafness phenotype.

1066 Deafness-Related Mutations in Gap Junction Protein Connexin 26 Have a Dominant Negative Effect on Connexin 30

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Numerous gap junction plaques within the mammalian cochlea contain both connexin 26 (cx26) and cx30 (Forge et al, 2002, Audiol Neurootol 7:141-145) and mutations in both genes have been linked to deafness. It is not clear however if there are interactions between these connexins and what role these may play in hearing. In this study, an in vitro expression system has been used to examine the effects of four mutant cx26 proteins on cx30. The mutants arise from point mutations associated with either non-syndromic (W44S, R75W) or syndromic (G59A, D66H, R75W) dominantly inherited deafness.

HeLa cells were transfected with cDNA constructs by microinjection. When co-expressed, cx26 and cx30 trafficked to the same gap junction plaques. These cells transferred the tracer Neurobiotin but not Cascade Blue, which passes freely through cx26 channels, suggesting cx30 affects the permeability properties of cx26. G59A and D66H had a perinuclear localisation when expressed alone but trafficked to the membrane when co-expressed with cx30. These results indicate that cx26 and cx30 can oligomerise to form heteromeric connexons.

Cells expressing cx30 showed high levels of communication, with 81 % of cells injected with Neurobiotin (N=16) transferring the tracer to more than one neighbour. This communication was significantly reduced by co-expression of cx30 with W44S, G59A or R75W ($p<0.05$; Chi-squared test), demonstrating a dominant negative effect of these mutants on cx30. W44S and R75W, both associated with profound, non-syndromic deafness, also had a dominant negative effect on cx26. Heteromeric cx26/cx30 channels may be present uniquely in the inner ear. The functional disruption caused by some cx26 mutations upon these heteromeric channels may underlie the non-syndromic nature of their effects.

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1067 Vestibular Adaptation in Squirrel Monkey Studied with a Prosthetic Semicircular Canal

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Peripheral vestibular dysfunction is a common and disabling problem. Since damage to the vestibular labyrinth and nerve is often permanent, we are investigating approaches that could provide a "prosthetic" afferent signal to the brain to compensate in part for abberant loss of peripheral vestibular function. We describe here preliminary results from one squirrel monkey.

The angular vestibulo-ocular reflex (VOR) was studied before and after the "prosthetic canal" was implemented. The intact animal had a normal horizontal VOR during sinusoidal yaw-axis rotation. After both lateral canals were plugged, the VOR response was minimal over the range of frequencies tested (0.01 to 2.0 Hz). The animal was then instrumented with the prosthetic device, which sensed angular head velocity about the yaw-axis and used that information to modulate the pulse rate applied to the lateral canal ampullary nerve via a stimulating electrode. The VOR was tested repeatedly for more than 8 weeks while the animal wore this device.

We found that a horizontal VOR was immediately measurable in the instrumented animal. While the gains were relatively low (peak gain was 0.12 at 2.0 Hz), they clearly contributed to eye stabilization during head motion, as the phase was compensatory for frequencies greater than 0.5 Hz. Further, the low frequency phase lead became smaller over time, suggesting that the central velocity storage mechanism was activated by the vestibular afference evoked by the chronic electical stimulation.

The results suggest that a measurable VOR can be produced by the prosthetic device and that central processing can modify the eye movement response produced by patterned electrical stimulation. These preliminary findings suggest that a canal prosthesis may be a useful way to study the adaptation that occurs after the vestibular afferent signal is altered, and that a canal prosthesis may eventually prove beneficial to patients with vestibular dysfunction.

1068 The Second Filter in the Crista Ampullaris

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Excitatory step displacement of the semicircular canal cupula elicits an increase in afferent discharge rate followed by a period of adaptation over which the discharge rate returns to the prestimulus background level. In the toadfish, *Opsanus tau*, the adaptation time constants approach 0.001sec in high-gain acceleration-sensitive afferents and exceed 1000 sec in low-gain velocity-sensitive afferents. These diverse time constants underlie the diverse response dynamics observed during

head rotations (Bode plots). We have shown previously that adaptation of hair-cell transduction currents is minimal relative to the afferents and does not underlie the afferent diversity or temporal neural code transmitted to the brainstem (Rabbitt et al., ARO Abstracts 2001). A second, yet unidentified, filter is therefore interposed between the hair-cell receptor current/potential and afferent discharge rates. We introduce here a new hypothesis that explains the additional signal processing based on the convergence of excitatory and inhibitory synaptic inputs from multiple hair cells. A simple mathematical model indicates that convergence could explain the broad-band mathematical differentiation carried out by hair-cell/afferent complexes. The hypothesis is supported by new evidence showing the presence of both GABA and glutamate in regions of the crista known to give rise to the acceleration-sensitive afferents (Holstein et al., ARO Abstracts 2003).

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1069 Distribution and Nature of Dihydropyridine-Sensitive and -Insensitive Calcium Currents in Hair Cells from Semicircular Canals of the Frog .

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The distribution of Ca2+ currents in hair cells from thin slices of frog crista ampullaris were studied using the whole-cell patch-clamp technique. Currents were recorded from hair cells positioned in peripheral, intermediate and central regions of the sensory epithelium. The size of the total Ca2+ current was always larger in intermediate cells (320 pA) than in central (160 pA) and peripheral (110 pA) cells. Almost all hair cells positioned in the central region of the crista and most of those from the intermediate and peripheral regions showed an inactivating Ca2+ current. About 35% of the total sensory cells from the two latter regions exhibited a non-inactivating Ca2+ current. Two different Ca2+ current components were found in cells showing the inactivating current: a sustained nifedipine sensitive current (L-type current) and a partially inactivating current insensitive to nifedipine (non-L-type current). L- and non-L-type Ca2+ current components were also found in cells exhibiting a total non-inactivating Ca2+ current. In all hair cells investigated, the L-type component represents about 70% of the total Ca2+ current. L- and non-L-type components activated close to –60 mV and reached a maximal value at –20 mV. The amplitude of these two Ca2+ current components varied among peripheral cells, where the current density gradually increased from the beginning of the region toward its end. No significant variation of L and non-L Ca2+ current density was detected in hair cells of either intermediate or central regions. Immunocytochemical techniques revealed the presence on hair cell basolateral membranes of two distinct classes of Ca2+ channels: an alpha1D and an alpha1B subunit. These results demonstrate the presence in frog crista ampullaris of regional and intraregional variations in the expression of L and non-L type Ca2+ currents. It is likely that such differential Ca2+ channel distribution represents a mechanism which sustains the wide variability in the gain of vestibular afferent neurons.

1070 Voltage-dependent Currents in Calyx Terminals Isolated from Gerbil Semicircular Canals.

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Whole cell patch-clamp recordings were made from calyceal terminals non-enzymatically isolated together with type I hair cells from the cristae of Mongolian gerbils. The mean input capacitance for calyces was 3.3 ± 1.6 pF (mean ± SD, n = 11). In voltage clamp, depolarizing steps from hyperpolarized potentials (> -80 mV) resulted in large rapidly activating, rapidly inactivating inward currents at membrane potentials above –60 mV. Inward currents showed voltage-dependent inactivation and were half-inactivated at -78.5 ± 5.6 mV, (mean ± SD, n = 10). Inward currents were absent when external Na^+ was replaced by choline, consistent with a Na^+ current. Depolarizing steps above –50 mV revealed an outward current which showed steady state inactivation and was half-inactivated at approximately –78 mV. Immunocytochemical localization of KCNQ4 channels in calyx terminals of the mouse vestibular system (*Kharkovets et al. PNAS*, 97:4333-4338, 2000) was supported by the sensitivity of a large component (76.9 ± 9.5 %, n = 4) of the outward current to the KCNQ channel blocker linopirdine (20 μM). Application of linopirdine revealed a small underlying outward current with rapid activation and inactivation kinetics.

In current clamp large action potentials could be observed in response to small hyperpolarizing current injections. The role of these voltage-dependent currents in shaping the action potential in calyceal terminals is currently under investigation.

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1071 Prenatal Acquisition of Voltage-Gated Conductances in Vestibular Hair Cells of the Developing Mouse Embryo

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The electrophysiological properties of prenatal mammalian hair cells have not been reported previously. Yet, for a comprehensive understanding of hair cell development, characterization of the pattern of acquisition of voltage-gated conductances is required. We used the whole-cell, tight-seal technique to record voltage-gated currents from hair cells of mouse utricles excised at several developmental stages. At embryonic day 16 (E16) depolarizing voltage steps evoked small outward currents with properties similar to those of outwardly rectifying potassium currents. The currents reversed at ~–60 mV. The V-half of activation was –21±4 mV (mean±S.D; n=10) with a slope factor of 9.2±2.7 mV. The maximal conductance was 15.2±10.6 nS. By E18 the conductance more than doubled (31.6±9.3 nS; n=5) and the V-half of activation shifted to –29±9 mV. The slope factor was not significantly different: 10.4±2.6 mV. Hyperpolarizing voltage steps evoked inwardly rectifying currents. The inward rectifier conductance was also larger at later stages: 2.3±1.1 nS (n=10) at E16 and 4.3±1.1 nS (n=3) at E19.

Interestingly, depolarizing steps that followed hyperpolarizing prepulses evoked rapidly activating (τ = 0.3±0.1 ms; n=8), rapidly inactivating (τ = 2.3±1.5 ms) inward currents. The currents had a V-half of activation at –40±7 mV and a V-half of inactivation at –82±6 mV. This current profile is consistent with the presence of a sodium conductance for which there is little evidence in postnatal hair cells. Indeed, the largest sodium-like currents we measured (~–400 pA) were from E16 and E18 hair cells; while at later stages (E19 and P2) the currents decreased to negligible levels. In current-clamp mode we noted that E16 and E18 hair cells displayed spike-like behavior (~70 mV amplitudes) following hyperpolarizing current steps.

1072 Mechanical Noise Enhances the Signal-to-Noise Ratio of Afferent Nerve Activity in the Bullfrog Sacculus

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Until recently noise was thought to degrade the performance of sensory systems. However, it has become clear that the detection and transmission of weak signals in sensory systems can be enhanced by noise via stochastic resonance (SR). In hair cells the quality of mechanoelectrical transduction is enhanced up to two-fold by nanometer level mechanical noise acting on the hair bundle. We wanted to know whether this gain could be preserved, perhaps even enhanced, as information flows across hair cell synapses, and into the stream of action potentials that conveys vestibular information to the central nervous system. To investigate this question we studied the effects of noise on the response of the VIII nerve to small mechanical stimuli directly applied to the amphibian sacculus. To this end, we approached the sacculus from a small opening on the ventral side of the otic capsule. The sacculus was moved by a small glass probed affixed

to the basolateral side near the macula. Afferent activity was recorded with a silver electrode hooked around the saccular branchlet of the vestibular nerve. The sacculus was stimulated with nanometer level periodic stimuli (typically 100 Hz) to which varying levels of low-pass filtered white noise were added, and the electrode's response was recorded using a dual-channel lock-in amplifier. We found that ~2.5 nm of mechanical noise enhanced the response of the saccular nerve to weak stimuli by up to four-fold. As expected for SR, noise degraded the SNR of afferent activity for large stimuli. These results suggest that the positive effects of low-amplitude mechanical noise result in improved transmission of vestibular information.

1073 Human Postural Sway Monitoring with Wearable Sensors

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Human postural sway is usually estimated from force plate (FP) center-of-force (COF) trajectories. But actual postural adjustments involve complex multi-linked segmented movements. We constructed wearable sensor packages (WSPs) to measure roll and pitch angular velocities and positions on a notebook PC. We compared FP results with those from WSPs positioned at two anatomical locations.

Ten normal subjects were tested with WSPs attached to foreheads and lower backs (near S2) while performing sensory organization tests (SOTs) on a Smart Balance Master (Neurocom International, Clackamas, OR). WSP roll and pitch angular responses were recorded. Equilibrium scores, sensory analysis data, and composite scores were computed from WSP data using formulae described for the SOT.

SOT composite scores (SOT-CS) were compared with scores from head-mounted WSPs (HSP-CS) and back-mounted WSPs (BSP-CS) for the 10 subjects. The BSP-CS differed insignificantly from the SOT-CS ($p<0.0012$). In contrast, the HSP-CS was lower than either SOT-CS or BSP-CS for all subjects.

SOT-CS results are derived from trigonometric transformations of COF trajectories. But WSPs measured angular data directly at the sensor sites. The relative agreement of SOT-CS and BSP-CS results is to be expected because anatomical locations of back WSPs were similar to estimated center of mass locations from SOT. Lower values of HSP-CS, compared with SOT-CS and BSP-CS, occurred because of additional head-on-body movement.

These results suggest that testing postural stability with WSPs provides additional information useful for accurately assessing the multi-linked segmental nature of human postural control.

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1074 c-Fos Expression in Brainstem After Unilateral Labyrinthectomy in Mutant Mice Deficient in delta 2 Glutamate Receptor Subunit

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The immediate early gene c-Fos is used as a marker of neuronal activation, because of its very limited expression in the normal state and quick appearance after stimulation. Expression of c-Fos has been previously reported in several regions of the brainstem after unilateral labyrinthectomy (UL), including medial vestibular nucleus (MVe), spinal vestibular nucleus (SpVe), prepositus hypoglossal nucleus (PrH) and the inferior olivary nucleus (IO).

The delta 2 subunit of the ionotrophic glutamate receptor (GluR delta-2) is specifically expressed only in cerebellar Purkinje cells in the nervous system, and mutant mice deficient in delta 2 proteins have been raised (Kashiwabuchi 1995). These mice did not exhibit long term depression (LTD), which is one form of synaptic plasticity (Hirano 1995). In the present study, we examined the c-Fos expression after unilateral labyrinthectomy in mutant mice deficient in GluR delta-2, to investigate the role of the glutaminergic system in vestibular compensation.

Twenty-four hours after UL, in wild type mice, c-Fos positive cells were observed in MVe, SpVe, PrH and IO, as noted in previous reports. However, in the mutant mice, c-Fos expression was significantly lower in PrH and IO than in wild type mice. PrH is thought to be the part of a neural integrator for vestibulo-ocular reflex, and the IO conveys error signals to Purkinje cells, and both are presumed to be involved in vestiblar compensation after UL. These findings suggest that GluR delta-2 plays important roles in vestibular compensation.

1075 A VOR Fixation Suppression Rotation Test to Facilitate Identification of Asymmetric Vestibular Function.

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Standard clinical rotation tests are sometimes unable to detect an abnormality or to provide an assessment of severity and side of lesion. Tests using larger amplitude stimuli (150–250°/s peak velocities) can facilitate identification of asymmetric vestibular function. However, nystagmus evoked by these larger amplitude stimuli is often difficult to analyze due to the difficulty in accurately measuring slow phase eye velocity. We were motivated to investigate if visual fixation could be used to suppress the amplitude of vestibular-evoked nystagmus in response to high amplitude rotational stimuli while still permitting identification of asymmetric vestibular function.

Normal and unilateral vestibular loss (UVL) subjects were rotated about an earth-vertical axis while viewing a chair-mounted fixation LED. The rotational stimulus consisted of 2 components. A lower frequency, larger amplitude "bias" component was used to drive the neural activity of most afferents innervating one canal of a semicircular canal pair to zero during a portion of the stimulus cycle. A 1 Hz, 20°/s "probe" component was added to the bias component to test the ability of the canals to encode the probe component motion throughout the entire bias component period. We predicted that a UVL subject would show 2 types of VOR fixation suppression asymmetries not present in normals. First, UVLs would be better able to visually suppress VOR during bias component rotation toward the dysfunctional ear than during rotation toward the intact ear. Second, VOR responses caused by the probe component would be absent during rotation toward the dysfunctional ear since canal afferent activity is silenced in the intact ear and the dysfunctional ear is unable to encode the probe component stimulus. Experimental results confirmed these predictions.

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1076 Characteristics of Horizontal Vestibulo-Ocular Reflex after Unilateral Plugging of Three Semicircular Canals in the Gerbil.

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Plasticity in the HVOR has been studied following various forms of peripheral injury in a number of species. Canal plugging attenuates the dynamic response of vestibular afferent neurons, particularly at low frequencies, without impacting the resting afferent discharge. The current experiments were undertaken to evaluate the to effect of canal plugging on the symmetry and amplitude of the HVOR in the gerbil; a lateral eyed rodent which normally demonstrates directional asymmetry in the HVOR. This experiment differs from previous experiments because we examined the left and right eyes independently and plugged all of the ipsilateral canals to avoid confusion from out-of-plane excitement of the vertical canals.

Seven gerbils were used in the current experiments. Three underwent bilateral plugging of all of the semicircular canals and 4 underwent unilateral plugging of 3 semicircular canals. Test protocols included horizontal optokinetic stimulation, horizontal rotation at 0.2, 0.5, and 1 Hz at peak amplitudes of 30, 60, or 90 degrees/sec, and stimulation of the otolith apparatus utilizing a centripetal acceleration vector rotated at .55 Hz through the head. Animals were tested 3 hours after surgery through 4 weeks.

Bilateral plugging eliminated the HVOR responses but the otolith-generated responses and optokinetic responses were normal. Unilateral plugging initially results in asymmetry that is dependent on which eye is being examined. At 3 hours, both eyes demonstrate greater gain for ipsilateral rotation, even on the side of the plugging. The poorest HVOR response was seen between 24 and 48 hours. Recovery to near normal HVOR gains was seen within 3 weeks of the lesion. The slowest recovery was seen in the contralateral eye for rotation towards the injured labyrinth. The data support a mechanism of initial depression of activity for several days, followed by plasticity over a period of weeks that permits return of function.

1077 Genotype-phenotype Correlations for the Horizontal VOR in Spinocerebellar Ataxia 1 - 8

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The autosomal dominant cerebellar ataxias are a group of neurodegenerative diseases, designated as the spinocerebellar ataxias (SCAs). Genetic studies have identified over 21 different subtypes. Characteristic features, due to degeneration of cerebellar and brainstem neurons, become progressively more severe and include instability of posture and gait, incoordination, ocular motor dysfunction, and dysarthria. Clinical observations suggest that eye movements and postural stability are universally but differentially impaired, presumably due to differences in the involvement of neuronal populations. The aim of the present work was to study the horizontal vestibulo-ocular reflex (hVOR) to identify characteristics specific for a given SCA subtype. Patients with genetically defined SCA1,2,3,4,5,6,7, and 8 were examined. The hVOR was recorded during whole-body rotations in the dark and while the subjects fixated on a light that rotated with them. The results for SCA5, 6, and 8 showed that both mild and severely affected patients had an intact VOR. However, there were differences in the gain among the severely affected. The SCA8 patients had a gain greater than 1 and higher compared to SCA5 and 6. Visual suppression of the VOR was severely reduced in SCA 6 and 8, but was strong in SCA 5. In contrast, the other SCA subtypes had a reduced frequency of the nystagmic quick phases that eventually resulted (in severely affected patients) in a total absence of quick phases. In that case there was only a slow phase (if there was a response), and the gain of the VOR during sinusoidal rotation was within normal limits or low. A linear discriminant analysis separated the SCA subtypes into distinct groups. These and other results will help to identify features that are diagnostic for SCA subtypes and provide new information about selective vulnerability of neurons controlling vestibular reflexes.

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