1 Hereditary Hearing Impairment

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There has never been a greater opportunity for research to make an immediate impact on human diseases and disorders than exists at this time. Remarkable progress has been made in determining the structure of the human genome, with thousands of useful markers mapped and about 50% of the sequence determined. Public databases contain a partial sequence from over 90% of all genes that are expressed. This infrastructure provides the fuel for discovering the genes that determine susceptibility to communication disorders such as hereditary hearing impairment.

It is estimated that one child in a thousand is born with hearing impairment that compromises the development of normal language skills. In the majority of cases, the cause of hearing impairment is mutations in genes which result in hearing impairment in the absence of any other clinical findings (nonsyndromic autosomal dominant, recessive, or X-linked hereditary hearing impairment). Within the last six years, scientists have determined the location in the genome of over seventy genes that cause nonsyndromic hereditary hearing impairment. Over twenty of these genes have been cloned. These genes encode proteins with varied functions, including unconventional myosins (intracellular motor molecules), transcription factors (gene regulatory proteins), cadherins and claudins that form specialized junctions between cells, and intra- and inter-cellular signaling molecules. These breakthroughs provide the tools for precise determination of the etiology of hereditary hearing impairment, leading to early intervention that will optimize development of language skills, as well as intervention strategies in cases where the hearing impairment is progressive. Understanding the genes whose mutation results in hereditary hearing impairment will provide a fundamental new understanding of the molecular and cellular functions that are essential for normal auditory function.

2 Stem Cells and Precursor Cells for Sensorineural Transplantation in the Auditory System.

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The loss of hair cells and/or primary sensory neurons is the most common cause of sensorineural hearing loss and peripheral vestibular disorders. Once lost, neither cell type regenerates in mammals. The possibility of transplantation to replace lost cells has received relatively little attention in the inner ear. However, there have been recent advances in our understanding of how hair cells and inner ear neurons arise and differentiate. In addition, stem cells of various origins are increasingly available. This suggests that it may eventually be possible to engineer stem cells into either sensory cells or sensory neurons. These cells might be derived from the tissues of a patient, allowing autologous transplants. To assess the potential for inner ear transplantation, we have explored the feasibility of transplanting stem cells, committed but undifferentiated precursor cells, and partially or fully differentiated cells in the inner ear.

Using in vitro models we have found that immature hair cells can be successfully transplanted into a previously damaged inner ear sensory epithelium. They can integrate into the sensory epithelium, and form new stereociliary bundles. Neither uncommitted stem cells from the sensory epithelium of the developing inner ear, nor neural stem cells derived from the central nervous system showed similar integration. Fully differentiated sensory cells derived from the adult inner ear also were not successfully transplanted. Immature sensory cells transplanted into the adult cochlea in vivo survived for weeks, but did not display integration into the organ of Corti.

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3 Plasticity and Refinement of Projections in the Auditory Nervous System

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In most mammals the auditory system is so immature at birth that neonates are deaf. This provides a valuable opportunity to study molecular and activity-dependent mechanisms contributing to formation of precise connections, emergence of specific functional capacities and plasticity. We have studied the development of auditory nerve (AN) projections to the cochlear nucleus (CN) by labeling small sectors of the cochlear spiral ganglion representing a narrow band of frequencies in kittens. Results show that projections from the basal cochlea exhibit clear tonotopic organization before birth, in kittens studied after C-section at 60-64 days gestation, prior to emergence of spontaneous activity in the AN and many days before the onset of functional hearing. However, our data also demonstrate that significant refinement of CN projections occurs during early postnatal maturation. Topographic restriction of fibers into frequency band laminae is less precise in all 3 CN subdivisions in perinatal kittens than in adult cats. Projections to the AVCN, PVCN, and DCN are 53%, 36% and 32% broader, respectively, in neonates than in adults when normalized for CN size. Preliminary data suggest that the precise organization of these projections is stable even when AN nerve activity is greatly reduced or abolished neonatally by ototoxic drug deafening. On the other hand, marked alterations in projections to the inferior colliculus (IC) occur after neonatal unilateral cochlear ablation, and stimulation by a cochlear implant in neonatally deafened animals can induce significant functional plasticity in the IC. Future studies will determine whether neuronal activity is essential for refinement of the AN projections to the CN and the extent to which these projections may be modified by aberrant input during the subsequent critical period.

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4 Experience and Auditory Brainstem Development: Signals, Cellular Events and Critical Periods

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Since the classical experiments of Hubel and Wiesel, a large variety of studies have shown that manipulations of sensory experience have profound influences on the development of sensory encoding pathways of the central nervous system. Yet little is known about the cellular mechanisms whereby changes in sensory system function influence the structure or integrity of CNS elements. We have used the brainstem auditory pathways of birds and mammals to investigate the early cellular events underlying deprivation- and deafferentation-induced changes in the structure and integrity of neurons and glial cells. I will discuss a series of in vivo and in vitro experiments which address three issues related to activity-regulated development and maintenance of cochlear nucleus neurons. What is the nature of the intercellular signals regulating structural integrity of postsynaptic neurons? What are some of the intracellular cascades of events underlying deprivation-induced changes in neuronal integrity? What biological mechanisms may underlie developmental differences in responses to peripheral manipulations (critical periods)?

5 Early Language Development: An Overview of Normal-Hearing Infants and Some New Findings with Deaf Infants After Cochlear Implantation

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Language development research over the past 30 years has revealed that infants are born with a remarkable capacity to acquire language. During the first year of life, normal-hearing infants learn much about the organization of sound in their native language. I will review findings from investigations showing that English-learning infants become sensitive to several properties in English that are important for segmenting words from fluent speech. For example, Jusczyk and colleagues have shown that sometime after six months, English-learning infants become sensitive to which sounds and sound sequences and which rhythmic patterns are likely to occur in English words. Recognition of these properties appears to play an important role in how infants begin segmenting words from fluent speech.

While much effort is devoted to delineating normal-hearing infants' speech perception and language skills, there is little currently known about these skills in congenitally deaf infants. A growing number of deaf infants are now receiving sensory aids, especially cochlear implants, at younger and younger ages. In order to evaluate the effectiveness of cochlear implantation during infancy and to better understand the effects of early sensory experience on language development, it is important to track the linguistic skills of deaf infants before and at regular intervals following cochlear implantation. We have adapted two methodologies that have been used extensively to study normal-hearing infants, the Visual Habituation (VH) procedure and the Preferential Looking Paradigm (PLP), to test infant cochlear implant recipients. I will report findings from our initial investigations, showing that VH and PLP are promising tools for tracking and assessing the early language development of this new population of infants.

6 Adult and Developmental Neuroplasticity Related to Vocal Behavior in Songbirds

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Songbirds produce a learned vocalization, called their "song", that is used in the context of reproduction to attract mates and defend territories. Song learning requires auditory experience derived from conspecifics (either early in ontogeny or in adulthood as well in some species). The learning, perception and production of song is controlled by a well-defined neural circuit that is unique in many ways to songbirds and exhibits a high degree of plasticity. Variation in the size and cellular properties of a key part of the circuit such as HVc (the high vocal center), both during development and seasonally in adulthood, correlates with the ability to produce song but the precise neural factors regulating the ability to learn song remain elusive. For example, in European starlings variation in the volume of HVc is related most closely to the ability of males to produce songs organized into long bouts rather than to learned repertoire complexity. The volume of HVc also varies seasonally with the largest volumes being observed in the spring but again this may be related to performance rather than to learning per se. Auditory areas involved in the perception of song exhibit a highly selective expression of an immediate early gene named ZENK in response to conspecific song as compared to heterospecific song. Female starlings prefer males who produce long-bout as compared to short-bout songs and exhibit higher ZENK induction when exposed to long-bout songs. Recent experience can influence this stimulus response-bias as we found by exposing wild-caught female starlings to one week of either short-bout or long-bout songs. Surprisingly, short-bout experience attenuated this stimulus response-bias in gene expression, whereas long-bout experience amplified it. Thus adult and developmental plasticity can be observed in relation to both the production and perception of birdsong.

7 Cochlear Implantation in Young Children

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This study examined the effects of age at implantation on the development of communication abilities in early-implanted children. All participants were prelingually deafened, received a cochlear implant before 5 years, and used current cochlear implant technology. Children were administered a battery of speech and language outcome measures preimplant and at successive six-month postimplant intervals. Word recognition measures were administered in the auditory-only modality; the children's responses were scored as the percent of words correctly identified. Language measures were administered in the child's preferred communication mode. One difficulty in examining age at implantation effects in children is finding the appropriate analysis technique. If the scores of children who differ in age at implantation are compared at a given postimplant interval (e.g., 12-months postimplant), some children will be older than others and may demonstrate superior performance as a result of maturation. Conversely, if participants are compared at the same chronological age, some children will have longer periods of device use, a factor that is known to influence performance. Therefore, we used a mixed model analysis to examine the rate of growth in word recognition and language skills as a function of age at time of implant separately for the OC and TC groups. Results revealed significant improvements in communication skills over time. Although spoken word recognition developed at similar rates in both age at implantation groups, children implanted before 3 years had significantly faster rates of language development than did children implanted after that time. Oral children appeared to demonstrate more rapid acquisition of word recognition abilities and language skills than did the children who used TC.

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8 Language Development in Prelingually Deaf Cochlear Implant Users

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Children with profound congenital or prelinguistic deafness encounter significant difficulties in the development of skills in an oral language such as English. However, their language development can be accelerated if they receive a cochlear implant, a sensory aid that facilitates language acquisition by providing important auditory information. The present study used a behavioral test (the Reynell Developmental Language Scales) and parent questionnaires (the MacArthur Communicative Development Inventory) to assess language skills pre- and post-implant in forty-four pediatric cochlear implant users. They were all profoundly-to-totally deaf, either at birth or before the age of three. They all received cochlear implants before the age of six and were programmed with state-of-the-art stimulation strategies (SPEAK or CIS) since the day of initial stimulation. The main finding was that post-implant language development proceeded at a pace that was not significantly different from normal. Thus, the language gap present at implantation did not increase after children start using the device, as it would if they had not received cochlear implants. Additionally, a new type of quantitative analysis of these data will be shown, which will address the effect of age at implantation on language development.

9 Compressive Nonlinearity in the Hair Bundle's Active Response to Mechanical Stimulation

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The auditory system's ability to interpret sounds over a wide range of amplitudes rests upon the nonlinear responsiveness of the ear. Whether measured by basilar-membrane vibration, nerve-fiber activity, or perceived loudness, the ear is most sensitive to small signals and grows progressively less responsive as stimulation becomes stronger.

Seeking a correlate of this behavior at the level of mechanoelectrical transduction, we examined the responses of hair bundles to direct mechanical stimulation. As measured after the attachment of a flexible glass fiber, an active hair bundle from the bullfrog's sacculus oscillated spontaneously. Sinusoidal movement of the fiber's base by as little as ±1 nm, corresponding to the application at the bundle's top of a force of ±0.3 pN, caused detectable phase-locking of the bundle's oscillations to the stimulus. Although entrainment increased as the stimulus grew, the amplitude of the hair-bundle movement did not rise until phase-locking was nearly complete. A bundle was most sensitive to stimulation at its frequency of spontaneous oscillation. Far from that frequency, the sensitivity of an active hair bundle resembled that of a passive bundle. Over most of its range, an active hair bundle's response grew as the one-third power of the stimulus amplitude. The bundle's sensitivity therefore declined in proportion to the negative two-thirds power of the excitation, a scaling behavior also found in the response of the mammalian basilar membrane to sound. This result signals the operation of an amplificatory process at the brink of an oscillatory instability termed a Hopf bifurcation.

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10 Understanding the Effect of Animal-Rights Activism on Biomedical Research

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All are aware that animal-rights activism has impeded biomedical research. A number of their activities are well known, some more deleterious to research than others. Demonstrations are more damaging the more they are directed at specific individuals. Not only does it damage the spirit of the victim, often making it difficult to work, other researchers are intimidated to the point that they will not acknowledge the important work they do. Destruction of facilities can set back research efforts when data are destroyed or valuable animals are released or stolen. This, to me, is more demoralizing than the simple destruction of laboratories, for money can cure the latter, but the loss of one's research program is absolutely devastating. This is how terrorism works.

Although animal care has greatly improved in the last twenty years, partly due to exposé's (generally over-blown in their claims), researchers face an increasingly bureaucratic effort to "do better." Increasing bureaucratic control does not necessarily equate with increased animal welfare. The demands of bureaucrats can be enervating. But at this point the healthiest response for the individual researcher is not to rail ineffectively against bureaucratic requirements but to consider them the cost of doing business. Research organizations are beginning to realize, though, that they should exert pressures on governmental bureaucrats to counter those they receive from the animal-rights community.

What can the researcher do? He or she should understand and develop a response to the philosophical tenets of the movement, the better to feel comfortable with one's work and to stand up to criticism. Read claims against the usefulness of research with animals and understand the dishonesty that underlies them. Then go forth and confidently educate the public in any forum.

11 Migration of bone marrow cells into the endolymphatic sac induced by microinjection of artificial endolymph into the cochlea

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Earlier studies in guinea pigs following microinjection of artificial endolymph into the cochlea of a quantity of artificial endolymph suggest that the volume of fluid in the endolymphatic sac (ES), and hence the volume of the entire membranous labyrinth, may be regulated by a dynamic relationship between active secretion and enzymatic degradation of a lumen-expanding substance that is intimately related to the intraluminal macrophages (Rask-Andersen et al., 1999). Morphological changes of the endolymphatic sac and surrounding petrous bone marrow space were analyzed after injections into the second turn of scala media with a micro-pump at a rate of 60-100 nl/min, lasting for a period of 4, 7.5, 15 or 18 min. For each procedure the contralateral (non-treated) ear served as a histological control. Following artificial endolymph injections for 7. 5 min or more there was an almost total absence of the normal intraluminal homogeneous substance (HS) on the injected side. In addition surrounding bone marrow with sinusoids reached into the ES. This included erythroblasts, neutrophils, promyelocytes, reticular cells and monocytes/lymphoblasts. Most of these cells were undergoing apoptosis and degradation through large reticular cells. Signs of a migratory pathway of monocytes/macrophages through the epithelial layer into the lumen of the sac could be observed. Our observations suggest that the disappearance of the HS occurs partly through macrophagic activity. These cells seem to be derived from surrounding bone marrow shuffled into the sac along anastomosing sinusoids. The mechanisms involved in the activation and stimulation of the bone marrow after endolymph injection remain to be elucidated.

12 Organ of Corti-specific SCF Complex - A Key Factor in Cochlear Homeostasis

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OCP1 and OCP2, two of the most abundant proteins of the organ of Corti (OC), have recently been identified, respectively, as an F-box protein and a homolog of Skp1, components of the SCF complex (Skp1, Cullin and F-box protein, plus ROC1), which target short lived proteins, such as cell cycle proteins, for ubiquitination and proteolytic degradation by the 26S proteasome. SCF specificity is imparted by the F-box protein, in this case OCP1. Although the target(s) of the OC-specific SCF complex have not been conclusively identified, involvement in cell cycle control is ruled out because of the postmitotic status of the OC. Instead, evidence is mounting that SCF^{OCP1} is involved in modulating the activity of the epithelial gap junction system (EGJS) of the cochlea, conceivably by controlling oligomerization and turnover of Cx26, known to be rapid. The prevailing notion is that the EGJS is essential for removal and recirculation of K+, exiting the hair cells as a consequence of mechanotransduction. Apart from several lines of biochemical evidence, an interaction of the SCF^{OCP1} with the EGJS is supported by: 1) Colocalization of OCP1 an OCP2 with Cx26 and Cx30. 2) Similar developmental expression patterns of all four

substances, closely correlating with development of the EP. It is of interest that certain components of the Wingless/Wnt signaling pathway are colocalized in the EGJS over the same developmental time course, in particular β-catenin and the adenomatous polyposis coli protein, APC, and two other proteins possibly involved in regulating β-catenin activity, presenilin 1 and δ-catenin (R. Killick, unpubl. observ.). Certain connexins are known Wnt target genes, and Wnt signaling can also modulate gap junction permeability in a transcriptionally independent manner. It is conceivable that the Wingless/Wnt pathway of the EGJS also interacts with the SCF^{OCP1} complex.

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13 Multiple Claudins are expressed in Outer Hair Cell – Supporting Cell Tight Junctions where they form a Unique Permeability Barrier and Adhesive Complex

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At the level of the reticular lamina, the apical surfaces of outer hair cells and supporting Deiters' cells are structurally interlocked and sealed by a distinct tight junctional complex. Claudins, a large family of transmembrane proteins, are the primary seal-forming elements of the extracellular space in tight junctions and presumed to be expressed in a cell and tissue specific manner. In a PCR screening of various mouse tissues, we found that multiple claudin species are expressed in the organ of Corti. Using an immunofluorescence assay with custom made antibodies to each of the known mammalian claudins, we observed that several claudins including 1,2, 3, 6, 9 and 14 are represented at the tight junction around the outer hair cells (OHC). A comparison of the intensity of the labeling signal indicates that claudin 9 is clearly the predominant isoform. We have further studied the structure and molecular content of this junctional complex using freeze-etching and immunogold labeling electron microscopy. Although adherens junctions and the adherens junction proteins E-cadherin, α- and γ-catenin are present in Deiters' cell-Deiters' cell junctions, they are not found in the OHCs-Deiters' cell junctions. However, the adherens junction proteins β-catenin and p120 were found not only between Deiters' cells but also in OHC-Deiters' cell junctions. Our observations lead us to conclude that the OHC-Deiters' cell junction is an enhanced, hybrid tight junction that functions as both permeability barrier and adhesion complex. This unique tight junction may derive its effective permeability and adhesive capacity from interactions between multiple claudin isoforms and the components of the β-catenin and p120 complex. Such unique molecular composition may account for this tight junction's ability to maintain its integrity despite the vigorous motion generated in the cochlear partition during sound transduction and OHC electromotility.

14 Connexins 31 and 45 are expressed on the mouse inner ear.

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The prevalence of deafness associated with mutations in genes that encode gap junction proteins, connexins, attests to critical roles played by gap junctions in the inner ear. Usually more than one form of connexin is present within a given connexon (gap junction) and it is thought that functional properties of given connexons are conferred by their connexin composition. Previous work has indicated the presence of connexins 26 and 30 within connexons joining all cochlear cells that are joined by gap junctions, although it is not established that all cochlear gap junctions contain these connexins. Hearing loss has been associated with mutations in connexin 26 and 30 genes. Connexin 43 has recently been associated with sensorineural hearing loss but the cells that express this connexin are not yet well characterized. Hearing loss has also been associated with connexin 31 mutations in humans and immunostaining of rat and mouse ears indicate that its distribution is limited to type II fibrocytes and cells of the spiral limbus. However, the presence of connexin 31 in the mouse inner ear has been questioned by Plum et al. This report reports the presence of mRNA encoding connexins 31 and 45 in the mouse cochlea. The presence of connexin 45 within the cochlea was indicated by positive findings using a restriction fragment differential display assay. This finding prompted acquisition of primers designed specifically for connexins 31 and 45 and performing RT-PCR reactions on mouse inner ear samples. Results with the specific primers confirmed the expression of both connexin genes within the mouse cochlea. The results question the supposition that mouse and human cochleas differ with regard to connexin content and indicate that further study of connexin 45 within the ear should add to the growing body of knowledge concerning the connexin family as critical cochlear components.

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15 Cation Absorption By Reissner's Membrane Via Epithelial Sodium Channels (ENaC)

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The epithelial cells of Reissner's membrane (RM) form most of the boundary of the cochlear duct. Little is known about the contribution of RM to the ionic homeostasis of endolymph. Several lines of evidence suggested a role in both Cl and cation transport. We have isolated RM and looked for evidence of electrogenic transepithelial ion transport with the vibrating probe. The lateral wall was dissected from the apical turn of adult gerbil cochleae, the stria vascularis removed and the attached portion of RM folded over the suprastrial portion of the spiral ligament and perfused at 37°C. The tip of the probe was positioned about 20 μm from the apical surface of RM and a current (Isc) was recorded flowing in the apical to basolateral direction. Isc was reduced by blockers of the epithelial sodium channel (ENaC). Benzamil inhibited Isc with an IC50 of 140 nM, amiloride with an IC50 of 1.2 μM, and EIPA with an IC50 of 70 μM. This sequence of potency is consistent with dependence of the current on ENaC and not on a Na/H exchanger. Isc depended on activity of the Na-pump since Isc was inhibited by ouabain. Isc was not due to other cells in the preparation, since removal of RM resulted in no detectable currents from the same portion of the spiral ligament. Previous reports by several groups suggested that RM contained a cAMP-dependent Cl pathway. Stimulation of this pathway by 10 μM forskolin in this preparation slowly decreased Isc but the underlying mechanism has not yet been identified. These results show for the first time that Reissner's membrane is capable of transporting Na out of cochlear endolymph via ENaC and may also provide a parasensory pathway for K efflux if the permeability of ENaC to this ion is sufficiently high.

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16 K^+ Secretion in Strial Marginal Cells and Vestibular Dark Cells is Controlled by the Extracellular Osmolarity

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The osmolarity of the basolateral fluid has been shown to control the rate of K^+ secretion and the activity of the apical KCNE1/KCNQ1 K^+ channel in vestibular dark cells (VDC) (Wangemann et al., 1995). The aim of the present study was to determine whether osmotic challenges control the rate of K^+ secretion in strial marginal cells (SMC) of stria vascularis. Morphometric measurements were made with videomicroscopy and K+ secretion was measured with the vibrating probe as current density in the vicinity of the apical membrane. Osmolarity changes were introduced by addition and removal of mannitol. A 10% osmotic challenge caused in stria vascularis within 60s no changes in cell width and nearly a 10% change in tissue height. In VDC no changes in cell width and a 3% change in cell height were

observed. The 10% hypoosmotic challenge induced in SMC and VDC a transient 50 and 42% increase in the current density near the apical membrane, respectively. Conversely, the 10% hyperosmotic challenge caused a transient 30 or 44% decrease in the current density, respectively. In conclusion, the data demonstrate that VDC regulate their volume. It remained unclear, however, whether SMC of stria vascularis regulated their cell volume. Both cell types, however, responded to osmotic challenges with a change in the rate of K^+ secretion that would be consistent with cell volume regulation. The similarity in the responses suggests an involvement of the apical KCNQ1/KCNE1 K^+ channel in both cell types. Interestingly, the osmotically induced changes in the transepithelial current exceeded those estimated to be necessary for cell volume regulation.

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17 Protein Identification, Glycosylation and Immunolocalization of β_1-Adrenergic Receptors in the Cochlea and the Vestibular Labyrinth

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β_1-adrenergic receptors (β_1-AR) have been found to stimulate K^+ secretion in strial marginal cells and vestibular dark cells (Wangemann et al., 1999, 2000). The aim of the present study was to identify β_1-AR protein, to determine the glycosylation status, and to localize β_1-AR in inner ear tissues. Crude membrane preparations of gerbil inner ear (lateral wall, modiolus and vestibular labyrinth) and kidney were prepared for Western blotting. Samples were digested with N-glycosidases, Endo H and PNGase F, and separated by SDS-PAGE. Proteins were detected by a rabbit polyclonal β_1 receptor antibody (AB), and visualized as chemiluminescence with an HRP-conjugated anti-rabbit AB. Cryosections were prepared from decalcified, zinc-formalin perfused, gerbil temporal bones. β_1-AR were localized by the rabbit polyclonal AB and visualized with an Alexa-488-conjugated anti-rabbit AB. Specificity of labeling was evaluated by preabsorption of primary ABs with the peptide the AB was raised against. Western immunoblotting of crude kidney membrane preparations revealed specific bands at 145, 90, and 63 kDa. Digestion with PNGase F (deglycosylating complex and high-mannose oligosaccharides) shifted the 145 kDa band to 120 kDa and the 90 kDa band to 75 kDa. Digestion with Endo H (deglycosylating only high-mannose oligosaccharides) shifted only the 145 kDa band to a 120 kDa band. Similar (not identical) bands and shifts were found for inner ear tissues. Confocal microscopy revealed specific staining for β_1-AR in strial marginal cells, spiral ganglia cells, inner and outer hair cells, Reissner's membrane and vestibular dark cells. These data demonstrate that β_1-AR occur as high-mannose and complex-glycosylated proteins and that they are localized in sensory and non-sensory cells of the inner ear.

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18 β_2-adrenergic receptors stimulate chloride secretion in semicircular canal duct epithelium

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The semicircular canal epithelium (SCE) is known to contribute to endolymph homeostasis (Milhaud et al., 1999). The aim of the present study was to investigate whether the transepithelial current I_{sc} is a) due to net anion secretion or cation absorption, b) under hormonal control and c) representative of the function of native neonatal and adult epithelium. Cultured neonatal rat SCE mounted in an Ussing chamber was used for I_{sc} and ionic flux measurements. Native neonatal rat and adult gerbil SCE were used for I_{sc} measurements with the vibrating probe and for cAMP measurements using a colorimetric immunoassay. In cultured neonatal rat SCE I_{sc} was carried by a net $^{36}Cl^-$ flux. Isoproterenol (ISO, 10 μM) stimulated I_{sc} and net $^{36}Cl^-$ flux but had no effect on $^{22}Na^+$ or $^{86}Rb^+$ fluxes. ISO and norepinephrine stimulated I_{sc} with an EC_{50} of 8.0 nM and 15 nM, respectively. Adenosine, histamine and vasopressin had no effect on I_{sc}. Forskolin stimulated I_{sc}, however, had no further effect after full stimulation with ISO or norepinephrine. The effect of ISO on I_{sc} was inhibited by the antagonists ICI118551 and CGP20712A with K_{DB} values of 0.2 and >10 μM. Similar K_{DB} values were obtained by measuring ISO-induced cAMP accumulation in native neonatal rat SCE. In native neonatal rat and adult gerbil SCE I_{sc} was stimulated by forskolin and ISO. In conclusion, our data validate the use of cultured neonatal rat SCE as a model for native neonatal rat and native adult gerbil SCE. The data demonstrate that the SCE contributes to endolymph homeostasis by secreting Cl^- and that Cl^- secretion is under hormonal control via β_2-adrenergic receptors.

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19 Endothelin-1 Induced Ca^{2+}-Sensitization of the Spiral Modiolar Artery is Mediated by Rho-Kinase and Reversed by CGRP

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The spiral modiolar artery (SMA) contains ET_A and CGRP receptors (Scherer et al., 2001, Herzog et al. 2001). Endothelin-1 (ET1) induces a transient increase in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]i$) and a long-lasting constriction suggesting an increase in the Ca^{2+}-sensitivity of the myofilaments. The aim of the present study was to determine whether the ET1-induced Ca^{2+}-sensitization is mediated by Rho-kinase and reversed by the vasodilator CGRP. A further aim was to visualize the actin cytoskeleton.

The Ca^{2+}-sensitivity of the myofilaments was evaluated by a correlation between $[Ca^{2+}]i$ increases and associated vasoconstrictions. $[Ca^{2+}]i$ and the vascular diameter were measured with fluo4-microfluorometry and videomicroscopy. Changes in $[Ca^{2+}]i$ were induced by changes in the extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) from 0 to 1, 3 and 10 mM. Increases in $[Ca^{2+}]_o$ caused increases in $[Ca^{2+}]i$ and constrictions. ET1 (0.1 nM) had no significant effect on $[Ca^{2+}]_o$-induced changes in $[Ca^{2+}]i$ but caused a significant increase in the magnitude of the associated constrictions. This observation suggests that ET1 increased the Ca^{2+}-sensitivity of the myofilaments. This ET1-induced Ca^{2+}-sensitization was prevented by the Rho-kinase antagonist 1 μM Y-27632 (n=6) and by 10 nM CGRP (n=6). The actin cytoskeleton was visualized by Alexa-488-conjugated phalloidin in fixed and permeabilized vessels. Confocal microscopy revealed that smooth muscle cells of the SMA contain a single layer of thick actin bundles.

In summary, these data demonstrate that ET1 induces a Ca^{2+}-sensitization of the myofilaments through activation of Rho-kinase and that CGRP causes a desensitization of the myofilaments.

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20 Ventral approach to inner ear in rat can preserve cochlear function

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Investigation of cochlear function is often hindered since the mammalian inner ear is difficult to reach without cochlea functional change, particularly in the rat and mouse. The cochlea of the rat and mouse is much smaller than that of guinea pig and rabbit. However, the rat and mouse offer more experimental advantages than other species such as guinea pig and rabbit since their genome is well characterised and in addition, there is significant homology between rodents and

human genome especially mouse. The goal of the present study was to develop a new surgical method to access rat cochlea. A ventral approach through the neck to access the undersurface of the tympanic bulla was performed. 10 rats were investigated in the study and a small hole was made in the cochlea at the scala tympani.. Auditory brainstem response (ABR) was measured from 4 kHz to 40 kHz at pre and post-surgery. We found that ABR thresholds were not statistically different between pre and post-surgery. Thus the ventral approach to the rat cochlea is safe and advantageous since it gives the surgeon more direct view of the cochlea and round window, and in particular it avoids injury of the stapedial artery as compared to post-auricular approach to the inner ear. Thus, the rat and mouse can be used as animal model to apply osmotic pump and round window catheter to deliver drug, and the information might be important for human.

21 A Flexible System for Measurement of the Compound Action Potential and Otoacoustic Emissions

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The compound action potential and otoacoustic emissions are commonly used measurements when assessing cochlear function in hearing research. However, the equipment setup required to do this either involves complicated in-house hardware and software development or the use of commercial equipment that often has limited flexibility. A software algorithm was designed to measure the compound action potential threshold, distortion product otoacoustic emissions, and the ipsilateral medial olivocochlear reflex response in guinea pigs. The software was written in MATLAB code and is run through a graphical user interface. This interface permits easy control of the frequency and amplitude ranges, as well as trial and repetition parameters. There is on-line graphical feedback representation of the electrophysiologic responses, to permit active user manipulation of the stimulus parameters as required. Data is stored in text files that are easily accessible for analysis by MATLAB, EXCEL, or other graphical software. A laptop computer runs the software, and drives Tucker-Davis Technology system III hardware via USB cable. The benefit of this system over what is currently available is that it is portable between different computers, can be run by less experienced personnel, and allows great flexibility in stimulus and recording parameters. This experimental setup could be easily adapted to most multi-user laboratories.

22 Trigeminal Ganglion Effects on Neurons in the Cochlear Nucleus.

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The trigeminal ganglion sends a projection to the ventral cochlear nucleus (VCN) of the guinea pig (Shore et. al., J. Comp. Neurol. 419:271-285) and to the cochlea (Vass et al., Neuroscience 84: 559-567). The synaptic terminals of the VCN projection end in the granule and magnocellular regions. We investigated the function of these pathways on VCN activity by electrically stimulating the trigeminal ganglion while recording spontaneous activity from neurons in the VCN.

A concentric, bipolar stimulating electrode was placed stereotaxically into the ipsilateral trigeminal ganglion (0.37 cm caudal to bregma, 0.45 cm lateral from the midline and 1.3 cm ventral to bregma). Electrical stimuli were applied as bipolar pulses, 100 ms per phase, at intervals of 200 ms. Current amplitudes ranged from 10-100 mA. After aspirating a portion of the cerebellum overlying the cochlear nucleus, responses from single units were obtained using a single shank, 16-channel electrode to enable simultaneous recordings multiple neurons. The majority of units recorded in the anteroventral and posteroventral divisions of VCN showed a complex response to trigeminal ganglion stimulation, consisting of an inhibitory phase followed by two excitatory phases. A small percentage of units showed no change, or decreased spontaneous rate. After applications of neomycin sulphate to the cochlear fluids, to eliminate the cochlear portion of the pathway, the excitatory components disappeared, leaving only an inhibitory component. Removing the cochlear portion of the pathway thus resulted in net inhibition of VCN units, suggesting that the trigeminal innervation of the cochlea is excitatory to VIIIth nerve fibers.

These results demonstrate that a projection from a predominantly somatosensory ganglion can influence the activity of second-order auditory neurons, and may play a role in integration mechanisms involving the cochlea and its central targets.

23 Effects of Trigeminal Ganglion Stimulation on the Central Auditory System.

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A projection from the trigeminal ganglion to the ventral cochlear nucleus (VCN) of the guinea pig was recently described (Shore et. al., J. Comp. Neurol. 419:271-285). The synaptic terminals of this projection end in the granule and magnocellular regions of the VCN. We investigated the effect of electrically stimulating the trigeminal ganglion on the central auditory system activity using 2-deoxyglucose (2-DG) autoradiographic techniques.

Guinea pigs were anesthetised with ketamine and Rompun and held in a stereotaxic device. Rectal temperature was monitored and maintained at 38° + 0.5°C. The bone overlying the cerebellum and posterior occipital cortex was removed to allow stereotaxic placement of a concentric, bipolar stimulating electrode into the ipsilateral trigeminal ganglion (0.37 cm caudal to bregma, 0.45 cm lateral from the midline and 1.3 cm ventral to bregma). Electrical stimuli were applied as bipolar pulses, 100 ms per phase, at intervals of 200 ms and amplitude of 100 mA. Control animals were not stimulated. 2-DG was administered by intramuscular injection. Following a 1-hour incorporation period, animals were sacrificed, the brains rapidly harvested, and prepared for autoradiography using standard techniques. Autoradiographs were analyzed using a computer-assisted video densitometry to determine film optical density in the central auditory regions of interest. The cerebellum was also sampled as a grey matter indifferent intra-brain control region.

Results showed systematic and significant differences between 2-DG uptake in the cochlear nucleus ipsilateral to trigeminal ganglion stimulation when compared to the contralateral side. Differences also were found between control and stimulated animals. These results demonstrate that a projection from a predominantly somatosensory ganglion can influence the activity of second- and higher order auditory neurons.

24 Interactions of excitatory and inhibitory synaptic inputs in the MSO.

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The medial superior olive (MSO) is a stack of bipolar neurons involved in sound localization. We investigated membrane properties of MSO cells in 4-15 day old gerbil brain slices, by using patch electrodes. Resting membrane potential (RMP) decreased linearly with age; at 4-9 days the average RMP was –44 mV, at 10-15 days the average RMP was –52 mV. MSO cells exhibited non-linear current vs. voltage (I-V) curves, with lower input resistance at depolarized voltages (206 vs. 164 Mohms). MSO cells were filled with lucifer yellow and neurobiotin and visualized after fixation by using immunocytochemistry. Well-filled cells were reconstructed in 3D using Neurolucida, modeled as

cylinders and imported into a neuronal simulation program (NEURON). The membrane of the model MSO cell was outfitted with Hodgkin-Huxley type sodium and potassium channels, and low-threshold potassium channels. Excitatory inputs were placed on the midway point of all second order dendrites. Interaural temporal delays (ITDs) were simulated by activating (at 100 Hz) one side of the cell prior to the other while recording EPSPs in the cell body of the model cell. ITD curves revealed half-maximal activation at 400 usecs ITD. EPSPs recorded from the model cell were imported into recording software (Clampex) and injected into a living MSO cell. Electrical activation of the MNTB and LNTB elicited IPSPs in MSO cells in the presence of glutamate receptor blockers (CNQX, APV and MK-801). IPSPs reversed at –87 mV and decayed with an average time constant of 2.4 msecs. In a few MSO cells, we coupled MNTB stimulation with injection of simulated binaural excitation through the recording pipette. Inhibitory inputs delayed MSO cell activation from the beginning EPSP cycles to later in the EPSP train. We are exploring the effects of temporal interactions of inhibitory and excitatory inputs on MSO activity patterns.

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25 Trigeminal Projections to the Cochlear Nucleus in Rats

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The auditory system has long been known to participate in cross-modality interactions, including those with somatosensory functions. It has been hypothesized that some somatosensory afferents provide information related to pinna and head position for the purpose of localizing a sound source in space (Young et al., J.Neurophysiol., 1995). It has also been speculated that somatosensory pathways which project into the auditory system are involved in the modulation of tinnitus (Levine, Am.J.Otolaryngol., 1999). One target of these somatosensory projections is the granule cell domain (GCD) of the cochlear nucleus (Wright and Ryugo, J.C.N., 1996). The GCD receives highly diverse inputs and projects to the dorsal cochlear nucleus(DCN), which in turn projects to higher auditory structures. Thus the GCD emerges as a multimodal, integrative nexus within the auditory system, and our goal is to identify the origin of cells projecting to it. This study focuses on inputs to the GCD from the trigeminal sensory complex.

The fluorescent retrograde tracer Fast-Blue was injected into the GCD lamina in 11 rats. After a 5-6 day survival period, the distribution of labeled cells was determined throughout the brain. Four rats did not exhibit injection sites limited to the cochlear nucleus and were thus discarded. In all remaining rats, labeled cells were observed in the trigeminal nuclear complex as well as the vestibular nuclei, gracile and cuneate nuclei, and pontine nuclei. The spinal trigeminal nuclei (pars caudalis,interpolaris, and oralis) contained many labeled cells, predominantly ispilateral to the injection site and mostly in pars interpolaris. Anterograde tracing experiments are underway to determine the synaptic nature of the projections. These data may provide a morphological substrate to observations of somatosensory influences in the perception of tinnitus.

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26 Synapses and Postsynaptic Targets of Type II Auditory Nerve Fibers in the Granule-Cell Lamina of the Mouse

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Type II auditory nerve fibers provide the afferent innervation of outer hair cells in the cochlea and project to the cochlear nucleus. The synaptic ultrastructure and postsynaptic targets of type II endings in the cochlear nucleus are not well known. We examined type II endings in the neuropil adjacent to the granule cell lamina (between the ventral and dorsal divisions of the cochlear nucleus), because in this area the swellings of type II axons become angular and complex-shaped and because the more numerous type I axons do not project here. Type II axons were labeled by extracellular injections of HRP into the cochlea of 4 mice, 7 type II axons were first drawn using the light microscope, and then portions of these fibers in the lamina were studied with serial-section electron microscopy.

A total of 27 synapses were observed, most commonly from angular or pedunculated en passant swellings, but not from ellipsoidal swellings or intra-swelling sections of the axons. Type II synapses are relatively simple--they had few, round vesicles, a synaptic cleft, and a meager postsynaptic density. The most frequent target was small dendrites or their spines; the soma of origin of these dendrites could not generally be identified. However, one small cell in the lamina with 3 small dendrites was a target, receiving a type II synapse on a proximal dendrite swelling. A less common target was large dendrites; although these dendrites could not generally be identified, somatic type II synapses were seen in 2 mice onto five moderately large neurons that produced large dendrites. These cells were located on the VCN edge of the lamina. Some large-dendrite targets had other, unlabeled synapses with postsynaptic bodies, suggesting that they also receive input from branches of medial olivocochlear axons. Overall, these results suggest that type II endings in the granule-cell lamina convey information to several types of neurons in the cochlear nucleus.

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27 Three-dimensional Reconstruction of Synaptic Structures in the Small Cell Shell of the Cochlear Nucleus.

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Still a challenge to current views of synaptic function, the synaptic nest was originally described in the medial geniculate body (Morest, 1971) as an aggregation of serial synaptic junctions without glial apposition, clearly distinguished in their structure from the glomeruli of the cerebellum and olfactory bulb. Similar structures, described variously as nests, glomeruli, or mitts, have been reported. The prevalence of such structures in the small cell shell of the cochlear nucleus provides an opportunity to analyze the key features of their synaptic organization in a relatively coherent region. Adult mice were perfused with an aldehyde fixative. Blocks were dissected from 200 μm thick coronal vibratome sections, osmicated, embedded in epon, and trimmed to 0.5 mm^2 faces. Serial thin (700 Å) sections were mounted in ribbons on sequential Formvar-coated slot grids. Serial photographic negatives were digitized for analysis with software developed at Boston Univ. (http://synapses.bu.edu). Reconstructions clearly differentiated individual synaptic sites apposed by glial processes from nest-like structures. The glomerulus (micronest) is organized around a single excitatory mossy fiber terminal without glial investment but flanked by terminal dendritic processes. The synaptic nest (macronest) is an accretion of many axonal terminals organized around at least one prominent dendrite and several smaller dendritic processes, all in direct contact without glial investment. Previously, we have reported (Josephson & Morest, 2000) that synaptic nests, especially macronests, lack high-affinity glutamate transporters suggesting an enhanced capacity to accumulate extracellular excitatory transmitter. If so, the nests may process neural information differently than classical synapses. Nests may also respond differently to overstimulation.

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28 Functional Input from the Inferior Colliculus to Cochlear Nucleus Neurons: an in vitro Whole Brain Study

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In addition to external acoustic information coming from auditory nerve (AN) fibers, the cochlear nucleus (CN) receives feedback inputs from central auditory structures and projections from non-auditory sources. The existence of these projections suggests an important role of auditory and non-auditory inputs in shaping the response properties of CN neurons, which in turn must subserve some fundamental functions. One of the major descending auditory pathways to CN neurons originates in the inferior colliculi (IC). However, the effects of this input on CN neurons are unknown. In the present study, using the isolated whole brain (IWB) preparation of the guinea pig, we directly assessed physiological responses of intracellularly recorded and stained CN cells to electrical stimulation of the IC. Stimulation of the contralateral IC evoked both excitatory (EPSP) and inhibitory (IPSP) postsynaptic potentials in CN cells as well as antidromic activation of some cells. Synaptic responses were observed in principal cells of all three subdivisions of the CN indicating a widespread influence of IC inputs onto the CN. The latencies of EPSPs and IPSPs were in the range of 5 - 9 ms suggesting oligosynaptic (di-, and trisynaptic) transmission. We propose that excitatory transmission to principal CN cells operate via direct IC projection to CN granule cells, whereas the inhibitory responses could be mediated by IC projections to ventral periolivary nuclei which project in turn to the CN. Additional experiments are needed to test the hypothesis for two distinct IC pathways exerting opposite effects on principal CN cells.

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29 3-Dimensional Reconstructions of Cochlear Nucleus Neurons in Guinea Pigs: Structure-Function Relationships Revealed in the *in vitro* Whole Brain Preparation.

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Our goal in this project was to reveal structure-function relationships in the cochlear nucleus by correlating the 3-dimensional structure of individual neurons with their physiological responses to various inputs. We used the in vitro whole brain preparation of the guinea pig to intracellularly record and stain neurons in the cochlear nucleus (Babalian et al., NeuroReport, 10:1913, 1999). While recording intracellularly, we tested the synaptic responses of CN neurons to electrical activation of inputs from each auditory nerve, the ipsilateral dorsal column nuclei, and the ipsilateral trigeminal nerve. At the end of each experiment, the brain was perfused with 4% paraformaldehyde, sectioned, and histologically processed using standard ABC procedures. We made 3-dimensional reconstructions of these neurons using a light microscope and computer software (Neurolucida v4.33). In this way, we were able to relate dendritic orientation, and physiological response properties to overall structure of the nucleus. Among the cell types reconstructed were multipolar, and octopus cells in the VCN. Octopus cells could be distinguished physiologically and structurally from other cell types: they never responded to dorsal column or trigeminal nucleus stimulation, exhibited "gradual spikes" and were oriented orthogonal to the isofrequency planes. Multipolar cells were of 2 basic types that could be determined accurately through 3-dimensional reconstructions: planar cells have their dendrites oriented parallel to the incoming auditory nerve fibers, whereas radiate cells have their dendrites lying across the path. Planar cells exhibit smaller IPSPs following stimulation of the ipsilateral auditory nerve compared to radiate cells, although there was no dramatic difference in their responses to somatosensory stimulation.

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30 Projections of Multipolar Neurons in the Ventral Cochlear Nucleus to the Lateral Superior Olive in Rats

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We are studying two types of multipolar neurons in the ventral cochlear nucleus (VCN) of the rat: planar cells and radiate cells. Planar cells have dendrites oriented parallel to VCN isofrequency sheets and project tonotopically to the dorsal cochlear nucleus (DCN). Radiate cells have dendrites oriented perpendicular to the isofrequency planes and project broadly to the DCN. In this study, we examined the projections of these neurons to the superior olivary complex. An extracellular injection of biotinylated dextran amine (BDA) was made into the DCN of each rat. After histological processing, we observed a mediolateral stripe of BDA-filled structures in the VCN that included planar cells. The stripe was in tonotopic register with the injection site. Labeled radiate cells were scattered across the tonotopic axis. We also observed a prominent stripe of labeled axons and terminals in the ipsilateral lateral superior olive (LSO) that was oriented parallel to the plane of isofrequency sheets. Our interpretation is that the LSO stripe is due to BDA being anterogradely transported by planar cell axons to their terminals. First, cutting the dorsal acoustic stria after the DCN injection (severing the DCN projection axons) failed to abolish labeling in the LSO. Second, thin collaterals from planar cell axons in the trapezoid body terminated in the stripe. Third, the position of the LSO stripe was systematically and topographically related to the position of the DCN injection site. Collectively, these observations imply that planar cells project tonotopically to the LSO. Multipolar cells correlate with the physiological class referred to as choppers, and choppers are thought to encode stimulus intensity. Our hypothesis is that planar cells provide intensity information to LSO cells that is used to compute interaural level differences for localizing sounds in space.

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31 Bidirectional synaptic plasticity in the dorsal cochlear nucleus

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The superficial layers of the dorsal cochlear nucleus (DCN) resemble the cerebellum and the sensory lobe of the electric fish (Mugnaini et al. 1980; Bell et al. 1997). Plasticity at parallel fiber synapses in these structures controls the gain of synapses at the interface between sensory and motor pathways. Cerebellar Purkinje cells show long-term depression (LTD) (Ito et al., 1982). Principal cells in the electric fish show both long-term potentiation (LTP) and LTD (Bell et al., 1997). Our results indicate that there is bidirectional plasticity at the glutamatergic synapses between parallel fibers and their targets in the mammalian DCN.

Whole-cell, voltage-clamp recordings were made from cartwheel and fusiform cells in coronal slices from mice. LTP was induced by high-

frequency synaptic stimulation (HFS; 100 Hz, 1 sec, twice) paired with depolarization from a holding potential of -80 to -30 mV. LTD was induced by low-frequency stimulation (LFS; 1 Hz, 5 min) paired with a similar depolarization. Stimulation of parallel fibers induced both LTP and LTD in both fusiform and cartwheel cells. In both cell types, LTP (LTD) could be reversed by subsequent LFS (HFS). Stimulation of the deep layer evoked EPSCs in fusiform cells that showed neither LTP nor LTD. To determine whether NMDA glutamate receptors and/or metabotropic glutamate receptors (mGluR) mediate plasticity, the sensitivity of LTP and LTD was tested to 100 μM APV and 200 μM LY341495, blockers of NMDA and mGluRs respectively. APV blocked LTP in 9/18 and LTD in 3/6 fusiform cells. LY341495 blocked LTP in 5/8 and LTD in 0/6 fusiform cells. In cartwheel cells, APV blocked LTD in 8/8 cells; LY341495 affected neither LTP or LTD. Thus both NMDA and mGluRs are involved in plasticity in the apical dendrites of fusiform cells, and NMDA receptors are involved in LTD in cartwheel cells.

32 SAM stimulus response of DCN single units indicates temporal integration and coincidence detection

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Previous researchers have examined temporal coding properties of single units in the cochlear nucleus. They documented phase locking as well as spike entrainment, in response to SAM stimuli. Ghoshal and Kim (1994) supported the hypothesis that these observations could be explained by interval regularity due to refractoriness. However, Joris et al (1994) have shown that leaky integrate-and-fire neuron models of temporal and spatial integration, sensitive to spike time coincidence in their inputs, exhibit strong spike entrainment accompanied by strong phase locking. Additionally, Yang et al. (1999) proposed spike time coincidence models that incorporate inhibition predominantly found in different centers of the auditory brainstem. To examine the significance of entrainment and phase locking as coincidence mechanism indicators, differences in the variation of entrainment and phase locking measures, between coincidence models and refractory models, need to be characterized.

We previously presented mathematical measures that aid in quantifying phase locking properties (Marangos et al. 1998) and differentiating between rate modulation and spike entrainment in single unit firing (Aggarwal et al. 1999). In this study, we quantify entrainment and phase locking for different coincidence models, and then compare the results with refractory neuron models. We also compare the results of this modeling study with DCN single unit recordings, where (i) most cells exhibit strong spike entrainment as well as phase locking, and (ii) temporal and spatial integration as well as inhibitory mechanisms have been reported in previous anatomical and physiological studies. This data analysis supports the hypothesis that temporal integration and coincidence detection mechanisms exist in the DCN, and are manifested in the SAM response properties of single units.

Comparison with ISI regularity analysis and intrinsic oscillation analysis results validates the SAM response analysis results.

33 Ventrotubercular Projections in the Rat Cochlear Nucleus: Identification of a Dimorphic Population of Small Cells

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Previous studies in our laboratory have demonstrated two populations of multipolar cells in the VCN that project to isofrequency regions in the ipsilateral DCN: planar and radiate cells. Planar cells lie within the VCN isofrequency sheet and may subserve narrow band processing. Radiate cells extend across VCN isofrequency sheets and may provide broadband information to the DCN. Recently, ultrastructural analyses of these cell populations has been performed and we identified a third, dimorphic population of cells that lie within VCN isofrequency sheets. In brief, biotinylated dextran amine (BDA) was iontophoresed into the DCN in Sprague-Dawley rats (n=7). After 24 hours survival, the brainstems were processed and sections with labeled cells were taken for electron microscopy. Each labeled cell body was photographed at 2.7k for area and perimeter measurements and at 14k for analyses of somatic synaptic contacts. Our results revealed a population of cells (n=11; area: 137.86um2 ± 25.6), not apparent in the light microscope, that is significantly smaller than both planar (n=10; 232.47um2 ± 59) and radiate cells (n=15; 333.89um2 ± 123.2). Analysis of somatic contacts revealed two subpopulations of small cells, those with low synaptic input (n=6, 25.96% ± 9.7 of cell surface apposed) and those with high synaptic input (n=5, 86.47% ± 2.9). Morphologically, several of the high apposition cells resembled chestnut cells, previously described only in the granular cell domain (Weedman et al., JCN, 1996). The small cells were evenly distributed along the medial-lateral axis of the nucleus and no small cells were identified outside the tonotopic stripe. The confinement of these cells to the tonotopic stripe suggests that they subserve functions related to narrow-band signal processing. Further studies will examine neurotransmitter properties of these cells.

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34 Developmental Patterns of Potassium Channel mRNA Expression in Rat Cochlear Nucleus

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Voltage gated potassium channels play critical roles in determining the responses of auditory brainstem neurons to acoustic stimuli, and individual classes of cells express specific patterns of channel subunits to accomplish their information processing tasks. In the present study, we examined the developmental expression patterns of potassium channels in rat cochlear nucleus, from postnatal days 3 to 56.

RT-PCR was carried out on total RNA isolated from the whole cochlear nucleus of individual rats, using separate RT and PCR steps. Relative quantification of mRNA levels was performed using a PE5700 thermal cycler, using primers and probes specific for KCNA1, KCNA2, KCNC1 and KCND2. Quantification was performed relative to 18S rRNA and GAPDH. The 18S and GAPDH levels exhibited an approximately constant ratio across the developmental time range that we examined.

KCNA1, KCNA2 and KCNC1 showed a monotonic increase in mRNA levels, from postnatal days 3 to 30. KCNA1 expression, relative to 18S rRNA, ranged from $1*10^{-6}$ at P3 to $1*10^{-5}$ at P30. There was a slight decrease in expression levels after P30, and levels were constant until P56. KCNA2 and KCNC1 showed parallel but smaller increases. In contrast, KCND2 mRNA showed a large amount of scatter between individual animals, but levels decreased by a factor of 2 after P30.

KCNA1 and KCNA2 can combine to form heteromultimeric potassium channels, and together are thought to be responsible for the low threshold potassium conductance in bushy and octopus cells. We observed a developmental shift in the relative levels of KCNA1 and KCNA2 mRNA. KCNA2 became less abundant by a factor of 2, relative to KCNA1, after P12. This suggests that there may be either a differential maturation of cells that express KCNA1 and KCNA2, or a developmental shift in the heteromultimeric composition of potassium channels containing these two subunits.

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35 Distribution Of Glutamate Receptors In The Cochlear Nuclei In Mice Lacking The AMPA Receptor Subunit, GluR2.

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AMPA-ionotropic glutamate receptors (AMPARs; 4 subunits, GluR1-4) mediate fast transmission in the CNS. These receptors are calcium permeable when GluR2 is absent. Since calcium acts as a 2nd messenger to potentiate synaptic responses, changes in AMPAR composition can alter normal synaptic function. AMPARs in synapses on spherical bushy cells of the anteroventral cochlear nucleus (AVCN) contain no GluR1, low GluR2 and high GluR3-4. In outer layers of the dorsal CN (DCN), synapses on apical dendrites of fusiform cells have only GluR2-3, while cartwheel cell dendrite synapses have all 4 subunits. The latter 2 synapses also have high delta GluRs. Outside of the DCN, such high levels are seen only in Purkinje cell synapses in cerebellum. In the AVCN of the GluR2 mutant (KO), levels of immunogold labeling for GluR2/3 antibody remain about the same as in wild type (WT) synapses. This is expected since only low GluR2 is found in normal animals, so that antibody labeling reflects mainly levels of GluR3; the latter apparently do not change much in KOs. In contrast, a significant decrease in GluR2/3 labeling (WT-0.82 [n=194] vs. KO-0.30 [n=141] gold/synapse) is seen in synapses of the KO outer DCN; this is expected since, in normal animals, these synapses contain substantial levels of GluR2. Interestingly, in these synapses, levels of delta increase significantly in the KO (WT-2.25 [n=165] vs. KO-3.04 [n=136] gold/synapse; or 7.76 vs. 11.69 gold/μm). A similar increase is seen for delta receptors in synapses of the cerebellum (Petralia et al., Soc. Neurosci. Abs. 2001), where delta receptors are involved in LTD in normal animals. Thus, we predict that synaptic function in the AVCN changes little in KOs, while profound changes are expected in the DCN. Also, the increase in delta receptors in KO synapses suggests that lack of GluR2 has altered the regulation of glutamate receptors.

36 The effect of random maskers on comodulation masking release in the cochlear nucleus

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Masking of a sinusoidal signal is considerably less when the masker has coherent level fluctuations across-frequency. This effect is called comodulation-masking release (CMR). In a recent study (Pressnitzer D., Meddis, R., Delahaye, R., and Winter, I.M. 2001, J. Neuroscience, 21, 6377-6386), using amplitude-modulated tones as the masker, it was shown that the responses of some units in the ventral cochlear nucleus of the guinea pig are consistent with the psychophysical CMR effect. This study investigates, if the type of neurons that showed CMR with amplitude-modulated tones as the maskers also show a CMR-like effect when noise maskers were used instead. In contrast to amplitude-modulated tones the noise masker waveforms will be different for each presentation. The experiments were performed on pigmented guinea pigs, anaesthetised with Urethane (1.5g/kg) and supplemented with Hypnorm (1 mg/kg). The target signal was a pure tone signal at the unit's BF. Three different conditions were investigated. In a reference condition, the masker was a 20-Hz noise band centred at the signal frequency (on-frequency band). In the comodulated and the deviant condition, the masker consisted of the on-frequency band and several flanking bands above and/or below the BF. In the comodulated condition all noise bands had the same temporal level variations whereas in the deviant condition the level variations were independent of each other. The response was measured for different signal-to-noise ratios. With non-deterministic maskers 25% of the units showed considerably higher d' values for the comodulated condition than in the deviant or reference conditions at the same signal-to-noise ratio. These units were classified as choppers or low-frequency units. In our current population of units, if the response of the unit showed the CMR effect with the deterministic masker, it also showed CMR with the non-deterministic masker.

37 Responses to Combinations of Tones in the Cochlear Nucleus of Awake Mustached Bats

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Acoustic behaviors including orientation and social communication depend on neural integration of information across the sound spectrum. Spectral integration is performed by combination-sensitive neurons, which show time-sensitive interactions (facilitatory or inhibitory) between distinct acoustic elements in complex signals. In the mustached bat (*Pteronotus parnellii*), combination-sensitive neurons are abundant in the inferior colliculus (IC). Facilitatory combinations-sensitive neurons are thought to be created in the IC, but the origin of inhibitory interactions is unclear. Because CN neurons project to combination-sensitive neurons in the mustached bat's IC, we examined whether neurons in the CN display the same combination-sensitive response properties that occur frequently in the IC. Using tracer-filled micropipettes and stereotaxic procedures, we recorded single unit responses in awake mustached bats to tones, noise, or combinations of tones or noise. Results are from 64 singles units localized to the three major divisions of the cochlear nucleus. The majority of neurons (36 units) were tuned to a single frequency band, with little evidence of two-tone interactions. Twenty-three percent (15 units) showed multi-peaked tuning curves. Twenty percent (13 units) showed inhibitory interactions that could not be described as sideband inhibition. No units showed facilitatory interactions similar to those observed in the IC. The multi-peaked tuning curves and inhibitory spectral interactions were found in all major subdivisions. These results demonstrate that spectrally complex response properties occur throughout the CN. However, the frequency range of interactions was different than what occurs among the majority of combination-sensitive neurons in the IC. This suggests that facilitatory and most inhibitory combinatorial response properties in the IC are created above the CN.

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38 Adaptation of evoked near-field potentials of the cochlear nucleus in response to pulsatile acoustical stimulation in awake rats.

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In order to study the properties of neural adaptation in response to acoustic stimulation, near-field evoked potentials were recorded from a chronically implanted electrode in the cochlear nucleus (CN) of awake Long Evans rats. Auditory thresholds were measured for each animal (n=8) and adaptation was investigated with repetitive 50 ms acoustical clicks delivered in trains of 250 ms duration separated by a silence of 250 ms. Eleven intra-train click rates were tested ranging from 100 to 2000 pulses per second (pps). The amplitude and the occurrence time of the first negative deflection (N1) of the averaged CN field potential were determined for each click in the train by using a subtracting method. As expected, a large response was obtained for clicks at the onset of the train followed by a fast exponential amplitude decrement and a plateau. This adaptation process was the more accurately fitted with a two time constants equation which allowed to describe a rapid, a short-term and the beginning of a long-term adaptation. At low click rate (100 pps), the rapid and short-term adaptation duration (respectively 24 and 74 ms) and time constants (respectively 6.7 and 41.1 ms) were in line with auditory nerve data (Westerman and Smith, 1984). At rates higher than 1000 pps, these latter constants did not vary

any more, an observation consistent with the absolute refractory period of the auditory nerve. For rates ranging between 400 and 1000 pps, constants of adaptation were very close to what was observed above 1000 pps, suggesting that the current adaptation was related to the relative refractory period of the auditory nerve. In conclusion, adaptation recorded in the cochlear nucleus well reflects the adaptive properties of the auditory nerve.

Westerman, L.A. and Smith, R.L., 1984. Rapid and short-term adaptation in auditory nerve responses. Hear. Res. 15: 249-260.

39 A Steady-State Auditory Response Evoked from Dichotic Tones.

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During recent years, a number of cochlear implant users have been fitted bilaterally. Benefits for adults have been shown to include improvements in localization ability. Psychophysical tests also suggest useful binaural processing abilities in at least some patients. Evaluation of the bilaterally implanted child may be problematic. There is therefore a possible need for an objective test of binaural processing.

Low frequency tones with differences of a few Hz presented dichotically elicit a perception of the difference tone (the binaural beats phenomenon). A search was made in 3 normal hearing subjects for a steady state evoked potential reflecting this difference frequency. Tone pairs of the order of 400, 600 and 800Hz were generated digitally. A trigger was generated digitally every 4th beat, to allow data capture by an evoked potentials recording system synchronized to the difference frequency. Recording was carried out with both diotic and dichotic application of the two tones. All tone pairs investigated elicited a discernible steady-state potential when applied diotically. Dichotic presentation yielded a small steady-state potential, discernible with long-term averaging of several thousand stimuli, at 3Hz and 40Hz difference frequencies, applied through insert phones at an intensity of approximately 60dBSL (+/-10dB). This reached an amplitude of 300-500nV, a factor of approximately 4-5 less than the potential evoked from the diotic stimulus.

Further studies will evaluate the dependence of the response on stimulus characteristics. The stimulus might also be encoded and applied through a cochlear implant, and the response characteristics correlated with binaural processing. However the test requires long recording times for the lowest difference frequencies, which may limit its potential usefulness as a clinical measure of binaural function.

40 Rapid acquisition of auditory brainstem responses with high frequency (8-14 kHz) tone burst trains

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Auditory brainstem responses (ABR) to three sequences of tone burst stimuli were compared in four groups of subjects; two normal hearing and two sensorineural hearing loss groups (N=20 or 30). The tone burst sequences were; 1) a single burst; 2) a five-stimulus sequence or train; and, 3) a fifteen-stimulus train. The tone burst center frequencies were at 6, 8, 10, 12 and 14 kHz. The ABR Wave V response latency & amplitude were used to determine the test-retest reliability and the probability of response detection at different frequencies and levels. For each sequence duplicate responses were obtained (in two different sessions), such that both intra- and intersession reliability could be determined. Reliability was similar among the three stimulus configurations. Statistically significant differences (ANOVA) in Wave V response latency were found between the single and the 15-stimulus train. The latency delay (about 0.2 ms) from the train indicate the presence of response adaptation when the frequencies of sequenced stimuli are closely spaced (1/3 octave or less), however, the significance of these differences depends upon the intended use of these stimulus trains. For many purposes these small differences are not biologically significant. For example, in the detection of ototoxicity, where a subject serves as their own control, a small amount of response adaptation does not preclude the use of closely spaced frequencies for serial monitoring. Stimulus trains are demonstrated to be efficient and suitable for the rapid acquisition of high frequency ABRs.

Oral presentation only

Audiovisual Requirements: Standard: one slide projector.

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41 Effects of Stimulus Repetition and Background Noise on Speech Evoked Auditory Brainstem and Frequency Following Responses in Normal and Learning Impaired Children

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This study investigated whether auditory brainstem and frequency following responses (ABR and FFR, respectively) to rapidly presented speech stimuli, presented in quiet and in noise, differed between normal and learning-impaired children. Subjects were normal-hearing school-age children. Normal subjects (NL) scored significantly better than learning-impaired subjects (LP) on standardized measures of reading and spelling and on a speech sound discrimination task. Responses were elicited by a 40 ms /d/, presented at 80 dB SPL. Stimuli were presented in trains of four repetitions, separated by 12 ms within and 30 ms between trains. Stimuli were presented in quiet and in white noise (S/N +15).

Addition of noise diminished most measures of response amplitude and synchrony, and increased all measures of response latency. Stimulus repetition resulted in increased latency of ABR wave V and decreased RMS amplitude of the FFR. The amplitude of a major FFR peak which occurred roughly 37 ms post stimulus onset was larger in NL than in LP subjects. Also, the 200-700 Hz frequency region of the FFR contained more energy in NL than in LP subjects. This finding, also shown by Cunningham et al. (2000), is intriguing; the group difference occurs in the same spectral region as does the first formant (F1) in stimuli along the /da/-/ga/ continuum. This stimulus component is crucial to behavioral discrimination. The amount of energy in the FFR over this spectral region correlated with behavioral discrimination along the /da/-/ga/ continuum.

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42 Hearing in "primitive" fish: brainstem responses to pure tone stimuli in the lake sturgeon, Acipenser fulvescens.

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We investigated the auditory capabilities of the lake sturgeon, Acipenser fulvescens. Sturgeon belong to the Acipenseriformes, an ancient group of fishes that arose early in the evolution of bony fish (late Triassic period). The purpose of this study was to test the hypothesis that basic functions of the auditory system developed very early in vertebrate evolution. We also selected lake sturgeon because the gross morphology of it's ear resembles that of teleost fishes and other vertebrates.

To test the hearing capabilities of lake sturgeon, we recorded auditory evoked potentials to pure tone stimuli of varying frequency and intensity using the auditory brainstem response (ABR) method. Our data show that lake sturgeons detect pure tones from 100 to 2000 Hz, with best sensitivity from 100 to 400 Hz.

We compared the sturgeon ABR data with responses obtained in two different species of teleosts, the oscar (Astronotus ocellatus) and the goldfish (Carassius auratus), using the same setup and data analysis as used in the sturgeon. The ABR data for the lake sturgeon are more similar to the goldfish, a hearing specialist that can hear up to 5000 Hz, than to the oscar, a hearing non-specialist that can only detect sound up to 400 Hz. Best frequencies of the goldfish are between 600 Hz and 1000 Hz, which is higher than the lake sturgeon. Whether the lake sturgeon can be considered specialized for hearing needs to be investigated further, particularly with respect to a potential relationship between the ear and any peripheral sound detecting structure. This result is of evolutionary interest since the lake sturgeon (belonging to an ancient group) seems to share brainstem response characteristics with teleosts, that are a highly successful and diverged group of fish. This indicates similar functions of the auditory system may already be found early in vertebrate history.

43 Brainstem Responses of American Shad, Alosa sapidissima, to Ultrasound

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Many toothed cetaceans use high frequency clicks (40-120 kHz) to identify objects in their environment, including prey. A number of species of clupeid fishes (herrings, menhaden and shads), are able to detect ultrasonic sounds to well over 100 kHz (e.g. Mann et al., 2001) suggesting that this ability evolved to avoid predation by marine mammals. However, the mechanism of ultrasound detection in these fish is still not understood. In this study, we investigated the response of brainstem nuclei of an ultrasound detecting species, the American shad, Alosa sapidissima, to learn about its response of ultrasonic versus sonic sound reception. American shad were presented with pure tone stimuli of 100 ms duration 20 dB above hearing threshold. Twenty-two units were recorded in eight experiments (14 multiunit and 8 single units). The units show several kinds of responses including tonic, phasic, and tonic-phasic. Twelve units responded in a phasic fashion to the stimulus, with two single units showing ON and OFF responses and two others showing inhibition during stimulation. Since ultrasound was used as a search stimulus, no purely sonic units were recovered. None of the units responded to frequencies below 10 kHz. Two types of units were found. One type responded to low ultrasound and sonic frequencies from 10 to 20 kHz, while the other type responded to sounds from 60 kHz to 97 kHz, with the strongest responses at 80 and 90 kHz. There does not seem to be any continuum for the whole ultrasound range in any unit. Since behavioral data (Mann et al. 1997) showed that American shad can detect a much wider range of frequencies than found in the units recorded, it is possible that different ultrasound frequencies are represented in different units, or different regions of the brain. There does not appear to be common processing of sonic and ultrasonic sound. This gives rise to the questions about which sensory organ is responsible for ultrasound detection.

44 A neural model for binaural coincidence detection using both excitation and inhibition

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The Jeffress model implies that the maximum of the rate-ITD curve is determined by the coincidence of excitatory inputs and the minimum by the dis-synchrony of the inputs. Although models of MSO activity can match available in-vivo data with purely excitatory inputs (Han and Colburn, Hear. Res. 1993), a variety of evidence indicates the presence of inhibition in these cells. This study explores the possible role of temporally synchronized inhibitory inputs within a multi-compartment model similar to that of Simon et al.(ARO 1999). Initial work focuses on comparisons between responses to low-frequency (100-1000 Hz) periodic stimuli that are in-phase, out-of-phase, and monaural. With only excitatory inputs and no spontaneous activity, firing rates consistently varied from lowest to highest for monaural, out-of-phase and in-phase inputs, respectively. Adding periodic inhibitory inputs with fixed delays D relative to the excitation, we found that rates for the out-of-phase case can drop below those for the monaural case only for a narrow range of D values. Further, if D was held constant while the frequency varied, we found that most out-of-phase rates dropped but only those frequencies that were "matched" with the delay were lower than the monaural rate. The rate-ITD curve exhibited an increase in sharpness and in some cases the peak rate was non-monotonic with the frequency of inputs (as opposed to the curve without inhibition). This study raises the question of whether the delay D varies with CF and whether the reduction of the out-of-phase rate relative to the monaural rate would depend on the frequency of the inputs.

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45 Responses Of Ferret Superior Colliculus Neurons To Stimuli Presented In Virtual Acoustic Space

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Auditory localization depends on the processing of monaural and binaural spatial cues. Traditionally, studies of auditory localization have involved presenting sounds from a speaker in free space or directly to the ears via headphones. More recently, virtual acoustic space (VAS) stimuli have been employed in both psychophysical and neurophysiological experiments. Such stimuli allow faster and more detailed mapping of the spatial response properties of auditory neurons. In addition, the sound signal may be manipulated digitally, so that the contribution of different acoustic cues can be assessed independently.

The superior colliculus (SC) in the midbrain is of interest because it is one of the few brain areas to contain a neural map of auditory space. Previous studies have emphasized the role of monaural spectral cues and interaural level differences (ILDs) in constructing this map in the mammalian SC. Interaural time differences (ITDs) are generally thought not to be involved. We investigated this issue by mapping auditory spatial response profiles of deep layer SC neurons in anesthetized ferrets, using VAS stimuli generated from the animal's own ears. Most SC neurons were broadly tuned within the contralateral hemifield and their receptive fields expanded with increasing sound level. In keeping with the results of free-field studies, the speaker angle producing the best response was fairly well aligned with the visual receptive field of superficial layer units mapped in the same electrode penetration, and both varied systematically along the rostrocaudal extent of the SC. Auditory responses were also examined with VAS stimuli that had ITDs set to an inappropriate value while the remaining cues were allowed to vary naturally. This had no clear effect on the units' best position or response magnitude. Our findings confirm that sensitivity to ITDs does not contribute to the map of sound azimuth in the SC, which is presumably based on ILDs and spectral cues.

46 Responses of Auditory Cortical Neurons Sensitive to Interaural Time Differences (ITDs) to Changes in Interaural Correlation

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Sensitivity to ITDs, a major cue for sound localization, can be more broadly described as sensitivity to interaural correlation. Consequently, the responses of ITD-sensitive neurons to changing interaural correlations are of interest. Previous reports have described such responses at the level of the IC and below in anesthetized animals. For this study, we recorded responses in the auditory cortex of unanesthetized rabbits.For each neuron, we first identified the tuning to ITDs using noise and binaural beat stimuli. These allowed us to identify a best and worst ITD and the responses as peak-, trough- or

intermediate-type. We then measured the response to changes in interaural correlation at the best and worst ITDs. As in the IC of owls (Albeck and Konishi, J. Neurophys, 74:1689-1700, 1995), some neurons had linear responses to interaural correlations that varied from +1.0 to -1.0, and others had non-linear responses that could be described as parabolic or ramped. When tested at best ITD, both of the non-linear types reached a minimum of firing as the correlation approached zero and the response declined slowly if at all for negative correlations. The responses at the worst ITD were usually the reverse, in that they had a maximum response at a correlation of -1.0 that declined to a minimum by zero correlation with little or no further change for positive values of correlation. The correlation values where the responses became asymptotic varied considerably across non-linear neurons. For most neurons of both peak and trough type the response at the best ITD and a correlation of 1.0 was greater than the response at worst ITD and a correlation of -1.0. In a few the reverse was true.

The greater effect of correlation on the best compared to the worst ITD suggests that the peaks provide a more salient cue for correlation than the troughs.

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47 Sensitivity of Inferior-Collicular Neurons to Interaural-Phase Difference (IPD) in the Presence of Preceding Sound with Various IPD

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Psychophysical studies have shown the localization aftereffect (LA; e.g., Kashino & Nishida, 1998; Carlile et al., 2001): The perceived location of a signal is biased away from the location of a preceding sound with relatively long duration. The present study examined neural sensitivity to interaural-phase difference (IPD) in the inferior colliculus (IC), using stimuli that were relevant to conditions that would produce psychophysical LA. We recorded activities of single-units or multi-unit clusters with low best frequencies (BFs; ≤1500Hz) from the IC of the anaesthetized gerbil. Binaural stimuli were presented to the animal, each of which consisted of a sequence of the *adapter* and the *probe* with a brief (typically 10 ms) gap between them. The adapter and the probe were BF-tone bursts with 200- and 50-ms durations, respectively, at 20 dB above the unit threshold. The adapter and the probe varied independently in IPD. In control conditions, the probe was presented alone. Generally, the adapter reduced the spike rates for the probe. For units with relatively large IPD selectivity (i.e. large spike-rate modulation by the probe IPD in control conditions), the reduction of the probe-driven responses tended to increase with decreasing separation of the adapter IPD and the unit's best IPD. For units with little IPD selectivity, the presence of the adapter unchanged or increased somewhat the IPD selectivity. Overall, it appeared that not the adapter IPD *per se*, but the strength of adapter-driven responses accounted well for the reduction size of prove-driven responses. In either unit type, the best IPD and IPD tuning width of (adapted) units were invariant with the adapter IPD. This indicates that the psychophysical LA is not caused by changes in IPD tuning of specific groups of neurons. Possibly, the LA is a result of modulated distribution of activation across neural populations.

48 Interaural Correlation Functions in the Inferior Colliculus of the Guinea Pig

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Models of binaural hearing based upon interaural correlation account for much of the human discrimination data and are consistent with the responses of inferior colliculus (IC) cells to interaural time differences (ITD) in tones and noise. However, there are few reports of direct measurements of sensitivity of neurons to interaural correlation in mammalian species. Interaural correlation versus spike count functions (ICFs) from –1.0 to +1.0 (0.1 steps) were measured in low-frequency IC cells of urethane anaesthetised guinea pigs at both zero and Best ITD using broad-band (50 to 5000 Hz) and narrow-band (1 ERB around best frequency), 50 ms long, noises at 20 dB above threshold. The noise was resynthesized on each presentation and the sample correlation was used in the ICF. The ICFs were classified as either linear, quadratic or ramp, depending upon whether a straight line, 2nd order polynomial or function comprising a constant section plus a sloping section best fit the data. At zero ITD, linear ICFs were most common (26/47 narrow-band noise, 13/26 broad) followed by quadratic (15/47 narrow, 11/47 broad). At Best ITD, linear and quadratic ICFs were nearly equally common (9/23 linear, 12/23 quadratic, pooled bandwidths). Ramp type ICFs were uncommon for both zero (6/47 narrow, 2/26 broad) and Best (2/23) ITD. Analyses of single-cell interaural correlation discrimination were difficult because of high variability in the data, but discrimination thresholds obtained were at least at least an order of magnitude greater than human thresholds. Thus although single IC cells provide sufficient information for discriminating the ITD of deterministic signals (i.e. tones; Shackleton *et al.* ARO abstracts 24: 210) they do not for the discrimination of interaural correlation of random signals.

49 Inhibition Sharpens Tuning to Interaural Time Disparities (ITDs) in the Inferior Colliculus of the Unanesthetized Rabbit

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A major cue for the localization of sound in space is ITDs. We examined the role of inhibition in the sharpening of ITD tuning seen in the inferior colliculus (IC) compared to the superior olivary complex. Specifically, we examined the effects of GABA, a prominent inhibitory transmitter, and bicuculline, a GABA antagonist, on IC neurons sensitive to ITDs. The agents were iontophoretically injected through a multi-barrel pipette while simultaneously recording from single IC neurons. The effects on firing rate and ITD tuning were evaluated before, during and after the application of the different drugs. If GABA-mediated inhibition is involved in sharpening ITD tuning in IC neurons, then applying additional amounts of this inhibitory transmitter should further sharpen ITD tuning. Indeed, for almost all neurons tested, GABA considerably sharpened the ITD functions and reduced the firing rate. If inhibitory inputs serve to attenuate and sharpen the ITD response, as suggested by our GABA experiments, blocking inhibitory inputs with bicuculline should enhance the firing rate and broaden the ITD response. As predicted, bicuculline often dramatically increased the activity of IC neurons sensitive to ITDs and broadened their ITD tuning. A major source of GABA-ergic input to the IC comes from the dorsal nucleus of the lateral lemniscus (DNLL). Our preliminary experiments indicate that inactivating the DNLL broadens the ITD tuning of IC neurons on the opposite side.

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50 Sensorimotor Activity of Midbrain Neurons During Head Orienting in Awake Barn Owls (Tyto alba)

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The barn owl is a well established model for sound localization. In response to faint sounds barn owls turn their head precisely towards the direction of the sound source. A map of auditory space has been described for the owl's midbrain and was tested for behavioral relevance using focal electrolytic lesions and direct electrical stimulation. These studies showed that activation of midbrain neurons is sufficient to evoke

head turnings towards contralateral directions. However, it remained unclear if auditory neurons are separated from premotor neurons, thus requiring auditory information to be relayed from sensory to premotor parts of the optic tectum (OT).

Here we recorded extracellular activity from OT units of awake, behaving barn owls. Owls performed a cue-directed selection paradigm where a visual cue pointed towards the side of the next relevant auditory target. Owls responded to auditory stimulation with a head turn towards the sound source. Head orienting was measured with a magnetic tracking system. We found that most neurons (71%) with auditory-evoked activity also showed movement-related activation that depended on the response side: Orienting towards contralateral directions was paralleled by an increase in spike rate and orienting towards ipsilateral directions by a decrease below spontaneous levels. A similar but less explicit activation pattern was found before movement onset, suggesting an influence of spatial attention. These results show that OT units act as sensorimotor interface neurons and that they are possibly also involved in the control of non-reflexive orienting behavior.

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51 Effects of SPL on Sensitivity to Interaural Intensity Differences in the LSO and IC

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The lateral superior olive (LSO) is dominated by neurons that are sensitive to interaural intensity differences (IIDs), in that they respond maximally to a particular range of IIDs. However, the responses of LSO cells are also influenced by other stimulus parameters such as frequency, duration, and overall intensity. The present investigation was aimed at assessing the influence of overall intensity on IID sensitivity in the LSO, and for comparison in the inferior colliculus (IC), which receives a strong projection from the LSO, and has many IID-sensitive cells. We recorded from single cells in the free-tailed bat, that were predominantly excited by stimulation of one ear, and predominantly inhibited by stimulation of the other ear (EI type). We generated IID functions by holding the intensity to the excitatory ear constant and varying the intensity to the inhibitory ear. We assessed the effects of overall intensity by taking 3 or more functions at different excitatory intensities, and quantifying changes in the 50% points of the functions. We found that the IID sensitivity of the majority of cells in both nuclei was greatly affected by overall intensity. However, the effects were much more profound in LSO, for both magnitude of effects, and for number of cells affected (LSO 94%, IC 68%). Also, LSO and IC cells showed different types of effects. The majority of LSO cells showed a systematic shift in sensitivity: at higher overall intensities, a greater relative intensity at the excitatory ear compared to the inhibitory ear was observed at the 50% points of the IID functions. Only 20% of the IC cells showed this type of pattern, with 44% showing non-systematic shifts. Finally, despite high variability among individual cells, a population analysis of the proportions of cells responding to various IID/overall intensity combinations showed a surprisingly invariant IID sensitivity across overall intensities, and invariance was greater in the IC than the LSO.

52 Localization in the anechoic environment and with virtual acoustical directional presentation investigated with a laser-pointing method

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Many scientific studies investigate the acoustical localization in the field of vision. Hence it is suitable to display the perceived auditory direction by a light point. In formerly known methods subjects use a hand-held light pointer or a pivoted pointer in front of them. However, the subject's motor system or the optical parallax may influence the results of those techniques. Instead, the newly proposed method utilizes a laser pointer with a deflection unit. Subjects enter the perceived direction with a trackball. The laser spot moves according to the rotation of the ball smoothly on a defined track. Localization results obtained by this method are presented. The reproduction of the acoustical direction was varied in three ways: free-field, headphones using individual HRTFs, and headphones using selected non-individual HRTFs. Furthermore, the influence of the initial laser spot position is investigated. The intuitive experimental operation and the high resolution of the laser-pointer method make it particularly suitable for localization research in audiology, psychoacoustics, and virtual acoustics.

53 Effect of Cuing on Sound Localization Accuracy in a Room

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A previous study of auditory attention examined how auditory localization accuracy in an ordinary room is affected when a test stimulus is preceded by an auditory cue from either the correct or opposite hemifield (Kopco, Ler, and Shinn-Cunningham, JASA, 109, 2377). Results suggested that the auditory cue does not improve localization accuracy, even when the cue is always informative. In fact, the presence of a preceding cue from either +90 or –90 degrees azimuth caused a consistent localization bias of the test stimulus (causing the test stimulus to be heard more towards the midline) for cue-test delays as long as 300 ms. In the current study, these findings are extended to determine how the azimuthal position of the cue stimulus affects localization bias. Acoustic analysis examines the extent to which localization bias can be explained by the reverberation in the room (which has a broadband T_{60} of roughly 450 ms), as opposed to perceptual effects (e.g., Carlile, Hyams, and Delaney, JASA 110, 416-424).

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54 Do Up/Down Reversals Really Exist?

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Interaural time and intensity cues are sufficient to specify a cone-of-confusion upon which a sound source may lie. The cone-of-confusion is thought to be resolved by spectral cues to sound-source elevation and front/back hemifield. The literature reports instances of front/back confusion, where a localisation response is in the wrong front/back hemifield. Presumably, these confusions arise when the cue to front/back hemifield is unreliable. Less frequently reported are instances of up/down confusion (where a localisation response is in the wrong up/down hemifield). We sought statistical evidence for localisation 'reversals', which are a type of confusion for which the true and perceived sound-source locations are symmetrical about the mid-frontal or mid-transverse plane. Up/down reversals suggest the availability of a cue to sound-source angle, in any direction, relative to straight ahead. Localisation response distributions, for a free-field localisation task, were analysed using mixture distribution techniques. A significant bimodal distribution across the front/back or up/down dimension with the two modes located symmetrically was taken as statistical evidence for localisation reversals. Not surprisingly, statistical evidence was found for front/back reversals. However, little evidence was found for up/down reversals. It was thought that this may be due to

the presence of a robust cue to up/down hemifield, possibly provided by the torso. A follow-up experiment using virtual-audio techniques was also conducted. The HRTFs for that experiment were measured with the head oriented directly up, causing any spectral influence of the torso to be symmetrical about the mid-transverse plane of the head. Again little evidence was found for up/down reversals.

55 Behavioral Performance in Binaural Tasks is Determined by Membrane Kinetics of Binaural Coincidence Detectors

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Phase-locked spike input to binaural coincidence-detector neurons in the superior olivary complex (SOC) underpins their physiological sensitivity to interaural time differences (ITDs). However, simulation of SOC neuron responses to ITDs, using a modified Hodgkin-Huxley model (Rothman et al., 1993, J. Neurophysiol. 70, 2562ff), reveals the dependence on stimulus frequency of ITD sensitivity to be different to the monotonic decrease in phase-locking with stimulus frequency, and its pronounced cut-off above 1 kHz (Johnson, 1980, J. Acoust. Soc. Am. 68, 1115ff). The simulations show a clear maximum of ITD-sensitivity, as determined by the extent to which their response is modulation with ITD, at signal frequencies around 350 Hz. This is in agreement with the frequency region over which the binaural masking level difference (BMLD) is maximal (Durlach and Colburn, 1978, in: Handbook of Perception. Vol. IV, fig. 50). The BMLD is a psychophysical phenomenon which is presumed to be directly related to ITD-sensitivity of such neurons. Further analysis of the simulations indicates that ITD sensitivity depends not only on phase-locking but to a substantial extent on the membrane kinetics of SOC neurons. A commonly asked question is why the frequency regions for maximum BMLD (350 Hz) and best ITD discrimination (approx. 800 Hz, see Durlach and Colburn, 1978, fig. 39) differ. When lateralisation is explained in terms of the balance between two spatially-tuned channels, as suggested by McAlpine et al. (Nature Neurosci. 4, 396ff), then the slopes of both overlapping channels determine ITD discrimination ability. With increasing frequency, the shorter periodicity of the auto-correlation function counteracts the decrease in steepness of these slopes as a result of decreasing ITD-sensitivity. Consequently, the frequency for which slopes of ITD functions are steepest (ITD discrimination) is above the frequency of maximum ITD sensitivity (BMLD maximum).

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56 Comparison of Relative and Absolute Measures of Sound Localization in Cats Obtained Under Identical Acoustic Conditions.

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Studies of localization use relative or absolute psychophysical paradigms. Relative tasks assess acuity by determining the smallest angle separating two sources, the minimum audible angle (MAA), that can be discriminated. The MAA varies with stimulus frequency, duration, level, and location. Absolute tasks measure the actual ability to indicate sound location. Changing stimulus parameters affects the accuracy and precision of the location estimates. But whether and how these two measures are related and whether or not the same neural mechanisms mediate performance on these two tasks is unknown. Here, we examine the relationship between absolute and relative measures of localization in cats. Cats were trained using operant conditioning to make orienting gaze shifts (combined eye and head movements) to acoustic targets from an initial fixation LED. Independent variables were the angle (horizontal or vertical) between the fixation LED and the source and the properties of the stimuli. Stimuli consisted of broadband noises (15ms-1sec durations), high- and low-pass noise, and 1/6 octave narrowband noise. The data were separated into two groups based on trial number. Data from one group were analyzed as an absolute task and measures of precision and accuracy of the final gaze were determined. The remaining data were analyzed as a relative task and only the initial direction of gaze, and not magnitude, was used to determine the percentage of "correct" shifts toward the target; gazes away and non-responses were considered "incorrect" responses. This method is similar to that used in infant localization studies. The MAA was computed using the bias-free measure, d'. In general, horizontal MAAs were smaller than vertical MAAs mirroring the smaller standard deviations of absolute localization estimates for horizontal than vertical targets. But the MAAs were larger than the corresponding standard deviations of the actual location estimates.

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57 The Headaim of Echolocating Bats Tracking Moving and Stationary Prey

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A long-standing question of interest to bat researchers is whether echolocating bats employ any predictive strategy when tracking moving prey. A predictive strategy would be revealed by orienting movements that are not simply directed towards the last known target position but use some information from previous echoes to anticipate a new target location. Another issue concerns the orienting behavior of the bat before and after detecting a target.

Here we study these questions by measuring the sonar beam pattern of flying echolocating bats (Eptesicus fuscus) as they attack tethered insect prey in a flightroom. Sixteen microphones arranged in a planar 'c' shaped array were used to measure sonar beam patterns. Head aim of the bats was inferred from the beampattern and used as an index of orienting behavior. Two high-speed infrared digital cameras recorded the bat's flight behavior and other relevant objects in the room. The bat's flight path and positions of any targets were reconstructed from the video.

Trials where bats attacked moving prey were studied to check for any predictive strategy being used. Specifically, the sonar beam aim [head aim] for each vocalization was analyzed and compared with the actual target location at that instant. In some trials, tethered prey was dropped into the flight space while the bat was searching. The time course of orientation to the prey and the accuracy of orientation over successive vocalizations was measured.

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58 Echolocation Behavior of Free-flying FM-bats Taking Tethered Insects in a Cluttered Environment.

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The FM-bat, *Eptesicus fuscus*, uses sonar to detect, localize, track and intercept insect prey on the wing. While this species is generally considered an open-space forager, recent reports indicate that *E. fuscus* also captures insects near clutter (Simmons et al., JARO, 2001), suggesting a wider repertoire of sonar-guided behavior than previously documented. Here, we present lab studies of insect capture behavior by *E. fuscus* and detail their adaptations in flight behavior and sonar vocalizations in the presence of vegetation clutter.

Bats were trained to capture tethered mealworms in a flight room lined with acoustic foam. Their behaviors were studied under open-room and clutter conditions. In clutter, the tethered insect was suspended 5-40 cm from leafy houseplants. Experiments were carried out using only long wavelength lighting to preclude the use of vision. Two gen-locked,

high-speed video cameras (240 Hz) recorded target position, bat flight path, and capture behavior. The resulting images were used to calculate the 3-D positions of the bat, target, and clutter. The bat's sonar cries were recorded with ultrasound microphones and digitized at 250 kHz over a time period that corresponded precisely to the video segment for a given trial.

With experience foraging in a cluttered environment, *E. fuscus* adapted their echolocation behaviors to successfully intercept insect prey positioned as close as 10 cm from vegetation. Beginning approximately 700 ms prior to insect capture and continuing for 300 ms after capture in clutter, bats produced groups of sounds with stable IPI, interrupted by longer intervals. Changes in the temporal patterning, bandwidth and duration of the bat's sonar signals accompanied acquisition of this task over a period of several days.

59 A Neural Network Sound Localization Model of Echolocating Bat, Eptesicus fuscus.

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Eptesicus *fuscus*, an echolocating bat, uses short duration (1ms-10ms) frequency modulated (FM) ultrasonic calls during the approach and terminal phases of insect pursuit and monitors the 3-D positions of objects in the environment from the acoustic information in returning echoes. Acoustic transformation of echoes by external ears, head, wings and torso at the ear canal can be mathematically described as directional transfer functions (DTF). DTFs provide unique cues for sound location, enabling a representation of the bats 3-D auditory scene.

It is widely accepted that interaural level differences (ILD) for bats are good indicators of sound source location in azimuth, whereas, cues for elevation are embedded in the monaural spectral structure of the DTFs. However, it is not known how these cues may be combined in the bat's sonar receiver to represent the locations of objects in 3-D space. A biologically plausible neural network model is a useful approach to investigate the available localization information without any constraint on the cues, thus providing new insights to bat sound localization.

We measured DTFs across 545 different positions, spanning whole frontal hemisphere from 4 different bat preparations. Ears were preserved in their natural position and the wings extended to the sides in a flight posture. 1mm diameter microphones were implanted at the position of the tympanic membrane. We are employing a time delay neural network (TDNN) for sound localization. DTF's and a cochlear model provide the inputs to the TDNN. Using the TDNN to extract the available localization information from its inputs, we are studying the effects of the sonar target position and signal characteristics (e.g. bandwidth, sweep rate) on model localization performance.

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60 Discrimination of azimuth in a forced-choice test is dependent on direction of sound incidence in a small passerine bird

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Laboratory experiments have often demonstrated that small birds are unremarkable in their sound localization abilities. In addition, small head size appears to limit interaural time and intensity differences (ITD and IID) available over biologically relevant sound frequencies. Despite these observations and apparent physical limitations, eastern towhees, Pipilo erythrophthalmus, (Emberizidae, Passeriformes) assess azimuth with surprising accuracy in natural habitat. To better understand this discrepancy, and obtain insights into sound localization mechanisms employed by small birds, we have begun conducting 2-alternative forced choice (2AFC) tests with eastern towhees in both the laboratory and in an outside aviary. Towhees in these tests can discriminate azimuth nearly as well as in the field (to within 10°), however, performance in tests described is highly dependent on head orientation (direction of sound incidence). Results suggest that interaural differences (ITD or IID) do not vary monotonically with direction of sound incidence. Instead, results suggest that interaural differences may oscillate asymmetrically as a function of azimuth within each lateral hemifield. We propose that towhees employ sound localization mechanisms that operate efficiently over long distances in natural habitat.

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61 Spatial unmasking of speech in simulated anechoic and reverberant rooms

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Masked speech reception thresholds were measured for a speech source in the presence of a speech-shaped noise masker for simulated anechoic and reverberant listening conditions. Both speech and masker sources were simulated using individualized HRTFs. The HRTFs were measured in a moderately reverberant room (T_{60}=450 ms) for sources at different distances (15, 100, and 200 cm) and directions (straight ahead and directly to the right of the subject). Reverberant simulations were generated using the full HRTFs (including reverberation), while anechoic simulations were generated by time windowing the full HRTFs to create pseudo-anechoic HRTFs. Speech and noise sources were then convolved with the appropriate HRTFs to simulate anechoic and reverberant simulations for different speech and noise configurations. For each spatial configuration, subjects were tested binaurally, monaurally with the "better" ear, and monaurally with the "worse" ear. Speech reception thresholds were measured adaptively, varying the target level while keeping the direct portion of the masker constant at the better ear. Results suggest that speech intelligibility improves and spatial unmasking increases when reverberation is included, at least for some of the tested spatial configurations. However, the binaural contribution to spatial unmasking is generally small and tends to decrease when reverberation is included.

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62 Investigations of the precedence effect in budgerigars (Melopsittacus undulatus)

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The precedence effect has previously been found in mammals, anurans, invertebrates, and one species of bird, the barn owl. Here, the precedence effect was measured in a small parrot, the budgerigar (Melopsittacus undulatus). The general hearing capabilities of budgerigars have been well examined. While they have unremarkable sound localization abilities, they do exhibit free-field binaural unmasking at amounts similar to those found in humans with much larger heads. Using operant conditioning procedures, we examined whether budgerigars exhibited the precedence effect in a manner similar to humans and other animals. Psychoacoustic methods were used to measure discrimination performance of click pairs from different locations in space and separated by a short delay, simulating a sound source and its echo. Localization dominance was found at interstimulus delays of 0.5 ms to 5.0 ms, where discrimination performance between click pairs was high because the echoes were suppressed. Discrimination performance was poor at shorter and longer interstimulus delays, during summing localization and past the echo thresholds. Further experiments showed that intensity differences

between a lead-lag stimulus pair could override time differences between the lead and lag, the timecourse of the aspects of the precedence effect changed with the intensity and duration of the stimuli but were not asymmetric with respect to leading stimulus location, localization dominance could be built-up and broken down, localization dominance occurred along the midline where minimal interaural time difference cues were available, and two other species of small birds also exhibited the aspects of the precedence effect. Most of these results are similar to those found previously in other animals, suggesting that the precedence effect is a general auditory mechanism for suppressing echoes in an animal's environment.

63 Auditory Velocity Aftereffects with Varying Interaural Time Differences

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Following prolonged listening to an adapting sound moving across the horizontal plane, a stationary test sound can be perceived as moving in the opposite direction, the effect known as the auditory motion aftereffect. Here we report another effect of adaptation to a moving sound, that is, changes in perceived velocity of a moving test sound, named auditory velocity aftereffect. Apparent sound movement was produced by varying only interaural time differences (ITDs). The subjective velocity of a test sound was measured using the constant method with a two-alternative forced-choice paradigm. In each trial, an adapting tone (500 Hz, approximately 10 s) was presented first, followed by a test tone (500 Hz, 1 s) and then a standard tone (250 Hz, 1 s), with 100-ms silent intervals between them. The frequency of the standard tone was chosen because our previous study indicated that frequency separation of an octave is enough to prevent the motion aftereffect. Subjects were requested to judge whether the test tone or the standard tone was perceived as moving faster. The velocity (i.e., ITD change rate) of the adapting tone was selected from eight values between 100 μs/s and 3600 μs/s and fixed through a session. The velocity of the test tone was selected randomly from seven values between 200 μs/s and 900 μs/s on each trial, and that of the standard tone was fixed throughout a session either at 300 μs/s or 700 μs/s. Psychometric functions were estimated using the maximum likelihood method. A test tone was found to be perceived as moving slower than the veridical value due to adaptation, and the largest aftereffect was observed when the velocity of the test tone was slightly slower than that of the adapting tone. These findings put constraints on models of auditory motion perception.

64 Stochastic Signals and the Franssen Effect

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The Franssen effect, the identification and localization of steady-state (slow onset) sounds as being the same as simultaneous transient (sudden onset) sounds, has been previously thought to be limited to pure tones in reverberant rooms. To examine the role of random signal variations (noise) in the Franssen effect, listeners heard transient/steady-state signal pairs from either one or two loudspeakers in a six-speaker array. Stimuli were Gaussian narrow-band noises with 2-4096 Hz bandwidths, centered at 250, 1000 and 4000 Hz. Initial transient RMS level was within 3 dB of maximum level. The breakdown of the Franssen effect -- perceiving the steady-state sound at its actual location -- did not occur for 1000-Hz-centered signals until the bandwidth was greater than 128 Hz, yet the steady-state portions of these signals were localizable. Responses to regular envelope fluctuations (sinusoidally amplitude modulated pure tones with equivalent bandwidths) did not exhibit the same breakdown of the effect as stochastic fluctuations. Room responses to narrow-band signals were analyzed; binaural cross-correlations showed little indication of the effect. Mannequin recordings of narrow-band stimuli in the same enclosure, presented to listeners over headphones, yielded similar responses. Results suggest that the Franssen effect is not wholly dependent on the localizability of the steady-state signal.

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65 Phonemic Contrasts in a Multiple-Talker Task with Normal Hearing and Sensorineural Hearing Loss Individuals

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Individuals with sensorineural hearing loss (SNHL) have difficulty understanding speech in adverse listening environments. Past studies have shown the effects of hearing loss and spatial separation on speech intelligibility. However, little is known about specific phonemic losses in speech intelligibility due to the combination of hearing impairment and spatial separation. The purpose of this study was to measure word identification ability of normal hearing and hearing-impaired individuals in a multi-talker task. Thirty-three listeners in each group participated in each test condition. The hearing-impaired listeners were below 60 years in age and exhibited a high frequency, cochlear hearing loss. The normal hearing listeners were age-matched to the hearing-impaired group. Recordings of the Modified Rhyme Test (MRT) were made with six male and six female talkers. The speech files were digitized and edited to time-align the test words and equalize sound levels. Multiple phrases from the MRT were processed through a TDT Power-DAC with non-individualized head related transfer functions and presented over Sennheisser HD600 headphones. The MRT phrases were presented at 0 dB SNR with and without spatial separation. The independent variables included two age-matched, equal size groups of normal hearing and SNHL listeners. The dependent variable was the phonemic contrasts within the word identification responses. Analysis of the data revealed a main effect for hearing loss and spatial separation. A difference in the type of phonemic contrasts between the two groups was found, especially for phonemes with high frequency content.

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66 Information Theoretical Analysis of Spike Count and Latency Codes for Acoustic Space in Primary Cortical Neurons

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When tested with brief noise bursts presented in virtual acoustic space, most A1 neurons will respond with brief "onset" bursts of action potentials. Brugge and colleagues (J Neurosci, 1996; 16: 4420-37) have proposed that A1 neurons may signal source position through variations in the timing (latency) of these onset responses, rather than through variations in spike count. However, it has been observed in a number of sensory systems that response strength and response latency may show a systematic relationship (stronger responses in terms of discharge rate tend to occur with shorter onset latencies). This relationship may arise naturally from the manner in which synaptic potentials are integrated within neurons. Consequently, response latency and spike count codes might be manifestations of the same underlying signalling mechanisms. When we applied an information theoretical analysis to the presumed spike count and spike latency codes, we found the amount of information transmitted through either of the candidate codes to correlate highly, but the amount of information transmitted by latency was in most cases slightly higher than that transmitted by spike count. We also found the two candidate codes to be highly redundant, in that little extra information could be gained by observing both spike count and latency over that obtained by observing only one of these variables alone. The high redundancy of the spike count and latency codes seems to support the notion that both these codes may be largely due to a

common encoding mechanism, but it seems likely that additional mechanisms must contribute to the the higher amount of information observed in response latency.

67 A Psychophysical Group Study of Developmental Tune-deafness

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Disorders of musical perception in the absence of deafness or brain condition have been described (Allen, 1878, Mind: 10, 157-167). Early descriptive studies describe deficits in pitch discrimination, tonal memory, and singing. We present a systematic assessment of such a tune-deaf sample.

Six subjects (2 male, 4 female: aged 35-56) described lifelong inabilities to perceive music. Pure tone audiograms and auditory filter widths (notched-noise method) were normal. Complex sound perception was assessed using a validated 2-AFC test battery (Griffiths et al, 2001, Hear Res: 154, 165-169). Estimated thresholds were determined from a Weibull fit to full psychometric function and compared with 17 age-matched controls. Perception of sinusoidal frequency modulation of a 500Hz carrier was normal for all subjects at 2Hz, but not at 40Hz and 120Hz. Perception of sinusoidal amplitude modulation of a 500Hz carrier was impaired for some subjects at 2Hz, 40Hz, and 120Hz. Within- and between-channel gap detection was normal for all subjects (excluding one). The threshold for the detection of regular interval noise was normal for all subjects (excluding one). Impairments were demonstrated for selective subtests of a validated musical test battery, (Liegeois-Chauvel et al, 1998, Brain: 121, 1853-1867). Prosody perception (Patel et al, 1998, Brain Lang, 61: 123-144) was normal for all subjects.

The findings support the existence of a developmental disorder of musical perception in the absence of peripheral deafness or neurological event. We show, further, that the disorder is associated with deficits in the perception of patterned sound.

68 Effect of Listening Experience on the Perception of Periodicity Strength in Chinchillas

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In stimulus generalization tasks, an animal is trained to respond to a particular stimulus, and responses are then measured to test stimuli that vary systematically along a stimulus dimension. A gradient in behavioral responses (1) suggests how closely test stimuli are perceived by the animal to resemble the training stimulus, (2) is consistent with the hypothesis that the animal possesses a perceptual dimension related to the stimulus dimension, and (3) can indicate what stimulus features are being analyzed during testing. Chinchillas trained to discriminate a cosine-phase harmonic tone complex (COS) from wideband noise (WBN) were tested in a generalization task with COS, random-phase complex tones (RND) and iterated rippled noises (IRNs). Preliminary generalization data indicated that chinchillas order complex sounds along a dimension related to stimulus periodicity strength (Shofner, 2001, JASA 109: 2465). Data from naive chinchillas suggested that the stimulus envelope had a large influence on the perception of periodicity strength, but data from one animal having previous listening experience with IRN indicated that the fine structure had a larger influence than envelope on this perception. To examine the effect of listening experience with IRN on the perception of periodicity strength, 3 of the above naive animals were retrained to discriminate IRN from WBN and were tested in the generalization task with IRNs, COS, and RND. Retrained animals now gave larger behavioral responses to test stimuli having small envelope periodicity strengths (i.e. RND, IRNs) than given previously in the naive condition. The results suggest that for broadband stimuli comprised of resolved and unresolved components, the chinchilla may normally analyze periodicity information in the stimulus envelope, but can learn to analyze information in the fine structure when trained with a stimulus having a small envelope periodicity strength.

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69 Interaction between envelope and carrier periodicity in tonal noises

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Psychoacoustic models for pitch detection and models for modulation detection are currently developing independently from one another. Does this dichotomy in psychoacoustic modeling reflect the existence of two independent subsystems for pitch and modulation processing? Modulated noises can also produce a tonal sensation and tonality is generally produced by the periodicity of the stimulus. To approach the idea of a common mechanism for periodicity detection, this study evaluates the extent of perceptual interaction between envelope and carrier periodicity. Envelope periodicity can be obtained by multiplying Gaussian Noise (GN) with a sinusoidal modulator. Carrier periodicity can be obtained by iterating a delay-and-add processing of GN, which generates Iterated Rippled Noise (IRN). Since IRN has a distinct pitch and does not show pronounced modulation in the envelope, it is particularly suitable to represent carrier periodicity in this study. To study the mutual influence of envelope and carrier periodicity, two sets of experiments were designed. One set dealt with the ability to detect envelope periodicity in absence and in presence of carrier periodicity. In the counter experiments, the listeners' task was to detect carrier periodicity in absence and in presence of envelope periodicity. The period of the modulator and the period of the corresponding carrier were always set to be the same. The results show that the sensitivity for envelope periodicity is significantly reduced in the presence of carrier periodicity, independent of the modulation period. However, the sensitivity for carrier periodicity is affected by the presence of envelope periodicity only for frequencies below about 50 Hz. Existing pitch and modulation models are tested, whether they can reproduce the results obtained in these psychoacoustic experiments.

70 The Role of Harmonic Resolution in Diotic and Dichotic Pitch Perception

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Two experiments investigated the relationship between the pitch salience of harmonic complexes and the resolvability of individual components. In each experiment, tone complexes with 12 successive equal-amplitude random-phase harmonic components of a 100- or 200-Hz fundamental frequency (F_0) were presented dichotically or diotically, for a total of four conditions. Under dichotic presentation, even and odd harmonics were presented to opposite ears, such that the peripheral spacing between components was $2F_0$. Under diotic presentation, all harmonics were presented to both ears, such that the peripheral spacing was F_0. The first experiment tested the resolvability of individual harmonics, by measuring listeners' performance in identifying whether a pure tone probe was higher or lower in frequency than the target harmonic of the complex, made perceptually more prominent by gating it on and off. Preliminary results indicate that approximately the first 10 and 20 harmonics are resolvable in the diotic and dichotic conditions, respectively, verifying that frequency

selectivity is limited only peripherally. The second experiment measured F_0 difference limens (DLs) as a function of lowest harmonic number. In all four conditions, F_0 DLs increased dramatically as the lowest harmonic number was increased from 9 to 15. The similarity of the results at both F0s provides further evidence that harmonic number, not absolute frequency, underlies the dramatic DL shift. The similarity of the results under diotic and dichotic conditions indicates that the auditory system, in performing F_0 discrimination, is unable to use the information provided by the additional resolved harmonics in the dichotic case. This result is consistent with a harmonic template theory of pitch, in which only harmonics that are normally resolved contribute in the calculation of pitch.

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71 A Systematic Clinical Battery for the Investigation of Pitch Sequence Processing

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We describe a systematic battery of tests for the assessment of pitch sequence processing. Functional imaging demonstrates that such processing involves cortical mechanisms beyond the primary auditory cortex. Research into the neural bases of sequence processing and music has uncovered dissociations between encoding the pitch interval sizes in a musical sequence, called 'local' processing, and extracting the pattern of rises and falls in pitch from note to note, often called 'contour' or 'global' processing (eg. Peretz, 1990). Here we suggest that a level of pitch sequence processing has been overlooked in these studies that represents a more 'global' level of auditory processing. This hypothesised level enables the extraction of any overall pattern in pitch, irrespective of the note-to-note pitch changes.

We present tests that assess processing at these three hypothesised levels and have the potential to unearth dissociations between them. At the first two levels the tests extend previous studies by Dowling to an atonal scale (Dowling, 1978). At the third level we introduce a new test that requires a same-different judgement on the overall pattern in pairs of six-element pitch sequences. These sequences are atonal and transposed in pitch between pairs. The overall pattern of the sequences is determined by a power spectrum (p) of the form, $p = \text{frequency}^{-1.4}$. The phase of each frequency component is randomised between sequences, except for the lowest frequency component, where the phase is either kept the same or inverted. This produces sequences with the same or different overall pattern, irrespective of the note-to-note pitch changes. This task can be accomplished by normal volunteers after a short period of training and is realistic for clinical use.

72 Effects of Masker Phase Curvature for On- and Off-frequency Simultaneous and Nonsimultaneous Maskers

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Repeating linear frequency sweeps (Schroeder phase stimuli) have been used previously to estimate the phase curvature of the auditory filters. As these harmonic stimuli have a constant phase curvature, one implicit assumption is that the curvature of the auditory filters can be approximated as being constant. Physiological measurements in various mammals have shown that the assumption of constant phase curvature is reasonable for frequencies within the passband of the auditory filter, but not for frequencies well below the center frequency (CF) of the filter. To investigate whether a similar change in phase curvature with frequency can be found behaviorally in humans, thresholds for a 2-kHz sinusoidal signal were measured in the presence of simultaneous harmonic tone complex maskers with cutoff frequencies of 1400 and 2600 Hz (on-frequency masker) or 200 and 1400 Hz (off-frequency masker). The phase curvature of the maskers was varied systematically to find the curvature that produced the minimum amount of masking. For the on-frequency masker, minimum masking occurred at a positive curvature, consistent with previous results. For the off-frequency masker, minimum masking occurred around zero curvature. This suggests that the response to frequencies well below CF exhibits no phase curvature, consistent with physiological data. The maskers producing the highest and lowest thresholds were selected from both the on- and off-frequency conditions and were used as forward maskers. For the on-frequency masker, large differences in threshold were apparent in forward masking also. For the off-frequency maskers, the difference was greatly reduced. This is qualitatively consistent with the expected effects of peripheral nonlinearity on forward masking.

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73 The Auditory Temporal Window in 9-10 Year Old Children, Adults and Dyslexic Adults

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It has been suggested that both young, normally developing children and older people with language impairment have poor temporal resolution - the ability to separate sounds in time. Work from this laboratory (Hartley and Moore, ARO, 2002) has offered an alternative interpretation, that poor processing efficiency in these groups leads to a wider variety of auditory impairments. This study examined these competing hypotheses by measuring the shape of the auditory temporal window, the time during which sounds are integrated (Moore et al, JASA, 83, 1102). Our experiments measured the rising arm of the temporal window using backward masking with variable delays between a target tone and a masking noise. Tone thresholds were estimated for 9 dyslexic and 12 control adults, and 12 children (9-10 y.o.), all untrained and audiometrically normal, using a two AFC task. Tone (1000Hz, 20ms including 10ms rise/fall) offsets occurred with a delay of 0, 10, 50 and 150ms before the onset of a noise masker (centred at 1000Hz, bandwidth 800Hz, 300ms). Presentation was monaural over headphones to the right ear. The children experienced more masking at every delay relative to the adults. This was greatest at the shorter delays (0ms = 15dB, 10ms = 16dB, 50ms = 7dB, 150ms = 5dB) and the shape of the curve was flatter than the control adults. This suggests a developmental change in the shape of the temporal window. The generally elevated masking seen in the children relative to the adults also suggests that there is a contribution from poor efficiency. The dyslexic adults also had elevated masking, but this was less marked than that in the children. However, the shape of the slope in the dyslexic adults resembled that of the children. This may indicate that temporal processing in adult dyslexics does not develop normally, and that both poor temporal resolution and poor efficiency may contribute.

74 Excessive Auditory Masking in Children with Language or Listening Impairments Interpreted as a Developmental Delay

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Eight percent of children have language or listening disorders. Affected children frequently have particular difficulty perceiving sounds in noise. We previously reported that 8-year-old children with specific language impairment (SLI) or central auditory processing disorder (CAPD) had normal detection thresholds for a 200-ms, 1-kHz tone simultaneously masked by a 300-ms bandpass noise (0.6-1.4 kHz; N0=40 dB SPL), but had higher thresholds than 8-year-old controls for a 20-ms, 1-kHz tone simultaneously, backward, or forward masked by that noise. Here we

report a different impairment pattern for 12-year-old children with CAPD. These children had simultaneous-masking thresholds for both long and short tones that were higher than 10-year-old controls, backward-masking thresholds equal to 10-year-old but higher than adult controls, and forward-masking thresholds equal to adult controls. Thus, by 12 years of age, children with CAPD appeared to have acquired an impairment in simultaneous masking for long tones, maintained their impairment in simultaneous masking for short tones, possibly reduced their impairment in backward masking, and eliminated their impairment in forward masking. A possible explanation for these results is that the hearing abilities of children with SLI or CAPD are developmentally delayed, and that this delay manifests itself as different sets of hearing problems at different ages because thresholds of controls decrease with age at different rates across our four conditions (e.g., Hartley et al. 2000, J. Sp. Lang. Hear. Res., 43, 1402-1415). Supporting this view, the thresholds of the 12-year-old children with CAPD were similar to those of 8-year-old controls. Thus, children with language and listening disorders may have difficulty perceiving sounds in noise due, in part, to auditory-system immaturity.

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75 Auditory Perception in Children with Dyslexia With and Without Attention Deficit/Hyperactivity Disorder

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The auditory temporal deficit hypothesis predicts that children with reading disability (RD) will exhibit a deficit in the perception of non-verbal acoustic stimuli that contain temporal cues. Tasks assessing auditory temporal and non-temporal function were administered to children 7 to 13 years old with RD (n = 42); Attention Deficit Hyperactivity Disorder (ADHD; n = 34); RD and ADHD (n = 39); and not impaired (n = 41). RD and ADHD were carefully measured through a series of neuropsychological tests. There were no group differences in age, race, gender, socio-economic status, performance IQ, audiogram or tympanogram. The hearing tests included tone-onset-time discrimination (TOT), interaural time and intensity difference limens (ITD and IID), gap detection, binaural masking-level difference (MLD), and temporal integration. All tests used a 4-interval, 2-alternative, forced-choice, 2-down, 1-up staircase procedure with training and feedback. TOT was made with 500 and 1500 Hz pure tones. IID, ITD and gaps were made with white noise. The MLD was a 500-Hz tone in half-octave noise. Temporal integration was measured between 512 and 32 ms with 500-Hz tones.

The pattern of deficits observed in children with RD could not be explained by a simple auditory temporal deficit. Children with RD/ADHD exhibited the most significant deficits, which were apparent across all tasks. The only salient finding in the RD group was a deficit in TOT. Children with RD were normal in our other tests of temporal acuity (ITD, MLD, gaps) and in our non-temporal controls (IID, N_oS_o, absolute thresholds to short and long tones). Relationships between auditory-function and phonological-processing measures were limited and confined to children with RD/ADHD. Findings suggest that deficits on non-verbal auditory tasks in children with RD may potentially be related to behavioral difficulties associated with ADHD.

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76 The temporal window model

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The temporal window model is a model of nonsimultaneous masking and other temporal resolution phenomena. The main assumption of the model is that temporal resolution is limited by the minimum integration time of the auditory system, represented in the model by the temporal window. The temporal window is an intensity weighting function that smooths the internal representation of stimuli over time, so that forward masking, for example, is explained as a persistence of the internal activity produced by the masker. Recent work has suggested that the main source of nonlinearity in simultaneous and nonsimultaneous masking is the basilar membrane response function. The latest version of the temporal window model includes a nonlinear front end based on the DRNL filter of Meddis and colleagues [Meddis, R., O'Mard, L.P., Lopez-Poveda, E.A. (2001). J. Acoust. Soc. Am. 109, 2852-2861]. The DRNL filter is intended to simulate the response characteristics of the basilar membrane. The new model can account for a wide range of nonlinear masking phenomena, including the upward spread of masking, the nonlinear growth of forward masking, the effect of forward masker duration, and two-tone suppression. The success of the model suggests that, subsequent to the basilar membrane, the auditory system may be regarded as linear in the intensity domain.

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77 Comodulation masking release for spectrally-complex signals

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When a spectrally-complex signal is presented in a masking noise, detection levels are typically lower if the masking noise is comodulated across frequency than if it is randomly modulated. Both within- and across- frequency-channel effects can contribute to this threshold difference. Establishing the contribution of across-frequency effects is particularly challenging when the spectrum of the signal completely overlaps that of the masker. Mendoza et al. (1998) found that the across-frequency phenomenon of comodulation masking release (CMR) is sensitive to the coherence of the masking noise even during periods when the pure-tone signal is absent. Specifically, CMR for gated maskers declines substantially when random maskers with the same long-term power spectra are presented before and after the segments of coherent maskers. The purpose of this experiment was to determine whether this effect could be used as a test of whether the threshold differences observed for a multi-component signal masked by a spectrally-similar masker incorporate an across-frequency CMR. Masked thresholds were measured for 400-ms tonal signals of 804, 1200, 1747, and 2503 Hz individually and as a complex. The masker consisted of 20-Hz wide narrow bands of noise centered on these frequencies, that were either comodulated or random with respect to each other. The masker was gated on and off with the signals, and thresholds were measured both in the presence and absence of additional bands of random noise that temporally surrounded the masker intervals. Results from six normal-hearing listeners indicated that the magnitude of masking release for the multi-component signal declined for most listeners when the gated, comodulated maskers were temporally surrounded by random bands. This suggests that the masking release was due at least in part to CMR.

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78 Modulation Rate Discrimination and the Continuity Illusion

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Studies of modulation rate discrimination typically investigate discriminability as a function of modulation depth, of stimulus features such as bandwidth or carrier frequency, and of type of modulation. The present study investigates modulation rate discrimination for a 1-kHz pure tone, sinusoidially amplitude or frequency modulated at a 20-Hz rate, as a function of duration. In addition, perceived duration is manipulated by physically increasing the duration of the stimulus, or by an illusory continuation of the modulated stimulus through a gap. In the latter case, two modulated tone bursts are separated either by silence, or by a low-pass noise. On average, there was little effect of duration per se on modulation rate discrimination. While listeners did tend to improve when the duration was increased from 100 to 200 ms, there was little or no further improvement with durations of up to 500 ms. There was an effect of adding noise to the discontinuous condition. On average, for both AM and FM, listeners performed better when a burst of noise was added into a silent gap between two modulated tone bursts, than when no noise was present. Listeners reported hearing the modulation as continuous through the noise, and the continuity facilitated rate discrimination performance over the discontinuous condition.

79 Does a Distortion Product Contribute to the Detection of Second-Order Amplitude Modulation?

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Sounds such as speech and music tend to have complex time-varying envelopes. Second-order amplitude modulation (AM) is a stimulus in which the depth of an amplitude modulation is sinusoidally varied as a function of time. The second-order modulation (modulation rate f_m' and depth m') produces sidebands around the first-order modulation (modulation rate f_m and depth m) at $f_m \pm f_m'$. In a recent study Lorenzi *et al*. (JASA, in press) suggested that second-order AM generates a distortion product via a nonlinearity at the difference frequency (f_m-f_m') that may contribute to the detection of second-order AM. Our aim was to test this hypothesis by masking the distortion product using a narrowband noise masker centred at the difference frequency. Using a 2I-AFC psychophysical procedure, we measured the threshold depth of second-order modulation (m') as the maximum peak-to-peak depth of the noise masker was varied between 0 and 0.5. The first-order modulation depth m was 0.25, f_m was 16 Hz and f_m' was 2 Hz (therefore the perceived difference frequency was 2 Hz). As a control we measured the effect of varying the noise masker depth on detection thresholds for first-order AM at a modulation rate of 2 Hz. Increasing the depth of the noise masker decreases the detectability of first-order AM with a modulation rate of 2 Hz but has little effect on the detectability of the second-order AM. These preliminary results suggest that either the distortion product is not processed by the mechanism that processes a first-order modulation of the same frequency, or the distortion product at the difference frequency does not contribute to second-order AM detection.

80 Monaural and Binaural Spectral Modulation Detection Interference

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Recent studies have shown similarities between spectral and temporal envelope perception including modulation transfer functions and masking patterns. In the present investigation, spectral modulation masking patterns were obtained in the presence of a second, interfering modulation frequency. This experimental paradigm closely follows studies of modulation detection interference (MDI) in the temporal domain. In Experiment I, the signal modulation (1.5 to 8.48 cyc/oct) was superimposed on a carrier from 800 to 1600 Hz. The masker modulation (3 cyc/oct) was superimposed on a second noise band that varied in spectral local across experimental conditions (either 400 to 800, 800 to 1600, 1600 to 3200, or 3200 to 6400 Hz). The masker modulation depth (spectral contrast) was fixed at 15 dB peak-to-valley difference, and the signal modulation depth was adaptively varied to determine a modulation detection threshold for each signal modulation frequency. In Experiment II, the masker (3 cyc/oct) and signal (0.75 to 8.48 cyc/oct) modulations were superimposed on two carriers having the same audio-frequency configuration, but presented to separate ears in a dichotic listening task. Noise carriers were 200 to 12800, 800 to 1600, or 6400 to 12800 Hz. Analyses of the data from both experiments indicated that the presence of the masker modulation interfered maximally with the detection of signal modulation when the two were close in frequency. This spectral modulation detection interference (SMDI) may reflect a perceptual fusion of spectral envelopes, and is consistent with an interaction within and/or among monaural and binaural domain-specific spectral envelope channels. These results are similar to those obtained in studies of temporal modulation detection interference (TMDI) and may reflect similar underlying mechanisms.

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81 Dichotic gap detection and speech recognition in noise

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In an earlier study, we found that speech understanding in a fluctuating background is related to temporal processing as measured by the detection of gaps in noise bursts. Fifty adults with normal or mild high-frequency hearing loss served as subjects. Gap detection thresholds in noise were obtained using a 150-ms noise burst with the gap placed close to carrier onset. NU-6 word scores for the subjects were obtained at a presentation level of 55 dB HL in competing babble levels of 50, 55 and 60 dB HL. A repeated measures analysis of covariance of the word scores examined the effects of age, absolute sensitivity, and temporal sensitivity. The results of the analysis indicated that word scores in competing babble decreased significantly with increases in babble level, age, and gap detection thresholds. The effects of absolute sensitivity on word scores in competing babble were not significant. These results suggest that in the absence of clinically significant hearing loss, age and temporal processing influence speech understanding in fluctuating backgrounds.

In a more recent study, gap detection thresholds were obtained in comparable dichotic conditions with similar groups of younger, middle-aged, and older listeners. The relationship between dichotically obtained gap thresholds and speech scores will be presented.

Finally, a third study was designed to determine the effect of moderate to severe hearing loss on temporal acuity and its relationship to speech discrimination in noise. Gap detection thresholds and word scores were obtained across a range of background noise levels in 13 young subjects. Five had normal hearing; eight had moderate to severe bilateral hearing losses. The relationship between gap detection thresholds and speech scores in younger adults with moderate to severe hearing loss will be discussed.

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82 Masking Patterns Using FM and Virtual Frequency (VF) Signals: Effects of transition direction and duration

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A frequency transition (virtual glide) is perceived by amplitude modulating a two-tone complex to dynamically change its spectral centroid (Lublinskaja, 1996; Anatharaman et al. 1997). Our previous work (Iyer et al. 2000; Iyer and Feth, 2001) investigated the processing of these VF signals by comparing them to "actual" glides – FM signals. We demonstrated that upward moving FM and VF signals were processed similarly in a temporal acuity task, but quite differently in a simple masking task. Several studies (Dooley & Moore, 1988; Madden & Fire, 1997; Gordon & Poeppel, 2000) have reported a perceptual asymmetry between upward and downward moving glides. In this study, we obtained masking patterns using FM and VF maskers as a function of direction of transition and duration. In the first experiment, threshold for a 20 msec probe was measured for "up" and "down" masker transitions. An "up" VF masker was generated by modulating two tones of fixed frequency such that the lower frequency was initially more intense than the higher frequency. A "down" VF masker was generated by reversing the modulation scheme. Similarly, "up" and "down" FM maskers were generated by modulating a sinusoid in a linear upward or downward trajectory. The probe was presented at 0, 115 or 230 msec after the onset of the 250 msec masker. In the second experiment, threshold for a probe was measured as a function of masker duration. The masker duration was either 125, 60 or 30 msec. The frequency separation between the two tones in the VF masker, and the frequency transition for the FM masker was set to 2, 5 or 8 ERBu. Results show that the masking patterns for the two classes of signals, VF vs. FM signals, are different. Effects of direction of transition and duration of maskers on masking patterns will be also be discussed.

83 Selective Attention To A Given Stream Affects The Segregation Of Simultaneous Speech-Analog Streams

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Complex sinusoids containing all harmonic components between 500 and 3500 Hz were bandpass filtered to generate single-formant signals. The center frequency of the filter was changed over a 100-ms-100-ms up-down or down-up trajectory to create single-formant signals with dynamic transitions. Simultaneous pairs of two such signals differing in fundamental frequency f0 (by 1/3 or 5/6 octave) as well as in the direction and extent of the formant change yielded two streams of non-speech signals having speech-like qualities. Subjects were instructed to discriminate the pattern of the formant change in one stream designated by presenting, before each block of trials, a complex tone having the f0 of the stream to be attended to. It has been shown (Divenyi, P.L., Proc. 17th Intern. Congr. Acoust., pp. 255-256 [2001]) that normal-hearing young listeners are able to segregate simultaneous streams of single-formant complex tones differing in f0 and the pattern of formant frequency change. However, it has not been ascertained whether this ability derives from evaluating the composite pattern of the two streams or from listening to the pattern of one of the streams. Results of the present experiments showed that selective attention to a stream is possible, although it is more difficult when the f0 difference is small. The degree to which the irrelevant stream has to be attenuated to allow segregation may be regarded as a measure of schema-driven segregation as defined by Bregman (*Auditory Scene Analysis*, MIT Press [1991])

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84 Auditory Analog of the Visual Flash-Lag Effect

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In vision, when a flashed stimulus is presented in physical alignment with a moving stimulus, the flashed stimulus appears to lag behind the position of the moving one. We have investigated this spatial localisation error in audition, and also cross-modally, pairing auditory motion with visual flashes, or visual motion with auditory flashes (sound bursts). Auditory stimuli for the cross-modal studies were: motion – a pink-noise source smoothly changing position along the horizontal mid-line; burst – a brief 1kHz tone. The visual stimuli were: motion – a Gaussian-shaped luminance peak translating across a computer monitor; flash – a brief stationary luminance peak. We find that the so-called flash-lag effect is present in both cross-modal pairings, as well as in both uni-modal pairings. In all cases, the transient stimulus is perceived to lag behind the moving stimulus. We have also investigated another auditory version, using spectral motion and a pure-tone burst, and found similar results. For an ascending sweep, when the ascending tone and the burst have the same frequency, the tone burst is perceived at a lower frequency than the sweeping tone. Conversely, the tone was perceived to be of higher frequency than the moving tone during descending sweeps. Purely visual theories cannot account for these results, which point to broader temporal differences in cortical processing of sustained and transient sensory stimuli.

85 The effect of temporal stimulus characteristics in maintenance of the acoustic reflex.

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In normal listeners acoustic reflex decay (ARD) typically occurs for high but not for low frequency tones. In patients with acoustic neuromas decay can be obtained at all frequencies, presumably due to poor neural synchrony. These observations have led us to hypothesize that resistance to decay is due to robust encoding of temporal fine structure of the eliciting stimulus. Using a 4-kHz stimulus, ARD is reduced by sinusoidal amplitude modulation (SAM) [Cook et al., Audiol. Neuro-Otol. 4(2):104-13, 1999], a result attributed to the low frequency pattern of SAM providing the temporal characteristics necessary to maintain the reflex. If this interpretation is correct, then further reductions in ARD should be seen for stimuli having temporal characteristics that even more closely resemble the neural response to low frequency fine structure. On the other hand, if other perceptual qualities of a SAM tone are responsible for the effect (e.g., rate pitch), then manipulations of perceived sound quality, rather than temporal characteristics per se, should produce similar effects. The experiment reported here included a reference condition, 1) 5 kHz pure tone, and three 'temporal' manipulations, comprised of a 5-kHz tone multiplied by: 2) a raised 100-Hz sinusoid, 3) a noise sample, low-pass filtered at 100 Hz, and 4) a half-wave rectified 100-Hz sinusoid. Additional conditions manipulated perceived pitch. Stimuli spanned 4.5 - 8 kHz, including a reference condition, 5) Gaussian noise, and a stimulus associated with a 100-Hz pitch, 6) iterated rippled noise. Results show the greatest reductions in ARD with the half-wave rectified stimulus, thought to most closely mimic the temporal characteristics of a low frequency tone. Little or no reduction in ARD was associated with the iterated rippled noise, suggesting that perceived pitch does not play an important role in maintaining the acoustic reflex.

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86 Identification of Unique Transcripts from a Mouse Full-Length, Subtracted Inner Ear cDNA Library.

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A mouse full-length cDNA encyclopedia is being developed from ~160 full-length enriched, normalized, subtracted cDNA libraries from a variety of tissues and development stages were constructed. A mouse inner ear subtracted cDNA library was constructed by using biotinylation of the cap structure (the 'CAP-trapper' method) coupled with the addition of trehalose to increase reverse transcriptase efficiency at high temperature by (Carninci et al., Genome Res 10:1617-1630, 2000). Sequence analyses of the 5' and 3' ends of the cDNAs were done on 8151 clones from this library. Using the predicted transcription start site from the 1,722 kb elongation factor 1α mRNA, a full-length rate of ~96% was achieved. The average size of cDNAs of know genes was ~2.5 kb with the majority being in the 0.5 to5.0 kb range. The initial 3' end sequencing identified 4027 groups (clusters) and of these 1302 cDNAs were unique in the RIKEN and GenBank databases with 485 representing new ESTs and 817 corresponding to new genes. Conservative estimates suggest that at least 600 previously unidentified genes are in this library. We have also sequenced 1508 clones from the 5' end, identifying 1231 clusters, 322 of which did not hit any sequence in the EST database and 458 of which were new and not represented by known genes. An *in silico* microarray containing around 5,000 representative cDNAs from each cluster is currently being produced. Expression analyses of subdissected fragments (spiral ganglion, organ of Corti, and the cochlear lateral wall (stria vascularis) will be done to classify each unique cDNAs 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.

87 EST Analysis of Gene Expression in the Mouse Organ of Corti at the Onset of Hearing

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In order to study the transcriptional repertoire of the organ of Corti during the onset of hearing, a database of expressed sequence tags (EST) is being generated from in depth sequencing of a non-amplified mouse organ of Corti cDNA library. Poly A+ RNA was extracted from a total of 364 finely dissected organs of Corti from 5 to 13 days old BALB/c mice cochleas. The cDNA fragments were directionally cloned into the pBluescript phagemid, using the Uni-ZAP XR system (Stratagene). Inserts of cDNA clones were sequenced, analyzed and clustered using specially written software (NEIBank, http://neibank.nei.nih.gov). Over 9,000 sequence reads yielded ~ 5500 unique gene clusters (potentially individual genes), of which 70% are genes with known or inferred function. Analyzes of the freqency of expression shows that 17 % of the gene clusters have more than 10 copies, 25 % have 3-10 copies, 15 % have 2 copies and 43 % have 1 copy. Among the highly expressed genes are those encoding the extracelllular matrix proteins Sparc (1.03% of all clones) and tectorin beta (1.01%). Other inner-ear specific mRNAs found in abundance included otoraplin (0.27%), tectorin alpha (0.18%), and otoconin (0.04%). We observed a large number of potentially novel genes and new splice forms of known genes. Transcripts previously shown to be essential for auditory sensory function as well candidates for deafness are also revealed. One of the most abundant transcripts (0.4%), encoding a novel 89 amino acid protein, was demonstrated to be expressed in inner and outer hair cells by in situ hybridization. The collection of ESTs with description and additional annotations (such as mouse and human chromosomal location) is being compiled as a web-based resource for public access. Clones from this collection will be available for further sequencing or for the construction of cDNA microarrays or other probes.

88 Serial Microanalysis of Vestibular Schwannoma

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Serial analysis of gene expression (SAGE) is a powerful new technique that allows detailed qualitative and quantitative evaluation of cellular gene transcript expression. Tissue in limited quantity ($5x10^4$ to $2x10^6$ cells) may be analyzed by a modified version of SAGE called microSAGE. The goal of this project was to determine if the microSAGE technique could accurately analyze the total gene expression profile of Schwann cells from a vestibular schwannoma specimen, given a small amount of vestibular schwannoma tissue (150-200 mg). Fresh, noncystic vestibular schwannoma specimen from an individual without the diagnosis of neurofibromatosis type II was attained intraoperatively and maintained in a sealed container at –80° C until the time of analysis. The tissue was analyzed according to the microSAGE protocol, using 180mg of vestibular schwannoma as starting material. The protocol resulted in the generation of a tag library which included many tags that correspond to known vestibular schwannoma transcripts. Many tags had no database match, indicating correspondence with novel or poorly characterized transcripts. This data demonstrates microSAGE is a useful technique for analysis of the gene expression profiles of vestibular schwannomas. Future studies will involve vestibular schwannomas with various growth rates, morphology, and neurofibromatosis type 2 status. Comparison of gene expression profiles from these different vestibular schwannoma subtypes may identify useful diagnostic or prognostic markers, as well as targets for therapeutic intervention.

89 Extraction of Mitochondrial DNA from Inner Ear Cells in the Cellodin Embedded Archival Temporal Bone by Laser Captured Microdissection and TaqMan PCR

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Several methods have been reported to extract DNA from the formalin-fixed, celloidin embedded archival temporal bone, which contribute to study the genetic basis of hearing impairment. However, during the process of fixation, decalcification, and celloidin embedding, the DNA in the celloidin-embedded temporal bone becomes fragile. Earlier studies have suggested that the maximum template length in these tissues is on the order of 471 base pair (bp). Recently mitochondrial DNA mutations such as A3243G, or A1555G, have been found to cause syndromic or non-syndromic sesnsorineural hearing loss. We report the trial of extraction of mitochondrial DNA in cellular level from the organ of Corti, stria vascullaris, and spiral ganglion cells using the technique of laser capture microdissection and TaqMan PCR. These techniques enable us to analize the extremely small amount of DNA by capturing a

few cells easily by laser beam and amplifying the short template length of DNA. These methods are expected to explicate the location and proportion of mitochondrial DNA mutation in cellular level and detect the impaired site genetically.

90 Towards conditional gene targeting in hair cells of mice

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Although the gene knockout technique is best for studying gene functions in vivo, it may result in embryonic lethality and cause complex pleiotropic effects that impede determination of the gene's functions in specific cells. The Cre recombinase (Cre) catalyzes recombination between two consensus 34-bp DNA recognition sites (LoxP sites) and this Cre-loxP system has been used successfully in many cells in mice for cell type specific gene knockout. In this study, we hope to create mice in which Cre is specifically expressed in hair cells as the first step towards conditional gene knockout in hair cells.

To target Cre expression in outer hair cells, we first isolated a 9-kb promoter fragment from mouse Prestin gene and inserted an IRES/Cre cassette after the first intron. Pronuclear injection was performed in the FVB/NJ strain. A total of three founders were identified. Genotyping of the founders and their offspring was determined by both PCR and Southern blot analysis using Cre. Secondly, we modified two mouse BACs containing Prestin by inserting an IRES/Cre cassette into the last exon of Prestin at the stop codon and the 3'UTR (Zuo et al, PNAS 96: 14100). To express Cre in both inner and outer hair cells, we are modifying BACs containing alpha 9 AChR with IRES/Cre. We will breed transgenic founder mice with a reporter mouse line, ROSA26R, to examine the expression of Cre in transgenic mice during neonatal development and in adulthood. These hair-cell specific Cre expressing transgenic mice will provide an invaluable tool for conditional gene knockout in hair cells for the hearing research community.

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91 Identification of transcriptional regulators of Prestin

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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 which is referred to as electromotility. The molecular motor of outer hair cells has been identified (Zheng, J. et al. 2000 Nature 405 149-155). We recently analysed the upstream region of the prestin gene in order to find elements for transcriptional regulation. Using the rat Genome Walker Kit (Clontech) we found a putative thyroid hormone response element (prestin TRE) in the 5` upstream region in relation to the ATG codon of prestin. Binding specificity of prestin TRE with thyroid hormone receptors (TR) was analysed using Electromobility Shift Assays (EMSA). Functional analysis of TR to transactivate a reporter gene via prestin TRE was performed using Luciferase Reporter Assays with a heterologous promoter. The studies verified the role of prestin TRE as transcriptional enhancer element of prestin and suggested a role of TRalpha or TRbeta acting as either monomer or heterodimer to enhance gene expression. Transactivation studies are used to identify the receptors acting as heterodimers together with TR. The same functional studies are performed to elucidate presumptive novel transcriptional elements of the prestin gene.

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92 Are genes for monogenic hearing impairment involved in age-related hearing impairment? An association study using Single-Nucleotide Polymorphisms (SNPs).

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The acuity of hearing declines with age. This hearing loss is bilaterally symmetrical, sensorineural and most pronounced in the high frequencies. There is a large variance of hearing thresholds at a higher age. Several studies indicate that this is due to a combination of environmental and genetic factors, whereby 35% to 55% of the variance of sensory age-related hearing impairment (ARHI) would be attributable to the effects of genes. None of these genes has ever been identified, but genetic research into hearing impairment has up to now concentrated on monogenic forms of hearing loss. At the moment, 20 genes for monogenic nonsyndromal hearing impairment and even more for syndromal hearing impairment have been cloned. These genes are good candidates to be involved in more complex forms of hearing impairment such as ARHI.

In this study, we looked for an association between common variants in deafness genes and the degree of ARHI, using a random sample of 153 Western European control persons. Hearing thresholds in the high frequencies were registered and expressed as the number of standard deviations from the mean hearing threshold in the otologically normal population (ISO7029 norm). Single Nucleotide Polymorphisms (SNPs) in 5 genes responsible for monogenic nonsyndromic HI (GJB2, KCNQ4, COCH, TECTA and DFNA5) were genotyped. However, none of the SNPs showed significant association with the degree of ARHI.

93 Influence of HLA in the pathogenesis of Meniere's Disease in Korean population

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To study the associations of Meniere's disease (MD) with human leukocyte antigen (HLA) alleles in Korean population, the distribution of HLA class I and class II alleles was examined in 39 patients with MD and 199 healthy controls. The frequencies of HLA-Cw*0303 (RR=2.5, $p<0.02$), and Cw*0602 (RR=3.7, $p<0.03$) were significantly increased in patients with Meniere's disease as compared with the controls. However, HLA-B44 (RR=0.2, $p<0.004$) and Cw*0102 (RR=0.3, $p<0.03$) were significantly decreased in the patients compared with the controls. When an association between hearing level and the presence of HLA alleles was evaluated, the frequencies of HLA-B13 (RR=7.4, $p<0.004$), B39 (RR=45.7, $p<0.003$), Cw*0303 (RR=4.5, $p<0.02$) and Cw*0602 (RR=6.5, $p<0.02$) were significantly increased and the frequencies of B44 (RR=0.1, $p<0.02$) and Cw*0102 (RR=0.1, $p<0.03$) were significantly decreased in patients with the state of mild to profound hearing losses, compared with the controls. HLA-B13 showed the different distribution between patients with and without hearing losses. In HLA-DRB1, DQA1, DQB1, and DPB1 allele frequencies, a significantly higher frequency of DRB1*15 was shown in the patients (RR=2.4, $p<0.03$). These results suggest that some HLA alleles may be a useful genetic marker in implying a prognosis in Korean patients with Meniere's disease.

94 QTL Mapping of a Suppressor of the *Eya1$^{bor/bor}$* Mutant

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Mouse models have provided significant insights into many of the complex molecular mechanisms underlying mammalian inner ear development. These models, in the form of spontaneous and induced mutations, also provide powerful tools for the study of a variety of homologous forms of human syndromic and nonsyndromic hearing loss.

Recently mutations in the EYA1 gene have been identified in some families with Branchio-Oto-Renal (BOR) syndrome. We have described a spontaneous mutation in the mouse orthologue, Eya1, providing a unique animal model for this human disease (Johnson et al. Hum. Mol. Genet. 84:645-653, 1999). BOR syndrome displays variable penetrance and expressivity, both likely the result of genetic background effects.

We have identified a modification of the originally described C3H mutant phenotype in F2 Eya1bor homozygous mutants resulting from C3H/HeJ X CAST F1 heterozygous matings. Unlike the parental (C3H/HeJ) mutants which are uniformly deaf, the F2 mutants display a suppression of the mutation such that there are normal hearing, hearing impaired and deaf mutants in roughly Mendelian ratios. We have analyzed 84 F2 mutants and have initiated QTL mapping of this modifier locus. The utility and potential pitfalls of this approach in the dissection of complex developmental pathways will be discussed.

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95 Genetic Linkage Analysis in a Pedigree with Progressive Low-Frequency Sensorineural Hearing Loss

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We are characterizing a large multigenerational family with moderate-to-profound bilateral sensorineural hearing loss. Auditory dysfunction in this pedigree is noticed typically in the low frequency ranges by the second decade of life and progresses to the mid and high-frequencies by the fourth decade. Some school age children in this family have reportedly failed their mandatory school hearing screening. One older family member is profoundly deaf across all frequencies and has undergone cochlear implantation.

The mode of inheritance in this large pedigree appears to be autosomal dominant. No disease-causing mutations were detected in the connexin-26 gene nucleotide sequence of affected family members. A candidate loci and genome-wide linkage analysis have been initiated on 28 DNA samples from this pedigree. Linkage to the low-frequency hearing loss loci DFNA1 and DFNA6 on chromosomes 5 and 4, respectively, have been excluded.

96 A Physical and Transcript Map of a Region Encoding Autosomal Recessive Syndromal and Non-Syndromal Sensorineural Hearing Loss on Human Chromosome 11q25

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We have previously described the use of autozygosity mapping in a large consanguineous family to identify a gene locus on human chromosome 11q25 causing histiocytosis with associated features of sensorineural deafness and joint contractures. The form of histiocytosis exhibited by this family does not fit readily into any of the recognised classes of the disease and appears to represent a novel form of familial histiocytosis demonstrating autosomal recessive inheritance. The existence of a novel non-syndromal autosomal recessive deafness locus (DFNB20) within this region was subsequently demonstrated by linkage analyses of 35 consanguineous families segregating non-syndromal sensorineural hearing loss with microsatellite markers from chromosome 11q25. An approximately 3cM region of homozygosity was observed in four affected individuals from a single family spanning the interval D11S1320-qter.

We are currently continuing this research using a positional candidate gene approach to identify and characterise the responsible gene(s) at this locus. We present a detailed physical and transcript map of the 11q25 region that includes ten known genes.

We also report the identification of novel polymorphic markers, which will allow us to further define the location of the histiocytosis syndrome and facilitate further refine mapping in this region.

97 Candidate Gene Analysis of Autosomal Recessive Non-Syndromal Sensorineural Hearing Impairment DFNB27

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We previously mapped the DFNB27 locus to chromosome 2q23-31 in a large consangineous family from the United Arab Emirates [Pulleyn L, *et al.*, (2000) Eur. J. Hum. Genet. 8:991-993]. The locus mapped to a 17cM region between markers D2S2157 and D2S2273, overlapping the dominant DFNA16 region.

Using the growing information on the human genome we have now refined the marker order in this region and narrowed it down to a 5 Mbase region between markers D2S1238 and D2S300. The region is now distinct from DFNA16 and contains 20 known genes plus several ESTs and predicted genes. Candidate genes in the region include ATF2, the knockout of which causes neurological defects including hearing impairment in mice. Also within the region is metaxin-2 which is thought to be involved in import of mitochondrial proteins, similar to the deafness-dystonia peptide which is responsible for the X-linked DFN1.

Mutation screening of these candidate genes is now underway and further families are being mapped to see if the region can be narrowed further.

98 A Novel Locus (DFNB30) for Progressive Nonsyndromic Recessive Hearing Loss Maps to Chromosome 10p in an Israeli Kindred.

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Non syndromic hearing loss is one of the most genetically heterogeneous traits known. A total of 71 autosomal non syndromic hearing loss loci have been mapped and 24 genes have been cloned. One of these is DFNB30, which we recently mapped in an Israeli family of Iraqi Jewish ancestry. In this four generation kindred, bilateral progressive non syndromic hearing loss begins in the second decade in the high frequencies. By the seventh decade, hearing loss is severe in high and middle frequencies, with some family members retaining partial hearing in the low frequencies. Vision and balance are normal. Inheritance of hearing loss appears recessive. We mapped the gene responsible for this hearing loss to a 10 megabase region of chromosome 10p12-p11. This genome region includes 17 known genes and at least 10 predicted genes. No part of the 10 megabase region was

homozygous in all affected relatives. In order to identify any regions of homozygosity in subsets of relatives, we created a dense genetic map from microsatellite and SNP markers. Genes with highest priority for evaluation are those which lie in regions of homozygosity in subsets of affected relatives and which are expressed in the inner ear.

99 Towards an identification of the DFNB17 gene

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Hereditary hearing impairment (HHI) accounts for over 50% of the approximately 1 of every 1000 newborn children with hearing loss. Although DFNB1 accounts for a large portion of HHI, many other loci are implicated. The DFNB17 locus has been mapped to an approximately 3-4 cM interval on human chromosome 7q31 in a large consanguineous Indian family. Twenty-two known genes and over 50 cDNAs are currently mapped to this interval. Markers D7S486, D7S2487, D7S655, D7S480 and D7S2529 anchor this region to publicly available physical maps and define a 7 Mb region for DFNB17. To further refine this interval additional polymorphic markers were analyzed against the pedigree. Over 6 highly polymorphic markers and 15 SNPs were informative and refined the centromeric and telomeric boundary to limit the DFNB17 interval to approximately 2Mb. We have determined the cDNA sequence and genomic structure for four genes that map to the DFNB17 interval. These include a putative actin capping protein, a potassium channel related protein, a putative transcription factor containing 12 alternately spliced exons with an HMG-containing DNA binding site and finally a second novel gene, termed “nexin-like” gene, containing 9 exons and having homology to the nexin family of serine proteases. All of these candidate genes were expressed in a human cochlear and mouse inner ear library. *In situ* hybridization studies of the nexin-like gene revealed a unique radial pattern of semicircular canal expression. To determine if mutations in these genes might be the cause of hearing loss at the DFNB17 locus, we screened the coding, splice-site and untranslated regions of the study subjects by direct sequencing. No disease-causing mutations were found in any of the four candidate genes. Analysis of other candidate genes in the refined interval is currently underway.

100 Mutations in GJA1 (connexin 43) cause non-syndromic autosoaml recessive deafness

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Among the more than 20 genes for non-syndromic deafness, connexins are the most common causes for genetic deafness in many populations. The involvement of several members of the connexin gene family in deafness suggests that others should be considered as candidates for non-syndromic deafness. To pursue this possibility, we initiated a study to determine whether there are mutations in GJA1, encoding connexin 43 (Cx43), which lead to deafness and to document the expression of Cx43 in the inner ear. We have found that alterations in a fifth member of this family, GJA1 (connexin 43), appear to cause a common form of deafness in African Americans. We identified two different GJA1 mutations in four of 26 African American probands and several polymorphisms. Three were homozygous for a Leu®Phe substitution in the absolutely conserved codon 11, while the other was homozygous for a Val®Ala transversion at the highly conserved codon 24. Neither mutation was detected in DNA from 100 control subjects without deafness. Connexin 43 is expressed in the cochlea, as is demonstrated by PCR amplification from human fetal cochlear cDNA and by RT-PCR of mouse cochlear tissues. Immunohistochemical staining of mouse cochlear preparations showed immunostaining for connexin 43 in non-sensory epithelial cells and in fibrocytes of the spiral ligament and the spiral limbus. To our knowledge this is the first alpha connexin gene to be associated with non-syndromic deafness. Connexin 43 must also play a critical role in the physiology of hearing, presumably by participating in the recycling of potassium to the cochlear endolymph.

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101 Nonsyndromic progressive hearing loss DFNA38 is caused by heterozygous missense mutation in the Wolfram syndrome gene WFS1

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Dominantly inherited progressive hearing loss DFNA38 is caused by heterozygosity for a novel mutation in WFS1, the gene for recessively inherited Wolfram syndrome. Wolfram syndrome is defined by juvenile diabetes mellitus and optic atrophy and may include progressive hearing loss and other neurological symptoms. Heterozygotes for other Wolfram syndrome mutations generally have normal hearing. Dominant deafness defined by DFNA38 is more severe than deafness of Wolfram syndrome patients and lacks any syndromic features. In a six-generation kindred from Newfoundland, Canada, WFS1 Ala716Thr (2146 G>A) was shared by all deaf members of the family and was specific to deaf individuals. The causal relationship between this missense mutation and deafness was supported by two observations based on haplotype and mutation analysis of the kindred. First, a relative homozygous for the mutation was diagnosed at age 3 years with insulin-dependent diabetes mellitus, the central feature of Wolfram syndrome. Second, two relatives with normal hearing had an identical haplotype to that defining DFNA38, with the exception of the basepair at position 2146. Other rare variants of WFS1 co-inherited with deafness in the family could be excluded as disease-causing mutations on the basis of this hearing-associated haplotype. The possibility that “mild” mutations in WFS1 might be a cause of nonsyndromic deafness in the general population should be explored.

102 Muted Mice Have Selective Inner Hair Cell Loss

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In muted mice, a spontaneous mutation, homozygotes typically have postural abnormalities, and are missing the otoliths in the saccule and utricle of one or both ears. Although an early report (Lyon and Meredith, 1969) states that these mice are not deaf, no systematic investigation of their hearing has been published. In the present study, we investigated the thresholds of homozygous (mu/mu) and heretozygous (mu/+) mice, and evaluated the inner and outer hair cell populations. Mu/+ and mu/mu mice were approximately 7 months in age at time of hearing evaluation. Mice were anesthetized (Avertin), placed on a homeothermic blanket with a rectal probe to maintain normothermia, and recordings were made in a single-walled sound chamber. Auditory brainstem responses (ABRs) were obtained using

TDT hardware and software. Free-field stimuli were presented at a rate of 21 Hz. Stimuli included .025 ms-duration clicks, and 3, 6, 12 and 24 kHz tonebursts (0.5 ms rise/fall time, no plateau, Blackmann window), that decreased in level from 90 dB pSPL in 10 dB steps. The lowest level at which an ABR was observed was called ABR threshold. Upon completion of the ABR protocol, each animal was sacrified, and cochleograms constructed. Vestibular end organs were also prepared as surface preparations and observed. Mean ABR thresholds were similar for both groups, with mean click, 12 kHz and 24 kHz thresholds of roughly 30 dB pSPL, 6 kHz thresholds near 35 dB pSPL, and 3 kHz thresholds of almost 70 dB pSPL. Cochleograms showed a moderate inner hair cell loss in the basal half of the cochlea, with little outer hair cell loss, for both mu/mu and mu/+ mice. Gross inspection of macula of saccules and utricles and cristae of ampulla showed apparently normal hair cell populations.

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103 The Mutations Diminuendo and Catweasel Affect the Hair Cells in the Sensory Patches of the Mouse Inner Ear

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Although many human deafness loci are known, few of these have a homologous animal model. However, recent mouse mutagenesis initiatives have provided new mutations that promise to be useful models of human deafness and vestibular dysfunction. We have begun detailed analyses of two new mutant lines, Diminuendo and Catweasel with initial assessment focussed on the integrity and patterning of the inner ear sensory patches using scanning electron microscopy.

Mutant mice were obtained from N-ethyl-N-nitrosourea (ENU) mutagenesis, and F1 offspring of mutagenised males were screened for new dominant mutations that affect hearing and/or balance. The mutant Diminuendo exhibits a progressive hearing loss whereby the Preyer reflex (an ear flick in response to sound), which is present from about 2 weeks of age, is lost thereafter (between weeks 4-6). This is associated with abnormal organisation of the hair-cell stereociliary bundles in the early postnatal organ of Corti followed by a progressive basal to apical loss of inner and outer hair cells. The mutant Catweasel exhibits behavioural abnormalities, including headtossing, suggestive of a vestibular deficit. In these mutant mice there appears to be a reduction in hair cell number in the utricle and stereocilia of existing hair cells appear to be less numerous and thicker than in the littermate controls. Backcrosses have been set up to map Diminuendo and Catweasel onto the mouse genome and, combined with further analysis of the hair cell changes described above, should help establish appropriate candidate genes for these mutations.

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104 Characterizing The Genetics Of Noise Resistance In 129S6/SvEvTac Mice

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129S6/SvEvTac (129S6) mice demonstrate robust noise resistance relative to CBA/CaJ mice (Yoshida et al., 2000; Kujawa et al., 2001). Toward identifying genes involved in noise resistance, F1 offspring of CBA/CaJ x 129S6 crosses were noise exposed at 4 wk (8-16 kHz octave band, 103 or 100 dB SPL, 2hr) and tested for permanent threshold shifts (PTSs; 4 to 45 kHz, via ABR) at 6 wk. All F1 offspring were CBA/CaJ-like with respect to noise resistance. Given this recessive inheritance pattern of 129S6 noise resistance, F1 mice were backcrossed to 129S6 (CBA/CaJ:129S6 x 129S6) to generate N2 progeny. One of 25 N2 mice noise exposed and tested showed the same hyper-resistance to noise-induced hearing loss (NIHL) as inbred 129S6.

We expect that genetic markers linked tightly to the extreme NIHL-resistance phenotype will be homozygous 129S6 in this NIHL-resistant N2 mouse (named 'Bud'). A full genome scan (160 polymorphic genetic markers spaced at 10 cM intervals across the 1600 cM mouse genome) has been initiated on tail DNA from Bud.

To narrow the homozygous 129S6 genomic areas containing the genes underlying the resistance phenotype, Bud was crossed with CBA/CaJ females to generate N3 offspring. One N3 mouse (the 14th daughter of Bud – 'DOB14') appears to have inherited some or all of these loci, as 4 of 12 test progeny from a cross between DOB14 and a 129S6 male displayed 129S6-like NIHL resistance. Tail DNA from DOB14 (which should narrow the homozygous 129S6 NIHL-resistant regions identified in Bud) and her resistant offspring (which should be heterozygous at the regions containing the NIHL-resistant genes) have been included in the full genome scan.

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105 Acoustic Startle and Prepulse Inhibition in Numerous Strains of Mice

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The acoustic startle response (ASR) and prepulse inhibition (PPI) were used as behavioral tests of responsiveness to acoustic stimuli in various inbred strains of mice. The ASR is elicited by bursts of intense noise (100 dB SPL), with the jerk-like motor reflex measured with a movement-sensitive (startle) device. PPI is a highly reliable behavioral phenomenon whereby a "prepulse" tone (70 dB SPL), not loud enough to produce a startle itself, is presented 100 msec before the startle eliciting stimulus; in neurologically normal subjects, there is a reduction of startle amplitude ("inhibition"). The baseline ASR and PPI are distinctly different measures: the ASR requires very intense sounds and is mediated by neural pathways in the lower brainstem; PPI measures the salience of moderately intense sounds and is mediated by neural pathways that involve the upper auditory brainstem and forebrain.

Approximately 40 inbred strains have been tested (10 males and 10 females per strain), as specified by The Jackson Laboratory Mouse Phenome Project. A wide phenotypic range has been demonstrated for both PPI and baseline ASR amplitude. Several inbred strains are characterized by very poor (virtually absent) PPI and poor ASRs, even though ABR thresholds indicate they have excellent hearing sensitivity. Others have poor PPI which is likely do to hearing impairment. Still others have unusually large ASRs and/or strong PPI. The phenotypic variations in PPI and/or ASR in normal-hearing strains (all reared under standard conditions) suggests that genetic factors are responsible for differences in suprathreshold auditory processing among strains. Some of these strains may be useful models to study mechanisms and genetics underlying individual differences in auditory behavior, central auditory processes, and the the ASR and PPI per se.

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106 Altered Susceptibility to Noise in Guinea Pigs with Unexpressed Hereditary Hearing Deficits

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There is a great variability in the resulting hearing loss found among individuals exposed to similar sound levels. A contributing factor could be unexpressed hereditary hearing deficits influence in the susceptibility to noise. The aim of this study was to investigate how the hereditary deficit in the German waltzing guinea pig affected the response to noise exposure. The German waltzing guinea pig is a new strain of animals expressing recessive hereditary inner ear degeneration (Ernstson and Ulfendahl, in prep.). In the homozygotes, which are deaf at birth, the Reissner´s membrane has collapsed upon the organ of Corti, resulting in a complete loss of scala media. The heterozygotes or carriers are symptom-free at birth but have been reported to express elevated reflex thresholds with increasing age (Ernstson & Ulfendahl, 1998).

Twenty-four animals were tested, 12 carriers of the waltzing guinea pig strain, and 12 wildtype guinea pigs as controls. The animals were exposed to narrow-band noise (1/3 octave, 2818-5623 Hz) at 110 dB SPL (at 4000 Hz) for six hours. Auditory brain stem responses (ABR) were measured at 4, 6.3, 8, and 12.5 kHz before and after noise exposure (24 hours, 1 week, 4 weeks).

All carriers had increased ABR thresholds prior to noise exposure when compared to control animals. Interestingly, the carriers were much less affected by the noise exposure than controls. The carriers of German waltzing guinea pig also recovered more rapidly, and after four weeks they had recovered to pre-exposure levels. The control animals, on the other hand, showed permanent threshold shift (PTS) at all four frequencies that were measured. Thus, the hereditary deficit in the German waltzing guinea pig seems to be resistant against noise trauma, at least up to four weeks after exposure.

107 Towards the Positional Cloning of the Late Onset Mouse Deafness Model *Junbo*

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A genome wide phenotype driven ENU mutagenesis approach was adopted by our facility to describe, among others, novel dominantly inherited single locus deafness disorders in the mouse. One such mutant *Junbo* (GENA 251) has become the primary focus of a positional cloning strategy aimed at describing the underlying molecular lesion. The founder BALB/c/C3HF1 *Junbo* mouse exhibited wildtype pinna reflex responses at P35 but failed to respond to the same stimulus when retested at P228. Analysis of an IVF derived backcross cohort showed the deafness phenotype to have an onset between P40 and P140 and to have a penetrance of approximately 75% on a C3H background. Gross morphological studies of the inner ear showed no evidence of cochlea, vestibular labyrinth or ossicle deformation. Affected mice were, however, shown to have effusive matter filling the middle ear cavity providing evidence that *Junbo* represents a single locus model of otitis media. Further phenotypic analyses have provided evidence of a low penetrance polydactyly of the front paws, craniofacial abnormalities and growth retardation. Following initial linkage to proximal mouse chromosome 3 over 200 meioses from affected *Junbo* animals have been haplotyped allowing the current non-recombinant region to be reduced to a 2cM section with conserved linkage to human 3q26.3. The Cornelia de Lange syndrome locus, CDLS, shares this map position and many phenotypic features with *Junbo* including deafness with high prevalence OME, limb and facial abnormalities and growth retardation. Detailed phenotype description, mapping refinement and gene candidate analyses on the *Junbo* mouse will provide crucial evidence of a putative common molecular lesion with CDLS.

108 Progress toward the molecular cloning of pirouette, a mouse deafness mutant.

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The mouse mutant pirouette exhibits profound hearing loss and circling behavior that are inherited as autosomal recessive traits. Analysis of cochlear pathology in pirouette homozygotes has previously indicated defects in actin-based structures during postnatal development of sensory hair cells. These include progressive deterioration of hair cell stereocilia, beginning in the first week of postnatal life, and the appearance of a 'cytocaud' in inner hair cells by postnatal day 3. Identification of similar defects in mice carrying mutations in Myo15 (shaker 2) has suggested related roles for MYO15 and the pirouette gene product in regulation of actin polymerization in hair cells (Beyer et al., J. Neurocyt. 29:227-239, 2000).

We have previously constructed high resolution genetic and physical maps around the pirouette locus on chromosome 5. Comparative mapping studies indicated that the human ortholog of pirouette is likely to be located at 4p12-14, and suggested this gene as a positional candidate for the DFNB25 nonsyndromic deafness locus. In addition, we have previously characterized two additional alleles at the pi locus, both induced by random transgene integration. Based upon analysis of mouse genomic sequence, and of human sequence from 4p12-14, we have found evidence for five genes within the approximately 1 Mb candidate region. We are currently performing mutation analysis on these genes, with emphasis on those that are located relatively close to the integration site in one of the transgenic alleles.

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109 The genomic structure and mutant alleles of Cadherin 23 (*Cdh23*), the gene mutated in waltzer and Usher Syndrome Type 1D

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Cadherins are components of adherens junctions and play critical roles during embryogenesis and organogenesis. They interact through the formation of anti-parallel dimers to mediate cell adhesion, migration and compaction. We recently showed that cadherins also play important roles in the inner ear; mutations in Cadherin-23 (*Cdh23*) disrupt stereocilia organization on hair cells leading to deafness and vestibular dysfunction in waltzer mice. Here we report our further characterization of the structure and function of *Cdh23*. The mouse *Cdh23* locus is comprised of two 5'-untranslated exons and 69 coding exons; together they cover a genomic distance of at least 350 kb. Amino acid sequence alignments and secondary structure-prediction suggest that Cdh23 ectodomains adopt a conformation similar to the classic cadherins. Nucleotide sequence analysis of six alleles of waltzer reveals a strong correlation between loss-of-function mutations and the deafness/waltzing phenotype. A *Cdh23* transcript with a spliced exon 68 is the predominantly expressed isoform in the organ of Corti. Age-related hearing loss (Ahl) is a non-syndromic trait in common inbred strains of mice associated with the *Ahl* locus on Chromosome 10. Sequence comparison of *Cdh23* between C57BL/6J and CAST/Ei identified ten amino acid polymorphisms. In the 5'- and 3'-untranslated regions we detected eleven single nucleotide polymorphisms. None of these sequence changes correlate with the *Ahl* phenotype. Our results provide the necessary framework for further characterization of *Cdh23* related hearing loss in mice.

110 Identification and characterization of the gene affected in the mouse deafness mutant spinner.

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The mouse mutant spinner exhibits profound hearing loss along with circling and head shaking behaviors. These defects are inherited as autosomal recessive traits and result from a spontaneous mutation on distal chromosome 9 that arose approximately 40 years ago. Our previous morphological analysis of homozygous spinner mice indicated progressive hair cell degeneration in the cochlea, with OHC loss apparent by postnatal day 25, followed by later IHC loss. Degeneration is preceded by defects in neuroepithelial cell morphology, including abnormal stereocilia bundles on hair cells.

Using a positional cloning approach, we have identified the molecular basis of the spinner phenotype. The spinner gene is completely deleted in the original spinner allele, and contains a C to T substitution within its coding region in a new spontaneous allele at the spinner locus. The single base pair substitution is expected to introduce a premature stop codon and truncate the predicted 153 amino acid product of this gene. The sequence of the product exhibits no significant similarities to known proteins. The protein is likely to be membrane-associated, based upon the presence of two predicted transmembrane domains near its N-terminus. The gene is expressed at varying levels in a range of tissues, including the inner ear. We are currently using in situ hybridization and immunocytochemical analyses to determine its site of expression within the cochlea.

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111 Distribution of Opioid Receptors in the Vestibular Periphery.

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We investigated the distribution of μ and δ opioid receptor (MOR and DOR) expression in the ganglia and cristae ampullares of rats using *in situ* hybridization (ISH) and immunohistochemistry (IHC). Young adult Long Evans rats were anesthetized with sodium pentobarbital and kept motionless for 45-60 minutes. Animals were perfused with 4% paraformaldehyde for ISH and light microscopy (LM) IHC studies or with 4% paraformaldehyde and 0.5% glutaraldehyde for transmission electron microscopy (TEM) IHC studies. The temporal bones were removed, the oval windows opened and the tissues were fixed for an additional 24 hours with the same fixatives. For ISH studies, cristae and Scarpa's ganglia were cryoprotected in 20% sucrose, sectioned at 10 μm with a cryostat and stored at –80C. For IHC studies, cristae were embedded in 4% agarose and sectioned at 50 μm with a vibratome. The antigen-antibody complexes were visualized with the peroxidase-diaminobenzidine method for LM, or with a colloidal gold-coupled secondary antibody for TEM. Finally, both DAB and immunogold processed sections were embedded in epoxy resin and sectioned at 2 μm thickness for LM or at 100 nm thickness for TEM. In the Scarpa's ganglia, both MOR and DOR mRNA and immunoreactivity were detected in both large and small neurons. In the cristae ampullares, no specific hybridization was detected while both MOR and DOR immunoreactivity was present in the central and the peripheral regions. Electron microscopy of the cristae revealed both MOR and DOR immunoreactivity in the outer membrane of calyces surrounding type I hair cells, adjacent to efferent nerve terminals. In conclusion, the pattern of expression of opioid peptides receptors in the vestibular periphery suggests that opioid peptides may be involved in efferent regulation of calyceal afferent responses.

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112 Vestibular Melanocytes; Study in vivo and in vitro

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INTRODUCTION: The aim of the present study was to demonstrate the reactions of melanocytes in the vestibular labyrinth under the influence of defined stress factors in vivo and in vitro. These melanocytes are located in areas of extensive energy consumption. Many studies could show that melanocytes play an important role in the regulation of Ca^{2+} ions of the endolymph.

MATERIAL AND METHODS: The present investigation was planned in two phases. First the vestibular labyrinths of 125 pigmented guinea pigs from the Wittmaack´s temporal bone bank were examined and analysed morphologically by computer systems (SIS ® and Sigma Plot ®). These guinea pigs had been treated with acoustic trauma and intoxication under well defined conditions.

Then the membranous labyrinths of 15 fresh sheep´s temporal bones were prepared for in vitro culture. Immunhistochemical markers (Melan A, MEL 5) and the proliferation rate under different conditions were observed.

RESULTS: The first part of the study revealed that due to different stress factors the labyrinthal melanocytes proliferate with considerable number of melanosomes with various deposits of melanin granules in the cytoplasm. These melanocytes posses only few dendrites. Many of the melanocytes of the labyrinth are found in well-vascularized areas of apparent secretory or metabolic importance.

The cultivation of melanocytes in different growth factor mediums showed that the

growth rate is higher in the inner ear than in cutaneous melanocytes and is influenced by the interaction between the subepithelial fibroblasts and the melanocytes.

CONCLUSION: With increasing knowledge of the melanocytes and their participation in the regulation of the inner ear's endolymph metabolism it might become possible to understand the nature of vestibular diseases and to find causal therapies.

113 Quantitative measurement of type I hair cell loss, ganglion cell loss and vestibular nystagmus after carboplatin

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Carboplatin selectively destroys type I hair cells and type I ganglion cells in the vestibular system of the chinchilla, and the loss of these cells is correlated with a reduction in vestibular nystagmus. The aim of this study was to quantitatively compare vestibular hair cell loss with vestibular ganglion cell loss, and to relate these anatomical changes to the duration of vestibular nystagmus. Five adult chinchillas were treated with 100 mg/kg carboplatin, and five others were used as normal controls. Vestibular nystagmus induced by caloric stimulation was evaluated before and 30 days after carboplatin treatment, then animals were sacrificed for evaluation of hair cell and ganglion cell loss. The duration of vestibular nystagmus was significantly reduced after carboplatin treatment, from 61.0 ± 7.26 to 13.5 ± 5.61 seconds. Carboplatin treatment resulted in a significant loss (approximately 30%) of type I hair cells in the striolar region of the macula in saccule and utricle and in the apical surface of the crista ampularis; however, type II hair cells and hair cells in the marginal regions of the sensory

epithelium remained normal. Ganglion cell loss was similar to the type I hair cell loss (approximately 25%).

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114 Calcium activated potassium current properties in cultured rat vestibular afferent neurons.

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We are not aware of previous reports dealing with the Ca^{2+} dependent K^+ current ($I_{K,Ca}$) in the vestibular system primary afferent neurons. The aim of this work was to characterize the $I_{K,Ca}$ in the vestibular afferent neurons in culture (< 24 hrs). The afferent neurons were isolated from wistar rats (P5-P9) and cultured in modified L-15 medium. The ionic currents were recorded using the whole cell patch clamp technique. The current-voltage curves obtained using voltage clamp ramps from a holding potential of -100 and -130 mV have the "N" shape characteristic of the $I_{K,Ca}$. By using $CdCl_2$ 200 µM (n = 5) and iberiotoxin (IbTx) 100 nM (n = 6) we found that $CdCl_2$ reduced the total outward current amplitude in a 31.8 %±1.3 % and IbTx reduced the outward current in a 21.4 ±2.8 %. The IbTx sensitive current activated at -50 mV with a time constant of 4.5 ±0.3 ms at 20 mV, and partially inactivated with a time constant of 260 ± 47 ms at -10 mV. Activation and inactivation time constants depended exponentially on the voltage between -10 and 50 mV. The maximal current was observed at 20 mV (= 95.7 ±0.009 pA/pF). The $I_{K,Ca}$ was found in all the studied neurons (n = 35).

Cultured neurons were also studied by using current clamp protocols. It was shown that IbTx (100 nM) application significantly modified the cultured afferent neurons response to current pulses. The firing frequency, the adaptation time course, the latency to the first potential, and the amplitude and duration of the action potential were modified by IbTx application.

These results indicate that vestibular afferent neurons express the $I_{K,Ca}$ very probably of the big K type and that, the $I_{K,Ca}$ significantly contributes to determine the response pattern of vestibular afferent neurons to electrical stimuli.

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115 Quantal and Nonquantal Junctional Transmission from Hair Cells to Afferents in the Turtle Posterior Crista: Computational Methods.

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To characterize quantal traffic from postsynaptic recordings, we adopted methods previously used by Rossi, Fesce and their colleagues. Characterization of such traffic requires the specification of quantal shape, size, rate and interval statistics. 1) Quantal shape is determined from the power spectrum of background activity, which is fit by a Lorenzian function corresponding to an alpha function, w(t) = C([alpha]t) ^{k}exp(-αt), t * 0, of unity amplitude. 2) To determine quantal size (q), records obtained during 0.3-Hz sinusoidal stimulation are hipass filtered with a corner frequency of 160 Hz. The third (skew) and second central moment (variance) are plotted against each other for 24 equally spaced points per cycle and averaged into a single cycle. From shot-noise theory, quantal size is estimated by the slope of the linear relation between the skew and variance. Instrumental noise is recognized as the variance at zero skew. 3) Quantal rates, r, during the background and during various phases of sinusoidal stimulation are computed from Campbell's theorem for the variances of the filtered record . 4) Quantal interval statistics are determined by subjecting the unfiltered records to Wiener (optimal) filtering. Computational methods were verified with simulated data, consisting of Poisson-distributed impulses of various rates convolved with typical alpha functions. Estimates from physiological data were confirmed by comparing the results of Wiener filtering of the actual data with simulated data whose parameters were obtained from spectral or shot-noise calculations. Estimates of w(t), q and r, the latter two parameters obtained from high-passed data, can be used to predict shifts in the membrane potential of the unfiltered record due to quantal traffic during low-frequency sinusoidal stimulation. Discrepancies with the actual shifts provide evidence for nonquantal transmission.

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116 Differences in Junctional Transmission Involving Calyx-Bearing and Bouton Afferents in the Turtle Posterior Crista.

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Intracellular recordings were made from afferent fibers near the crista. Activity was modulated by sinusoidal indentation of the canal duct. To study synaptic traffic in isolation, spikes were blocked with TTX. Both quantal and nonquantal transmission were observed. As reported previously (Soc. Neurosci. Abstr. 26: 1121), quantal transmission is indicated by the presence of discrete potentials of 0.1-0.5 mV, which peak in 1-3 ms and then decline in 5-15 ms. The potentials have the properties of mEPSPs: they are TTX-insensitive, are blocked in low Ca-high Mg solutions, and are randomly timed. mEPSPs are abolished by CNQX, but are unaffected by AP-5, suggesting that transmission involves AMPA glutamate receptors. Shifts in membrane potential during sinusoidal stimulation are larger than can be ascribed to modulation of quantal rate, implying the presence of nonquantal transmission. The implication is confirmed by the observation that potential shifts persist after quantal transmission is blocked. In the present study, we have been able to distinguish calyx-bearing (CD) afferents from bouton (B) afferents by their characteristic responses to electrical activation of efferent fibers. The two kinds of fibers differ in both their quantal and nonquantal transmission. mEPSPs are smaller and briefer in CD units. Quantal rates are similar in the two fiber classes with background rates of 500-2000 /s. Excitatory identations typically double the rate, while inhibitory identations can silence quantal activity. Stimulation results in a peak-to-peak potential shift of 3 - 4 mV. On average, the nonquantal component makes up 60% of the total shift in CD units as compared to 20% in B units. The mechanisms of nonquantal shifts has yet to be determined. But as it is the potential shift which results in the modulation of afferent discharge, the nonquantal component would appear to be of functional importance especially in CD units.

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117 A Cellular and Pharmacological Analysis of the Responses of Turtle Posterior Crista Afferents to Efferent Activation

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In the turtle posterior crista, there is heterogeneity in the responses of afferents to electrical stimulation of efferent fibers. Many bouton afferents are inhibited whereas calyx-bearing afferents are excited. Intracellular recordings were made from both types of afferents close to the posterior crista to record synaptic potentials during and after efferent activation. Our results show that efferent-mediated inhibition in bouton afferents most likely results from the presynaptic (i.e. efferent to hair-cell) activation of alpha9/alpha10-containing nicotinic receptors (α9/10-nAChRs) functionally coupled to the activation of small-conductance, calcium-dependent potassium channels (SK). Consistent with this suggestion, the inhibition is 1) associated with a reduction in the

frequency of mEPSPs; 2) completely antagonized by cholinergic antagonists known to block α9/10-nAChRs, and; 3) converted into an excitatory response following superfusion with selective SK blockers. During SK blockade, efferent stimulation produces a large depolarizing postsynaptic potential (PSP) that is also completely blocked by α9/10-nAChR antagonists. A portion of the efferent-mediated depolarization may be postsynaptic (i.e. efferent to afferent) because the PSP is not completely abolished by glutamate-receptor antagonists that appear to eliminate quantal transmission from hair cells. Since the remaining efferent-mediated depolarization is also completely antagonized by α9/10-nAChRs blockers, the same nicotinic receptors may underlie both the presynaptic and presumed postsynaptic components. Efferent-mediated excitation of calyx afferents is characterized by a large postsynaptic depolarization without an appreciable change in mEPSP frequency. Unlike efferent responses in bouton afferents, the excitation of calyx afferents is not very sensitive to α9/10-nAChR antagonists.

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118 Position-dependent expression of L and non-L Ca^{2+} currents by hair cells of frog semicircular canals

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The properties and the distribution of Ca^{2+} currents were studied using the whole-cell variant of the patch-clamp technique in hair cells of frog crista ampullaris. The currents were recorded in thin slice preparations and examined in peripheral, intermediate and central cells of the sensory epithelium. Two classes of cells were found: one exhibiting partially inactivating Ca^{2+} currents and one displaying sustained Ca^{2+} currents. Almost all central cells showed inactivating currents, while about 35% of intermediate and peripheral cells displayed non-inactivating currents. Ca^{2+} current magnitude was in average invariably larger in intermediate cells (320 pA) than in central (160 pA) and peripheral (110 pA) cells. In cells showing inactivating currents two Ca^{2+} current components could be detected: a sustained L current sensitive to 5 μM nifedipine and a partially inactivating non-L component insensitive to conotoxin GVIA, conotoxin MVIIC and agatotoxin IVA. Similarly, cells exhibiting non-inactivating Ca^{2+} currents showed a L and a non-L component. In all cells the L component represented the maximal current (70%). Both L and non-L component activated close to –60 mV, showed rapid time course of activation (tau-L, 0.60 ms; tau-non-L, 0.45 ms) and reached a maximal value at –20mV. The magnitude of L and non-L currents significantly varied among peripheral cells. Current density was very small in cells located at the beginning of the peripheral region and increased gradually becoming maximal at the opposite end. No significant gradient of Ca^{2+} current density was detected among intermediate and central cells. These results demonstrate the presence in frog crista ampullaris of regional and intraregional variations in the expression of different types of Ca^{2+} channels. It is likely that such differential distribution represents a mechanism which sustains the variation in the gain and in static and dynamic properties of vestibular afferent discharge.

119 Differential Expression of Voltage-Dependent Currents by Hair Cells from the Frog Utricle and Canal

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Within each vestibular sensory epithelium, currents expressed by hair cells vary from the center to the periphery. This regionalization has been well studied in the semicircular canal, but much less in the otolithic organs. In the pigeon, type II hair cells from corresponding regions of canal and utricle epithelia appear to express similar currents (Weng and Correia 1999). However, since other vestibular hair cell properties differ between amniotes and anamniotes, this similarity may not be conserved through evolution. To test this hypothesis, we compared the whole-cell currents expressed in hair cells from the frog utricle and canal.

Our results suggest that, in the frog utricle, the expression pattern of IA, ICa, and IK(Ca) are similar as in the canal, with IA being largest in the periphery and ICa/IK(Ca) in the striola. On the other hand, all other currents differed in distribution (IK1) or in both distribution and nature (INa, delayed rectifiers) from those of canal hair cells. Two currents (INa, IKvs) appeared to be unique to the utricle, whereas the main delayed rectifier (IKva) was pharmacologically similar the IKv expressed in the saccule. Differently from the canal delayed rectifiers, IKva was largest in the extrastriolar cells.

120 A Voltage-dependent Sodium Channel in Type II Cells of the Rodent Utricle

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Previously it has been shown that a relatively TTX-insensitive sodium (Na) channel is present in type II cells of the semi-intact mouse utricle (1) and in both type I and type II hair cells isolated from the rat crista (2). These currents are almost completely inactivated at the resting potential of type II cells (~-60 mV). In semi-intact utricular epithelia excised from neonatal mice (postnatal days, P, 1-4), whole-cell recordings from type II hair cells revealed a Na current that can be activated from -64 mV, suggesting a more positive voltage range of inactivation. The Na current available for activation from -64 mV was 57.7 ± 8.6% of that available from -124 mV (n=10, mean ± S.E.M.). Activation curves had a half-maximal activation voltage ($V_{1/2}$) of -35.7 ± 3.8 mV (n=10). This current may correspond to the TTX-sensitive Na current that has been reported in type II hair cells in the neonatal rat utricle (3).

One candidate for the α-subunit for TTX-*in*sensitive, negatively inactivating Na channels is the cardiac SCN5A subunit. Preliminary RT-PCR experiments show that SCN5A message is present at P1 and P21 in rat vestibular organs (anterior and lateral cristae and the utricle). Further electrophysiological and molecular biological experiments will be undertaken to investigate more closely the development and identities of Na channels in rodent vestibular hair cells.

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121 Hair Cell and Afferent Counts in Rodent Otolith Organs

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Though much is known regarding the anatomy of the utricular macula (Fernández et al., *JNP* 60:167, 1990), precise estimates of hair cell and afferent numbers are lacking. Our project elaborates utricular macular morphology by counting the numbers of type I and type II HCs in mouse, rat, and chinchilla using the disector method. Labeled cells were also counted in samples of mouse and rat maculae stained using calretinin immunochemistry, which was shown to label calyx afferents in chinchilla (Desmadryl and Dechesne, *EBR* 89:105, 1992) and in 5 other species, and type II HCs in mouse and rat (Desai et al., *Soc. Neurosci. Abst.* 26:6, 2000).

Utricular maculae were dissected from mouse (N=3), rat (N=3), and chinchilla (N=3), 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 and type II HCs, and HCs stained with calretinin, were determined.

Numbers (mean ± SE) of type I (I) and type II (II) HCs and epithelial areas (A) follow: mouse (I, 2466 ± 190; II, 1907 ± 291; A, 0.20 ± 0.0 mm^2; N=3), rat (I, 2380 ± 285; II, 1854 ± 188; A, 0.34 ± 0.02 mm^2; N=3), and chinchilla (I, 8045 ± 786; II, 8353 ± 476; A, 1.00 ± 0.06 mm^2; N=3). The ratios of type I to type II HCs in the medial extrastriola (M), striola (S), and lateral extrastriola (L) for each of the species are: mouse (M, 55:45; S, 60:40; L, 56:44), rat (M, 54:46; S, 64:36; L, 56:44), and chinchilla (M, 45:55; S, 56:44; L, 50:50). The overall ratio of type I to type II HCs is 50:50 in the chinchilla, while the mouse and rat have slightly more type I HCs than type II HCs (55:45). In the mouse and rat, particularly in the extrastriolar region, less than 10% of type I HCs and 30-40% of type II HCs stained with calretinin.

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122 Simulation of the effects of otolith curvature on 3D otoconia displacements by linear accelerations

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We have performed a finite element simulation of realistic otolith displacement by static linear accelerations. The simulations were based on accurate measurements of the surfaces of human utricular and saccular maculae, which indicate a clear curvature of these surfaces. The results show that this curvature, a feature found in all mammals, leads to a significant, direction specific reduction of the linear displacements: utricle and saccule show a reduction of the displacement along the direction of the strongest curvature by 60%. The other two directions are only slightly affected. In addition, the curvature causes a small rotation of the otolith membrane, even for purely linear accelerations. These rotations can account for up to 20% of the overall displacement of the otoconia. Based on the displacements, we also calculated the activation patterns on the otoliths epithelia that are caused by the displacements of the otoconia. These patterns give for the first time an overall image of peripheral otolith activity. The individual activation patterns for selected locations during static pitch or roll tilt correspond well with single cell recordings of actual peripheral otolith neurons.

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123 Modeling Current-Displacement Relations of Utricular Hair Cells .

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Hair bundle deflections trigger transduction by opening mechanically sensitive channels at stereocilia tips. Individual transduction channels behave stochastically. It is less clear how combined action of individual channels generates whole cell transduction currents. We are using finite element models to predict whole cell transduction currents for hair cells in the utricle of a turtle, and to analyze their dependence on bundle structure. Our models predict the tension that develops at transduction channels as the bundle is deflected by a force ramp. We input these tensions to a Boltzmann function to predict channel open probability as a function of force/deflection and the resulting whole cell transduction current.

We have simulated tensions at transduction channels and current-displacement (I(X)) relations for bundles in the medial extrastriola (MES) and the dominant bundle type in the striola (S); these two bundle types differ markedly in structure (number and heights of stereocilia, array geometry, etc.). Simulations of channel tensions for a given input deflection force suggest that they decrease with distance from the kinocilium. In the MES bundle, 31% of channel tensions exceed 0.1 pN when the kinocilium tip is deflected by a small (20 pN) force (vs. 17% in the S bundle). A larger (200 pN) force brings 67% of channels to exceed 0.1 pN tension and produces a large, nearly saturating deflection. In the S bundle, 21% of channels fail to achieve 0.1 pN tension even at 800 pN applied force, suggesting that this bundle can transduce very large accelerations. Simulations suggest that I(X) relations for S bundles have a greater asymptotic amplitude and steeper slope than do those for MES bundles. Support: NSF IBN-9724123 and NIH DC05063.

124 Structure of Otolithic Membranes in the Utricular Striola

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In vertebrate utricles, movement of otolithic membranes is thought to be the proximate stimulus for hair bundle deflection. To characterize this stimulus we need to know the structure of the otolithic membranes (otoconial, compact gel, and column filament layers; Kachar et al., 1990) and how they are coupled to utricular hair bundles. As a first step we are using confocal microscopy to examine relations between the otolithic membranes and underlying hair bundles in the utricle of a turtle, *Pseudemys (Trachemys) scripta*. We incubated fixed utricles in EDTA to dissolve the otoconia and visualized otolithic membranes and hair bundles *in vitro* with fluorescent dyes conjugated, respectively, to wheat germ agglutinin (WGA) and the actin probe phalloidin.

Otoconia over the striola are smaller than those over the medial and lateral extrastriola. Beneath the otoconia, the surface of the WGA-labelled gel layer appears to be perforated by irregularly-shaped openings (unstained regions). In the striola, these openings are especially large (< 10 μm^2 to > 100 μm^2). At successively deeper levels of the gel, these large openings gradually give way to a meshwork of numerous, smaller (2-30 μm^2) openings within a stained extracellular matrix. Phalloidin-labelled hair bundles occupy unstained compartments in the column filament layer and deep surface of the compact gel layer. Some of these compartments are continuous with channels that reach the outer surface of the striolar gel layer. Each channel houses one or more hair bundles; within these channels, bundles are eccentrically placed. Our data suggest that the structure of otolithic membranes in the utricular striola varies with distance from the neuroepithelium and that a subset of bundles occupy channels that are open to the otoconial mass.

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125 Displacement of the Semicircular Canal Cupula for Sinusoidal Stimuli

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Displacement of the horizontal semicircular canal cupula in response to sinusoidal mechanical indentation of the canal was measured in the toadfish, Opsanus tau. Fluorescent polystyrene microbeads of 1-micron

diameter, with WGA lectin surface modification, were adhered to the utricle-side surface of the horizontal canal cupula. Sinusoidal indentation of +/-15 microns amplitude and frequencies ranging from 0.3 to 30 Hz was applied. The microbead markers were imaged using epi-fluorescence microscopy, and marker positions were captured using stroboscopic illumination and a large-format CCD camera. Results show that the displacement gain of the central region of the cupula increased slightly with stimulus frequency, ranging from 0.07 to 0.2 microns per micron of indent (or 0.018 to 0.05 microns per deg/sec of head rotation velocity). The displacement phase with respect to head rotation velocity was ~10 degrees advanced and showed no frequency dependence. Displacement results and previously measured differential pressure results were empirically fit and used to derive a complex-valued volumetric impedance for the cupula. The modulus of the impedance showed a gradual increase in stiffness and a constant damping across the frequencies tested. Measurement of displacements at different locations along the length of the crista revealed no differences in phase response. Displacements at various positions on the surface of the cupula were also measured, providing the deflected shape of the cupula during sinusoidal canal indentation.

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126 A mathematical model of the mechanical coupling and the hair cell dynamics in the semicircular canals

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The object of our work was to develop a model of the semicircular canal (SC) hair cell response to mechanical stimuli. We introduced a model of the SC such that cupula was modeled both as a piston or as a diaphragm. The SC was modeled as a toroid with two main components: i) a duct and ii) a widening that forms the ampulla and the utricle. The endolymph motion was described by the Navier-Stokes equations. The analysis of the model demostrated that the cupular dynamics under periodic stimulation is equivalent in the piston and diaphragm models. Based on these, a simplified model in which the detailed cupular structure is not essential is presented. By combining mathematical methods with the morphological analysis of the SCs of the axolotl (Ambystoma tigrinum), a differential equations system describing cupular displacements as a function of angular acceleration was obtained. The total ionic current and voltage responses to current injection were studied in isolated hair cells from the axolotl SCs. The total ionic current of the hair cells was dominated by an outward-going current that partially inactivates. The maximal conductance, activation and inactivation time constants and its voltage dependences were considered as characteristics of the total current. A Hodgkin-Huxley type model of the hair cell was developed. The data obtained from the experimental measurements were used for the definition of model parameters. The mathematical analysis of the model showed the absence of a limit cycle and the presence of only one asymptotically stationary steady regime. For the mechanoelectrical transduction process the model developed by Marking and Hudspeth (1995) was used in this work. On these basis we proposed an approximate hair cell mechanical receptor model that allowed for the analysis of hair cell response to angular accelerations.

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127 Three-dimensional Biomechanical Model of Semicircular Canalithiasis and Cupulolithiasis

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A morphologically descriptive 3-canal mathematical model was developed to study the biomechanical origin of gravity-dependent semicircular canal responses observed under pathological conditions of canalithiasis and cupulolithiasis. Results are relevant to the origin and treatment of Benign Paroxysmal Positional Vertigo (BPPV). The mathematical model describes the influence of calcium carbonate debris (particles) located in a canal lumen and/or adhered to a cupula on the time-dependent responses of the ampullary organs. Each semicircular canal was modeled as a curved tube filled with endolymph, considered as a Newtonian viscous fluid undergoing unsteady Stokes flow. The particles were modeled as spheres free to move in the membranous labyrinth lumen (canalithiasis) or adhered to a cupula (cupulolithiasis). The total force acting on each particle was modeled as summation of gravitational force and the viscous drag interaction of the particle(s) with endolymph. The complete model consists of a coupled set of second-order differential equations to predict the position(s) of the particle(s) as well as the endolymph volume displacement in each canal. The model was applied to predict debris movement in response to reorientation of the head relative to gravity and to predict the influence of the debris on canal responses to angular motion stimuli.

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128 A Computational Model of the Caloric Test Based on Gravitational Fluid Physics

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Robert Bárány (1907) first described the characteristic positional nystagmus provoked by caloric stimulation of the external auditory canal as a method to assess vestibular labyrinthine pathology. He proposed a mechanism based on convection forces that develop when the adjacent endolymphatic fluid rises (warm stimulus) or sinks (cold stimulus), causing an ampullopetal or ampullofugal deflection of the lateral semiircular canal (LSC) cupula with a resulting nystagmus. However, when the caloric test was performed in microgravity (SpaceLab), the direction of the resulting nystagmus was identical to that seen on Earth. This observation prompted a reassessment of Bárány's hypothesis as the sole mechanism of the caloric response.

We used direct numerical simulations (DMS) based on gravitational fluid physics to describe the endolymphatic flow expected in the LSC under normal (1G) and microgravity (μG) conditions. Aatomical measurements of human temporal bone specimens were used to construct a three-dimensional finite element model of the LSC. Caloric test data in 1G were used to test the model's predictions of nystagmus resulting from varying the intensity of therml stimuli and canal orientation to gravity.

We found the model predicts that the direction of endolymphatic fluid flow and LSC cupula deflection in μG does not differ from 1G. We conclude that the Bárány hypothesis of endolymphatic fluid flow as the ource of the caloric nystagmus is supported by more sophisticated DMS methods based on modern gravitational fluid physics

129 Ototoxicity: An historical note and a reason for hope

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Ototoxicity as a side-effect of drug treatment was hardly mentioned in early medical writings, but Avicenna (980-1037) warned that when mercury vapor was used to kill head lice, the host could be deafened by the treatment. In 1696 Morton wrote that temporary deafness was the only ill-effect of using cinchona bark in treating fevers. By the mid-19th century both quinine and the salicylates were known to cause tinnitus and impaired hearing, but the unwelcome symptoms usually did not last and were thus of little concern to patient or physician. The action of the anthelmintic oil of chenopodium was more serious, because deafness and vestibular problems could be permanent. Ehrlich's antisyphilitics atoxyl and arsacetin could also affect hearing and balance, but it was not until the late 1940s, when prolonged and successful use of streptomycin against the scourge of tuberculosis frequently impaired both sensory systems that ototoxic drugs presented a serious problem for medicine and for society. They can be of value in the treatment of Menière's disease, but that use has yet to be fully established. Recent studies have revealed and elucidated--at least in part--the biochemical basis of the action of the aminoglycosides on the inner ear. The findings have led to a viable and readily available means of preventing ototoxicity that has been successfully tested in the clinic.

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130 In Vivo Uptake of Fluorescently-conjugated Gentamicin by Avian Cochlear Hair Cells

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Vertebrate sensory hair cells are pharmacologically-sensitive to aminoglycoside antibiotics used in life-threatening Gram-negative bacterial infections, e.g. meningitis. The nephrotoxicity and ototoxicity of aminoglycosides are well-known, but the rate of drug uptake in vivo remains poorly understood. We have identified intracellular locations of fluorescently-conjugated gentamicin in hair cells (nuclei, mitochondria, Golgi bodies, endoplasmic reticulum, and throughout the cytoplasm) in vitro. However, little is known about the rate of drug uptake in vivo. In this study using post-natal chicks, we find that fluorescently-conjugated gentamicin is preferentially taken up by hair cells 6-9 hours post-injection, in a cochleotopic gradient (from high-to-low frequency regions).

Post-natal day 14 chicks were injected with 300 mg/kg gentamicin solution containing a 300:1 molar dilution of gentamicin-Texas Red (GT-TR) conjugate. The inner ears were excised at 3 hour intervals post-injection and fixed with formaldehyde and subsequently labeled with FITC-phalloidin. GT-TR uptake in other organs was also tracked. Cochleae were mounted and observed using confocal microscopy.

GT-TR fluorescence was observed in blood, bone, liver, and muscle at 3 hours but no label was seen in the cochlea. GT-TR was preferentially taken up by proximal cochlear hair cells within 6 hours, and at progressively later time points, increasing numbers of more distal hair ells displayed GT-TR labeling. Supporting cells displayed negligible GT-TR fluorescence.

Uptake of GT-TR by avian cochlear hair cells occurs in a cochleotopic gradient, similar to the pattern of hair cell death in the avian cochlea, where proximal high frequency hair cells die before the more distal hair cells in lower frequency regions. The implications of this uptake pattern are discussed.

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131 Simulation of gentamicin distribution in inner ear fluids with round window application

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Local drug application to the inner ear is gaining wider clinical acceptance. As a result, it is important to establish how drugs are distributed in the ear as a function of time for different delivery methods. This study presents an analysis of prior data documenting gentamicin concentrations in the inner ear fluids of the chinchilla following round window (RW) application. The analysis utilized a simulator program that takes into account inner ear dimensions and drug dispersal processes, including diffusion, clearance and inter-compartmental exchange. In order to simulate data from chinchillas accurately, cochlear fluid spaces of the chinchilla were quantified using 3-dimensional magnetic resonance images. The published time course of gentamicin in vestibular perilymph was closely approximated by the adjustment of parameters defining RW membrane permeability, drug clearance and inter-scala exchange. The time course was consistent with gentamicin spreading from scala tympani to scala vestibuli in the basal turn by local communication processes, and could not be explained by diffusion via the helicotrema. In addition, a comparison of different RW delivery methods was performed. Gentamicin levels and spatial distribution in the ear were markedly influenced by the time the applied drug remained in the middle ear. Drug levels at different locations were compared using peak level (C_{max}) and area under the curve (AUC), both of which varied with the delivery method. The relative distribution of drug in the ear was influenced by the delivery method when quantified by C_{max} but not when quantified by AUC. It can be concluded that in the development of local inner ear drug application strategies as clinical therapies it is necessary to consider inner ear pharmokinetic characteristics, the specific drug delivery method and the therapeutic range for the applied drug.

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132 Reactivity of copper complexes with gentamicin

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Gentamicin is one of the aminoglycoside antibiotics. A major disadvantage of this drug is its oto- and nephro-toxicity. The underlying mechanism of this toxicity is postulated to result from gentamicin-induced free radical formation via iron complexes [1]. Aminoglycoside are also know to be strong chelators for Cu(II) ions and resulting complexes mediate oxidative reactions [2]. We therefore determined whether gentamicin can share these properties and whether its copper(II) complexes may contribute to aminoglycoside toxicity.

Coordination of Cu(II) to gentamicin and redox activity of the resulting complexes were studied by UV-Vis, CD and EPR spectroscopies. Only mononuclear complexes (species with one Cu(II) ion), exhibiting two types of coordination modes, were detected in the range of pH 5-12. At low pH copper(II) is coordinated to gentamicin through 3" nitrogen and 4" oxygen – {N, O} coordination mode. A second coordination mode {2N, 2O} corresponds to involvement of the next nitrogen (2') and oxygen (5') in Cu(II) binding.

Conjugated diene formation from arachidonic acid in the presence of copper(II)-gentamicin complexes and H2O2 at pH 7.4 clearly indicate activation of H2O2 towards free radicals formation. The reporter molecule for hydroxyl radicals, N,N-dimethyl-p-nitrosoaniline indicated the presence of hydroxyl radicals in the Cu(II)-gentamicin-H2O2 system. Interaction of gentamicin with Cu(II) and resulting oxygen activation may contribute to free radicals formation by this drug.

Thus, copper ions might play a role in the biological activity of gentamicin contributing with iron to the in vivo toxicity of aminoglycosidic antibiotics. Relative importance of Cu(II), Fu(II) and Fe(III) will dependent on bio-availability of these ions.

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133 Kanamycin Increases Reactive Oxygen Species And Apoptosis In The Mouse Cochlea

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The generation of reactive oxygen species (ROS) has been implicated in the toxic side effects of aminoglycoside antibiotics. Aminoglycosides enhance iron-catalyzed ROS formation and lipid peroxidation in vitro, and antioxidants or iron chelators attenuate aminoglycoside-induced threshold shifts in vivo. Since ROS are well established effectors of signal transduction pathways for apoptosis, a causal link between ROS and ototoxicity can be hypothesized.

In this study, we determined ROS formation and apoptotic pathways in a recently established mouse model of aminoglycoside ototoxicity (Wu et al., Hear. Res. 158:165-178, 2001). CBA mice received kanamycin (700 mg/kg body weight bid) for 3, 7 or 15 days. The 15-day regimen will result in a threshold shift of approximately 50 dB at 24 kHz. Cochlear lipid peroxidation (measured as thiobarbituric acid-reactive substances, TBARs, and 4-hydroxynonenals, 4-HNE) and peroxynitrite formation (determined via nitrotyrosine) increased with the duration of treatment. Immunoreactivity to nitrotyrosine and 4-HNE was found in inner and outer hair cells, supporting cells and spiral ganglion cells. Caspase-3 activity, a marker of apoptosis, was significantly enhanced by kanamycin treatment. The number of TUNEL-positive apoptotic nuclei also increased in spiral ganglion and supporting cells, outer hair cells and, to a lesser extent, inner hair cells. Concurrent treatment with 2,3-dihydroxybenzoate, which protects against kanamycin ototoxicity, attenuated the increases in TBARs, 4-HNE, caspase-3 activity and the number of apoptotic cells in the cochlea.

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134 Translocation of cytochrome c in hair cells of gentamicin-treated avian basilar papilla

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Sensory hair cells exposed to aminoglycosides have been shown to undergo an apoptotic-like death. Recent evidence implicates a class of proteases called caspases as crucial mediators of hair cell degeneration (Forge & Li, 2000, Hear Res, 139:97). One key activator of caspases is cytochrome c (cyt c), which is translocated from the mitochondria to the cytoplasm when pro-apoptotic molecules (e.g. bax) overwhelm anti-apoptotic molecules (e.g. bcl-2). Although mechanisms by which cyt c promotes cell death are unclear, its translocation is important in neuronal cell death (Deshmukh & Johnson, 1998, Neuron 21:695).

We previously reported caspase-3 activation in gentamicin (gent) - damaged hair cells in chick basilar papilla (BP) and used a general caspase inhibitor (z-vad) to protect hair cells against gent. The experiments reported here were designed to characterize: 1) the subcellular localization of cyt c in normal and gent-damaged hair cells; and 2) the effect of caspase inhibition on cyt c translocation. Whole organ BPs from 5- to 10-day-old chicks were exposed to 0.5mM gent for 6 hrs with or without 100μM z-vad. Cochleae were then immediately processed for TuJ1 and cyt c immunoreactivity. Gent-treated BPs contained statistically significantly more hair cells with translocated cyt c (mean=82.8) than did control tissues (mean=1.4). The number of hair cells with translocated cyt c further significantly increased with co-treatment with z-vad and gent (mean=211). Our data indicate that cyt c translocates to the cytoplasm in dying hair cells and suggests that this redistribution of cyt c is an upstream event to caspase activation. Previous research on neuronal cell death suggests that the increase in cells with cyt c translocation following caspase inhibition is due to survival of the cells having this condition. Ongoing experiments are testing this hypothesis by examining the distribution of cyt c at earlier and later times after aminoglycoside treatment.

135 Alteration of E-cadherin expression during the occurrence of hair cell apoptosis in mouse vestible

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E-cadherin, Ca^{2+}dependent homophilic cell-cell adhesion molecule, is known to be a major adhesion molecule in the inner ear sensory epithelia. In the organ of Corti, the distribution of E-cadherin and its reorganization following aminoglycoside treatment has been demonstrated. However, the expression of E-cadherin in the vestibular epithelia and its alteration aftert aminoglycoside treatment is unknown. On the other hand, E-cadherin is reported to be associated with regulation of apoptosis in other systems. In this study, we examined alteration of E-cadherin expression in mouse vestibular epithelia during induction of apoptosis in hair cells.

ICR mice were used. The animals administrated a neomycin solution by injection into to the posterior semicircular canal of the left ear. The utricles were extracted at one day, three days and five days after treatment. Utricles of the right ear were used as the control. The specimens were fixed with 4% paraformaldehyde. We then performed immunostaining for E-cadherin and TUNEL staining in whole mount.

In the control specimen, TUNEL positive cells were found in specimens treated with neomycin. TUNEL-labeling was most prominent at the third postoperative day. Degradation of E-cadherin expression was observed in parallel with induction of hair cell apoptosis. However, at 5 days after treatment, E-cadherin expression, which was poor-organized, restored.

These findings suggest that E-cadherin-mediated cell-cell contacts between hair cells and hair cells or supporting cells temporally degrade during induction of hair cell apoptosis.

136 Unilateral Transtympanic Gentamicin Activates Apoptosis Cascade in Both Ears

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Recent research in aminoglycoside ototoxicity has focused on the deleterious effects of these drugs on neuroepithelial and support cells. However, earlier studies noted damage to inner ear ganglion cells. We used a chinchilla model to further explore the effects of aminoglycosides on the vestibular and spiral ganglion. We performed either unilateral transtympanic (TT) bolus (230 μl) or minipump (Alza 2002, 1 μl /hr) injections of gentamicin (GENT, 10 mg/ml). After a 4 h to 14 d exposure, animals were euthanized and transcardially perfused with 10% phosphate buffered paraformaldehyde. The temporal bones were decalcified, embedded in paraffin and sectioned in a mid-modiolar horizontal plane. Alternate sets of sections were stained immunohistochemically for GENT (anti-GENT, Sigma) and markers of apoptosis cascade activation (anti- cleaved caspases 3, 7, 9 and intact PARP, Cell Signaling Technologies). Intense GENT immunoreactivity (ir) was observed within 4 h in spiral (Sp) and Scarpa's (Sc) ganglia cells, stria vascularis, and connective tissues of the injected ear. Sp and

Sc neurons still showed intense GENT-ir after 14 d of exposure by either method. In the contralateral ear, GENT-ir was apparent as early as 4 h after injection and persisted 14 d. Sp and Sc neurons in both ears also expressed PARP and cleaved caspase 3, 7, and 9. These results demonstrate that TT GENT is retained bilaterally by Sp and Sc neurons for at least 14 d in chinchillas. The Sp and Sc GENT uptake also appears to activate pro-apoptotic signaling pathways, which could lead to ganglion cell death.

137 A mouse experimental model for hair cell apoptosis

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Mouse experimental models have several advantages including availability of transgenic or knockout animals over other animal models. However, there have been few reports describing degeneration of the inner ear using mice for resistance of mouse tissues against ototoxic agents and difficulties in surgical treatments. In this study, we examined three mouse models treated with local application of aminoglycosides. Gentamicin application through the round window membrane caused hair cell apoptosis, but the numbers of apoptotic hair cells were too small for analysis of mechanisms for apoptotic processes. A single injection of gentamicin into the cochlear second turn produced numerous apoptotic hair cells in the cochlea and vestibule. However, individual differences among experimental animals were large. In addition, degeneration of sensory epithelia was too severe for investigation of subsequent repair processes. A single injection of neomycin into the posterior semicircular canal induced moderate damage of sensory epithelia. The numbers of apoptotic hair cells in this model were sufficient for analysis of apoptosis. In addition, individual differences among animals could be ignored. Using sham-operated specimens, we confirmed that apoptosis observed in this model was caused by aminoglycoside toxicity, not by surgical damage. Apoptosis in hair cells in this model could inhibit by supplementation of a caspase inhibitor. Moreover, immuhistochemistry for cytochrome c revealed that redistribution of cytochrome c occurred in apoptotic hair cells. These findings indicate that the experimental model using injection of neomycin into the posterior semicircular canal was suitable for analysis of hair cell apoptosis and subsequent repair processes in mouse inner ear.

138 A Computational Model of Hair Cell Loss: Effects of Ototoxic Drugs and Acoustic Trauma

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When hair cells are subjected to stress, a complex system of signaling pathways and genetic networks are activated. This system is made up of many subsystems, some related to cell repair, others to apoptosis. Whether a cell recovers or dies will depend on the level of stress as well as the previous history of stress and other environmental factors and the complexity of the stress response makes it difficult to predict the impact of an arbitrary insult.

We have begun developingt of a comprehensive computational model for the hair cell stress response in the chick basilar papilla (BP). At any instant in time, each hair cell is assumed to be in one of several states which correspond to activation or inactivation of various stages in the stress response. Each state is associated with specific molecular markers (e.g. TIAR, cytochrome-c, caspase-3) so that the model can be compared directly with experimental results. To model the effects of ototoxic drugs, the cell-stress model is coupled to a pharmacokinetic model that predicts the time course of drug concentration in extracellular and intracelluar compartments. To model the effects of acoustic trauma, the cell-stress model is coupled to a model that predicts the acoustic power delivered to each cochlear region.

To calibrate the model, groups of birds are either exposed to traumatizing tones or are given single subcutaneous injections of gentamicin. Animals are then sacrificed at regular intervals and whole mounts of the BP are prepared for assessment of hair cell damage and protein expression/activation. The time course and extend of damage and expression is measured and compared to model predictions. The model parameters are then adjusted to bring the model into better agreement with the experimental data. The approach is an interactive one in which we cycle between experiments and model refinement.

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139 Inner Ear Cell Lines as an In Vitro System for Drug Ototoxicity Screening

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Little is known about the cell and molecular basis of drug ototoxicity. Experimental evidence suggests that some well-known ototoxic drugs such as aminoglycosides, may be able to induce apoptosis in sensory hair cells. Here, we report the derivation of two inner ear cell lines, one from the organ of Corti (HEI-OC1) and the other from the vestibular sensory epithelia (HEI-Ve1) of Immortomouse™, that undergo apoptosis when exposed to ototoxic drugs. HEI-OC1, HEI-Ve1 and NIH3T3 (fibroblast-control) cells were exposed to different concentrations of gentamicin, streptomycin, cisplatin (pantoxic), or penicillin (non-ototoxic) for 24 hours. Apoptosis was assayed by measuring caspase-3 activity. We found that inner ear cell lines were, on average, 5-fold more sensitive to ototoxic drugs than to penicillin. In addition, we used the HEI-OC1 cells to evaluate the protective effect of L-carnitine on cisplatin-induced apoptosis. We found that a 48-hour pre-incubation of HEI-OC1 cells with 2mg/ml of L-carnitine, significantly diminished the cytotoxic effect of 0.5 mM cisplatin (24-hour incubation). Altogether, our results suggest that these cell lines could be used as an in vitro system for drug ototoxicity screening. The availability of conditionally immortalized inner ear cell lines, sensitive to ototoxic drugs, will facilitate the elucidation of the molecular mechanisms of drug ototoxicity and will thus help to devise better strategies to prevent and/or reverse ototoxic drug-induced sensorineural hearing loss. The cell lines can also be used in automated, high throughput platforms to simultaneously evaluate large numbers of drugs, to determine toxic profiles, and to investigate potential synergistic interactions.

140 The chinchilla as a model of rapid spiral ganglion cell degeneration

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Research on topics related to the survival of spiral ganglion neurons (SGNs), such as the effects of neurotrophins, electrical stimulation, and various pharmacologic agents, are aided by the use of animal models in which SGNs degenerate in a predictable fashion over a well-defined time course. One method for producing an animal model of SGN degeneration is to co-administer a large dose of gentamicin (GM) and ethacrynic acid (EA), which causes rapid and complete destruction of hair cells in the cochlea. Most SGNs survive the initial GM/EA insult but subsequently die. In animal models such as cats and monkeys, SGN degeneration is a slow process that can take more than a year to complete. The slow time course of degeneration is a major drawback in the use of these species. Our data suggest that the chinchilla may be a more practical model, because SGNs degenerate very rapidly following GM/EA treatment. We injected 17 adult chinchillas with a single concurrent dose of GM (125 mg/kg, IM) and EA (40 mg/kg, IV) and measured SGN loss after 0.5, 1, 2, 4, and 6 months of survival. Cochleograms showed that all of the inner and outer hair cells were

missing 2 days after GM/EA treatment. At 0.5 months, most SGNs were present but morphologically abnormal. Approximately 70% of SGNs were missing at 1 month post-treatment; 95% were missing at 2 months, and 99-100% were missing at 4 and 6 months. The rapid time course of SGN loss in the chinchilla should make this animal a more practical model for studying mechanisms of cell loss and survival. Intervention strategies aimed at preventing the delayed loss of SGNs are currently underway.

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141 Effect of gentamicin on isolated cochlear outer hair cells with and without liver extract.

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The cause of gentamicin ototoxicity has been reported to be mediated by free radicals such as nitric oxide, superoxide, and peroxynitrite. Gentamycin otic drops are commonly used not only for otitis externa but also for otorrhea in the presence of tympanostomy tubes or tympanic membrane perforations. Many studies have demonstrated the ototoxicity of common otic preparations such as Cortisporin.® otic drops. Surprisingly, we observed that gentamicin, when exposed alone to isolated outer hair cells (OHCs), did not demonstrate as much ototoxicity as expected. The purpose of this study was to assess the relative ototoxicity of gentamicin with and without liver extract by direct exposure to isolated OHCS. OHCs from adult chinchilla's cochlea were exposed to standard bathing solution (control), gentamicin alone (1:40 dilution), gentamicin?(1:40 dilution) with liver extract, and liver extract alone. The cells were observed and recorded using an inverted microscope, and then analyzed on the Image Pro-Plus 3.0® program. The ototoxicity was based on the results of two different observations: the time to cell death and the change in cell length of the OHCs. The time to cell death observed in the gentamicin(n=14), liver extract (n=5), and gentamicin + liver extract (n=5) were 50.0±.00, 53.4.±9.1, and 28.0±5 minutes respectively. The gentamicin group was significantly different from the gentamicin + liver extract group (p=0.006008). The percent change in length observed in the gentamicin, liver extract, and gentamicin +liver extract were 85.6±4.6, 87.9±.6.4, and 54.8±.6.4 pixels respectively. The gentamicin group was significantly different from the gentamicin + liver extract group (p=0.004074). Our study suggests that gentamicin was activated by liver extract producing significant cytotoxicity towards isolated cochlear OHCs.

142 Morphological and functional changes in the guinea pig inner ear during intracochlear administration of gentamicin by osmotic pump

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Transtympanic administration of gentamicin has been reported to be one of the useful therapy for vertigo, such as Meniere's disease. But it is difficult to determine the accurate concentration of gentamicin in clinical therapy. To investigate the effective and safety concentration of gentamicin, various concentration of gentamicin was administered to the guinea pig inner ear by osmotic pump, and we observed caloric nystagmus duration changes as a marker of vestibular function, and ABR threshold changes as a marker of cochlear function. Fourteen days after treatment, 40 mg gentamicin caused canal paralysis, but ABR remained moderately. Four milligrams gentamicin showed no obvious change in both vestibular and cochlear function. Histopathological examination revealed severe cytoplasmic damages in both vestibular and cochlear endorgans in guinea pigs given 40 mg gentamicin after 14 days. In guinea pigs given 4 mg gentamicin, no significant change was observed in vestibular and cochlear endorgans histopathologically. Our results indicate the possibility that appropriate concentration of gentamicin may act as an effective agent for the treatment of vertigo.

143 Responses to Communication Calls in the Inferior Colliculus of the Mustached Bat

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In the mustached bat inferior colliculus (ICC), many neurons have facilitatory or inhibitory frequency response areas. Many neurons also have multi-peaked frequency tuning curves with one curve often near 20 kHz. These complex response properties make the neurons well suited for analyses of communication calls. Many communication calls of the mustached bat are multi-harmonic with fundamental frequencies around 20 kHz. Here, we assess if single unit responses to communication calls in the ICC can be explained based on neuronal excitatory, facilitatory and inhibitory response areas. We tested excitatory areas by varying the frequency of a single tone between 10-100 kHz. 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. Rate-level tests were performed with 14 calls. Half the units responded best to one call, with responses to 2 or 3 other calls having higher thresholds. Other units responded to 6-13 calls, but thresholds varied, suggesting intensity is important for selectivity. Many responses could be explained by excitatory frequency tuning. Units with multi-peaked tuning curves showed less selectivity, and units tuned to low frequencies (10-20 kHz) responded to many calls. This was not surprising as tuning to the lower frequencies tended to be broad. Thus, many calls had energy falling within the tuning curve of the unit. Facilitatory interactions increased selectivity. For example, one unit was tuned to 57 kHz and facilitated by a 28 kHz tone. This unit only responded to one call, an upward frequency-modulated sweep with narrow bands of energy in the 28 and 57 kHz ranges. Even though other calls had energy at 57 kHz, the combinatorial response provided for selectivity to one call. The results show that some neurons in the ICC respond selectively to communication calls, and that complex spectral integrative properties underlie the selectivity.

144 Role of Inhibition in Shaping Neural Responses to Complex Stimuli in the Inferior Colliculus of Mustached Bat

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Many neurons in the mustached bat's inferior colliculus (IC) exhibit time-sensitive interactions (facilitatory or inhibitory) between distinct spectral elements in sounds. Prior work showed the importance of glycinergic inhibition in facilitatory interactions. In this study we compare the roles of glycine and γ-aminobutyric acid (GABA) in creating both inhibitory and facilitatory interactions. Single unit responses were tested before and after application of bicuculline (GABA-A receptor antagonist) and/or strychnine (glycine receptor antagonist). The neurotransmitter antagonists were applied individually and simultaneously using 5-barrel micropipettes, while simultaneously recording with a piggy-back single-barrel micropipette. "Facilitatory" units exhibit a nonlinear increase in the response to one spectral element in the presence of a second spectral element at a particular delay (the "best delay"). "Facilitatory" units often showed inhibitory interactions at delays shorter or longer than the best delay. Strychnine by itself or with bicuculline eliminated the time sensitive facilitation, but bicuculline, by itself, did not. Bicuculline or strychnine, together or individually, did not eliminate inhibition at shorter delays. Inhibition at longer delays is

eliminated by bicuculline in some units. These results suggest that: a) facilitatory interactions are dependent on glycine and not on GABA, b) there is little interaction between the glycinergic and GABAergic inputs in creating facilitation, and c) inhibition at shorter delays may not be created in the IC. Among "inhibitory" units, the response to one spectral element is suppressed by a second spectral element presented simultaneously. Bicuculline and strychnine, by themselves or together, did not block the inhibitory interaction. This suggests that this interaction is not glycine- or GABA-dependent and could be a property of neurons projecting to the IC.

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145 Forward and reverse engineering of mechanisms that shape selectivity for FM sweeps.

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Frequency-modulated (FM) sweeps are prominent features of the communication and echolocation signals of many species. Studies of selectivity for FM sweeps have provided important information about how the central auditory system wires itself to extract spectrotemporal information. This report approaches this issue in two ways; unraveling the components of selectivity for the rate and direction of FM sweeps in adults, and tracking the ontogeny of selectivity in the developing auditory system. The pallid bat is useful because its auditory system contains a high percentage of neurons that respond selectively to the downward FM sweep of its echolocation pulse; in the inferior colliculus, one third of neurons tuned to the echolocation pulse respond exclusively to its direction and velocity. Young bats exhibit a selectivity for the pulse immediately after representation of their full audible range, but prior to experience with echolocation, suggesting a strong genetic contribution to response selectivity. The present study uses a two-signal, spectrotemporal analysis approach in which delaying the onset of either excitatory or inhibitory sounds is used to reveal the timing of the inputs that shape selectivity for this dynamic signal. Results suggest that the differential arrival times of inhibitory inputs generated by most or all frequencies within the FM sweep are responsible for creating selectivity for direction and rate of frequency change. Early arrival of inhibitory input by lower frequencies in the sweep may shape direction selectivity by suppressing responses to upward sweeps, while later arrival of inhibition from higher frequencies may shape selectivity for rate of frequency change.

146 Representation of Communication Signals in the Inferior Colliculus of the Guinea Pig

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The responses of individual neurons in the inferior colliculus (IC) to guinea pig vocalization calls presented in free-field conditions were evaluated. Single unit activity was extracellularly recorded in guinea pigs anesthetized with a mixture of ketamine and xylazine. Four types of guinea pig communication signals were used for stimulation: purr, chutter, chirp and whistle. The spectrotemporal discharge patterns of the IC neuronal population were clearly related to the spectrotemporal acoustic patterns of vocalization sounds. The areas of elevated neuronal activity in population response maps matched the areas of the occurrence of energy in spectrograms of the calls, but the response patterns were not simply replications of the acoustic patterns. The similarity of peri-stimulus time histograms (PSTH) to the energy of the call in the near-CF frequency band suggests that the temporal pattern of the near-CF frequency band plays a dominant role in the unit response. Interestingly, the response patterns of units with similar CFs can be fundamentally different, suggesting different coding mechanisms. Each population PSTH obtained as an average PSTH over all units was correlated with the sound envelope of the call with the exception of whistle, since this call is acoustically highly structured with both frequency and amplitude modulation. The importance of sound temporal features was confirmed by the observation that the time-reversed version of whistle elicited significantly weaker responses (by 24 % on average) than the response to the natural version of whistle. Calculation based on instantaneous firing rates clearly indicated the position of the main spectral peaks, but did not always reflect their relative magnitude. On the other hand, the synchronization indices calculated for sounds consisted of many repeated phrases (i.e. purr and chutter) showed a weak relationship to sound spectra.

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147 Responses of Inferior Colliculus Neurons to SAM Tones Located in Inhibitory Response Area

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Responses of single neurons in the inferior colliculus of chinchilla to sinusoidally-amplitude-modulated (SAM) tones alone and the presence of steady-state tones were obtained. The carrier frequency of SAM tone was set to either the characteristic frequency (CF) or a frequency in the inhibitory response area (RA) of a studied neuron. When a SAM tone was presented alone in inhibitory RA, a neuron showed no response or a very weak response to the modulation. However, when a steady-state CF tone was presented simultaneously with the SAM tone, modulated responses were observed. The response to the off-CF SAM tone paired with a CF tone was 180 degrees out-of-phase with the response to a modulated CF tone, suggesting that the response to the steady-state tone was inhibited by peaks in the SAM tone. When the CF tone and the off-CF SAM tone were equal in intensity, responses showed lower synchronization compared with responses to on-CF SAM. Temporal modulation transfer functions (tMTFs) were also measured, with and without a steady-state CF tone. For those neurons showing band-pass tMTF, the best modulation frequency could be different and the bandwidth of the tMTF tended to be narrower when an off-CF SAM tone was paired with a CF tone. The results suggest that the responses of IC neurons can be altered by modulation that is located outside the excitatory RA.

148 Representation of Amplitude Transients in the Inferior Colliculus of Decerebrate Cats

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The sensitivity of the auditory system to the temporal structure of the sound amplitude envelope has been extensively demonstrated both psychoacoustically and physiologically. Recent physiological studies of anesthetized preparations suggest that neural responses to onset ramps are shaped by the dynamics of the onset envelope, rather than by the level of the sound or the onset duration per se. In the current study we use linear amplitude ramps that rise above a pedestal to investigate the representation of amplitude transients in the ICC of unanesthetized decerebrate cat. The ramped pedestals are 600 ms BF tones with 10 ms onset/offset time and 190 ms of plateau level (0-30 dB above the unit's threshold) before and after the ramp. The 200 ms ramp starts 200 ms after the pedestal onset and has variable rise/fall duration (2-50 ms) and variable level above the pedestal (6-20.8 dB). Units are classified as suggested by Ramachandran et al. (1999).

The responses of each unit to the ramp above the pedestal were divided to two components, transient and sustained, the transient component usually defined by the first 25-45 ms of the response. The ramp rise-time, step-size and slope were compared for best predicting the transient and sustained components of the response using multiple linear regression and cluster analysis. We show that the ramp slope is the best predictor for the transient component of all the Onset units (11/11) and for some of the O-type units (4/13). By contrast, The ramp step-size is

the best predictor for the transient component of all the I-type units (8/8) and for the majority of the O-type units (9/13). The ramp step-size is the best predictor for the sustained component of all the units that have significant sustained responses. This suggest a dichotomy between primarily transient-sensitive, broadly tuned neurons (onset) and primarily level-sensitive, sharply tuned neurons (type I).

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149 Spectro-temporal weighting functions determined using pseudorandom and white noise stimuli in the inferior colliculus

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We describe two new methods for characterizing an auditory neuron's sensitivity to combinations of sound energy in time, frequency, and between the two ears. These new methods are based on previous methods of deriving spectral weighting functions with steady-state stimuli, and differ from spectro-temporal receptive fields (STRFs) in that they directly estimate spectro-temporal weights, in spikes/dB/sec (Yu and Young, 2000). These weights can be applied to predict a neuron's response to novel complex stimuli, as a measure of the model's validity. The first method uses pseudorandom, "checkerboard" stimuli with a random, time-varying spectrum. The second method uses gaussian white noise decomposed for analysis into a multiresolution spectrogram with time and frequency resolutions as in the auditory nerve. Through multivariate linear regression, time-varying firing rate is related to spectro-temporal content in these stimuli to estimate spectro-temporal weighting functions (STWFs).

STWFs were estimated binaurally for single units in the ICC of the unanesthetized, decerebrate cat. For both methods, 5 out of 12 type I and type O units showed long-lasting, on-BF inhibition lasting up to 20 msec after on-BF excitation and off-BF inhibition. This inhibition in STWFs from both white noise and spectro-temporally rich stimuli is consistent with results of STRFs obtained using ripple stimuli in the ICC of the cat (Escabi et al, 2001). The mean-squared error (MSE) of prediction and mean standard error (SE) of time-varying rate were calculated and normalized by the mean-squared response power (P) to allow comparison across units and methods (MSE/P, SE/P). Preliminary results show smaller MSE/P and larger SE/P values for the multiresolution method compared to the checkerboard method. This suggests that the multiresolution method has slightly higher prediction accuracy, but requires more repetitions for rate estimates to converge.

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150 Segregation of Spectral Information Processing among Neural Populations in the Inferior Colliculus

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Previous studies have shown that neurons of the ventral (VCN) and dorsal (DCN) cochlear nuclei transmit different rate representations of an acoustic spectrum. While VCN chopper neurons encode spectral level within a narrow band about best frequency (BF), DCN principal neurons use wide-band mechanisms to detect the presence of spectral notches. VCN chopper and DCN principal neurons transmit this information to the central nucleus of the inferior colliculus (ICC). The current study investigates the extent to which such spectral representations are maintained by ICC neurons in unanesthetized cats. For each neuron, the transformation of spectral level into rate was quantified by a weighting function, an estimate of the neural receptive field. Functions were derived from responses to wide-band stimuli with random spectral shapes. Their generality was then tested by predicting responses to 99 virtual space stimuli (VSS) that contain realistic spectral cues. ICC rate responses to VSS contain 1.1 to 2.6 bits of information about stimulus identity, a range comparable to that of cochlear nucleus neurons. This suggests that VSS are encoded by ICC neurons in an organized manner. The transformation of spectral level into rate is a nearly linear function in 16 Type I units (Ramachandran et al., 1999). Weighting functions suggest that Type I units are driven by strong on-BF excitatory inputs originating from the contralateral ear. Transformation of level into rate is most linear for 10 Type I units that are inhibited by ipsilateral noise. This property makes these neurons most suitable for transmitting information about interaural level differences. The responses of 7 Type O and 9 Type V units--neuron types that are driven by strong contralateral inhibitory inputs--are less predictable. The sensitivity of 1 Type O unit to sharp spectral features was created by a second-order transformation of spectral level; however, the remaining units fail to respond to VSS spectra in an obvious manner. It is possible that these neural populations contribute to spectral feature detection in ways that are not yet understood.

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151 Modeling Spectrotemporal Integration in the Central Nucleus of the Inferior Colliculus

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Neurons in the central nucleus of the inferior colliculus (ICC) are known to exhibit numerous nonlinearities. Here we use a spectrotemporal integrate fire (STIF) model neuron to study nonlinear spectrotemporal integration in the ICC. The STIF model is obtained by first fitting neuronal STRFs with a sum of spectrotemporal Gabor functions. Next, the modeled STRF (STRFm) is used to predict the intracellular current in response to rippled stimuli. This current is injected into the integrate fire (IF) compartment which models the neuron's membrane dynamics and threshold nonlinearity. The effects of various intrinsic parameters are tested including: threshold level, time constant, refractory period and signal to noise ratio (SNR). Comparing modeled results with neuronal data, we find that differences in response to dynamic ripple (DR) and rippled noise (RN) stimuli can be explained by the model and these appear to be most affected by the neuron's threshold and SNR. Furthermore, we find that high intracellular thresholds are likely responsible for selectivity enhancement of the response of some neurons to DR. The presented findings outline rules for spectrotemporal transformations that may arise from intrinsic mechanisms.

152 Complex Sound Perception: Binding Harmonics within Single Combination-Sensitive Neurons

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Perception of complex communication sounds is a major function of the auditory system. Spectral representation of sounds performed by the basilar membrane "place-coding" mechanisms is then maintained up to the higher levels of the auditory system. Nevertheless, in order to create a coherent percept of complex sounds consisting of multiple harmonic frequencies, the auditory system should instantaneously group or bind the individual harmonic components (spectral grouping). This perceptual strategy may simplify further processing of complex sounds and facilitate their meaningful integration with other sensory inputs. Based on experimental data and our realistic neural network model, we propose that associative learning of combinations of harmonic frequencies and nonlinear facilitation of responses to those combinations, also referred to as "combination-sensitivity", may provide a mechanism for spectral grouping. The neural network was created with commercially available software (Neuroimitator^{TM}) allowing phenomenological simulation of electrophysiological properties of classical neurons and synapses. It had seven layers for processing auditory inputs between the periphery and the midbrain and

included a parallel tonotopic input that converges and diverges within the network. We simulated combination-sensitivity of the auditory neurons using Hebbian and associative types of synaptic plasticity. After associative learning of harmonic combinations, neurons in higher-order layers of the network exhibited an emergent property of multifrequency tuning that is consistent with experimental findings. Spectral grouping was demonstrated as the capacity of the network to "recognize" the fundamental frequency of a harmonic complex even when the fundamental frequency itself was missing.

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153 Neural Correlates of the Huggins Dichotic Pitch

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Dichotic pitch is a phenomenon in which changes in the interaural phase relationship of dichotically presented noise produce a tonal percept. One example is the Huggins pitch (HP), in which the interaural phase difference (IPD) of the noise has a given value, ϕ, in a narrow signal band and is ϕ-π at all other frequencies. These stimuli produce a tonal percept at the center frequency of the band, regardless of whether the band is in phase (ϕ=0, HP_+) or antiphasic (ϕ=π, HP_-).

To search for neural correlates of dichotic pitch, we presented HP_+ and HP_- stimuli to anesthetized cats and recorded the responses of delay-sensitive units in the inferior colliculus (IC). The overall interaural time difference (ITD) of each stimulus was set to the best and worst ITD of the unit, and responses were recorded as a function of signal band center frequency. The bandwidth was always 8% of the center frequency. In conditions where the signal band was positioned at a favorable ITD (HP_+ at the best ITD or HP_- at the worst ITD), the vast majority of units showed a peak in firing rate when the band center frequency matched the unit CF. On the other hand, when the signal band was at an unfavorable ITD (HP_+ at the worst ITD or HP_- at the best ITD), most units showed a notch in the firing rate when the signal band matched CF. For a few units, responses were measured as a function of ITD. The majority of these showed a significant decrease in ITD sensitivity (both a drop in rate at the best ITD and an increase in rate at the worst ITD) when the signal band center frequency coincided with CF. These results are consistent with an interaural cross-correlation model for delay-sensitive IC neurons, and generally support existing models of dichotic pitch.

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154 Are pitch neurones the result of difference tones on the basilar membrane?

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One theory of pitch suggests that neural maps of periodicity (quantified by sensitivity to amplitude modulated, AM, tones) exist in the inferior colliculus (IC). Low characteristic-frequency (CF<2.0kHz) IC neurones, acting as pitch extractors, respond to high-frequency AM tones outside their traditional frequency-vs-level response area with preferred best modulation frequencies (BMFs) matching their CFs. These "pitch neurones" appear similarly responsive to stimulus fine structure and stimulus envelope structure, suggesting they receive input from neurones of different (higher) CFs, tuned to AM rates equal to their own CF. An equally plausible explanation for such behaviour, however, is that pitch neurones respond to difference tones produced by non-linearities of the cochlea in response to high-frequency AM tones. To test this possibility, single-neurone responses were recorded in the right IC of anaesthetised guinea pigs to monaural and binaural stimulation. A high-frequency tone at moderate levels (<65 dB SPL) outside the pure-tone response area was presented to the left ear with 100% AM at a rate equal to CF. The hypothesis that neural activity to this stimulus results from difference tones produced by the AM was examined by presenting a tone (CF minus 1Hz) to the opposite, right, ear evoking a binaural beat response. This response is assumed to result from coincidence detection of low-CF inputs in the lower brainstem. We further tested the hypothesis by cancelling the presumed internally-generated difference tone in the left ear with an external tone. The phase and amplitude of the cancellation tone was estimated by presenting a tone (CF minus 1Hz) to the left ear to beat monaurally at 1Hz with the (CF) difference tone. As expected, addition of the cancellation (CF) tone to the left ear abolished the binaural beat response. These data are consistent with the notion that pitch neurones are responding to low-frequency information present on the basilar membrane.

155 Binaural Bandwidths of Inferior Colliculus Neurones Measured using Interaurally-Delayed Noise

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Low-frequency neurones in the inferior colliculus (IC) respond to interaural time differences (ITD) of tones with cyclic functions with a period equal to the stimulus frequency. For broad-band stimuli, the ITD functions are quasi-periodic: a neurone's frequency tuning determines the periodicity. The attenuation of the side-peaks of the noise delay function (NDF), relative to the central peak, is a measure of a neurone's binaural band-width. We quantified the binaural band-widths of neurones in the inferior colliculus of anaesthetised guinea pigs using interaurally-delayed broadband noise. A NDF was obtained over ±1.5 cycles of the period of the best frequency (BF). The noise level was 15 to 25 dB above the binaural noise threshold. Each NDF was fitted with a Gabor function by minimising the mean square error between the NDF and the Gabor: a good fit was obtained for 315 neurones. The parameters of the fitted Gabor functions provide metrics of 1) the peak ITD, 2) the symmetry of the NDF, 3) the most effective binaural frequency (EBF), and 4) the binaural bandwidth. EBFs for neurones with BFs of 350-450 Hz deviated only minimally from BF. EBFs for neurones with lower BFs tended to be higher than BF, whilst EBFs for neurones with higher BFs tended to be lower than BF. This suggests that 350-450 Hz are the most effective frequency components for modulating the output of IC neurones to interaurally-delayed broad-band noise. The binaural bandwidth describes the attenuation of the side-peaks of the NDFs and is a measure of neural bandwidth. Across all neurones, the average binaural bandwidth, expressed as an equivalent rectangular bandwidth, was 0.96 ± 0.76 octaves re. EBF, and 0.98 ± 0.93 re. BF. Those neurones for which BFs and EBFs were most similar, showed the narrowest binaural bandwidths (i.e. least damping of side peaks of NDFs), with binaural bandwidths broadening (greater damping of side-peaks of NDFs) as BF and EBF became more disparate.

156 Coincidence of Amplitude Input/Output Functions by Expressing Stimulus Level as Rate of Pressure Change Fails for Short Noiseburst Risetimes

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Noiseburst input/output (I/O) functions from the auditory nerve and inferior colliculus (IC) show changes in slope when noiseburst risetime is varied. However, if stimulus level is respecified as rate of pressure change at sound onset (in Pa/s), then the I/O functions across noiseburst risetime overlap (Phillips et al., 2001). Data from the chinchilla IC and auditory cortex (AC) showed that these respecified I/O functions for the amplitude data did not overlap for lower level noisebursts with risetimes of less than 1-2 ms (Burkard et al., 2001). The present investigation

evaluated the effects of noiseburst risetime and level from the chinchilla IC and AC, using risetimes shorter than 1 ms.

Chinchillas had chronic electrodes implanted in the IC and AC. Following a recovery period, animals were placed in a passive restraint, and recordings were made from the right IC and AC. Stimuli were noisebursts ranging in level from 78 to 0 dB SPL in 6 dB steps. Risetimes included 0, .125, .25, .5, 1, 2, 4 and 8 ms.

For both IC and AC, latency I/O functions for the various risetimes were superimposed when stimulus level was expressed as rate of pressure change (Pa/s). The amplitude I/O functions, with stimulus level in Pa/s, did not result in a superimposition of the various risetime functions: for lower stimulus onset slopes (in Pa/s), the functions for shorter risetimes fell below those for the longer risetime data. The shorter the risetime, the higher the stimulus slope (in Pa/s) at which these functions diverged, and in both the IC and AC data. It appears that a physiologic filter with a finite time constant limited responses to the slope of noiseburst onset. This effect is most prominent for lower signal levels, suggesting an origin in the acoustically most sensitive cochlear sector.

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157 Gap Responses from Chinchilla Inferior Colliculus and Auditory Cortex Across Noiseburst Level

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The purpose of the present study was to investigate the effects of noiseburst level on gap detection. Six adult chinchillas were anesthetized and tungsten electrodes were surgically placed in the auditory cortex (AC) and inferior colliculus (IC). Following a recovery period, AC and IC responses to single noisebursts or noiseburst pairs were recorded from unanesthetized animals. Noisebursts were 50 ms in duration (0 ms risetime), and presented at levels of 30, 55 and 80 dB SPL. Gap times included 0.25 , 0.5, 1, 2, 4, 6, 8, 12 and 16 ms. Responses were amplified (10,000X) and filtered (10-3000 Hz), with each response the average of 100 stimulus presentations. A response time epoch of 180 ms was used. The dependent variables were the latencies and amplitudes of onset 1 (onset response to first noiseburst), offset 1 (offset response to first noiseburst), onset 2 (onset response to second noiseburst), and offset 2 (offset response to second noiseburst), as well as gap thresholds (shortest gap at which a response was present). For onset 1 and onset 2 data, latencies decreased and amplitudes increased with stimulus level for both IC and AC. Also, onset 1 and 2 latencies increased and amplitudes decreased with decreasing gap time, for both IC and AC. The gap threshold (shortest gap at which a response was present) of the offset response to noiseburst 1 was substantially longer that of the gap threshold of the onset response to noiseburst 2. This was true for both IC and AC data, for all noiseburst levels. For offset 1 and offset 2 data, both latencies and amplitudes increased with increasing noiseburst level, for both IC and AC. Furthermore, the amplitudes of offset 1 of IC increased when gap time increased from 8 to 16 ms.

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158 The Effects of Broadband Noise on the Envelope Following Response from Chinchilla IC and AC

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In the present three studies, adult chinchillas were used to examine the effects of broadband noise on the amplitude of the envelope following response (EFR) from chinchilla inferior colliculus (IC) and auditory cortex (AC). Chinchillas were anesthetized, and tungsten electrodes implanted. After a recovery period, unanesthetized chinchillas were placed in a passive restraining device and put in a sound-attenuating chamber. Recordings were made from the right IC or AC, while the left ear was stimulated with a two-tone stimulus consisting of F1 and F2, and broadband noise. F1 was constant at 2000 Hz while F2 varied between 2040, 2080, 2160, or 2320 Hz. Stimulus and noise levels varied across the 3 experiments. The noise and the stimulus were acoustically mixed for the first and the third experiment, and electrically mixed for the second experiment.

Results in all three experiments showed a decrease in EFR amplitude from both IC and AC for noise levels at or above 40 dB SPL. For higher stimulus levels, in both the IC and the AC, there was EFR amplitude enhancement at moderate noise levels. The greatest amount of enhancement occurred at 160 Hz in the IC, and at 80 Hz in the AC. The first study showed enhancement at 80 dB pSPL for 60 dB SPL of noise. The second study showed EFR enhancement at 80 dB pSPL for noise levels from about 40-60 dB SPL. We again saw enhancement in the third experiment at 75 and 90 dB pSPL for moderate noise levels. We also saw some evidence of enhancement at various stimulus levels for the 20 dB SPL noise condition.

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159 The Effects of Ipsilateral, Contralateral and Binaural Masking Noise on the Envelope Following Response from the Inferior Colliculus and Auditory Cortex of the Chinchilla

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We examined the effects of broadband noise on the onset response and the envelope following response (EFR) from the inferior colliculus (IC) and auditory cortex (AC) of the adult chinchilla. Tungsten electrodes were implanted in five chinchillas under anesthesia. After a recovery period, unanesthetized chinchillas were placed in a passive restraining device, in a sound-attenuating chamber. Recordings were made from the IC and AC while presenting the left ear with 60 dB SPL toneburst pairs (F1 and F2). F1 was held constant at 2000 Hz and F2 was varied between 2040, 2080 and 2160 Hz. Broadband masking noise was continuously presented to the same ear as the paired tones (ipsilaterally), to the opposite ear as the tones (contralaterally) or to both ears (binaurally), at levels ranging from 0 to 80 dB SPL in 10 dB steps. Dependent variables included the latency and the amplitude of the onset response, as well as the amplitude of the EFR. EFR amplitude was obtained by windowing the waveform, doing a Fast Fourier Transform, and obtaining the amplitude of the spectral component at the F2-F1 difference tone.

Onset response and EFR amplitudes from both the IC and AC decreased for masker levels at and above 30 dB SPL. This decrease in amplitude was greatest for ipsilateral and binaural noise. Although smaller than the effects seen for ipsilateral and binaural noise, contralateral noise did produce a decrease in IC and AC onset response and EFR amplitudes. Onset response latency increased at or above 40 dB SPL ipsilateral or binaural masking noise, with little effect of contralateral noise on IC or AC response latency. For some stimulus conditions, EFR amplitude from the IC and AC did not monotonically decrease in amplitude with increasing level of contralateral noise.

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160 Influence of bicuculline on two-tone masking and the processing of repetitive stimuli in the primary auditory cortex of the gerbil

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Temporal characteristics of cortical auditory processing are important for two tone masking as well as for neuronal response to repeated presentation of the same stimulus. To test the extent to which GABAergic inhibition could play a role in shaping corresponding properties of cortical neurons, in two-tone masking experiments a suppressor tone of variable frequency and intensity was presented 30ms

prior to a probe tone at the neurons best frequency at 10-20dB SPL above threshold. The neurons response to the probe tone is changed such that suppression of activity in the area of excitatory tuning curve and at lateral sidebands could be observed. To assess if masking, especially in the lateral sidebands, is mediated by intracortical GABAergic inhibition or already produced by subcortical mechanisms, we compared the suppressive effects caused by the masker before and during iontophoretic application of the GABAA antagonist bicuculline(BIC) in primary auditory cortex neurons of anesthetized gerbils. A minority of neurons showed a decrease of the suppressive effect of maskers presented within the excitatory tuning curve during the application of BIC. In most of the neurons, BIC had no significant influence on the areas of two-tone masking and on the neuronal response to the probe tone, suggesting a subcortical rather than an intracortical inhibitory mechanism. We also investigated the neuron´s ability to follow a pure tone presented at increasing repetition rates of 1.25-9.09Hz before and during application of BIC. Again, in a minority of neurons a tone evoked response could be elicited at higher repetition rates during BIC application, in the majority of cells there was no significant effect of repetition rate. The data emphasize that temporal processing below the level of the auditory cortex is strongly involved in shaping cortical responses to multiple stimuli.

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161 Responses of Neurons in Cat Primary Auditory Cortex to Bird Chirps and their Modifications

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To study the representation of complex sounds in primary auditory cortex, we used natural sounds extracted from field recordings (© the Library of Natural Sounds, Cornell Laboratory of Ornithology, Ithaca, New York). The chirps consisted of amplitude- and frequency-modulated tones with some lower-energy noise components such as echoes and unrelated background sounds. Bird chirps with an acoustic context (about 200 ms of sound preceding and following the chirps) were used as the basic stimuli. These stimuli were gradually simplified. A segment containing only the chirps and their simultaneous background was extracted from the basic stimuli (this stimulus is called Natural below). Each Natural stimulus was separated into the clean chirp (Main) and the remaining Noise. The simplest stimuli consisted of frequency modulated tones that roughly followed the trajectory of the clean chirps, but with a constant amplitude (Artificial). Responses of well-separated neurons were recorded in primary auditory cortex of halothane-anesthetized cats. Each simplification step significantly changed the neuronal responses. There were only weak correlations between the responses to the basic stimuli and to the Natural stimuli, and between the responses to the Natural stimuli and to the Main stimuli. Many neurons responded to the Natural stimuli in the same way that they responded to the Noise stimuli, altough the Main component in Natural was 15-20 dB louder. There were also large differences between the responses to the Main stimuli and the Artificial stimuli, which were caused by differences in their temporal envelopes, mostly at onset. Thus, neurons in auditory cortex are very sensitive to small acoustical perturbations of their input. Such sensitivity is not expected in models based on simple linear frequency filtering such as the spectro-temporal receptive field.

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162 Comparison of Response Characteristics in Auditory Cortex of the Awake and Anesthetized Ferret

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We recently developed an awake preparation for chronic physiological recording in the ferret (ARO Abstracts, Fritz et al, 2001) and described neural responses in auditory cortex in the awake animal to a variety of acoustic stimuli including tones, noise, ripples and their combinations. Previous neurophysiological studies of the ferret auditory cortex in our laboratory (Depireux et al, 2001) have used an anesthetized preparation which has yielded valuable insight into auditory function but may have caused depression of neural activity and inhibited cortico-cortical interactions. Hence we have continued to explore responses in auditory cortex of the awake animal in order to understand the full range of dynamic information flow, and have recorded from over 300 single units. We characterized and correlated the activity of single units from neighboring cells in a cortical column in response to these stimuli.

As we have shown in previous research, units in auditory cortex are well characterized by their responses to the envelope of moving ripples. An important property of a spectro-temporal receptive field (STRF) is its separability, i.e. whether it can be decomposed into the product of two 1-dimensional functions (temporal and spectral). We have characterized the STRFs from cortical neurons in the awake and the anesthetized preparation analyzing separability in the two populations and found an overall similarity of the STRFs in both conditions. However, we observed differences in the response pattern to pure tones in the awake vs anesthetized ferret (increased sustained on-responses and vigorous off-responses in the awake preparation) and so discuss the apparent disparity in the effects of anesthesia on cortical responses to pure tone and dynamic ripples.

163 Effects of Reversible Inactivation of the Ferret Primary Auditory Cortex on the Localization of Brief Sounds

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Lesions of the primary auditory cortex (A1) in primates and carnivores impair auditory localization. These studies have so far been restricted to measurements of horizontal localization in the frontal hemifield. We have examined the effects of reversibly inactivating ferret A1 on localization judgements made throughout the horizontal plane and in the midsagittal plane.

Ferrets were trained on an approach-to-target task to localize broadband noise bursts presented from one of 12 loudspeakers located at 30° intervals around the perimeter of the testing arena. Vertical localization was measured by training animals to discriminate between sounds presented from one of two speakers that were separated by different angles in the midsagittal plane. A1 was inactivated bilaterally over long periods with Elvax implants releasing the $GABA_A$ receptor agonist muscimol. The spatial extent and time course of inactivation were examined by recording single-unit activity, both in A1 through a hole in the Elvax and at various distances from the edge of the Elvax.

Muscimol-Elvax implants reversibly impaired the animals' ability to localize short-duration noise bursts in both the horizontal and vertical planes and led to a significant increase in the number of front-back errors made. These data confirm that activity in A1 is needed for normal sound localization and suggest that this cortical area may be involved in the analysis of spectral cues, which are used for vertical localization and for resolving the spatial ambiguities inherent in binaural cues.

164 Is the perceptual orthogonality of pitch and timbre based on orthogonality of periodotopy and tonotopy?

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Harmonic sounds, particularly voiced speech sounds and animal communication signals, are characterized by periodic envelope or amplitude modulations. The periodicity information is coded in spike intervals in the auditory nerve and by different temporal response patterns in the nucleus cochlearis. A neuronal model has been suggested that utilizes these temporal informations for a correlation analysis of signal periodicity in the midbrain assuming delayed responses and coincidence of delayed and undelayed responses as basic processing elements (Langner 1983, Exp. Brain Res. 52: 333; Langner 1992, Hearing Res. 60: 115).

Units at different locations in the central nucleus of the IC were found to be tuned not only to a certain frequency, but also to different modulation frequencies represented in topographic maps for a wide range of modulation frequencies (Schreiner and Langner 1988, J. Neurophysiol. 60: 1823). Similar maps have been found with electrophysiological recordings in the auditory forebrain of mynah birds (Hose et al. 1987, Brain Res. 422: 367) and in the cortex of gerbils (Schulze and Langner 1997, J. Comp. Physiol. 181: 651), with optical recording in cat cortex (Langner et al. 1997a),with 2-deoxyglucose mapping in ICC and cortex of gerbils (Langner et al. 1999 Assoc. Res. Otolaryngol: 180), and with magnetoencephalography in the auditory cortex of humans (Langner et al. 1997, J. Comp. Physiol. 181: 665)

It is remarkable, that in each case tonotopic and periodotopic organizations have similar and approximately constant gradients which are orthogonal to each other. Therefore, one should consider the periodotopic axis as the 2nd neural axis of the auditory system The orthogonality of tonotopy and periodotopy in the auditory system may relate to the fact that in psychophysics timbre, a percept largely defined by the spectral content of a signal and pitch, which has its temporal correlate in periodicity, are to a certain extent orthogonal or independent perceptual variables (Plomp & Steeneken 1971, 7th Int. Congr. on Acoust.: 1602).

165 Phase-locked responses to pure tones in the guinea pig primary auditory cortex

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We have previously shown evidence of phase-locking to pure tones in the ventrorostral belt area of the guinea pig cortex. We now show that many units in the primary area (AI) also phase-lock to pure tones. Binaural and monaural stimuli (100 - 200 ms tone pips with 8 ms rise/fall times) were presented in a closed sound system to guinea pigs anesthetised with a ketamine/xylazine mix at a level sufficient to maintain areflexia. Recordings were made with glass-insulated tungsten electrodes and typically were from small clusters of 2 or 3 units, although some single units were also recorded. Responses to pure tones at 100 Hz were obtained from 145 clusters/units. Of these 105 did not phase-lock at 100 Hz (mean characteristic frequency (CF) of 365 Hz). The remaining 40 did phase-lock at 100 Hz and also over a range of 60 -300 Hz (mean CF 379 Hz). These phase-locking clusters were recorded in seven tracks oriented approximately orthogonal to the cortical surface. Phase-locking units occurred at depths of 0.1 to 1.27 mm (layers I-V). Strongest phase-locking occurred in layers III and IV, where the thalamic afferents terminate. Within any one track all the phase-locked units responded at the same phase. The strength of phase-locking varied with the stimulation frequency and was sometimes sharply tuned. It also varied with sound intensity as some units only showed phase-locking at close to threshold, whereas others preferred stimuli at least 20 dB above threshold. There was often an exponential decay in the discharge rate with each successive cycle of stimulation and most units did not respond for more than 140 ms after the onset of the stimulus. When a guinea pig vocalisation was presented, five of the units gave phase-locked responses to the fundamental frequency of 270 Hz. Thus these units may be important in analysing naturally occurring communication calls.

166 Comparison Of Local Field Potentials And Unit Cluster Activity In Primary Auditory Cortex And Anterior Auditory Field In The Cat

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In order to study neural transformations between thalamocortical inputs and cortical unit activity we recorded simultaneously, and at the same electrode, the multi-unit activity (MU) and the local field potentials (MU) in the primary auditory cortex (AI) and the anterior auditory field (AAF. Recordings were made from 161 recording sites in 53 juvenile and adult cats under light ketamine anesthesia. Responses were obtained to gamma tones for frequencies between 625 Hz and 40 kHz, at different intensities covering the range from 75 dB SPL to threshold in 10 dB steps. We compared the response properties of MU and LFP, in terms of characteristic frequency (CF), threshold at CF, minimum latency and frequency tuning-curve bandwidth. We found that, on average, thresholds at CF were lower for LFP events compared to those for MU spikes (4.8 dB for AI, and 3 dB for AAF). Minimum latencies were shorter for LFP events than those for MU spikes (1.5 ms in AI, and 1.7 ms in AAF). Frequency tuning curves were systematically broader for LFP events than those for MU spikes (1.0 octave in AI, and 1.3 octaves in AAF). In contrast, the CF was not significantly different between LFP events and MU spikes. The LFP results indicate that cortical neurons receive convergent sub-cortical inputs from a broad frequency range. The sharper tuning curves for MU activity compared to those of LFP events are likely the result of a combination of volume conduction effects and intracortical feed-forward inhibitory processes.

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167 Complex Sound Perception: Modeling Response Coherence in the Activity of Neural Ensembles

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Whereas neural mechanisms such as combination-sensitivity represent a specialization that can be used to extract a combination of stimulus parameters, they do not adequately account for the final perception of complex sounds. For this, it is important to consider the activity of a neuronal population as described by representational, dynamical and vector models of stimulus coding. Using commercially available software (Neuroimitator™), we have developed a new model for stimulus driven spiking in cortical neurons. Our population model consists of a parallel-hierarchical network of neurons that uses associative learning to create coherent activity within neural ensembles. In our model, presentation of tone pairs resulted in a two- to three-fold increase in correlation of the response pattern of output neurons over that due to single tones. To test this model, we recorded neural activity with tungsten micorelectrodes from the posterior pole (AIp) of the primary auditory cortex of awake mustached bats, Pteronotus parnellii. AIp consists of a tonotopic antero-posterior (high-low) axis. We obtained single unit responses to pure tones and species-specific call syllables in the central region of AIp. Recordings from 60 neurons in 6 animals showed that many AIp neurons at different levels of the "tonotopic" axis exhibit two to four peaks (at approximately 12 kHz, 24 kHz, 35 kHz and 42 kHz) of excitatory tuning. Tone pairs corresponding to a combination of these frequencies, however, neither

facilitated the response of neurons nor affected the response latencies (5 to 25 ms). Rather, the response to tone pairs showed increased coherence that was evident in the low variance of the response pattern of multiple neurons. This coherent response to harmonics contained within the excitatory tuning of a population of neurons may help to signal a familiar call.

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168 Effects of Quinine on Auditory Cortex Networks Growing on Microelectrode Arrays

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Quinine is known to cause modulation of central auditory neurons resulting in tinnitus-like activity. *In vivo* electrophysiological studies have suggested that quinine significantly increases spontaneous firing rates in selected auditory cortex neurons. In this study we have used spontaneously active cultured auditory cortex neuronal networks as a dynamic platform for detection of activity alterations brought about by quinine.

Neurons dissociated from auditory cortices of 14 day-old mouse embryos were grown on substrate integrated microelectrode arrays with 64 electrodes. Healthy looking 3-4 week old cultures were mounted on a recording chamber, and the spontaneous extracellular spike and burst activities were recorded. To evaluate the acute effects of quinine, serial addition of the compound was undertaken to reach final concentrations ranging from 0.5 to 40 µM. Auditory cortex networks showed an increase in mean spike and burst rates with addition of quinine at concentrations ranging from 0.5 to10 µM. Further increases in quinine concentration brought about a decrease in spike and burst rates. In addition, 10 to 20 µM quinine also increased the regularity of bursting in the networks. All of these changes were completely reversible with medium changes. Quinine-induced increases in spike rate and burst rate along with increased synchronization of bursting seen in cultured networks correspond to some of the changes seen *in vivo* studies, which are thought to be related to the perception of tinnitus.

169 Organization of Inhibitory Sidebands in the Low-Frequency Region of the Pallid Bat Auditory Cortex.

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The pallid bat listens passively to low-frequency (1-30 kHz), prey-generated noise for prey-localization, while reserving high-frequency (30-60 kHz) echolocation for obstacle avoidance. In the auditory cortex of the pallid bat, the majority of neurons with best-frequency (BF) below 30 kHz prefer broadband or bandpass noise over pure tones. Neurons that prefer bandpass noise are found in the dorsal part of each isofrequency contour (IFC). Neurons that responded equally well to both broadband and narrowband noise are located within the ventral part of each IFC. In this study, the organization of inhibitory sideband frequencies was determined within each IFC using the simultaneous two-tone inhibition paradigm. The main result of this study is that the complexity of inhibitory sideband frequency organization varies systematically within each IFC. Neurons within the ventral part of each IFC did not have any inhibitory sidebands, while neurons recorded dorsally showed the presence of sideband inhibitory frequencies. Organization of inhibitory sidebands was correlated with the organization of bandpass selectivity. Differential organization of inhibitory sidebands within IFCs has been previously documented in the cat and ferret auditory cortices, suggesting that it might be a common feature of auditory cortical organization. As suggested in these systems, the differential organization of inhibitory frequencies may be involved in spectral cue processing.

170 Thalamocortical Connections of Functional Areas in the Mustached Bat's Auditory Cortex

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The auditory cortex of the mustached bat consists of multiple functional areas, of which many are specialized to encode particular information conveyed by the biosonar signal. These neurons come in three main types: Neurons sensitive to target range are facilitated by combinations of frequency modulated (FM) sounds in the biosonar signal and are tuned to particular echo delays (FM-FM neurons); neurons sensitive to target velocity are facilitated by combinations of constant frequency (CF) sounds in the biosonar signal and are tuned to particular Doppler-shifts (CF/CF neurons); and neurons sensitive to an initial contact with insect targets are facilitated by combinations of FMs and CFs and are tuned to both echo delay and frequency (FM-CF neurons). Each type is found in multiple areas, and areas of similar response type are the most strongly interconnected.

Neurons with similar response types are also found in the auditory thalamus and inferior colliculus (IC). However, multiple areas of similar response type have not been described at either level. Thus, a transformation occurs between the IC and cortex. To investigate this transformation, we traced thalamocortical connections of physiologically defined regions of auditory cortex. Injections in different cortical areas containing FM-FM neurons all had separate labeling patterns in the thalamus with some overlap. Similarly, areas sensitive to CFs had distinct labeling patterns with some overlap. Labeling from CF and FM areas also overlapped. This overlap is consistent with thalamic physiology that shows considerable intermingling of response types. Thus, segregation of response types into separate subsystems has begun by the level of the auditory thalamus but is less complete than seen in the auditory cortex.

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171 History of the early development of the cochlear implant

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The cochlear implant has revolutionized otology and the treatment of deafness. This research explores both the contributions and the contributors to cochlear implant progress during the time period between the first reports of direct cochlear nerve stimulation in the 1950's and the systematic study by Bilger et al. in 1977 of the first 13 cochlear implant patients (Bilger RC, Ann Otolaryngol 86 (Suppl. 38), 1).

Stimulating the cochlear nerve with electrical impulses and bypassing the cochlea in order to produce useful hearing had its inception in the 1950's in France, where the collaboration between Andre Djourno and Charles Eyries yielded the first report of such direct stimulation. Since Djourno detested commercialism and Eyries lacked interest in the project, this group did not pursue this line of research further. Their results failed to garner significant interest from either the scientific or clinical communities until three Californian otologists resumed work in the 1960's where Djourno and Eyries left off. Support for the work from both the clinical and scientific community during this time was meager, as the usefulness of implant hearing was questioned. Interestingly, this early work was executed outside of the University setting and without extramural funding.

Progress on the cochlear implant required involvement of both university-level research efforts and commercial industry. Yet despite significant improvements in implant design and surgical implantation, speech recognition with the device was unobtainable throughout the 1970's. These early years established, rather, that the auditory nerve

could be stimulated chronically and that the implant offered patients some useful hearing.

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172 Auditory Capacity: Developmental and Societal Implications

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Early identification and dramatic advances in technology—hearing aids and cochlear implants—have combined to provide very young children with all levels of hearing loss with the potential to fully access spoken language. In May, 2001, two-thirds of newborns in the United States were screened for hearing loss; this compares with an average age of identification of 2 ½ years as recently as five years ago.

The long-term implications of early intervention are complex and are still being studied and documented. Early work has shown that children who receive intervention for hearing loss prior to 6 months of age have markedly different language skills than those who receive intervention later. Besides the more obvious expected outcomes of elevated levels of listening and speaking, a deaf child with access to spoken language enjoys a range of other "life opportunities" such as higher levels of literacy and educational achievement, full employment potential, and mastery of social-emotional skills. These are benefits for the individual child's quality of life but also for the larger community which enjoys quantifiable contributions in the workplace and beyond.

173 Discrimination of Speech Sounds in Deaf Infants Following Cochlear Implantation

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Deaf infants' hearing loss can now be identified at earlier ages as a result of recent universal newborn hearing screening laws, and clinicians are now providing cochlear implants (CIs) for infants under the age of two years. To evaluate the benefit of CIs during infancy, we have adapted the Visual Habituation (VH) procedure, which is a technique that has been used successfully to assess speech perception skills in normal-hearing infants.

We tested ten normal-hearing (NH) 6-month-olds and five deaf 8- to 24-month-olds 1-month following CI. The youngest-implanted deaf infant (CI-1) was also tested at six months post-CI. On each trial, infants were presented with a visual display of a checkerboard pattern. During the Habituation Phase, half of the trials ("sound trials") included a repeating speech sound (e.g., "hop hop hop") while the other half were silent ("silent trials"). The Test Phase included one trial with the original speech sound and one trial with a novel speech sound (e.g., "ahhh"). Both sounds were paired with the visual display.

Results: NH 6-month-olds and CI-1 at six months post-CI oriented to the visual display longer during the sound trials than during the silent trials. However, at one month post-CI, deaf infants showed no difference in their orientation times. Likewise, the NH infants' oriented, on average, longer during the novel speech sound trial than during the original one. Again, deaf infants did not display the same pattern at one month post-CI, while CI-1 at the 6-month interval did. These findings suggest that important speech perception skills may emerge sometime between one month and six months following CI. We are currently tracking the progress of the other four deaf infants who received CIs to see if these speech perception skills will show a consistent pattern and emerge within six months after CI.

174 Aetiology of Hearing Loss versus Language Outcome after Cochlear Implantation in Children

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Aims: Our aim was to examine the relationship between the aetiology of hearing loss and language outcome after cochlear implantation in children.

Study Design and Setting: We conducted a retrospective study by reviewing the case notes of children who had received a cochlear implant in the Great Ormond Street Hospital Cochlear Implant Programme between 1992 to 2000.

Patients: We identified 106 children who had received cochlear implants between 1992 to 2000, with at least 1 year's experience with the cochlear implant at the time of the study.

Methods: We recorded information on aetiological investigations of the hearing loss. We examined the relationship between the aetiology of the hearing loss, the presence of additional medical disability to the hearing loss, and the yearly improvement in Receptive Language Scores and Speech Intelligibility Scores.

Results: Children with a syndromic aetiology had significantly lower improvement in receptive language scores at year 4 and year 5 after implantation and in speech intelligibility scores at year 5 after implantation than children with a hereditary-non syndromic diagnosis or an unknown diagnosis. Vision abnormalities and balance problems were significant negative predictors for improvement in both speech and receptive language scores.

Conclusion: The presence of a syndromic diagnosis or of vision or balance problems may be correlated with poorer improvement in language and speech scores in children who receive cochlear implants. It is important to investigate thoroughly the cause of the hearing loss and additional disabilities in the child cochlear implant candidate, as these factors may predict outcome.

175 Use of the Speech Processor and Classification of the Results in Cochlear Implant Children aged 7 to 18 Years

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At the Medical University of Hannover (MHH) 297 patients received a cochlear implant at the age of 7 to 18 years between 1986 and 2000. Most of them were prelingually deaf and got their rehabilitation at different rehabilitations centers.

All rehabilitation data were collected and retrospectivly evaluated. For this study the Categories of Auditory Performance by Sue Archbold (1995) were chosen to compare the data of all clinics. These Categories are a Classification Scheme by means of which every child can be classified and which we adapted to the German language and situation. Categorie 1 is the lowest level and refers the child`s possibility to differenciate sounds, categorie 6 is the highest level and shows that the child is able to use the telephon with familiar speakers.

A part from these data the datas from the nonusers were also collected.

In order to evaluate for age-dependent effects the patients were divided into three age groups on the basis of their age at implantation; group 1: 7 to 11 years, group 2: 11 to 14 years, group 3: 14 to 18 years.

18 patients use their speech processor less than 5 hours a day. To verify the use of the speech processor the nonusers were questioned.

The results of the classification and the data of the nonusers will be presented.

The conclusion is drawn after comparing important data such as age of deafness, age at implantation between users and nonusers and the risk factors.

176 Preoperative Electric ABR's in young children

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The purpose of this study was to characterize the configuration of the electrically-evoked ABR (EABR) obtained with transtympanic promontory stimulation in children 2 years and younger at the time of implant surgery. EABR's were obtained from twenty 1-2 year-old patients using biphasic current pulses ranging in level from 200-900uA delivered via a transtympanic needle electrode. These measurements were carried out in the operating room, prior to cochlear implant surgery for the clinical purpose of ascertaining the electrical excitability of the ear to be implanted. Due to the presence of the stimulus artifact, the electric equivalents of waves I and II (EI and EII) could not be identified. Peaks EIII, EIV and EV were all present in the majority of the subjects, with the following mean latencies: EIII=2.63/0.13;EIV=3.62/0.33;EV=4.53/0.45 ms. The presence of these ABR 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 lemniscus is functional in these mostly prelinguistically-deaf patients in spite of reduced or absent auditory input. Response latencies obtained preoperatively with transtympanic stimulation will be compared to response latencies obtained postoperatively with cochlear implant stimulation.

177 Speech Reception In Nucleus CI24M Cochlear Implant Users With Processor Settings Based On Electrically Evoked Compound Action Potential Thresholds

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This research concerns the possibility of processor adjustment based on a profile derived from measurements of the electrically evoked compound action potential (ECAP) thresholds across the electrode array, followed by adjustment of the overall level of the profile to the hearing threshold (NT) and maximum comfortable loudness level (NC) using live voice. The new method yields a threshold, NT, much lower than the conventional T-levels and an NC somewhat above the conventional C-levels such that the new dynamic range is about twice the conventional dynamic range. The results for CVC word lists show that speech reception is quite insensitive to this new dynamic range setting. On average, the speech score decreases by 7%.Considering habituation to the initial processor setting of at least 6 months, the small decrease in CVC scores with the new setting suggests that a more user-friendly adjustment procedure can be developed. However, the speech score appears to be sensitive to an increase of NC above the C-levels at the basal electrodes. In some individuals this reduced the speech score considerably.

178 A Comparison of EAP and EABR Thresholds in Nucleus CI24R Cochlear Implant Recipients

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The primary purpose of this study was to evaluate the differences between EAP and EABR measurements within Nucleus CI24R cochlear implant recipients. The internal electrode array of the CI24R is pre-curved to provide more direct stimulation of neural elements of the modiolus. Experimental evidence has indicated that as the internal electrode array of a cochlear implant is placed closer to the modiolus, behavioral and electrical threshold levels are decreased (Kawano, Seldon, Clark, Ramsden, & Raine, 1998; Shepherd, Hatsushika, & Clark, 1993; Tykocinski, Saunders, Cohen, Treaba, Briggs, Gibson, Clark, & Cowan, 2001). Providing more direct stimulation to the neural elements may have a number of practical and clinical advantages. EAP and EABR growth functions for electrodes 5, 10, 12 and 20 were obtained on ten postlingually deafened adults. Additionally, behavioral threshold levels for a 250 pulses/s stimulus were obtained on all subjects. Results revealed no significant differences between EAP and EABR threshold levels. This result is in contrast to previously published findings by Brown, Hughes, Luk, Abbas, Wolaver, & Gervais (2000) with the straight electrode array, the CI24M, showing EABR thresholds lower than EAP thresholds. Additionally, noticeable differences between electrically evoked threshold levels obtained with the CI24R and the straight electrode array, the CI24M, were not indicated. The data also suggest that EAP and EABR threshold levels are closer to behavioral upper comfort level than to the behavioral threshold level. Possible explanations for these findings are presented. Clinical suggestions for obtaining valid EAP responses with the CI24R implant also are provided.

179 Comparison of Psychophysical and EABR Thresholds for Biphasic Current Pulses of Varying Leading Phase

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Physiological responses to single biphasic electrical pulses are significantly affected by the polarity of the leading phase. This result suggests that the different leading phase polarities stimulate different neural structures and result in responses of different thresholds and latencies. Psychophysical thresholds show similar polarity dependent effects. EABR studies were performed in a group of four unilaterally-deafened, implanted, behaviorally-trained cats to determine the extent to which systematic changes in EABR thresholds and response latencies might be used to infer the relative sites of excitation underlying the psychophysical responses. EABRs were collected for symmetric biphasic pulses of 200, 400, and 800 ms/phase, for both anodic- and cathodic-leading phase polarities. Two different investigators visually estimated threshold by viewing EABR intensity series in 1 dB steps using three different forms of waveform analysis: 1) scaled stimulus artifact template subtraction from EABR waveforms; 2) the raw EABR, using the first identifiable wave, and; 3) the raw EABR using only wave III.

EABR threshold estimates were well correlated with those taken from psychophysical tests (R = 0.6 for all 3 methods) and accurately estimated the polarity-dependence of the behavioral thresholds. Systematic errors in threshold estimates, however, were evident across the analysis techniques. Analyses based on template subtraction often estimated EABR thresholds for 200 or 400 ms/phase stimuli to be more than 5-dB below behavioral thresholds, but provided the closest estimate to behavioral threshold for the 800 ms/phase stimuli. Both analyses based upon the raw waveforms estimated thresholds for 800

ms/phase stimuli to be 5 dB above behavioral thresholds. In general, the best agreement with behavioral data was obtained by using raw waveforms for threshold estimates of 200 and 400 ms/phase stimuli and template subtraction for 800 ms/phase stimuli.

180 The estimation of auditory sensitivity in cochlear implant candidates using steady-state evoked potentials (SSEP)

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The precise measurement of residual hearing in prospective cochlear implant candidates is essential. Steady-state evoked potentials are scalp-recorded potentials elicited in response to sinusoidal amplitude and/or frequency modulated tones (Picton et al., 1987). The purpose of this study was to determine the use of steady-state evoked potentials in the objective assessment of hearing in young children suspected of significant hearing loss. Pilot data have been obtained from ten subjects ranging in age from 6 to 40 months. Results indicate that 1) some subjects who have no response to the ABR click stimulus have present SSEP thresholds at higher stimulus intensities, and 2) SSEP thresholds indicate ear differences that were not suggested by ABR measures. The correlation between SSEP thresholds and tone burst ABR thresholds for all frequencies and subjects tested indicates a strong relation ($p < 0.05$) between SSEP and tone burst thresholds for this sample. At the time of this meeting, the complete data set will be presented. Very young children, and those with significant hearing loss represent the ultimate challenge for accurate prediction of hearing and appropriate recommendation for cochlear implant candidacy and ear selection.

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181 Variations in Neural Adaptation among Nucleus CI24 Cochlear Implant Users and its Relationship to Psychophysical and Behavioral Measures: Preliminary Results.

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Despite major developments in the area of cochlear implants in recent years, there is still a significant amount of variability in word recognition performance among cochlear implant users. A number of studies have attempted to determine which preoperative factors and/or postoperative electrophysiological measures may be related to postoperative word recognition performance. While the results of the individual studies are not always consistent, the two preoperative factors that appear to correlate with performance most strongly are duration of deafness and preoperative sentence recognition scores. To date, no single post-operative electrophysiological measure has been shown to correlate strongly with post-operative performance.

One factor that has not received much attention in the literature and that may be related to postoperative performance is neural adaptation. Electrophysiological techniques have been used to assess adaptation to electrical stimulation in animals; however, to date no study has been published exploring the effects of adaptation on EAPs in human cochlear implant users.

In this study we measured neural adaptation of the whole nerve action potential in a population of Nucleus cochlear implant users using Neural Response Telemetry. Adaptation was measured using a range of stimulation rates from 10Hz to 350Hz. Variations in adaptation were observed for different users. Correlations between adaptation, MAP programming levels and word recognition scores will be discussed.

182 Late and cognitive auditory potentials in patients with cochlear implants: a parametric study

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The purpose of this study was to evaluate speech-evoked late auditory and cognitive potentials in adult patients with cochlear implants and age-matched normal-hearing subjects under different conditions. The effects of presentation level (roving vs.constant loudness) and presence or absence of an assigned task on exogenous and endogenous component peak latencies and amplitudes were assessed within subjects in both groups. All response components (N1 through P3) were present in both the task and no-task as well as roving and constant loudness conditions in both the normal-hearing and implanted groups. There was a trend for prolonged N2 and cognitive component peak latencies in the no-task condition relative to the task condition, with some statistically significant differences. This was more pronounced in the normal subjects than in those with cochlear implants. Amplitudes were larger in the task condition, especially for P3 in both normal-hearing and implanted subjects. There were both amplitude and latency differences between the roving vs. the constant loudness conditions especially in the normal-hearing subjects, but htese were not systematic.

183 Comparison of High Resolution vs. Conventional Sound Delivery in the CLARION CII Bionic Ear

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The benefit afforded by cochlear implants is related directly to improvements in technology and in the capability to process and transmit more sound information to the auditory system. The CII Bionic Ear (introduced in March 2001) incorporates an entirely new electronics platform with improved input amplitude resolution (12-bit analog-to-digital converter), enhanced frequency resolution (70,000 samples/sec), and a wider input dynamic range (82 dB) compared to existing systems. The CII has a digital processing capability of 100 MIPS, and can transmit and deliver acoustic information at a resolution of 92,800 digital words per second. The maximum stimulation rate of the CII electronics is 1,000,000 updates/sec (simultaneous stimulation) or 250,000 non-overlapping biphasic pulses/sec (non-simultaneous stimulation). In combination with the HiFocus II electrode, the 16 independent output circuits in the CII can provide 16 channels of information in monopolar mode, 15 channels in adjacent bipolar mode, and 31 channels in multipolar mode. The CII system has FDA approval for use as a standard cochlear implant, wherein only a portion of the electronic capability of the device is used. A clinical trial of the CII Bionic Ear system is in progress to investigate the benefit associated with a first version of High Resolution sound delivery. Comparisons of consonant recognition (quiet and noise), word recognition, and sentence understanding (quiet and noise) between conventional sound processing and High Resolution sound delivery have been made. Results show significant improvement in total information transfer and in transmission of place, manner, duration, nasality and voicing cues when patients are switched to High Resolution processing, especially in the presence of competing noise. Improvements on conventional measures of speech recognition also are evident with High Resolution sound delivery, particularly in noise.

184 Psychophysical Data and Speech Percpetion Results with the CII Bionic Ear ™ System

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The advanced capabilities of the CII Bionic Ear™ System allow for various modes of operation including output on multiple channels at extremely narrow pulse widths thus yielding extremely high rates of stimulation. Published studies have indicated that increased stimulation rate can convey an increase in cues such as manner of articulation.

Subjects systematically tested with these parameters were those enrolled in the study, The High Resolution Speech Processing Strategy in Adults with Severe to Profound Hearing Loss (IDE G990146-CII). Prior to evaluation in this new mode of operation, all subjects had been fitted with non-investigational (control) strategies (e.g. SAS) for 3 months. Subjects were evaluated over a 3-day period. The vehicle for implementing this High Resolution mode of stimulation, Bionic Ear Programming Software (BEPS), which allowed for a large number of combinations of pulse widths and numbers of channels yielding a variety of pulse rates (e.g. 2840Hz at 11us/ph for 16 sequential channels). Further, programs not only took the form of simple sequential stimulation but also involved various electrode groupings (e.g. paired groupings).

Psychophysical data (thresholds, comfort levels, loudness-growth functions, and place-pitch ranking results) and speech perception data have been collected on 25 subjects to date. This poster describes the results of parameter manipulations on performance and discusses the issues involved in fitting these processors, including the conversion of patients from their previous strategies.

Patient performance was assessed acutely via the use of the Iowa Consonant Tests delivered in quiet and at a +10dB SNR via direct connection to each subject's sound processor. Results indicated a significant improvement in total information transfer as well as the individual transmission of the cues of place, manner, duration, nasality and voicing in the presence of competing background noise.

185 The Investigation of the Binaural Effect in Bilateral Cochlear Implant Users

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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 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 USA was initiated in September 2001.

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.

186 Bilateral Cochlear Implant and Bimodal Hearing with Cochlear Implant and Hearing Aid

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After successful cochlear implantation on one ear, some patients continue to use their hearing aid at the opposite side. They report an improved understanding of speech especially in noise as well as a better perception of music when hearing aid and cochlear implant are worn in combination. A survey with 11 bimodal and 4 bilateral supplied subjects was carried out to assess speech understanding and localization ability. The bimodal subject group was provided with the same type of hearing aid. A week after the initial fitting a fine tuning of the hearing aid program was performed and speech tests were conducted subsequently. The localization tests were carried out in an anechoic room in complete darkness. To minimize non-binaural hints a roving level paradigm was used. Subjects pointed to the direction of sound incidence by use of a trackball with a computer-controlled laser-pointer. The high precision of the method permits the discussion of the displayed localization results in terms of the accuracy of localization.

Results: The additional usage of a hearing aid improved speech understanding in 9/11 subjects of the bimodal supported group. Two subjects with substantial residual hearing of the bimodal group also showed improved localization ability, five subjects were able to differentiate the side of sound origin. All subjects of the bilateral cochlear implant subject group displayed localization ability with varying precision. One bilateral supplied subject showed a localization accuracy of 10 degrees. An explanation for these remarkable results is difficult as the bilateral cochlear implants are controlled by two independent speech processor devices running without a common clock, thus interaural time difference cues seem to be distorted. Interaural level differences seem to carry enough directional coding information although different compression schemes might distort the interaural envelope differences to some extent.

187 Recovery of Glucose Metabolism in Left Posterior Inferior Temporal Region after Cochlear Implantation in Deaf Children

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Improvement of hearing and speech ability after cochlear implantation (CI) is hard to predict especially in deaf children. Previously we reported that hypometabolism of primary and secondary auditory cortex in pre-CI deaf children was correlated with the improvement of speech perception following CI (Lee et al., 2001). The functional neuroanatomy underlying the recovery of speech perception following CI, however, was not yet fully understood. Here, we studied the change of glucose metabolism and those that are associated with the speech perception, after CI. 18F-FDG positron emission tomography (PET) and speech perception test given both before and after the CI were analyzed. Eight deaf patients (three female and five male; four prelinguial and four perilingual) underwent 18F-FDG PET scan and Korean version CID test before (average age = 5.7 yr) and after (average age = 8.24 yr)

CI. Average score of CID test was 0 before and 42±38 (range from 0 to 96) after CI. Voxel wise comparison was made while CID test scores were served as a covariate and age at the time of scan as a nuisance variable. The voxels were identified as showing significant difference or correlation if they passed the height threshold $p < 0.005$ (uncorrected). Cochlear implantation in deaf children resulted in increase of glucose metabolism in visual association cortex such as left middle occipital gyrus and bilateral precuneus. However, functional recovery of speech perception was correlated with increase of metabolism in higher associative areas in the left side, such as middle/lateral occipital gyrus (BA 37/39), left middle/inferior temporal gyrus (BA 21/37). These findings suggest that functional recovery of left posterior inferior temporal region is critical for gaining speech perception capability in young deaf patients after CI.

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188 Auditory and Visual Stimuli for fMRI in Hearing Impaired Subjects

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Functional MRI using auditory stimuli is a useful research tool. However, subjects with severe hearing loss are limited in possible stimuli due to loss of hearing sensitivity and word discrimination. Activating auditory and language cortex by fMRI in hearing impaired subjects may have potential for such uses as predicting cochlear implant success or determining individual language areas prior to neurosurgery for tumor removal, etc.

This paper shows methodology and results of auditory fMRI in normal and severely hearing impaired subjects using speech and non-speech stimuli. Results show good activation of auditory cortex in severely hearing impaired subjects when using auditory stimuli of either speech or non-speech stimulus types. Examples are also shown of cortical language activation using visual stimuli. Visual stimuli may provide an alternative approach to cortical language activation in hearing impaired subjects.

189 Spiral Ganglion Cell Populations in Children with Normal and Pathological Ears

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The degree of survival of spiral ganglion cells in cochlea is considered to be of primary importance for success in cochlear implantation. However, there is no systematic survey to determine whether spiral ganglion cell populations differ among children with different pathological conditions or whether the postnatal change of populations is present during childhood. This study analyzed features of total and segmental spiral ganglion cell populations in children with normal and various pathological conditions. Sixty-three human temporal bone specimens (from 43 children 4 days to 9 years of age) were studied histopathologically. These specimens were divided into 5 diagnostic groups: Group 1, normal ears; Group 2, congenital infectious diseases; Group 3, chromosomal aberrations; Group 4, hereditary or genetic craniofacial anomalies; and Group 5, asphyxia. Eighteen of the 63 ears had documented profound deafness. In either normal or various pathological conditions, the total number of ganglion cells did not change as a function of age. The total number of ganglion cells was significantly larger in Group 1 (33702) than in each of Groups 2-5 ($p<0.01$), and the number was significantly larger in Group 2 than in each of Groups 4 and 5 ($p<0.01$ and 0.05). The ratio of basal to apical ganglion cell populations remained constant in both normal and pathological ears. Each ratio of the number of basal and apical ganglion cells in Groups 2-5 to the mean number in Group 1 was at least 40 %. The mean total number of ganglion cells in ears with documented profound deafness was approximately 40 % of those present in normal ears. Our results suggest that, normally, cochlear neurons are completely present at birth and minimally regress during the first decade of life. In addition, although intergroup differences among various pathological groups were present, the majority of pathological ears had over 10000 spiral ganglion cells present.

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190 Information-theoretic distance measure of the neural discrimination of stop consonants

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This study examined the auditory nerve discrimination of voiced stop consonants using information-theoretic distance measures (Johnson et al, 2000). These measures quantify the information content of changes in neural responses to manipulations of a stimulus parameter without making a priori assumptions about the nature of the coding mechanism. The magnitude of the distance measure indicates the fidelity of encoding and therefore can be used to infer neural discrimination. This analysis was applied to the encoding of complex speech sounds by recording the responses of auditory-nerve fibers (ANFs) to five synthesized CVC_CV segments (e.g. /dad_da/). The consonants within the speech segments represented a continuum of formant frequencies from /b/ to /d/; the vocalic portions of the segments were identical. Distance measures were computed by contrasting responses to each stimulus relative to the /b/ endpoint. The most rapid growth of the distance measure was correlated with F2 formant differences and was observed among ANFs with best frequencies (BFs) near F2. Syllable initial positions were better discriminated than syllable final positions. The distance measures also suggested high information content during the release bursts of the consonants. Low spontaneous rate ANFs encoded formants with greater fidelity than high spontaneous rate fibers at 70 dB SPL, but these differences were less apparent at 50 dB SPL. Additional modeling experiments suggest that our estimation of the neural discrimination of stop consonants can be improved by not limiting information sources to tonotopically localized responses and by distinguishing responses that are made during upward and downward formant transitions.

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191 Recognition of Filtered Speech in Noise at Higher-Than-Normal Levels: Decreases in Scores With and Without Increases in Masking

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With signal-to-noise ratio held constant, speech recognition in noise decreases as speech level increases above conversational levels. The magnitude of this effect may vary with speech material and masker spectrum and the mechanism remains unclear. In a previous study, word recognition in speech-shaped maskers of normal-hearing subjects declined slightly but systematically with increasing speech level while a constant signal-to-noise ratio was maintained across masker levels. Using the articulation index, the deterioration in word recognition was attributed to increasingly high thresholds in the speech-shaped maskers. Here, to assess the decline in word recognition for low- and high-frequency speech and noise, 18 young adults with normal hearing listened to filtered speech and speech-shaped maskers in one of two conditions: (1) low-pass filtered at 1.41 kHz or (2) high-pass filtered at 1.41 kHz. This cutoff frequency was selected to achieve equal articulation indices (and equal word-recognition scores) for the two conditions. An additional low-level broadband noise was always present to equate subjects' thresholds. Pure-tone thresholds at one-third-octave

intervals were measured in quiet and in all maskers. For both low- and high-pass-filtered speech and noise, with signal-to-noise ratio held constant, word recognition decreased with increasing speech level. For low-frequency speech and noise, the decline was attributed to reduced speech audibility resulting from nonlinear growth of masking, as was observed previously for broadband speech and noise. However, for high-frequency speech and noise, scores declined with increasing signal levels despite linear growth of masking. These findings may relate to differences in low- and high-frequency speech information and/or differences in responses of low- and high-frequency auditory nerve fibers at high signal levels.

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192 The effect of vocalic context, manner of articulation, and voicing on the perception of the place of articulation feature in noise

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In the perception literature, it is common to analyze perceptual results in terms of underlying phonetic features, especially voicing, manner, and place of articulation. In our work, we will show the large effect vocalic context, voicing and manner of articulation can have on the perception of the place of articulation feature in noise. I will report on perceptual experiments which examined the saliency of the labial and alveolar place of articulation features for syllable-initial plosive and fricative consonants in the presence of background noise. Stimuli sted of naturally-spoken CV syllables where the consonant was one of /b,d,p,t,f,s,v,z/ and the vowel, one of /a,i,u/. The syllables were spoken by 2 males and 2 females with 4 repetitions each, added to varying levels of additive white Gaussian noise, repeated twice, and presented to listeners. Four normal-hearing listeners, 2 of each gender, participated in the experiments. In each experiment, a pair of consonants which only differ along the place of articulation feature (e.g. /p, t/) were presented to subjects in forced choice identification tasks.

Results show that perceptual thresholds are highly influenced by vocalic context, manner of articulation and voicing. For example, voiced plosives are more robust than voiceless ones except for the /Ci/ case. The difference was greatest for the /Ca/ context where a 14 dB difference in threshold (between the voiced and voiceless plosives) was observed. The opposite was true for the fricatives; the place of articulation was more salient for the voiceless fricatives /f,s/ than the voiced ones /v,z/ consistently. Detailed results, comparison with previous work, and implications of the work on theories of speech perception and on how perceptual experiments are often analyzed will be presented.

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193 Functional imaging study of speech perception in different masking contexts

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The comprehension of speech can be disrupted by continuous, unmodulated white noise, with a relationship between the level of the masking noise and the amount of disruption. Speech is also masked by the sound of another speaker, but this is relatively independent of level, suggesting that the masking speech signal is not simply affecting the acoustic processing of the attended speech, but also has some informational effect (c.f. the cocktail party effect). In this functional imaging study (using PET), we contrasted different levels of two different masking stimuli (white noise and speech) on the neural correlates of speech processing. Masking levels were set that meant speech was perceived in each condition (approx. 80% intelligibility). Four different masking levels were presented for each masker type, and each condition was repeated.

The results indicated that the level of the masker in the speech-in-noise conditions correlated with activity in left supplementary motor area. Consistent with the literature, there was no level dependent response for the speech-in-speech conditions. Comparing the speech-in-noise over the speech-in -speech condition revealed left rostral prefrontal areas and parietal regions, indicative of the increased attentional demands of detecting the speech in noise. In contrast, the neural activity associated with the speech-in-speech condition over the speech-in-noise condition demonstrated extensive bilateral activation of the superior temporal lobes, associated with the processing of the unattended speech. This ran lateral and anterior to primary auditory cortex, to the anterior superior temporal sulcus on the left. The unattended speech is apparently being processed as far as meaning in the left anterior STS, a pattern consistent with 'selection by meaning' in auditory selective attention.

194 An fMRI Study of the Neural Correlates of Plasticity in the Processing of

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Speech is a complex acoustic stimulus, and there is much redundancy in the normal speech signal(Shannon, 1995).This study attempts to investigate plasticity in the normal speech perception system, by contrasting the neural systems activated by noisevocoded speech, before and after training.

We used a novel learning design, using sparse sampling fMRI. Subjects were presented with a single 6 channel vocoded sentence every 19 seconds, at the offset of which they made a rating of how well they had understood the sentence. After a number of presentations of noise vocoded stimuli, subjects underwent a training phase, when each presentation of a sentence was accompanied by a written version of that sentence. After this there was another presentation of sentences, without accompanying text. Thus, the stimuli(6 channel noise vocoded speech)remained constant throughout the experiment, but the subjects' ratings of how well they understood the sentences went up from "no understanding" to "understood as well as normal speech" before and after training.

Our analysis looked at areas in the brain, which paralleled the subjects behavioural ratings, and uncovered a network localised in the left temporal lobe. As the stimuli were identical throughout the experiment, they act as their own control; we therefore suggest that areas showing significant activation are involved in processing intelligible speech, and are showing plasticity in this due to learning. This has implications for the recovery of function after aphasic stroke, and of neural plasticity associated with cochlear implants.

195 Plasticity in Speech Perception: Spectrally-Rotated Speech, Revisited

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There is currently much interest in the extent to which listeners can learn to perceive speech under various transformations that render it initially unintelligible. One extreme example is spectrally-rotated speech [1,2], in which the spectrum of low-pass filtered speech is instantaneously rotated around a middle frequency (here 2 kHz). Thus, low frequencies become high, and high frequencies low. Intonation and

speech rhythm remain approximately intact under spectral rotation, as do contrasts of periodicity vs. aperiodicity. Spectral shape and its dynamics, however, are completely altered.

Four adults underwent 6 hours of training with spectrally-rotated speech from a single female speaker using both audio-visual and audio Connected Discourse Tracking [3]. In audio sentence tests with a different female speaker, performance by the trained group increased from a mean of 5% key words correct prior to training to 42% at the end of training. Performance with a male speaker increased to a lesser degree, from 9% to 26%, suggesting that a part of the training may be specific to the sex of the training speaker. Four controls who received no training showed no improvements in any test. Even the trained group showed little or no improvement in the identification of intervocalic consonants, and vowels in a bVd context. This is somewhat surprising in the light of strong evidence from other tests with various signal transformations that the trained listeners adapted to a significant degree to altered spectral dynamics in connected speech.

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196 English Phoneme and Word Recognition by Nonnative English Speakers

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Listeners whose first language was Spanish were tested with English phonemes, words and sentences. Listeners were divided into four categories according to experience with the second language. Speech was presented in a sound treated booth at a level of 70dBA. Listening conditions included noise (SNR of 15dB, 10dB, 5dB, 0dB and –5dB) and reduced spectral information (2, 4, 6, 8 and 16 frequency bands). Plomp's (1986) and Boothroyd and Nittrouer's (1998) models were applied to the data. The distortion factor *'D'* defined by Plomp was found to increase with age of learning of the second language. An additional *'distortion'* seems to be introduced when a second language is learned at a later age. The model of Boothroyd and Nittrouer was applied to assess the relative contributions of phonemic and lexical processing as a function of language experience. The *'k'* factor defined by this model was found to be a decreasing function of the age of learning of the second language, i.e., the use of contextual information increased with language experience. One surprise was that even for bilingual listeners the use of contextual information was poorer than that of native listeners. Preliminary data show that nonnative listeners had more difficulty understanding vowels, words and sentences. Surprisingly, English experience had less effect on word and sentence recognition than on vowel recognition. Significantly lower performance on vowel recognition was seen even for fully bilingual listeners with reduced spectral resolution. In hearing impairment and cochlear implants, which can result in reduced spectral resolution, setting the parameters of the prosthetic device should consider the conflicting vowel spaces of the two languages for bilingual patients.

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197 Invariance of Linguistic Context Use Across Modality

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Speech recognition depends on access to sensory data and the use of linguistic context. Clinically, speech recognition is assessed using words presented in isolation or sentences. In intervention for adults with hearing loss, it is important to separate problems of access to sensory data from linguistic context use. Problems with access to sensory data may require changes to signal processing parameters in sensory aids. Problems in linguistic context use may require training. Boothroyd & Nittrouer (1988) propose the use of simple probability theory to separate sensory from linguistic context effects. Two factors are derived:(1)j-from the recognition probability for wholes and for parts; and, (2) k-from the recognition probability for units presented with and without linguistic context. While this approach may have clinical utility, an underlying assumption used in the derivations must be examined - the assumption that j and k are constants – that they are not affected by the underlying recognition probabilities for the no-context situation. Previous research indicated j-factors differed significantly when sensory data were manipulated by input modality (i.e., hearing alone, lipreading alone, and the two combined), but not when manipulated by use of different talkers (Hnath-Chisolm & Boothroyd, 1998). These findings suggest different processing strategies might be used when the type of sensory data differs. The present study examined the invariance of k-factors across modality. Results obtained from 30 young adults (normal hearing/vision) indicate k-factors for the use of lexical context, obtained by comparing recognition of phonemes in real and nonsense words, were significantly higher in the lipreading alone than in the combined condition. Similarly, k-factors for the use of semantic context, obtained by comparing the recognition of words in syntactically correct, semantically anomalous and in real sentences, varied significantly across modality. NIDCD RO3 DC03990

198 Audiovisual integration of speech in children with learning disabilities: the McGurk effect

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Numerous studies have demonstrated that speech perception is related to reading ability. In addition, it has been shown that subgroups of children with reading disabilities exhibit impaired audiovisual integration of nonlinguistic stimuli. To more directly characterize the relationships among speech perception, audiovisual integration and reading, this study examined the McGurk effect and its relationship to other measures of speech perception, cognitive processes and auditory neurophysiology in learning disabled children. The McGurk effect is an auditory illusion that occurs when people see and hear a talker producing conflicting speech sounds. The majority of adults report hearing a combination or fusion of the acoustic and visual stimuli.

Children in the study were 8-14 years old and either had been diagnosed with learning disabilities (LDs) or had no learning problems (NLs). The stimuli were /aka/, /apa/ and /ata/ presented unimodally (auditory or visual), and in congruent (auditory=visual) or conflicting (auditory*visual) audiovisual combinations. Subjects heard the stimuli binaurally through headphones while they watched the speaker's face on a computer monitor. Children were instructed to report what they heard, and the tester coded the response as an auditory, visual, combination, fusion or "other" response.

LD and NL groups showed similar patterns of responses for congruent audiovisual stimuli in quiet and in noise. In quiet, LDs reported a higher

proportion of auditory only responses to conflicting stimuli compared with NLs. Furthermore, LD children with an onset latency of a /da/-elicited ABR within a normal range reported fewer combination and fusion responses to conflicting stimuli than the NL group or LD children with a delayed onset latency.

These findings indicate that distinctive patterns of perceiving audiovisual speech may underlie the reading and learning deficits of some children.

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199 Spectral Analysis Reveals Lateralization of Thalamic Responses to Complex Signals

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It is well established that there is left-hemisphere specialization for speech. In our laboratory, onset responses from the auditory thalamus in guinea pigs have shown lateralization to complex stimuli (King et al., Neurosci Lett 1999). In addition, lateralization of responses to steady-state portions of complex signals has also been noted (King et al., ARO abstr 2001).

In this study, components of synthesized speech stimuli were used to assess which aspects of these signals result in lateralized responses. A 2 kHz pure tone, previously shown to evoke symmetric responses, served as a control. Speech tokens (/a/ and /da/) were deconstructed into time-varying sinusoidal (TVS) forms of /a/ and /da/ by removing the fundamental frequency (glottal pulse). In addition, the 2 kHz tone was modulated by a sawtooth wave matching the fundamental frequency of /a/ and /da/. Finally, two other tone complexes were constructed to determine the contribution of formant spacing on lateralization.

Subjects were albino guinea pigs. Depth electrodes were placed in left and right ventral subdivisions of the medial geniculate nuclei. Stimuli were presented monaurally to each ear and contralateral responses were analyzed. Amplitudes of onset and steady-state responses were assessed. Frequency content of the steady-state responses was determined by FFT analysis.

Similar to previous findings, onset and steady-state amplitudes of the responses lateralized to /a/ and /da/, but not the pure tone. The same pattern of results was seen for all derivatives of the speech stimuli. New information from this study focused on describing lateralization of spectral content of the steady-state responses. Analyzing the waveform in this manner is a promising technique for investigating lateralization of steady-state activity. In addition, it provides a means of assessing the extent to which onset and steady-state responses reflect related vs independent processes.

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200 Intelligibility of Speech Recorded from the Cochlea of Fetal Sheep in utero

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The intelligibility of speech stimuli recorded from the inner ear (cochlear microphonic, CM) of fetal sheep in utero and when outside the uterus was determined perceptually using a group of untrained judges. A fetus was prepared for acute recordings during a surgical procedure. Two separate lists, one of meaningful and one of nonmeaningful speech, were spoken by a male and a female talker, delivered through a loudspeaker to the side of a pregnant ewe at 95 and 105 dB SPL, and recorded with an air microphone, a hydrophone placed inside the uterus, and an electrode secured to the round window of the fetus. Perceptual tests generated from these recordings were played back to 139 judges. The intelligibility of the phonemes recorded in air was significantly greater than the intelligibility of these stimuli when recorded from within the uterus. Similarly, intelligibility recorded from CM when the fetus was ex utero was significantly greater than from CM with the fetus in utero. Overall, male and female talker intelligibility scores recorded within the uterus averaged 91% and 85%, respectively. When recorded from the fetal CM in utero, intelligibility scores averaged 45% and 42% for the male and female talkers, respectively. An analysis of the transmission of consonant feature information revealed that "voicing" was better transmitted into the uterus and into the fetal inner ear in utero than "manner" or "place." Information regarding voicing, manner and place was better preserved in the fetal inner ear in utero for the male than for the female. Spectral analyses of vowels showed that the fundamental frequency (F0) and the first three formants (F1, F2, and F3) were well preserved in the uterus recordings for both talkers, but only F0, F1, and F2 (< 2000 Hz) were identified in CM recordings from the fetal inner ear in utero.

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201 Tactile and Auditory Feedback in Speech Motor Control

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We examined tactile and auditory feedback effects in ongoing speech motor control by observing compensatory response to an unexpected perturbation of the oral cavity. A mechanical device was used to change dynamically the thickness of an artificial palate; the thickness of the un-inflated artificial palate was increased. The palatal perturbations were introduced under conditions of normal and masked auditory feedback. Acoustic, perceptual, and articulatory and EMG activity changes were examined in response to the palatal perturbation. An electro-magnetic articulographic system was used to look for evidence of compensatory articulation during the utterance of repeated CV syllables containing fricatives and affricates. Muscle activities of the tongue were measured by using surface electrodes on the tongue. When perturbation by palatal inflation was randomly given just before the initial syllable in the repeated fricative syllable in the normal auditory-feedback condition, compensation by the tongue for the unexpected palatal inflation became evident with time lag between 76 ms and 300 ms from the tongue-palate onset, and changes in intrinsic muscle activities related to the rapid compensation by the tongue was observed. Even when auditory feedback was masked, rapid compensation of the tongue and the related EMG changes were observed, but speech errors randomly occurred in the succeeding syllables.□@These facts suggest that the tactile feedback gathered by sensing contact between tongue and inflated artificial palate is primarily used to develop the rapid compensation, and that the auditory feedback is used in finely adjusting articulation with a longer time lag.

202 An Immunocytochemical Evaluation of the Vascular Innervation of the Human Posterior Cricoarytenoid Muscle

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The posterior cricoarytenoid muscle (PCA) has a crucial role in respiration and is recruited during phonation. It is composed of muscle fibers that differ physiologically and histochemically from non-laryngeal muscles and have a higher mitochondrial volume and oxidative enzyme activity, and more type 1 fibers than other laryngeal muscles. These extreme oxidative specializations are supported by blood flow rates that are higher than in other respiratory or laryngeal

muscles. Oxidative metabolism produces damaging free oxygen radicals. Since the PCA is highly oxidative, it may be more vulnerable to age-related free radical damage. Given that the mechanisms controlling muscle fiber size, strength and fatigue resistance are interdependent with adaptations for oxidative metabolism, age-related changes in the relationship between oxidative metabolism and blood flow may play an important role in the pathogenic mechanisms underlying age-related PCA remodeling.

Understanding laryngeal vascular control is essential to understanding the physiology of airway maintenance, phonation, responses to injury and the role of age-related remodeling of the PCA. There have been few studies of laryngeal vasculature innervation and none have examined aging. Data show an age-related reduction of arterial sympathetic fibers and changes in superior cervical ganglion neurons. Since laryngeal sympathetics are derived from this ganglion, this age-related loss of sympathetics may contribute to the reported age-related decrease of PCA blood flow. Using immunolabeling, this study will determine the types of innervation on the vasculature of the human PCA. These basic data will be used to select variables to be assessed in studies of age-related changes in laryngeal vascular control mechanisms.

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203 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 almost entirely 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 as well as of fiber type-specific contact lengths in the aging TA.

The results indicate no age-related change in the length density of capillaries in contact with muscle fibers or in the length density of capillaries within connective tissue. However, there was a significant age-related increase in the length of capillaries in contact with type 2 fibers referenced to the volume of type 2 fibers, but not in the type 1 fiber capillary length to fiber volume ratio. The mechanism underlying this age-related increase in the capillary supply to type 2 fibers is presently unclear. However, since this change would support an increase in the maximal oxygen flux to the volume of type 2 fibers, it may be related to an increase in the oxidative capacity of type 2 fibers. This increase in the adaptation for fatigue resistance in type 2 fibers may be the result of an increased recruitment of type 2 fibers as a compensatory response to the reported selective age-related loss of type 1 fibers in the human TA muscle.

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204 Effects of chronic obstructive pulmonary disease on auditory function in humans

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Chronic obstructive pulmonary diseases (COPDs) can lead to hypoxemia and hypercapnia. Although much is known about acute hypoxia, particularly in animals, little is known of the effects of longstanding low-PO2 levels on auditory function in humans, hence the purpose of this investigation. Sixty patients with COPD were referred for study from the Department of Chest Diseases; their results were compared to a non-COPD control group of 30 subjects. All subjects had negative histories of hearing loss or risks. Age was limited from 20 to 50 years. Subjects were evaluated using pure-tone audiometric, click-evoked OAE, noninvasive electrocochleographic, and ABR testing. The independent variable in this study was blood-gas PO2 (statistically different between groups). Results revealed significant differences between groups for all auditory measures. Collapsing data across groups, significant correlations were found between HTL (+), OAE (+), ABR I-V (-) and PO2, but not for SP or AP. Lastly, 2x2 contingency tables were constructed for criterion PO2 and correlated auditory measures. Chi-squared analyses revealed statistical significance for HTL and OAE, but not ABR I-V, suggesting a critical level of PO2 for completely normal auditory sensitivity (i.e. some 70 mm Hg). More work is needed, however, to completely assess clinical significance, including consideration of possible involvement of the vestibular system and/or predisposition of COPD patients for audiovestibular dysfunction from concomitant risk factors.

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205 Auditory Findings in Children with Hyperbilirubinemia who Subsequently Developed Kernicterus

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Kernicterus (K) and auditory neuropathy (AN) have been associated with significant hyperbilirubinemia (HB). We surveyed a convenience sample of 16 children with moderate-to-severe K. IRB-approved surveys were completed by parents at child's mean±SD (range) age of 5.5±3.8y (0.67-12). RESULTS. Birthweight was 3215±661g (1705-4318); gest age 36.9±2.5wk (30-40); 11/16 were male. Jaundice was 1st noted at 27.3±13.4h of age (0-48). Peak total serum bilirubin was 34.1±8.3mg/dl (24.0-54.4mg/dl). Cause of HB was ABO blood group incompatibility (4), rare antigen (2), and unknown in 10. 15 were treated with phototherapy, and 10 also with exchange transfusion. K was diagnosed at 7±4.3mo of age (0.33-16). All had disorders of muscle tone, movement or coordination, 12/15 gaze paresis, and 11/15 dental dysplasia. Auditory brainstem responses (ABR) were done in 15/16. All were abnormal initially: 11 "failed," 4 "moderate to severe," and 1 "inconclusive. ABRs improved in 3/11 with 2 or more ABRs by 10±8 mo (2-18) and remained abnormal in 8 at 32.6±32.5 mo (5-96). AN was diagnosed in 6 and "unsure" in 2; all with hearing impairment; 1 with no impairment but absent ABRs at 14d, 4mo and 5yrs was not diagnosed AN. OAE results were reported in 4, all normal. Hearing impairment was reported in 12, all with abnormal initial ABRs. Of 4 reporting no hearing loss, 1 failed ABRs at 12 and 19ds and passed at 10 mo, 1 failed at 14d, 4mo and 5yrs, 1 had ABRs "inconclusive" at 6

and 12 mo and "OK" at 18 mo; 1 was never tested. CONCLUSION. AN, abnormal ABRs and hearing impairment are commonly found in HB infants later diagnosed with K.

206 Sources of the Amplitude Modulation Following Response

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The scalp-recorded amplitude modulation following response (AMFR) is gaining recognition as an objective audiometric tool, but little is known about the neural sources that underlie this potential. We hypothesized, based on our human studies and single unit recordings in animals, that the scalp-recorded AMFR reflects the interaction of multiple sources. We tested this hypothesis using an animal model, the unanesthetized Dutch-Belted rabbit. Based on changes in the AMFR under cocaine, ketamine, sodium pentobarbital and cortical spreading depression, and based on local AMFR recordings in multiple structures, we conclude that the surface-recorded AMFRs is indeed a composite response from multiple brain generators. For example, depressants or cortical inactivation selectively attenuated the large amplitude, long latency AMFRs associated with low modulation frequencies(<~100 Hz). Moreover, the attenuated responses now had latencies that aligned with those associated with higher frequency, shorter latency responses. Consistent with local AMFR recordings and single unit responses, the latency and the frequency limits of the AMFR are such that the responses to low (< ~100 Hz), and high (> 100 Hz) modulation frequency regions reflect a primary contribution from cortical and subcortical generators, respectively.

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207 Transmission Properties of Bone Conducted Sound in Humans

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In the past, only a few investigations have presented the vibration at the cochlea with bone conduction (BC) stimulation: dry skulls were used in these investigations. Here, objective results of the transmission properties of BC sound in humans is presented, measured as vibration at the cochlea in 6 intact cadaver skulls.

On one side of the skull, a miniaturized triaxial accelerometer measured the cochlea vibrations and on the other side, the cochlea vibrations were measured with a laser Doppler vibrometer (LDV). A transducer that was rigidly attached to threaded holes in the skull provided the stimulation. Three bands between the two ears were defined on the skulls: neck, vertex, and forehead. The stimulation was provided at 9 positions equally spaced over each band. Stimulation was also provided close to the otic capsule. In total, BC sound was applied to the skulls at 29 positions.

The LDV and the accelerometer gave comparable results. Individual vibration levels were affected by the resonances and anti-resonances of the skulls: the results were usually within 10 dB of each other. Cochlear vibration levels for the three directions were normally within 5 dB. When the stimulation position was close to the cochlea the stimulation direction became the dominant vibration direction. The phase rolls off faster the longer from the cochlea the stimulation position was. With mastoid stimulation, the time difference for BC sound between the two cochleae was around 0.2 ms above 1 kHz.

The accelerance level of the ipsilateral cochlea with mastoid stimulation was around 0 dB re 1 $ms^{-2}N^{-1}$ below 500 Hz; above 500 Hz it rises with 30 dB/decade up to 10 kHz. The transcranial attenuation was close to 0 dB up to 700 Hz, above which it increased with 12 dB/decade. With forehead stimulation the vibration energy level was the same as with mastoid stimulation below 400 Hz, it then falls off and becomes around 15 dB lower than at the mastoid from 2 kHz and above.

208 Speech Information Transmitted By Four Amplification Systems To Listeners With Severe Hearing Loss

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Much research has investigated the benefits of nonlinear amplification for listeners with mild to moderately-severe loss, but little is known about the benefits of this amplification for listeners with severe loss. Particularly, it is not yet known how nonlinear processing alters the intelligibility of specific consonant features (such as voicing, manner, and place of articulation) for this group of listeners. Some features may be more susceptible to the effects of nonlinear amplification than other features.

Eleven adult subjects with severe sensorineural loss participated. Nonsense syllable identification was measured on a 23-alternative forced-choice task. Tokens were digitally processed with a laboratory-based amplification system to simulate four hearing aid types: linear peak-clipping, compression limiting, two-channel wide-dynamic range compression, and three-channel wide-dynamic range compression. Stimuli were presented monaurally via an earphone to the listener in a sound-treated booth. Frequency-gain response was selected using the Desired Sensation Level prescription (Cornelisse et al, 1995).

Significant overall percent-correct differences were found among the four amplification systems, with compression limiting providing better consonant recognition than three-channel compression. Sequential information analysis (SINFA), which examines the amount of information transmitted for each consonant feature, determined that all the amplification systems transmitted the same voicing information, but differences were seen among the systems for manner and place information. The SINFA analysis may be more sensitive to differences than an analysis of overall error rate. The results will help us to understand the effects of nonlinear amplification on specific consonant recognition by listeners with severe hearing loss.

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209 Exclusion criteria in semi-implantable hearing aids

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Semi- implantable hearing aids can provide better audiometric results and a higher satisfaction in patients and therefore be an alternative method of improving hearing compared to conventional hearing aids. However patients have to be carefully selected which limits the number of available implantation candidates (Junker et al., 2001) .

We present a case of a 55 y old engineer with insufficient functional gain after conventional hearing aids fitting. Preoperatively, all clinical data showed that he could become a good implant candidate.

After implantation, a gap between tone and speech audiometric data occured and progressed rapidly. The patient descriebed that he would hear, but not really understand words. Speech audiometric results over the time course, MEMR, otoacoustic emission, ABR, EcochG, Carhart - Test, CT, PET and neurological data are presented.

The observed data indicate that the patient suffer from a lesion of the auditory pathway. The location of the lesion is discussed on the base of the observed data.

Before fitting with semi- implantable hearing aids, exclusion of a retrocochlear lesion/ neuropathy (e.g. means of Carhart- test) is strongly recommended.

210 Application of binaural beat phenomenon to evaluation of diplacusis binauralis dysharmonica

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Patients with sensorineural hearing loss sometimes complain of diplacusis binauralis dysharmonica which is the phenomenon of hearing the same tone at a different pitch in each ear. The problem of diplacusis involves the shift of pitch perception. Binaural beats are the appearance of subjective fluctuations in the loudness of two dichotically presented sinusoids of constant amplitude that differ only in frequency. The appearance of beats is the result of neural interaction at a central nervous system receiving afferent fibers from both ears. Several conditions, such as beats or roughness, can be distinguished depending on intraural frequency difference ([Delta]f), the same as two tones presented in one ear. Within the "binaural critical bandwidth," two tones are often perceived as one image. Furthermore, [Delta]f eliciting beats or roughness varies as a function of the standard frequency (f1) employed.

We compared the probability of detection of binaural beats and roughness as a function of[Delta]f and f1 between patients with sensorineural hearing loss and controls in order to measure the shift of pitch perception. The binaural beats phenomenon can be observed only with low tones, so we selected subjects having low-frequency hearing loss. In some cases of endolymphatic hydrops, the amount of diplacusis seemes to vary in degree with amount of hearing loss, becoming more significant when the hearing acuity decreased and less noticeable when the hearing improved. We investigated whether appearances of diplacusis correspond to the change of binaural beats detection. When the change of[Delta]f to elicit binaural beats shows the shift to a higher pitch than in normal ear, the perception of the low tone in the impaired ear occurs at a place normally associated with high frequencies. When transient diplacusis disappeares though hearing loss remains, the shift of pitch perception in the impaired ear is suggested to be compensated by plasticity of central nervous system.

211 The Effect of Word Duration on Cortical Auditory Evoked Potentials in Passive and Active Listening Conditions

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The amplitude and latency of obligatory and cognitive components of CAEP vary with respect to acoustic properties of the speech signal as well in different listening conditions. Understanding the way in which these stimulus and cognitive conditions affect the CAEP is critical to adapting them for use as clinic or research tools. CAEP were measured in response to synthesized CV words tokens, /bu/, /du/ and /gu/, that varied with respect to duration in both passive and active listening conditions. Two different CV durations were used, 150 ms (long) and 50 ms (short), and were presented in an odd-ball paradigm. The latency and amplitude of obligatory CAEP components, P1, N1, and P2 were measured along with the cognitive components, MMN for the passive conditions and P300 for the active conditions. Fifteen normal hearing adult subjects were tested and evoked potentials were recorded from a 32 channel electrode-array. The longer duration tokens yielded larger amplitude and longer latency obligatory components compared to those to the short duration stimuli. During passive listening to the odd-ball stimulus trains, the shorter tokens yielded larger MMN components, and MMN reached statistical significance only for the short /du/ contrast. For active listening conditions, the P300 components were of larger amplitude and shorter latency for the 50 ms tokens compared to the 150 ms tokens. The results of this experiment suggest temporal integration as a mechanism in determination of obligatory component amplitude. For MMN, it appears that a storage or comparator mechanism may be very short-acting, because consistent MMN was obtained only for the short stimulus tokens. Similarly, the larger amplitude earlier latency P300 components for the short duration stimulus indicate that the perceptual mechanisms underlying this response are sensitive to overall stimulus duration and less so to the acoustic phonetic contrast present in the initial consonant position.

212 Diplacusis, Hearing Thresholds and Otoacoustic Emissions During Temporary, Sudden Onset, Unilateral Cochlear Hearing Loss.

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There is limited data available in the literature on diplacusis. The mechanism underlying diplacusis is presumed to involve a shift in the cochlear travelling wave pattern although the mechanism is not well understood.

Data from an intensively studied single episode of sudden mild hearing loss, probably caused by a viral infection, is reported. There was associated binaural diplacusis and the hearing loss was not conductive. A treatment of steroids was administered for one week. This episode provided the opportunity to look in detail at the effect of the hearing loss on diplacusis and otoacoustic emissions and to trace the recovery on a day-by-day basis.

The hearing threshold deteriorated by 10 dB at 1 kHz and 20 dB at 4 kHz upwards. The inter-aural pitch difference was up to 12 % at 4 and 8 kHz. DPOAE level reduced by up to 20 dB and a greater change was seen using a wide frequency ratio than a small frequency ratio with both the 2f1-f2 and 2f2-f1 DPs. There was also a frequency specific change in TEOAE level. Frequency shifts seen in the 2f2-f1 DPOAE fine structure corresponded to changes in the diplacusis. Complete recovery to previous levels was observed for TEOAE, DPOAE and hearing threshold. The diplacusis recovered to within normal limits.

Both 2f1-f2 and 2f2-f1 DPOAE levels (believed to be dominated by the 'wave fixed' and place fixed' modes respectively when f2/f1>1.1) reduced by the same amount during the episode. So there is no evidence in these data of a difference in sensitivity to hearing loss of the wave and place fixed DPOAE modes.

The timecourse of the TEOAE, diplacusis and hearing threshold were significantly different. The TEOAE level appeared to recover first (most notably in the 4 kHz band). Diplacusis continued to deteriorate for the first week after the hearing change was first noticed, whereas hearing threshold itself did not, suggesting that the cochlear mechanisms involved may not be identical.

213 Neonatal Auditory Brainstem Responses (ABR) in Some Congenital Syndromes

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Congenital abnormalities such as craniofacial and chromosomal anomalies are risk factors in infant hearing loss. ABRs were collected on several infants with congenital syndromes. These included 9 infants with Fetal Alcohol Syndrome/Fetal Alcohol Effects (FAS/FAE) and one each with Down's Syndrome, Fragile X Syndrome, and Dandy-Walker Syndrome. ABRs were collected using 70 and 40 dBnHL clicks.

FAS/FAE is characterized by craniofacial anomalies, mental disabilities, peripheral and central hearing disorders. Of the 9 infants with FAS/FAE, 6 infants (67%) had abnormal ABRs. Abnormalities consisted of elevated ABR thresholds, prolonged peak and/or interpeak latencies, and/or reduced amplitudes. Down's Syndrome is characterized by mental retardation, craniofacial anomalies, and hearing disorders. The Down's Syndrome infant had relatively small ABR amplitudes, short peak and interpeak latencies. Fragile X Syndrome is characterized by mental retardation and craniofacial anomalies. The Fragile X Syndrome infant had prolonged peak latencies, elevated ABR thresholds, and reduced amplitudes. Dandy-Walker Syndrome is characterized by hydrocephaly of the 4th ventricle and mental retardation. The Dandy-Walker syndrome infant had small amplitudes and poor reproducibility of the ABR's wave V. These results suggest that neonatal ABR screening on infants with congenital syndromes is effective in the early identification of hearing and/or neurological disorders. This is particularly important in the case of FAS/FAE where 6 of the 9 infants went undetected in the newborn nursery. The detection of these 6 FAS/FAE infants (and the Fragile X infant) was only made later during follow-up evaluations at Children's Hospital. Other implications are (a) that infants with the Fragile X Syndrome or the Dandy-Walker Syndrome are at risk for peripheral and/or central hearing disorders and (b) that unusually small ABR amplitudes can be indicators of CNS abnormalities. (NIH/NIAAA grant AA10941).

214 Discrimination of Competing Stimuli in Electrical Stimulation

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In cochlear implantation, speech intelligibility is prone to be influenced by spectral compositions among perceptually related acoustic variables. This may be, in part, due to the difficulty in discriminating vowels and consonants of similar structures in electrical stimulation. These components in a speech sound can affect each other significantly. We investigated effects of these response interactions to competing stimulus features in cochlear implantees. Stimuli consisted of simultaneous and sequential combinations of two tones synthesized from vowel and consonant test sets. While selected acoustic features were systematically varied, subjects were asked to choose answers in a closed set test and correct responses were scored in the scale of intelligibility. Participants showed various levels of intelligibility in the measured scale. These results had clinical implications that better performances in speech discrimination appeared to have an equivalent degree of correlation with those in simple feature discrimination. We propose this method of searching effective ranges of stimulus parameters as a quantitative tool of refining the mapping strategies in the electrical stimulation of residual auditory nerves.

215 Cell Therapy for Wound Healing: Survival and Function of Transplanted Fetal and Adult Fibroblasts

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The environment of the wound is both complex and dynamic, with incompletely understood spatial and temporal organization. However, it is fairly well accepted that early events (and interventions) in the wound healing process determine subsequent outcomes. Therefore, we are investigating the potential applications of cell therapy for optimization of wound healing, utilizing a cutaneous wound model in the adult rabbit. Several types of fibroblasts were used as the donor cells: autogenic adult, allogenic adult, allogenic fetal and xenogenic fetal fibroblasts. Previously we reported that these cells survive and proliferate in the intact, nonwounded skin of adult rabbits without induction of an immunologic or inflammatory response. For this study the cells were labeled with a fluorscent dye, suspended in a hyaluronic acid gel and introduced into full thickness wounds in the dorsal skin of the animals. Wound biopsies were taken at 7, 14, and 28 days and frozen immediately. Specimens (5um) were cut with a cryostat and analyzed by light and fluorescent microscopy to assess cell survival and distribution within the wound bed and the surrounding wound margins. Analysis of 7 day and 14 day wound samples indicate that transplanted labeled cells were detectable in 92% of the wounds. In these wounds there was good survival with decrease in cell numbers at later time points. This decrease is consistent with the normal downregulation of cellularity in the wound as healing progresses to resolution. As for nonwounded skin, the transplanted cells did not incite an immunologic or inflammatory response in the more metabolically active wound tissue. Histologic and tensiometric data were collected to determine the functional outcome of various transplanted fibroblast phenotypes. Taken together, these results provide support for the effective application of cell therapy to regulate the wound healing response. Future studies will focus on the qualitative outcome of healing.

216 Patterns of Cartilage Structural Protein Loss in Human Tracheal Stenosis

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ABSTRACT

Objective: To identify which of the major structural proteins in tracheal cartilage are lost in the inflammatory process. Also, to determine if damaged cartilage shows signs of regeneration and if this is an age–dependent phenomenon. Study Design/Methods: Immunohistochemistry of archival, human tracheal and subglottic stenosis segments, removed for the treatment of airway compromise were investigated for differential loss of collagen I, II or aggrecan. Results: Specimens were found to have preferentially lost collagen I and aggrecan in areas of severe disruption of the cartilage ring. Collagen II was preserved. In addition, areas of apparent cartilage regeneration were identified based on increased collagen II and aggrecan relative to baseline levels in uninjured sections of the rings. Regenerative capacity was present in most of the specimens investigated and was not age-specific. Conclusions: Collagen I and aggrecan are lost in areas of severe ring compromise indicating that at least one of these two molecules are responsible for structural integrity. The remaining cartilage has some regenerative capacity, but it is small relative to the degree of cartilage damage. No new collagen I was identified in the cartilage ring indicating that although an intense inflammatory reaction occurred, fibroblasts did not deposit new collagen I as seen in other scar tissues.

217 The Response of Laryngeal Fibroblasts to Hyaluronic Acid and Chondroitin Sulfate

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Normal voice production requires that the extracellular matrix (ECM) of the vocal folds possess a certain structure that optimizes the viscoelastic properties of the vocal folds. Injury to tissue leads to abnormal biomechanical properties and voice. To determine the effects that hyaluronic acid (HA) and chondroitin sulfate (CS) have on scarring in laryngeal cells, the expression of several key genes known to be important in wound repair and scarring was determined in cell cultures containing HA, CS, and a combination of HA and CS. The genes studied included CD44, decorin, elastin, fibromodulin, fibronectin, HA synthase 2, hyaluronidase, matrix metalloproteinase 1, and procollagen 1.Two cell lines, one taken from the normal vocal folds of a 73-year-old male and the other taken from the tracheal scar of a 16-year-old male were used in this study. Each cell culture was grown to confluence for the third passage using standard cell culture techniques and were then split into four groups, which were then each treated with the appropriate

media (normal media, HA, CS, or HA+CS). After one week of treatment, the cells were lysed and the mRNA levels determined using RT-PCR. There was limited differences in gene expression suggesting that neither HA nor CS produces a predictable change in the mRNA gene expression of several key wound healing genes. Probable reasons for the lack of gene response include a treatment time that was too short and inadequate modeling of the true *in vivo* environment. Future research incorporating shorter treatment times and better experimental models will hopefully allow an unambiguous determination of the gene expression response of laryngeal fibroblasts to HA and CS.

218 Acoustic Rhinometry: Influence of the paranasal sinuses

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Introduction: Acoustic rhinometry (AR) is an established diagnostic tool in rhinology. The cross sectional area (CSA) of the nose is plotted versus the distance (derived from the time delay in the acoustic response) providing clues on nasal morphology. The aim of this study was to test the hypothesis that paranasal sinuses are a main factor for inaccuracy of AR in the posterior part of the nose.

Method: We studied the influence of paranasal sinus volume on AR measurements in "box models", nasal cast models from corpses, one human cadaver and seven human individuals. Paranasal sinuses differing in volume between 0 and 20ml were fixed and the models measured varying the length and diameter of the joint between the models and the paranasal sinuses. The maxillar sinuses of the cast and the patients were punctured and systematically filled with saline, AR performed meanwhile.

Results: In the box model the measured apparent cross-sectional area in the posterior cavum decreased with the volume of the paranasal sinuses. This effect was limited by the length and the diameter of the paranasal joint as well as the concha. Little or no influence on the posterior CSA was seen in the anatomical nose models. The tests on cast and subjects proofed that there is no significant modification of the posterior area distance curve within the nose.

Conclusion: Acoustic rhinometry shows reproducible measurements up to 4cm in the anterior part of the nose that correspond well with the actual nasal CSA. The paranasal sinuses appear not to account for the inaccuracy in the posterior section of the area distance curve. Further studies on the deficiency of the method are needed.

219 Development of a Murine Model to Study Cisplatin (CDDP)-Induced Ototoxicity.

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Cisplatin is approved for the treatment of several different solid tumors, and we are using it in a multi-agent regimen to treat children with medulloblastoma. Our data has shown the dose-limiting toxicity for this agent is ototoxicity, thus, we are investigating the use of amifostine as an otoprotective agent. To gain insights into the mechanisms by which amifostine may prevent CDDP-ototoxicity, and to further optimize treatment with cytoprotective agents, we developed a mouse model for CDDP-induced ototoxicity. We determined the lethal CDDP dose injected ip which kills 50% of mice within 96 hours was 22.5 mg/kg. We utilized a (Zuo PNAS 1999) transgenic mouse model in which green fluorescent protein (GFP) was expressed specifically in hair cells in FVB mice. These mice were treated with CDDP 24 mg/kg or saline single injection ip, and perfused at 96 hours. Thereafter, the cochlea were sectioned and OHC viewed via confocal microscopy. OHC loss was most prominent in the basal hook region, followed by the remainder of the basal turn. Minimal OHC loss was seen in the medial turn. Whole mount TUNEL staining revealed positive apoptotic cells after CDDP treatment. In conclusion, we have shown OHC loss in the basal turn of the cochlea, as well as positive apoptotic cells after CDDP treatment. These results will allow us to proceed with the evaluation of otoprotective agents and to define molecular mechanisms for CDDP-induced ototoxicity.

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220 Dural cell cuture - a new approach to study duraplasty

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Background: Repair of CSF-fistulas has been performed with increasing success endonasally by insertion of various grafts. Current knowledge of wound healing after graft insertion, however, is still limited and only few animal studies are available. The process of wound healing after dura repair using a degradable transplant is thought to happen by graft replacement due to endogenous tissue.

Material and Methods: Harvested dura pieces from minipigs were perforated to mimic central dura lesions and investigated in a cell culture environment on various conditions (graft material, growth factors) for cellular closure of the perforation.

Results: Cellular outgrowth from the dura into the central perforation was noticed on collagen coated surfaces and to a diminished grade in collagen gels, but missing in case of poly-L-lysin or laminin coating. Immunohistochemistry defined the outgrowing cells mainly as fibroblasts with some intermingled epithelial cells. Scanning electron microscopy proved cellular closure of the defect in case of dura placement on non-crosslinked collagen transplants. Lesser cellular outspread was observed on PDS sheets, while no cells migrated into the central dura perforation in case of placement on a cartilage substitute. Cell counting indicated enhanced cellular closure of the dura opening after substitution of insulin or FGF. EGF didn't reveal acceleration of cellular dura closure.

Conclusions: Our study succeeded in establishment of a cell culture model for duraplasty and indicated cellular outgrowth from the dura borders at the site of defect during the wound healing process. The presented cell culture model depicted among the tested materials for dura closure collagen grafts as best suited for duraplasty. In accordance with the immunohistological finding of mainly fibroblast outspread from the dura borders additional application of fibroblast stimulating growth factors accelerated cellular defect closure.

221 Penetrating Injuries of the Face: A Case Review and Approach to Treatment

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Educational Objective:

At the conclusion of this presentation, the participant should be able to articulate an understanding of the effects of low and high velocity penetrating injuries to the midface. In addition, upon the completion of this presentation, the participant should be able to formulate an algorithm that allows for the strategic evaluation and treatment of penetrating injuries of the face.

ABSTRACT

Background:

Penetrating cranio-facial injuries are not uncommon and often result in significant morbidity and mortality. The evaluation and treatment of these injuries requires a pragmatic and organized approach to achieve the best outcome.

Case Presentation:

We present a 36-year old groundskeeper who sustained a penetrating cranio-facial metal bolt injury. This mechanism has characteristics of both a high and low velocity projectile. Radiologic imaging confirmed the presence of a 15cm metallic object piercing the nasal bones with its tip being lodged in the infratemporal fossa. Head and neck angiography revealed no evidence of vascular injuries. The foreign body was successfully removed in the operating room without any secondary damage.

Case Discussion:

In the case of penetrating injuries of the face, knowledge related to the shape of the foreign body and its relationship to surrounding structures is imperative. In the hemodynamically stabile patient, complete radiographic imaging including early angiography is essential. Long term follow-up is recommended to evaluate for delayed traumatic injuries to cranial vessels and the paranasal sinuses.

222 Research Funding in Otolaryngology – Current Trends

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OBJECTIVES: The evolution of diagnostic and treatment options in the care of otolaryngology patients is dependent on the resources available for meaningful research. We looked at recent trends in funding for clinical as well as basic science research within ENT and how this compares to the level of funding across other specialty fields.

METHODS: Computer-Assisted Medline Search (CAMS) was utilized in data collection. The proportion of federal and nonfederal support was studied by searching a permutated index through OVID. Search fields were limited to 'financial support' or 'support, u.s. gov't, p.h.s.' or 'support, u.s. gov't, non-p.h.s.'

RESULTS: Hearing Research was used as a representative journal for basic science funding, demonstrating a 14% decrease in National Institutes of Health (NIH) funds. The number of journal articles with non-NIH sources of funding remained stable. Archives of Otolaryngology and Laryngoscope were used as surrogates for the clinically focused journals. Neither NIH funding nor non-NIH research support changed significantly. Additionally, twelve ENT journals were reviewed to compare sources of funding between the periods studied. Sixty-six per cent of ENT journals demonstrated a reduction in the number of NIH funded manuscripts, while 42% demonstrated a decline in total funding. A small increase was noted in total funding for ENT research compared to other specialties, as well as a small increase in NIH funding. However, in the period from 1986-2000, ENT was noted to have 26% of total articles funded, with 11% coming from the NIH. All but one of the other medical and surgical specialties reviewed noted lower total funding than that of ENT.

CONCLUSIONS: Tracking sources of funding for research is helpful for determining which avenues are best to pursue for obtaining future support. Additionally, it is possible to make comparisons to other specialty fields with regard to trends in funding across departments.

223 Physical mechanisms of otoacoustic emission generation and propagation: Transiently evoked, distortion product and stimulus frequency OAEs.

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The mechanisms responsible for otoacoustic emission generation and propagation are discussed in the context of a general semi-analytic basis function formulation recently developed by Talmadge et al. [J. Acoust. Soc. Am. 104, 1517-143 (1998); 108, 2911-32 (2000)]. This formulation may be used to describe both the effects of small, distributed discontinuities, which manifest themselves as cochlear fine structure, and the effects of cochlear nonlinearity, which make the fine structure level dependent as well being a source of distortion. A theory of the generation of transiently evoked, distortion product and stimulus frequency OAEs is presented, which includes effects of both nonlinearity and resonance on OAE fine structure. Experimental measurements in humans and other mammals provide further support to this theoretical framework. The main qualitative differences between OAEs in humans and other species can be traced to differences in the length of the cochlea, the cochlear place-frequency map and the frequency response of the middle ear.

224 Applications of OAE-based tests of efferent reflex strength to the study of olivocochlear function in animals.

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The sound-evoked olivocochlear reflex is binaural: efferent feedback to one ear can be evoked by sound in either (or both) ear. The strength of both the ipsilaterally and contralaterally evoked efferent reflex in both awake and anesthetized animals can be assessed via a number of OAE-based tests, including contralateral suppression of ipsilateral OAEs, as well as measurement of the post-onset adaptation of the ipsilateral DPOAE at 2f1-f2. The post-onset adaptation test is based on the idea that the primary tones used to elicit distortion products also elicit feedback efferent activity. Thus, the time constant and magnitude of ipsilaterally evoked efferent activity can be revealed by tracking, with fine time resolution (~10 msec /point), the DPOAE magnitude after primary tone onset. This monaural post-onset test is particularly useful in the study of awake animals, since it does not require acoustic stimulation of both ears. This review will summarize the applications and limitations of these OAE-based techniques to the study of efferent function in animal models, including recent work 1) demonstrating that post-onset adaptation is efferent mediated, 2) documenting the effects of anesthesia on efferent reflex strength and 3) suggesting that pre-exposure measure of efferent reflex strength is highly predictive of the vulnerability to acoustic injury.

225 Medial olivocochlear effects on OAEs in humans: contralateral reflex, left/right asymmetries and influence of benzodiazepines

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Efferent fibers of the medial olivo-cochlear system (MOCS) synapse with the sides and bases of the outer hair cells. Studies have shown that suppression of the amplitude of otoacoustic emissions (click and tone evoked otoacoustic

emissions, spontaneous OAEs, and Distortion Product OAEs) is induced by the MOCS neurons during contralateral (and also ipsi- and

bi-lateral) stimulation, presumably by modification of OHC motility. The influence of acoustic crosstalk and middle-ear reflexes has been studied in patients with unilateral deafness and in patients with absent acoustic reflex. Vestibular neurectomy inhibits this suppression effect, presumably by transecting the

olivocochlear bundle. The suppression effect is intensity-dependent and frequency-specific. The most efficient contralateral acoustic stimulus for suppressing OAEs is noise, and broad-band more than narrow-band; conversely, continuous pure tones inhibit only moderately except if amplitude- or frequency-modulated. More recently, several asymmetrical effects have been revealed. In right handers, contralateral inhibition is greater in the right ear (i.e., probe in right ear and noise in left), but such asymmetry is not found in left-handers. This asymmetry seems to be related to central auditory asymmetry. Indeed, the descending auditory pathway includes fibers coming from the auditory cortex that could influence the superior olivary complex, and thus the medial efferent system. Some clinical cases of patients recorded before and after surgical removal of Heschl's gyrus have shown reduced MOCS effectiveness in both ears but especially contralaterally to the resection. Administration of Benzodiazepines (BZD) has been shown to have an asymmetrical effect on the MOCS: in right-handers, an inhibition of the suppression effect is obtained in the right but not the left ear. Moreover, a recent PET-scan study has shown significantly stronger BZD binding in the left Heschl's gyrus. This BZD receptor asymmetry could be the substrate of the functional asymmetry seen with the contralateral suppression of OAEs. Thus, these results are consistent with the view that there is a modulation of peripheral auditory activity by the auditory cortex in humans.

226 Efferent effects in humans measured with SFOAEs: Methodological issues and results for ipsilateral, contralateral and binaural reflexes

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We will review the use of stimulus frequency otoacoustic emissions (SFOAEs) as a means of measuring efferent-induced mechanical effects in humans. Although somewhat more complicated to measure than distortion product or transient-evoked otoacoustic emissions, SFOAEs provide good signal/noise ratios and have the advantage of requiring only a single low-level tone that evokes little or no efferent activity by itself. We will consider the technical problems associated with measuring efferent effects using SFOAEs, especially the confounding effects of middle-ear-muscle contractions. For all kinds of OAE efferent tests, we will review and consider the adequacy and ease of use of the various methods that have been used to assess whether middle-ear-muscles are producing, or contributing to, putative "efferent-induced" changes.

A second focus will be to review work relevant to comparing ipsilateral, contralateral and binaural medial-efferent reflexes. We will consider the extent to which the human data conform with the expectation from animal work that the ipsilateral reflex is twice as strong as the contralateral reflex and the binaural reflex is the sum of ipsi and contra reflexes. We will review data showing the extent to which the relative effects produced by ipsi, contra and binaural medial-efferent reflexes depend on subject, test frequency, elicitor: type, level, duration and bandwidth, and past history of stimulation.

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227 Adaptation of distortion product otoacoustic emission: Comparison of humans and animals and theoretical interpretations

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Time-dependent changes in level of distortion product otoacoustic emission (DPOAE) were found to be partly effects of ipsilateral reflex of the medial olivocochlear (MOC) system in anesthetized cats (Liberman et al., JASA, 1996). Analogous DPOAE adaptation was observed in normally-hearing awake humans (Kim et al., JARO, 2001). Time course of DPOAE level in animals and humans was characterized by a 2-exponential function. The median fast and slow time constants of DPOAE adaptation in humans were 69 ms and 1.51 s, respectively. Time course of DPOAE level was often biphasic which is represented by a combination of fast and slow components of level change where one is positive (decreasing level) and the other is negative (increasing level). One measure of the ipsilateral MOC reflex strength is the sum of the absolute amounts of the fast and slow level change. This measure ranged from near zero to about 3 dB (median, 1.1 dB) in humans, and from near zero to about 6 dB in cats. Data for humans, awake rabbits (Kim et al., ARO Abstr., 2002) and other anesthetized or awake animals show similarities and differences. Adaptation of DPOAE involves a change not only in level but also in phase. A complex-valued 2-exponential function was found to characterize time courses of both level and phase of DPOAE where each of the fast and slow components has a time constant and amounts of level and phase change. Phase change was lagging, leading, or biphasic. It is hypothesized that DPOAE has two components and that interactions between the two components, together with a change in cochlear mechanics produced by MOC reflex, underlie complexities in time courses of DPOAE level and phase. Basic information about DPOAE adaptation in humans should contribute to the development of practical applications such as identifying people at risk of acoustical injury and a clinical test of the functional status of the MOC system.

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228 Clinical Studies of Medial Olivocochlear Function

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Studies of suppression, using contralateral, ipsilateral, and binaural efferent elicitors have shown:The MOCS effect or suppressing TEOAEs, which is both reliable and valid, exists separately from the middle ear muscle reflex but can be confounded with it if care is not taken. Binaural noise is the most effective elicitor, three times as effective as contralateral noise, and about twice as powerful as ipsilateral noise in forward masking conditions. Thus, using continuous contralateral noise is the easiest way to see the effect but the least powerful. TEOAE-suppression is seen mainly for low–to-mid-frequencies, and between 8-18 msecs after the emission begins. Suppression is related to both frequency bandwidth and intensity and is most effective with broad-band stimuli. Infants and adults show similar inter-subject variability, but no correlation between the amount of suppression and the amplitude of TEOAEs. MOCS function appears by 28-30 weeks of gestational age, and reaches a maximum within a few months after birth, declining with age. The binaural advantage of suppression disappears by about age 70, even though emissions remain. MOCS function depends to a great extent on afferent synchrony because patients with bilateral Auditory Neuropathy (dys-synchrony) show no suppression, while patients with a unilateral synchrony disorder show suppression in BOTH ears so long as the good ear is being stimulated. Hyperacusics show much more suppression than normals, while most normal hearing adults show more suppression in

their right ears than in their left ears. Language-impaired children show more suppression in their LEFT ears until after Fast ForWord, at which point the suppression asymmetry normalizes. Professional musicians may have more suppression than non-musicians.

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229 The semicircular canal microphonic

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Present experiments were designed to quantify the alternating current (AC) component of the semicircular canal microphonic for angular motion stimulation as a function of stimulus frequency and amplitude. The oyster toadfish, Opsanus tau, was used as the experimental model. Calibrated mechanical indentation of the horizontal canal duct was used as a stimulus to generate hair-cell and afferent responses reproducing those present during head rotation (Rabbitt et al., J. Neurophysiol. 73(6), 1995). Sensitivity to polarization of the endolymph DC voltage re: perilymph was also investigated. Modulation of endolymph voltage was recorded using conventional glass electrodes and lock-in amplification over the frequency range 0.2-80 Hz. Access to the endolymph for inserting voltage recording and current passing electrodes was obtained by sectioning the anterior canal at its apex and isolating the cut ends in air (Highstein et al., J. Neurophysiol. 72(2), 1996). For sinusoidal stimulation below ~10 Hz, the horizontal semicircular canal AC microphonic was nearly independent of stimulus frequency and equal to approximately 4 microV per micron indent (equivalent to ~1 microV per deg/s). A saturating nonlinearity decreasing the microphonic gain was present for stimuli exceeding ~3micron indent (~12 deg/s angular velocity). The phase was not sensitive to the saturating nonlinearity. The microphonic exhibited a resonance near 30Hz consistent with basolateral current hair-cell resonance observed previously in current-clamp records from semicircular canal hair cells (Highstein et al, ARO Abstract 135, 1997). The magnitude and phase of the microphonic exhibited sensitivity to endolymphatic polarization consistent with electro-chemical reversal of hair cell transduction currents.

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230 Mechano-Electric Transducer Adaptation Kinetics Alter Streptomycin Affinity

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Fast adaptation of mechano-electric transducer (MET) channels is believed to underlie a mechanical tuning mechanism in turtle auditory hair cells. The mechanism for the establishing the tonotopic distribution in fast adaptation remains to be elucidated. Dihydrostreptomycin, (DHS) a known blocker of MET channels was used to probe for the mechanisms involved in establishing the tonotopic gradient. DHS had a greater efficacy for low frequency MET channels (IC50 14±2μM than high frequency channels (IC50 75±5 μM) under equivalent conditions. Adaptation slowed as MET channels were blocked suggesting summation, mostly likely of intraciliary calcium, was important for establishing the tonotopic differences. DHS efficacy could be altered by manipulations that changed the kinetics of adaptation. Lowering external calcium slowed adaptation and increased DHS efficacy. DHS efficacy in high frequency cells was increased by elevating intracellular calcium buffering while DHS efficacy in low frequency cells was reduced by lowering calcium buffer concentrations. This data suggest that the kinetics of adaptation were responsible for DHS efficacy. Stationary noise analysis supports the hypothesis that there are kinetic differences between MET channels at high and low frequency positions.

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231 Mechanotransducer channel kinetics in turtle auditory hair cells

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In the first step in auditory transduction motion of a hair cell's ciliary bundle causes gating of mechanically-sensitive ion channels located in the stereocilia. Faithful transduction will be limited among other things, by the kinetic properties of these channels. We have measured the kinetics of activation and adaptation of transducer channels in turtle cochlear hair cells using both macroscopic currents and single-channel recordings. A hair cell in the intact basilar papilla was voltage-clamped and its hair bundle stepped by a fast piezoelectric stack actuator (time constant about 22 microseconds). Fits to the onset of the transducer current gave a principal time constant (in 2.8 mM Ca) that decreased with stimulus amplitude from about 0.4 ms to 0.05 ms (Crawford et al., 1989). Lowering Ca concentration to 0.05 mM increased both the activation and adaptation time constants. The results were extended by measurements on single transducer channels, which were obtained by brief exposure of hair bundles to 0.2 micromolar free Ca buffered with 2 mM BAPTA. Channels responded to bundle deflection towards the kinocilium with an increase in probability of opening, which during maintained displacement declined again in a manner resembling adaptation in the macroscopic current. Reducing external Ca from 2.8 to 0.05 mM increased the channel's mean open time and slowed the average response to bundle stimulation. The results may help to distinguish different channel gating schemes and suggest that calcium ions, along with bundle displacement, act directly on the transducer channels to modulate their probability of opening.

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232 Calmodulin Modulates Myosin-1c Interaction with Hair-Bundle Receptors

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Myosin-1c (Myo1c; formerly known as myosin I-beta) plays an essential role in adaptation of hair-cell mechanoelectrical transduction. Because Myo1c is the sole identified component of the transduction apparatus, we sought to characterize Myo1c receptors in hair cells, with particular focus on those at tips of stereocilia, the location of the transduction apparatus. Using recombinant fragments of Myo1c with its associated calmodulin light chains, we examined binding of these complexes to stereociliary receptors. Surprisingly, this interaction does not depend on the C-terminal tail of Myo1c, long proposed to be the cargo-binding site of the molecule. Instead, binding of recombinant Myo1c fragments to stereociliary receptors depends upon the first two calmodulin-binding IQ domains of Myo1c. Interaction of Myo1c fragments with stereociliary receptors occurs in the presence of EGTA and is blocked by calmodulin, which presumably exerts its effect on Myo1c by occupying a previously unoccupied IQ site. The calcium-sensitive binding of calmodulin to Myo1c may, therefore, modulate the interaction of the adaptation motor with components of the transduction apparatus.

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233 Heterogeneous K^+ Conductances in Vestibular Type I Hair Cells.

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In rodent type I utricular hair cells, conductance-voltage curves are often best fit by the sum of two Boltzmanns, one with a negative $V_{1/2}$ between -80 and -100 mV ($g_{K,L}$) and the other with a $V_{1/2}$ value between -60 and -30 mV ($g_{DR,I}$). Kharkovets (2000)found that an antibody to KCNQ4 specifically labeled type I hair cells and proposed that $g_{K,L}$ comprises KCNQ4 subunits. Rennie(2001) reported that KCNQ blockers are poorly effective on $g_{K,L}$. We also found that the KCNQ blockers, linopirdine and XE991, block $g_{DR,I}$ more reliably than $g_{K,L}$. In only 2 of 6 cells, XE991 (10 μM) shifted $g_{K,L}$'s activation range positively. Here we present evidence that $g_{K,L}$ comprises subunits from the ether-a-go-go (eag) superfamily.

Members of the eag superfamily share several features with $g_{K,L}$, including Cs^+ permeability. The superfamily consists of three subfamilies: erg , elk, and eag. WAY123,398 blocks the entire superfamily and E-4031 is specific for the erg subfamily. In 6/7 type I cells, WAY123,398 (10-50 uM) blocked $g_{K,L}$ substantially (65±12%). The more selective E-4031 (10 uM), completely blocked $g_{K,L}$, but had no effect on more positively activating K conductances. These data suggest that type I K conductances include both eag-superfamily subunits and KCNQ subunits.

To investigate expression of mRNA of the eag superfamily in vestibular epithelia, we isolated and reverse transcribed mRNA from P18-21 utricular sensory epithelia. We obtained PCR products for erg1 and erg2, but not erg3. Syeda et al.(this meeting) found that an antibody made against erg1 labeled type I hair cells in the rodent utricle and crista and that the strength of labeling varied with region in the epithelium. This suggests that the heterogeneity in type I K conductances observed in isolated rat type I hair cells may have a regional basis.

234 Regional Variation in K^+ Channels in Rodent Vestibular Sensory Epithelia

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Type I vestibular hair cells have a very negatively activating conductance, $g_{K,L}$. Kharkovets et al. (*PNAS* 97:4333, 2000) found that an antibody to KCNQ4 strongly and specifically labelled type I hair cells and proposed that $g_{K,L}$ comprises KCNQ4 subunits. As shown at this meeting (Hurley and Eatock), however, $g_{K,L}$ can be blocked by drugs selective for K channels in the eag superfamily. Here we show that antibodies against erg1, a member of the eag superfamily of K channels, label type I hair cells and calyces in the peripheral zones of rat cristae and maculae.

We performed Western blots on vestibular endorgans and ganglia, cochleas, brain and pituitary with an antibody against part of the C-terminus of Herg1 (gift of J. Nerbonne, Pond et al., *J Biol Chem* 275:5997, 2000). Bands at ~120, 165 and 205 kD were seen for all tissues. The 120 kD band is near the unglycosylated weight of eag superfamily members, and the higher MW bands correspond to the principal bands seen by Pond et al. (2000). Pre-adsorbing the antibody with C-terminus peptides to erg2 and erg3 blocked staining, showing cross-reactivity within the erg family.

To investigate the cellular location and regional distribution of erg1 and KCNQ4 subunits, we did immunocytochemistry with antibodies to erg1 (Chemicon; and gift of J. Nerbonne) and KCNQ4 (gift of T. Jentsch, Kharkovets et al. 2000) and examined the sections in the confocal microscope. The pattern of labeling varied with region in the epithelium. Erg1 staining was chiefly localized to the cell membranes of type I cells and calyces in the *peripheral* zones of the cristae and in the *extrastriolar* zones of macular endorgans. KCNQ4 labeling extended much further along the basolateral membranes of type I cells and/or calyx endings in the *striolar* and *central* zones. Together with the pharmacological data from Hurley and Eatock (this meeting), these results suggest that the K+ channels of type I hair cells vary with region.

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235 An Immunocytochemical Investigation of the Distribution of Calretinin in the Basilar Papilla of Turtle

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Calcium plays an important role in the regulation of fast events in the sensory hair cells of the inner ear. The calcium buffering capacity of high frequency hair cells in the turtle cochlea (basilar papilla) has been shown to be greater than that of low frequency ones. Calretinin has been reported in frog saccular hair cells where it has been proposed to act as the main calcium buffer. We have therefore been investigating its distribution in the turtle basilar papilla. For this, we have performed immunofluorescence labelling of whole papillae from the red-eared turtle, Trachemys scripta elegans, using a monoclonal antibody to calretinin (SWant). The papillae were fixed using 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4), then permeabilised using 0.5% Triton-X 100 in phosphate-buffered saline (PBS). They were blocked in 10% goat serum in PBS, incubated in primary antibody overnight (1:100-1:500) at 4oC in 1% bovine serum albumin-PBS followed by a secondary antibody conjugated to FITC or TRITC (1:20), then examined using an MRC BioRad confocal laser system.

The hair cells but not the supporting cells were clearly labeled for calretinin, with the highest levels of fluorescence being observed at the basal end of the papilla. The nerve terminals of the auditory nerve fibres also appeared to be labeled and fluorescence could also be seen in nerve fibres leaving the papilla at the high frequency end. As a control, immunolabeling was also performed using the same primary antibody preadsorbed with calretinin; this completely blocked the labeling. These findings agree with the physiological data showing a higher concentration of diffusible calcium buffer in the high frequency hair cells. However, calretinin may not be the only calcium buffering protein in turtle hair cells. We are now therefore comparing its distribution with that of others such as calbindin.

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236 Molecular Features Specific to Calcium Channels in Vertebrate Hair-Cell Organs

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Evidence suggests that at least two types of pore-forming voltage-gated calcium channel (VGCC) subunits exist in hair cells, an alpha-1D and an alpha-1B. In the current work, we compared the apparent hair-cell-specific molecular features of these channel subtypes for a trout saccular hair-cell sheet and a rat organ of Corti fraction. Hair cell samples and, for additional comparison, brain tissues, were homogenized in 4M guanidine thiocyanate. RNA was extracted, treated with DNase, and reverse-transcribed with oligo-dT, random hexamer, or

RACE primers. A trout brain cDNA library was also constructed in lambda ZAP II vector and used to determine teleost-specific primer sequences. The alpha-1 subunit cDNA from trout saccular hair cells revealed apparent hair cell-specific splicing, compared to sequence from trout brain. Like the alpha-1D sequence from the chick cochlear sensory epithelium (Kollmar et al., Proc. Natl. Acad. Sci. USA 94: 14883-14888, 1997), the alpha-1D sequence from the trout saccular hair cell has an insert of 26 aa in the I-II loop and a 10-aa insert in the IVS2-IVS3 loop compared to brain. However, in the trout, unlike the chick, the splice choice in the IIIS2 region is identical between hair cell and brain. In addition, there is a deletion of 9 amino acids in the carboxyl terminal of trout hair-cell alpha-1D. The rat organ-of-Corti alpha-1D, compared to rat brain alpha-1D, contains a 28-aa insert in the I-II loop, an alternative splice in the IIIS2 region, and an alternative splice in the IVS3-IVS4 region. For the trout hair-cell alpha-1B subunit, PCR studies indicate the presence of two splice variants in the I-II loop region, one incorporating an insert of 11 aa relative to brain, and the other lacking the insert. In all, the data suggest that the octavolateralis calcium channels are evolutionarily conserved across the vertebrates, subserving specific hair-cell functions.

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237 Auxiliary Subunits of Voltage-Gated Calcium Channels in Teleost Saccular Hair Cells

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The voltage-gated calcium channel (VGCC) is a heteromeric complex composed of an alpha-1 pore-forming subunit and auxiliary subunits, alpha-2-delta, beta, and gamma. The auxiliary subunits are hypothesized to modify the alpha-1 subunit properties and render functional diversity by expression of various subunit combinations. Previous work from our laboratory has demonstrated that the mouse cochlea expresses alpha-2-delta-1, beta-1, beta-3, and beta-4 auxiliary subunits, but not gamma-1 (Green et al., J. Neurochem. 67: 37-45, 1996). In the present investigation, a trout saccular hair-cell layer was employed to determine VGCC auxiliary subunit expression in the mechanosensory cell. PCR primers were designed utilizing sequence data from GenBank, targeting highly-conserved regions. Trout brain cDNA was chosen as a positive control containing transcripts for most of the VGCC subunit isoforms. Specific primers were applied to saccular hair cell cDNA for PCR amplification and partial sequences were extended with RACE. The hair cell sheet from the rainbow trout sacculus was found to express homologues of the mammalian alpha-2-delta-2, beta-1, beta-2, and gamma-2 VGCC auxiliary subunit genes. A third beta transcript was identified with homology to regions of beta-4. Data suggest that the beta-2a splice variant is expressed in the hair cell. This finding is of particular interest since beta-2a inhibits voltage-dependent inactivation of VGCCs (Restituito et al., J. Neurosci. 20: 9046-9052, 2000). It is also of interest that gamma-2 is expressed in saccular hair cells; the stargazer mutation of the gamma-2 gene in mice gives rise to a phenotype of seizures, ataxia, and sensorineural deafness (Letts et al., Nat. Genet. 19: 340-347, 1998). These findings suggest that the hair cell could be one of the affected sites in the stargazer mutant.

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238 Simulating Inner Hair Cells of Mice: The BK Current Suppresses Neonatal Voltage Oscillations

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Inner hair cells (IHCs) in the organ of Corti transform acoustical vibrations into transmitter release. The composition of different ionic currents has been recently targeted by several studies. Apart from the transduction currents, the major currents in adult IHCs are carried by delayed rectifier potassium channels, large conductance calcium activated (BK) potassium channels (Kros et al., 1998, Nature, 394:281-284) and voltage gated class D L-type calcium channels (Platzer et al., 2000, Cell, 102:89-97).

Neonatal IHCs of mice already express delayed rectifier potassium channels (although with different characteristics compared to the delayed rectifier in the adult) but currents flowing through the BK channels appear later, during maturation around the onset of hearing. Before that the cells react with slow voltage oscillations upon current injection. With the development of the BK current, the voltage oscillations are suppressed and the IHCs show a graded receptor potential.

Neglecting in first instance the transduction mechanism and exocytosis we developed an electrophysiological model for the mouse IHC. A model incorporating the calcium current and a delayed rectifier corresponds to a neonatal IHC. Such a model shows slow voltage oscillations upon current injections, whereas the incorporation of a BK current suppresses the oscillations, in agreement with experimental findings of Kros et al. (1998).

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239 2 photon Imaging of FM 1-43 Uptake Reveals Membrane Uptake, Trafficking and Fusion in Mammalian Inner Hair Cells

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Using confocal and 2-photon imaging of the membrane marker FM 1-43, we have studied membrane retrieval and trafficking in inner hair cells (IHCs) of the guinea pig organ of Corti. Using an in situ preparation of the cochlea, we studied apical uptake by bath-applying FM 1-43 (5 μM) to the scala media. Initial apical FM 1-43 uptake was rapid (< 2s) and calcium dependent. Uptake kinetics remained unchanged by pharmacological block of the mechanotransducer channel. From defined excitation voxels of 2PCLSM, we estimate the uptake rate at about 1500 vesicles/s. Fluorescence first appeared in apical structures. Within approx 180 seconds, labelled membrane was transported to the base of the cell by kinesin-dependent trafficking and targeted to regions (about 5 -10 μm length) and to hotspots (1 μm diameter) associated with the basolateral plasma membrane. These hotspots could be destained by transcellular stimulation. 250 μA, 20 Hz pulse trains applied across the tissue led to a weak decrease of fluorescence. However, when apex to base trafficking was blocked by a kinesin inhibitor, the same stimulation led to a significant loss of basal fluorescence by about 40%. Using a strip preparation of the organ of Corti, where FM 1-43 was either applied to the basal or apical pole of IHCs, we found that IHCs possess basolateral uptake 0.1 of the apical rate. Potassium-induced depolarisation (40 mM) of IHCs in this system increased apical membrane retrieval. This latter effect was significantly reduced when cadmium (100 μM) or nimodipine (10 μM) were present during depolarisation. Calcium may thus raise apical uptake directly or, by triggering synaptic release, cause compensatory apical endocytosis.

Our results suggest that IHCs might use their capacity for rapid membrane retrieval from the apical membrane, where the rate can be adjusted to the activity of the cell, to refill membrane pools associated with release sites at the base.

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240 AP-5 blocked the increasing of free Ca^{2+}induced by glutamate in isolated cochlear IHC

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To investigate the effect of Ap-5 on the increasing of free Ca^{2+} concentration($[Ca^{2+}]_i$) that was induced by glutamate in isolated cochlear inner hair cell (IHC), and to detect the Glu autoreceptors on the membrane of IHC. We used the laser-scan confocal microscope (LSCM), and fluo-3, a fluorescent probe for $[Ca^{2+}]_I$ to observe exogenous glutamate (Glu) -induced $[Ca^{2+}]_i$ change in isolated IHCs and OHCs of guinea pig cochlea. The exogenous glutamate (Glu) -induced changes in $[Ca^{2+}]_i$ of isolated IHCs were recorded under LSCM when AP-5(an antagonist of NMDA) and CNQX(an antagonist of AMPA) were administrated. The IHCs could be identified according to their unique flask shape with a distinct neck and spherical base and a large spherical nucleus. Normal cell shapes could be maintained about two hours in most cases. The images of $[Ca^{2+}]_i$ from LSCM were similar to those from inverted microscopy. Fluorescence of Fluo-3 distributed in the isolated IHCs with brighter staining in the nucleus. In the presence of low concentration of Glu (3.85μmol/L), there was an increase of $[Ca^{2+}]_i$ in IHCs, but no change in OHCs. The increases of $[Ca^{2+}]_i$ were observed in 9(n=10) IHCs and there was no change in 1 IHC. For 10 of the observed OHCs, 7 OHCs showed no $[Ca^{2+}]_i$ change and only 3 OHCs showed minor reduction of $[Ca^{2+}]_i$. However, Glu did not induce corresponding increase of $[Ca^{2+}]$ in IHCs when AP-5 was administrated previously. The CNQX did not blocked increase of $[Ca^{2+}]$ in IHCs. These results suggest that exogenous Glu is capable of increasing $[Ca^{2+}]_i$ concentration of IHC by acting on NMDA autoreceptor of IHC in a positive feedback manner.

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241 Transmitter Release at the Inner Hair Cell Ribbon Synapse

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The aim of this study was to investigate the release mechanism at the ribbon synapse between inner hair cells (IHCs) and afferent fibers in the mammalian cochlea. To record postsynaptic activity from afferent fibers we used whole cell recording with the patch pipette directly contacting the terminal swelling of an afferent fiber at the base of an IHC in the neonatal rat cochlea (postnatal day 7-13).

Spontaneous excitatory postsynaptic currents (EPSCs) carried by AMPA receptors were observed in 16 of 21 boutons recorded in saline containing 5.8 mM K^+ (holding potential -90 mV). EPSCs could be elicited in silent boutons or their spontaneous occurrence accelerated by elevating extracellular K^+, thereby depolarizing the IHC. EPSC frequency changed with IHC depolarisation: from 1.5 ± 1.1 per second in 5.8 mM K^+ (n = 4 boutons) to 27.2 ± 22.1 per second in 40 mM K^+ (n = 5 boutons). One afferent fiber recorded in 5 mM K^+ showed bursts of EPSCs due to Ca^{2+}-action potentials in the IHCs. EPSC frequency within the bursts was 148 per second.

Although afferent fiber frequency changed with depolarisation of the IHC (5 mM K^+, 40 mM K^+ and during Ca^{2+}-action potentials), EPSC amplitude distributions in all three conditions stayed quite similar: Amplitude distributions were highly skewed with a maximum at about 36 pA (at -90 mV). Amplitudes varied over a wide range between 15 and 775 pA in every recording with mean amplitudes between 130 and 190 pA.

Given the wide range of amplitudes in the single bouton recordings, we propose that the hair cell ribbon synapse operates by multivesicular release.

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242 The Effects of CNQX, DAA, and MK-801 Perfusion on Avian Auditory and Vestibular Compound Action Potentials

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Glutamate (or a glutamate-like substance) is currently the primary candidate for the hair cell transmitter in the sensory endorgans of the inner ear. Kainic acid (KA), an excitotoxic glutamate receptor agonist, has been shown to eliminate both auditory and vestibular compound action potentials (CAPs) in the bird. These and other findings support a role for glutamate/kainate receptors in afferent synaptic transmission in the avian auditory and vestibular systems. However, owing to its toxicity, KA may act indirectly on afferent terminals to block synaptic function in the auditory and vestibular periphery. To better identify receptors, we used perilymphatic perfusion to deliver three glutamate antagonists to the inner of the chicken (*Gallus domesticus*, n=36). We tested CNQX (6-cyano-2,3, dihydroxy-7-nitroquinoxaline), a potent competitive antagonist for non-NMDA glutamate receptors, DAA (D-α-aminoadipic acid), and dizocilpine maleate (MK-801) both NMDA-specific antagonists. Recordings of auditory and vestibular CAPs were used to evaluate transmission at the hair cell primary afferent synapse. DAA (1mM) failed to significantly alter auditory and vestibular responses. At high doses, MK-801 (1mM) significantly reduced (p=0.035) auditory CAPs but had no effect on vestibular CAPs. CNQX had no effect on vestibular CAPs at doses below 100μM. In contrast, CNQX reduced or eliminated auditory responses at doses as low as 1μM. The effective concentration (EC_{50}) for auditory responses was ~30μM. These findings support the hypothesis that non-NMDA glutamate receptors mediate auditory responses in the bird. The effectiveness of CNQX on vestibular receptors was considerably less than that on the auditory. The results may reflect the existence of different receptor types for auditory and vestibular sensors or reduced drug exchange rates between the perilymph and vestibular receptors.

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243 The Influence of Static Pressure on Vibrations of the Tympanic Membrane in Animal Ears

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Laser-Doppler measurements of sound-induced velocity of the centers of the pars tensa and pars flaccida of the tympanic membrane of gerbils were made while manipulating the middle-ear static pressure. The measurements indicate a difference in the effect of static pressures on the acoustic response of the two tympanic-membrane segments; most notably, the pars flaccida's response to low-frequency sound stimuli is

more sensitive to changes from zero static pressure. At frequencies above 2 kHz, however, the velocity of the center of the flaccida and of the center of the tensa are similarly effected by static pressure. The pressure-induced changes in the two point velocities have some features in common with human tympanometry including an asymmetry in the response to positive and negative static pressures and a directionality of the response. The asymmetry appears as a strong difference in the sensitivity of the velocities to middle-ear static pressures of different polarity. Negative middle-ear pressures generally produce decreases in velocity that are much larger than those induced by positive middle-ear pressures. Indeed, with sound stimuli of 4000 Hz and greater the sound-induced velocity with positive pressures often is larger than that of the ambient condition, while the velocity is always reduced by negative middle-ear pressure. The directionality appears as a shift in the location of the static pressure that produces the peak velocity that depends on whether the pressure is stepped from – to + or vice versa. Comparison of these point-velocity measurements to acoustic-admittance measurements in the literature, suggest a significant difference in the sensitivity of the velocities and the middle-ear admittance to static pressure. This suggestion is being investigated.

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244 Eardrum displacement under static pressure

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Quasi-static pressure differences between the middle ear (ME) cavity and the external auditory meatus play an essential role in middle ear mechanics. These pressures cause deformation of the eardrum and displacements of the ossicles which are huge in comparison to sound induced effects. The ME mechanical system succeeds in transducing vibrations with pico-meter amplitude while dealing at the same time with quasi static displacements in the millimeter range, and protecting the cochlea from high low frequency pressure inputs.

To improve the basic understanding of this complex mechanical system, we have developed a dedicated optical apparatus, based on moiré interferometry, to study the deformation and volume displacement of the eardrum. With our apparatus we have performed in-vitro shape and deformation measurements of the human and gerbil eardrum. The results allow us to obtain many mechanical parameters, such as volume displacement and translation and rotation components in the movement of the malleus. We will present full field high resolution measurements of eardrum deformation as a function of pressure. The data are an important input for the development of highly realistic mathematical models of the ME mechanical system. Static pressures are also associated with many pathologies of the ME and the eardrum. In the gerbil, we have measured volume displacement of the pars tensa and the pars flaccida (PF) as a function of ME pressure. The results obtained on the PF were used to make a parametric model for the volume displacement, and to determine the possible role of the PF in ME pressure regulation. In contradiction to some traditional concepts, our data show that the PF only can have an extremely small contribution as a buffer for large ME pressure changes. On the other hand we found that the PF reacts to extremely small pressure changes, which may be an important aspect of the toltal ME pressure regulation process.

245 Mechanics of the ossicular chain at static pressures, its influence on sound transport and the behaviour of ossicular and piston prostheses in the human ear

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The ear functions as a very sensitive pressure receptor, detecting sound pressures with vibrational amplitudes of molecular dimensions. This delicate detector is also permanently exposed to the million times larger variations of atmospheric pressure changes in daily life of up to several 100 mm water (daPa), as they occur with gushes of wind, sneezing, blowing one's nose, diving and flying as well as with tympanometry and pneumatic otoscopy. Evolution has developed a middle ear construction that provides a functioning of the sensitive sound transporting apparatus in these ambient air pressure changes without damage. This complex demand is answered by different constructions in the mammalian (also human) and reptile middle ear. Many anatomical details in the construction of the human middle ear demonstrate this function which is consigned to static pressure demands, like the gliding in the ossicular joints, the function of the middle ear muscles, the arrangement of ossicular ligaments, the design of the ossicles etc.. Temporal bones experiments show the different mechanics of the tympanic membrane - ossicular chain during sound transport and with ambient air pressure acting on the middle ear. Further experiments with reconstructions of the middle ear demonstrate the behaviour of ossicular prostheses and piston prostheses at variations of atmospheric pressure. These findings not only help to better understand the construction principles of the middle ear, but they also give information on the stability and reliability of modern surgical reconstructions of the middle ear in daily life with its permanent variations of ambient air pressure, but also in diving and flying.

246 Finite-Element Modelling of Nonlinear Behaviour of the Middle Ear

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In the finite-element method, a complex and irregularly shaped physical system to be analysed is divided into a number (often large) of discrete elements that are individually easy to analyse. Most or all of the model parameters have very direct relationships to the structure and material properties of the system. A finite-element model generally has relatively few free parameters whose values need to be adjusted to fit the data.

General issues related to finite-element modelling include mesh generation to obtain a topologically correct mesh of well-shaped elements; testing for convergence to choose a trade-off between accuracy and computation time; and the specification of material properties. When displacements are small, finite-element simulations involve the linear calculation of static responses, natural frequencies and modes (eigenfrequencies and eigenvalues), time-domain responses and frequency responses.

When deformations are large, however, responses generally become nonlinear, as with tympanometric pressures in the middle ear. The nonlinearity may be due to geometric effects, nonlinear material properties, and/or changes of boundary conditions (e.g., contact). Quasi-static pressures also lead to viscoelastic effects such as creep and hysteresis. A general approach to nonlinear simulation involves incremental, step-by-step solutions, determining the equilibrium state at one time-step or load condition before advancing to the next. For example, a pressure can be applied in small increments up to the final pressure, with iterations being performed at each step to compute the deformation at that pressure. This is conceptually straightforward but numerically delicate and computationally expensive. Some of the issues can be illustrated by nonlinear simulations of the cat eardrum.

247 Pressure-Volume Relations of the Human Middle Ear with a Focus on Pitfalls in Tympanometric Determination of Middle Ear Pressure

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The middle ear system (MES) is subjected to larger pressure variations due to environmental and physiological factors, which may be involved in the development of middle ear disorders. The behaviour of the MES in response to such pressure loads can be investigated measuring its pressure-volume relationship (PVR), which shows non-linearity and hysteresis. Based on 76 normal ears the general mechanical properties are demonstrated.

These pressure loads induce volume displacements of the tympanic membrane (TM), which change the middle ear pressure (Pm). Further, hysteresis was found subject to a significant variation. These two factors will affect indirect tympanometric determination of Pm to some degree, and hence, our results have led to a focus on these sources of inaccuracy.

Problems addressed are: 1) model experiments, where middle ear volume (Vm) is decreased, show exponentially increasing negative tympanometric Pm despite a normal model Pm, 2) model experiments, where Pm is altered in both negative and positive direction, show a systematic numeric overestimation of tympanometric Pm, and 3) PVR's in ears with effusion show significantly increased hysteresis suggesting that the peak pressure difference found in bidirectional tympanometry increases; this was corroborated by tympanometry in such patients. These factors can lead to negative overestimation of tympanometric Pm amounting to more 100's daPa's.

In conclusion, indirect tympanometric measurements of Pm are higly susceptible to the presence of middle ear effusion due to both the viscosity of the fluid behind the TM and the depletion of the expandable air filled Vm. Hence, tympanometry should not be considered reliable in such pathological ears, where measurements of Pm are of special interest. Direct measurements of Pm should be preferred in such cases at least when the scope is basic science.

248 Tympanic membrane stiffness loss – otitis media sequel and a precursor to retraction pockets and cholesteatoma? Experimental data from static pressure measurements of the gerbil tympanic membrane.

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Otitis media has in its various forms a very high incidence in children. A small number of these individuals develop later a severe form of chronic retraction pocket disease: cholesteatoma. The pato-physiological mechanisms involved are not clear, although it is obvious that the tympanic membrane plays an important role.

We have used static pressure measurements for assessment of the tympanic membrane properties in different forms of experimental otitis media, i.e. acute, purulent otits media, otitis media with serous and with mucoid effusion, as well as cholesteatoma. Further more, measurements have been carried out on ears from animals that have been exposed to short term sniffing-simulation.

The results show general losses of the overall stiffness in the tympanic membrane, and sometimes week spots or zones were observed. Similar changes were obtained from cholesteatoma ears. These changes may be precursors to the development of retraction pockets, possibly in conjunction with other disturbances in the middle ear. Histology sections showed some disturbances of the lamina propria, that may explain the stiffness changes.

After short term sniffing-simulation no obvious stiffness changes were obtained.

249 Why Do We Find Positive Middle Ear Pressures in the Morning?

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The pressure regulation in the physiologically closed middle ear, as in the supine position, is of special interest since the regulatory effect of the Eustachian tube has been emphasized for decades. A closed Eustachian tube should result in gas resorbtion from the middle ear and a fluid filled cavity due to negative pressure. However, studies of morning pressure in the middle ear after several hours in bed showed positive pressures in most middle ears, as did serial tympanometry during rest in the supine position. Gas diffusion seemed to be bidirectional with different directions and rates for the different gases during changing physiological conditions. This process has been studied further by gas partial pressure measurements (mass spectrometry) and evaluation of the predictive power of some mathematical models regarding the net result of the diffusive process.

250 On the control of middle ear pressures

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The Middle Ear (ME) is a gas pocket loosing gas into the tissues and regaining it back through the Eustachian Tube (ET)- supposedly as a passive flow. In "Chronic Otitis" the ME presents a negative pressure, which is supposed to act on gas flow through the ET. Several observations suggest however that ME gases economy is a multifactorial active process. Thus when Atelectatic ear turn hyperinflated when inflated with gas the Tympanic Membrane (TM) will return exactly to its initial atelectatic position after a time which depends on the diffusion coefficient of the inflated gas. This demonstrates a feedback mechanism and the influence of diffusion. Long term follow up of a large cohort of Atelectatic ears shows that only 1% will turn Cholesteatomatous while in 46% the TM will stay in the same steady state and in 44% the TM will return back to the physiological position-some ears turn (spontaneously) hyperinflated. A steady state of the TM can be found in any position below or above the annular level of TM for shorter or longer times. Direct relation exists between the mastoid size and the prognosis and dynamics of this waxing and waning ME pressure. Pressure measurements showed that gas passes through the ET on swallowing in the order of magnitude of I mm H2o but this happens in an inconsistent manner .At times swallowing elevates ME pressure at, others it elevates aninitial "above atmospheric pressure" even further. Circa 30% of the swallowing acts show no opening of the ET. These variations in the mode of gas passing the tube suggest an active process which explains the variations of ME pressures i.e Atelectasis and Hyperectasis. The interpretation of the above views the ME as an actively regulated system controlled through a feedback mechanism. Pressure changes are influenced by several factors such as variations in the perfusion diffusion. Pressure control seems to be more effective in the presence of a large Mastoid and may fail in its absence.

251 "Alport Syndrome" mice exhibit sensitivity to acoustic overstimulation

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Several animal models of human diseases associated with high frequency hearing loss exhibit an abnormally thick basement membrane in capillaries of the stria vascularis. The underlying molecular mechanism for matrix accumulation in the strial capillary basement membrane of mouse models for Alport syndrome, diabetes mellitus and systemic lupus erythematosus has not been completely delineated. The thickened basement membrane in strial capillaries of the mouse mutant 129SvJ-Col4a3, a model of Alport syndrome, is due to increased deposits of type IV collagen and laminin. The matrix accumulation occurs in the presence of increased MMP9, an extracellular protease that degrades basement membrane components. The present investigation was undertaken to determine if matrix protein accumulation in the strial capillary basement membrane renders the subject more susceptible to environmental stress, resulting in the high frequency hearing loss observed clinically.

Young 129SvJ-Col4a3 mice and their normal littermates were exposed to a 8-16 kHz noise band presented continuously at 106 dB SPL for 10 Hr. Auditory thresholds were monitored using the auditory brainstem response technique (ABR). Immediately after noise exposure, a mild threshold shift was noted at all test frequencies (8, 16, 24 and 32 kHz) in control animals. In contrast, significantly greater threshold shifts were noted at 16 and 24 kHz in the Alport mice. While thresholds in both the control and Alport mice returned to pre-exposure levels by 5 days post-exposure, the Alport mice recovered more slowly. Following the final ABR measure, the amount of Na,K-ATPase specific activity in the stria was determined. A trend toward lower activity in the Alport mice was noted. The data suggest that accumulation of matrix proteins in the strial capillary basement membrane compromises function of the cochlear lateral wall so that addition of environmental stress causes increased susceptibility to hearing loss.

252 Characterization of Prion Protein (PrP)-Induced Hearing Loss and its Pathophysiology in Transgenic Mice

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Prion diseases are fatal neurogenerative diseases that can present as genetic, sporadic, or infectious disorders. In these illnesses, the normal, cellular prion protein (PrPc) undergoes a posttranslational modification to generate the pathogenic isoform (PrPsc). A trangenic mouse model, Tg(tTA:PrP), that expresses large amounts of PrPc under control of the tetracycline transactivator, has been created to study prion diseases and to help gain an insight on the normal function of PrPc. Tg(tTA:PrP) mice develop a progressive ataxia at roughly 2 months of age unless PrPc production is suppressed by oral doxycycline administration. In addition, the mice also express circling behavior characteristic of mutant mice with inherited hearing loss.

The aims of this study are to determine the time course of the onset of hearing dysfunction, and to characterize the histopathology of the peripheral auditory system correlating it with PrP transgene expression. Tg(tTA:PrP) mice kept off doxycycline for 10 weeks, thus expressing the PrP transgene for the duration of that period, were found to have severe to profound hearing loss, reduced densities of cochlear spiral ganglion neurons (SGNs), intense PRP expression in the spiral ligament, using rabbit anti-PrP polyclonal antibody, moderately increased expression in SGNs and increased SGN death, determined with Tunel-staining. In contrast, when PRP expression is suppressed by continual administration of doxycycline, mice had normal amount of SGNs, minimal or no PRP expression and no cell death. The results of this study contribute significantly towards understanding the mechanism by which PrP overexpression can cause auditory dysfunction.

253 Eph-receptor Deficiencies Lead to Altered Cochlear Function in Mice.

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Ephrins and Eph receptors are a family of molecules that have been implicated in many developmental processes including neuronal network formation, guidance of cell migration, and axonal pathfinding. These molecules exhibit the unique ability to send bi-directional signals following ligand-receptor interactions resulting from cell-cell contacts. Gene-targeted knockout mice of B-class Ephrins and Eph receptors have been shown to display phenotypic responses that correlate with anatomical defects. For example, disruption of the Eph B2 receptor leads to defects of the vestibular system, including pathfinding abnormalities in efferent axons and reduced endolymph production. Such developmental distortions lead to deficiencies in ionic homeostasis and repetitive circling behaviors. The present study demonstrated that B-class Ephrins and Eph receptors are expressed in cochlear tissues, suggesting that they may play a role in auditory functions. To determine whether Ephrins and Eph receptors have a functional role in the auditory system, we compared distortion-product otoacoustic emission (DPOAE) levels, collected across a broad frequency range, between groups of mice expressing different Eph receptor genotypes. Eph B1 and Eph B3 receptor knockout mice exhibited significantly diminished DPOAE levels as compared to wild type littermates. These results indicate that these Eph receptors are necessary for normal cochlear function.

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254 Individual Susceptibility to Noise Induced Hearing Loss; Measurements on the Cochlea Before and After Compulsory Military Service

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Could measurements of the status of the outer hair cells and the efferent system show who will be more susceptible to noise induced hearing loss? About 200 conscripts, 19-21 years of age, most of them normal-hearing, doing their service in tanks or in a military orchestra were tested twice - at the very beginning of the service and after 7 or 11 months. Control groups, 80 men of equal age and hearing span, were also tested.

TEOAEs and DPOAEs with and without contralateral suppressor noise were measured. Measurements of thresholds for brief tones at the peaks and in the valleys of intensity modulated octave band noise, were performed at 4 kHz, 10 Hz modulation frequency, at various noise levels. The non-linear level dependence of these thresholds, the PMTF-

curves, (part of the Psychoacoustical Modulation Transfer Function) gives information about the outer haircells.

The results were first analysed by group: Individuals belonging to the same military group and the same time span between measurements, i.e. with roughly the same noise exposure were grouped. There were very few significant changes in hearing thresholds for groups, the worst effects were noted for the musicians. In this group there were several cases of tinnitus. For some individuals there were considerable increases in thresholds at 6 and 8 kHz. Among individuals with regular noise exposure, it was difficult to find a common indicator explaining individual susceptibility, when looking at results before exposure. However some individuals were exposed to incidents, e.g. impulse noise from shots occurring when their ears were unprotected. Although the number of individuals is not sufficient for statistical proof there are indications that PMTF-curves might be valuable predictors for noise susceptibility. Certain shapes of the non-linearity of the PMTF-curves, possibly caused by impulse noise, seems to open up for detoriation of thresholds in continous noise.

255 8-*iso* Prostaglandin F_{2a}, a Product of Noise Exposure, Reduces Inner Ear Blood Flow

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We have previously shown the formation of 8-isoprostane (8-*iso* Prostaglandin F_{2a}), a marker for free radicals (reactive oxygen species), in the cochlea in response to noise exposure (Ohinata Y. *et al*., Brain Res. 878, 2000). Since 8-isoprostane is a vasoactive compound in other systems, we are now assessing its potential as a regulator of cochlear blood flow (CBF). The probe of a laser Doppler flowmeter (Periflux 4001) was placed on the basal cochlear turn of pigmented guinea pigs for recording CBF. 0.1ml doses of 8-*iso*PGF_{2a} (10-50 µg/kg) were infused into the right jugular vein. There was rapid and concentration-dependent change in blood pressure (BP) and CBF, followed by recovery toward baseline. BP was allowed to return to or near baseline before the next higher dose. The ratio of CBF to BP, as a specific measure of cochlear vascular conductance (VC), showed a strong dose-dependent decrease, indicating a constriction of the cochlear vasculature, reducing CBF. In order to isolate the cochlea from systemic BP effects, a syringe pump was used to infuse 8-*iso*PGF_{2a} into the anterior inferior cerebellar artery (AICA) via a micropipette. This attempt yielded limited results, due mostly to frequent thrombus formation at the pipette tip. However, in animals with successful AICA infusion of ~25 µg/kg over 2 min and minimal BP change, there was a rapid 25-30% decrease in CBF and VC. A similar pattern of VC decrease was also noted in animals in which there was an increase in BP during AICA infusion. Given the observation that 8-isoprostane is produced in the inner ear with high levels of noise exposure, we may expect that its vasoconstrictive effect contributes to noise-induced damage. Prevention of isoprostane-mediated cochlear vasoconstriction may provide a route of intervention to reduce noise-induced hearing loss.

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256 Heat shock factor 1 in the rodent cochlea

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Heat shock proteins (Hsps) enhance cell survival in response to stress. Their expression is regulated by the heat shock transcription factors (Hsfs). Upregulation of Hsp70 has been reported by several groups following cellular trauma to the cochlea. We now examine the role of Hsf1, the stress-responsive member of the Hsf family, in the cochlea of the rat and mouse, using cerebellum as a positive control. Semi-quantitative RT-PCR showed constitutive expression of Hsf1 in the normal rat cochlea. The α spliceform was predominant over the β spliceform in all tissues examined, and Hsf1 was more highly expressed in the sensorineuralepithelial and lateral wall subfraction of the cochlea than in the modiolus. Immunocytochemistry in unstressed mouse and rat revealed Hsf1-like staining in the nuclei of inner and outer hair cells of the organ of Corti, in marginal and intermediate cells of the stria vascularis, and in spiral ganglion cells. Exposure of rats or mice to a 41-42 C, 15 minute heat shock, resulted in Hsf1 activation in the cerebellum and cochlea of both species, as measured by Western blot. The role of Hsf1 in the cochlea following stress was examined using Hsf1 knockout mice. Hsf1 knockout mice and their wild-type littermates were exposed to a 98 dB, 2-20 k, 100% duty cycle noise exposure for 2 hours and auditory brainstem responses (ABRs) were measured at 3 hours, 3 days, and 2 weeks following cessation of the noise. Mice lacking the Hsf1 gene exhibited less recovery of hearing than their wild-type littermates. These studies suggest a potential role for Hsf1 in protecting the cochlea against stress. We are currently assessing the potential role of Hsf1 in the preconditioning by heat stress against noise overstimulation, shown by Yoshida et al (1999).

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257 Hypoxic Conditioning Reduces Noise-Induced Hearing Loss (NIHL) in Mice.

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Conditioning by moderate sound exposure and hyperthermia have been shown to confer protection against subsequent NIHL (Canlon et al. 1988; Yoshida et al. 1999). We examined whether acute hypoxia is also protective. CBA/CaJ mice (n=16, 3.5 mos) were placed into an 80x40x50 cm chamber infused with either room air (n=8) or 8% oxygen/92% nitrogen (n=8) at a rate of 2.0 liters/min for 4 hrs. At 24 hrs post-treatment, animals were exposed in pairs to broadband noise (4-45 kHz) at 110 dB SPL for 1.5 hrs. ABR thresholds were obtained 2 weeks post-noise-exposure at 5, 10, 20, 28.3, and 40 kHz. Comparison of pre- and post-noise-exposure ABR thresholds revealed significantly less NIHL in the hypoxia-treated group ($p < 0.001$, 2-way ANOVA). Threshold shifts for room air-treated mice were >20 dB at 20-40 kHz, while those for hypoxia-treated mice were < 15 dB. A separate test in 7 mice showed no significant threshold shift from hypoxia at the time of noise exposure, so that acute threshold shift from hypoxia does not account for our results. Ongoing experiments in our lab are aimed at determining the optimum conditioning duration, interval, and age, as well as identifying the underlying mechanisms (e.g., upregulation of growth factors, heat shock proteins, antioxidant enzymes).

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258 Evidence that the Inner ear Supporting Cell Antigen (IESCA) is CTL2, a member of the Choline Transporter-like Family

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The monoclonal antibody (KHRI-3) raised against inner ear tissue binds to a cell surface antigen on inner ear supporting cells, causes hearing loss, and precipitates a protein doublet of 65 and 68 kDa under non-reducing conditions and 68 and 72 kDa under reducing conditions. We used the KHRI-3 antibody to immunoaffinity purify the IESCA from

guinea pig inner ear. The affinity-purified material was isolated by SDS PAGE and subjected to MS/MS sequencing. Ten amino acid sequences were identified. All were identical to sequences in human CTL2, choline transporter-like protein 2. To confirm that the KHRI-3 defined IESCA and the CTL-2 protein are the same, we synthesized a peptide based on an antigenic region of CTL-2 protein sequence and raised rabbit antiserum (CTL-2 antibody) against it. Western blot and immunoprecipitation experiments of guinea pig inner ear with CTL-2 and KHRI-3 antibodies showed that both antibodies identify the same protein bands. RT-PCR using RNA from human and guinea pig inner ear demonstrated that CTL-2 is expressed in the inner ear in both species. CTL2 has 10 membrane spanning domains, with cytoplasmic N and C termini, and asparagine residues in the extracellular loops that are consistent with our data that KHRI-3 binds N-linked carbohydrates on the IESCA. Furthermore, multiple cysteine residues imply that intra-chain disulfide bridges maintain the conformation. Our results show that CTL-2 is identical to the KHRI-3 inner ear supporting cell antigen and thus is a strong candidate to be the target of human autoantibodies in autoimmune hearing loss. Inhibition of choline transport may be the mechanism of antibody induced hearing loss.

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259 Superoxide dismutase mimetic M40403 protects hair cells in gentamicin treated cochlear cultures

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Reactive oxygen species, such as the superoxide anion, are believed to contribute significantly to the ototoxic effects of aminoglycoside antibiotics. Superoxide anions are normally inactivated by a family of superoxide dismutases (SOD); however, under conditions of stress, SOD defenses can be overwhelmed leading to oxidative damage. Partial protection from oxidative damage can be achieved with SOD enzymes; however, their clinical utility is limited by their immunogenicity, short half-life, and instability. Recently, a nonpeptidyl mimetic of SOD, M40403, was found to be stable, to enter the brain and to protect against ischemia reperfusion injury. To test the hypothesis that M40403 would protect against aminoglycoside ototoxicity, we prepared cochlear organotypic cultures from P3 B10 mice. After 1 day in culture, gentamicin or gentamicin combined with M40403 was added to the medium. One day later, the tissue was fixed, stained with rhodamine labeled phalloidin and examined with a fluorescence microscope to obtain hair cell counts in the middle cochlear turn. In the presence of 1 mM of gentamicin, outer hair cell (OHC) survival decreased to 7% of control values; addition of 5, 10 and 30 mM M40403 increased OHC survival to 75%, 85% and 60% respectively. Treatment with 1 mM of gentamicin decreased IHC survival to 3% of control values. Addition of 5 mM, 10 mM or 30 mM of M40403 to the culture medium increased IHC survival to 74%, 87% and 74% respectively with 10 mM again providing the optimal protection. Significant hair cell loss also occurred with 0.5 mM of gentamicin, and the addition of 10 mM of M40403 increased OHC and IHC survival to 93% and 87% respectively. These results indicate that M40403 provides significant protection against gentamicin ototoxicity. Since this small molecule is stable, has a long half-life and crosses the blood brain barrier, it might prove to be a potent antidote against aminoglycoside ototoxicity in vivo.

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260 Caspase Activation in Adult Mouse Utricles Exposed to Neomycin

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We are investigating the mechanism(s) of hair cell death following aminoglycoside exposure. We previously reported that inhibition of caspases, a class of proteases involved in apoptosis, reduces hair cell death in adult mouse utricle following neomycin exposure in vitro. Caspases are present in normal cells as inactive proenzymes (zymogens), and they are activated by cleavage of the prodomain. Upstream caspase–8 and –9 are activated by ligation of a death domain-containing cell surface receptor and mitochondrial damage, respectively. Once activated, these upstream caspases activate downstream caspases (-3, -6, and –7), which then destroy the cell by cleaving proteins necessary for cell survival (e.g., cytoskeletal proteins, inhibitors of DNAses). We have used immunohistochemistry to identify the procaspases expressed by hair cells. The data indicate that upstream procaspases-8 and –9 as well as downstream procaspases-3 and –7 are present in undamaged hair cells. We have used both immunohistochemistry and in vitro substrate activation to determine which caspases are activated in hair cells exposed to neomycin. Activated downstream caspases include caspase-3 and caspase-7. Upstream caspase-9 is activated in hair cells in response to neomycin, suggesting that a mitochondrial signal is involved in aminoglycoside-induced hair cell death. Preliminary evidence suggests that inhibition of caspase-9 alone is sufficient to prevent hair cell death following neomycin exposure. These data indicate that caspase-9 is an important regulator of aminoglycoside-induced hair cell death.

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261 Hair Cell Survival Following Aminoglycoside Treatment with Caspase Inhibitors *in vivo*

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Sensory hair cells (HCs) undergo apoptosis after ototoxic insult. Many neuronal apoptotic pathways culminate in the activation of caspases, leading to cell death. Hair cell survival following ototoxic insult can be enhanced by treatment with caspase inhibitors *in vitro* (Forge and Li, Hear. Res. 139:97). We examined the effects of caspase inhibition on HC death following systemic injections of aminoglycosides and infusion of caspase inhibitors into the inner ear.

Chickens were implanted with osmotic pumps containing 50 µM or 100 µM zVAD (general caspase inhibitor) or carrier. The catheter attached to the pump was implanted into the vestibule (Roberson *et al*., Hear. Res. 141:155). Beginning one day following the surgery, the animals received 5 daily IM injection of either streptomycin (1200 mg/kg) or saline. Utricles were removed, fixed, and HC's were identified by immunoreactivity to calretinin. HC's were quantified in 10,000 μm^2 regions.

Direct infusion of zVAD into the vestibule increased HC survival in both the striolar and extrastriolar regions of streptomycin-treated utricles. Following treatment with 50 µM zVAD and streptomycin, sampled regions contained 55 ± 2 HC's in the striolar region and 144 ± 13 HC's in the extrastriolar region. Treatment with 100 µM zVAD and streptomycin resulted in 69 ± 4 HC's in the striolar region and 176 ± 4 HC's in the extrastriolar region. Following treatment with carrier and streptomycin, sampled areas contained 28 ± 6 HC's in the striolar

region and 76 ± 8 HC's in the extrastriolar region. Similar densities were obtained with animals receiving streptomycin treatment alone. Animals that received saline alone had 75 ± 3 in the striolar region and 153 ± 3 HC's in extrastriolar region. These results suggest that caspase inhibitors promote hair cell survival following systemic treatment with streptomycin.

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262 Prevention of Aminoglycoside-induced Hearing Loss by Aspirin: Preliminary Data from a Clinical Study

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Aminoglycosides are still among the most commonly used antibiotics worldwide but no clinical therapy exists to prevent their ototoxic and nephrotoxic side effects. Animal experiments (in guinea pigs and mice) have shown that various antioxidants or metal chelators may reduce the magnitude of aminoglycoside-induced auditory and vestibular damage, including salicylate. This latter finding (Sha and Schacht, 1999) suggested that aspirin, whose active metabolite is salicylate, might become an antidote to aminoglycoside-induced ototoxicity in patients.

195 patients were evaluated in a double-blind randomized placebo-controlled study. Patients hospitalized for infections and diagnosed to receive short-term (5 to7 days) treatment with gentamicin were additionally given either a placebo dosing or 3 x 1 gm of aspirin per day during and for one week following the gentamicin treatment. Audiological evaluation at frequencies of up to 8 kHz were given before the first dose of gentamicin, again at day 7 (day of discharge form the hospital) and at a follow-up session 5 to 7 weeks later. The patient groups matched for age and total dose of gentamicin received and pretreatment thresholds were also similar in both groups. The criterion for gentamicin-induced "hearing loss" was set as a threshold shift of > 15dB at both 6 and 8 kHz in the same ear. By this criterion, there were significantly fewer adverse events in the group receiving aspirin than in the placebo group. The results give hope that antioxidant treatment in general and aspirin in particular may be beneficial in attenuating hearing loss in patients receiving aminoglycoside therapy.

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263 The Role of Lateral Wall Pathology in Hearing Loss of Aged Animals.

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Age-related hearing disturbance (presbycusis) is one of the most common causes of human hearing loss. Although the factors involved in presbycusis have not yet been clarified, there is some evidence to suggest that disturbance of inner ear fluid homeostasis may be related to auditory dysfunction. Disruptions to homeostasis may be caused by pathologic changes within the lateral wall of the cochlea. Increased oxidative stress, decreased heat shock protein (Hsp) levels and other pathologic changes in stria vascularis and spiral ligament may be important causative factors. The aim of the present study is to investigate the pathophysiology of cochlear lateral wall that may cause age-related hearing loss in animals.

In the present study, expression of inducible nitric oxide synthase (iNOS) and Hsp70 in young and aged animals was compared by immunohistochemistry. The effect of sodium nitroprusside (SNP) on intracellular Ca^{2+} in cultured marginal cells was assessed using fluorescence ratio imaging. Hearing threshold changes in chinchilla after application of SNP through round window was also assessed. Functional changes in young and aged animals were compared by measuring auditory brainstem response(ABR).

Intracellular Ca^{2+} in cultured marginal cells increased. In immunohistochemical staining for Hsp 70, positive staining was observed in the stria vascularis of both young and aged rats. Immunoreactivity was stronger in the young rats compared to aged rats. For iNOS, positive immunoreactivity was seen in stria vascularis of aged rats. After SNP application to the round window of the chinchilla, ABR change was observed

In conclusion, these findings indicate that lateral wall components may play an important role in the maintenance of homeostasis in the inner ear, and may be an important determinant in the pathophysiology of age-related hearing loss.

264 The Mouse Ahl Gene May Not Affect the Cochlear Lateral Wall, and Does Not Lead to Reduction of the Endocochlear Potential (EP).

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Ahl is a gene that causes age-related hearing loss (ARHL) in at least 10 mouse strains (Johnson et al. 2000). In C57BL/6 mice, where it is best characterized, it has been associated with progressive hair cell loss, neuronal loss, and degeneration of stria vascularis/spiral ligament (Spongr et al. 1997; Ichimiya et al. 2000; Hequembourg and Liberman, 2001). Ahl therefore appears to lead to a 'mixed' form of ARHL, according to Schuknecht's criteria (Schuknecht, 1974), and has important implications for how this condition arises. If it is to be useful for our understanding of human ARHL, the 'essential' phenotype associated with Ahl must be determined. This issue can be addressed by direct comparison of different inbred mouse strains that carry Ahl. We examined cochleas of 5 such strains: C57BL/6, BALB/c, C57BR/cd, BUB/Bn, and 129S6/SvEv. ABR thresholds, hair cell counts, EP recordings (lower basal turn), and mid-modiolar sections were obtained in mice up to 17 mos of age. In keeping with previous reports (Johnson et al. 2000), each strain showed progressive hearing loss, particularly at high frequencies. Although histological analyses are ongoing, C57BL and BALB mice were found to differ with regard to the extent of degeneration of the lateral wall. In contrast to C57BL, both stria and spiral ligament appear normal in BALBs to at least 13 mos of age. Moreover, EP measures in 3 Ahl strains (C57BL, BALB, 129S6) show no reduction for ages up to 17 mos, despite severe ARHL. Average EPs by strain were: 106±9 mV in C57BL (n=7); 109±2 mV in 129S6 (n=4); and 100±6 mV in BALB (n=13). Based on our results to date we propose the following: 1) Degeneration of stria vascularis/spiral ligament is not necessarily associated with Ahl. 2) Age-related degeneration of these structures in C57BL mice may arise from gene(s) other than Ahl. 3) Degeneration of stria vascularis/spiral ligament in C57BL mice is not severe enough to account for their hearing loss.

265 An Augmented Acoustic Environment Delays Age-Related Hearing Loss in C57BL/6J Mice as Revealed by DPOAEs and Cochlear Histopathology

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Willott and Turner (Hear Res, 1999) reported that an augmented acoustic environment (AAE) delayed age-related hearing loss (AHL) in C57BL/6J (C57) mice. The present study assessed cochlear function at

the outer hair cell (OHC) level by measuring distortion-product otoacoustic emissions (DPOAEs) in both AAE and control mice. These results were subsequently correlated with cochlear histopathology. Beginning at 25 d of age, C57 mice were exposed nightly, for 12 h, to a 70-dB SPL broadband AAE (200-ms pulses at 2/s), centered at 10 kHz. DPOAEs were recorded in the form of DP-grams (DPOAE level as a function of primary-tone frequency), with geometric–mean (GM) frequencies ranging from 5.6-48.5 kHz (f_2=6.3-54.2 kHz), in 0.1-oct steps. DP-grams were collected at three primary-tone levels (L_1=L_2=55, 65, 75 dB SPL). ABRs were recorded at 6, 8, 12, 16, 24, and 32 kHz to confirm the AAE effect. At 6 mo of age, both cochleas from 4 control and 3 AAE mice were post-fixed in OsO4, dehydrated, embedded in plastic and dissected as flat preparations. Hair-cell, supporting-cell and nerve-fiber losses were determined from apex to base and cytocochleograms prepared for all cochleas. Function-structure correlations were made by overlaying the final DP-gram on the cytocochleogram. By 4 mo of age, clear differences between AAE and control DPOAEs were observed. Control C57s showed average DPOAEs that were near noise-floor levels between GM frequencies of 25-48.5 kHz, while the AAE ears exhibited moderately reduced DPOAEs over a restricted range of GM frequencies between 25-35 kHz. The functional data matched well with the histopathology in all ears. As a group, the controls had worse function and more hair-cell loss than the AAE mice. However, in both groups, there was large variability across mice. The ears of the AAE mice were more variable than the controls and 2/3 had pronounced asymmetries between ears.

266 Aging, DPOAEs Across f2/f1 Ratio, and Ultra-high Frequency Hearing

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Human auditory aging studies often match hearing sensitivity (8kHz and below) of young & older subjects to control for peripheral hearing loss. OAEs and ultra high frequency (UHF) hearing sensitivity may provide more sensitive representations of cochlear status. Age-related reductions of DPOAE amplitudes in subjects with normal hearing thresholds have been reported. However, no study has examined the status of UHF thresholds while studying the effects of f2/f1 ratio on the same, normal-hearing, younger and older human subjects. Two normal-hearing, gender-balanced groups of subjects were used: young (18-30 years) and b), older (60-74 years). DPOAEs were measured with the ILO-88 (v.5.6). Primary-tone ratios ranged from 1.05 to 1.8, with L1 = 70 and L2 = 60 dB SPL Primary-tones ranged from 1 to 6.3 kHz. Hearing thresholds were obtained for the range of .25-20 kHz using a Grason-Stadler 61 audiometer (TDH-50 and Sennheiser HDA 2000 earphones). Results: Older subjects, with normal thresholds below 8 kHz, had elevated thresholds above 8 kHz. Few had measurable thresholds above 12.5 kHz, and mean thresholds were >60 dB higher than young subjects at 12.5 kHz. DPOAEs for both age groups showed the largest amplitude responses for f2/f1 ratios of 1.2 to 1.3, and both groups showed very small DPOAE amplitudes for ratios of 1.1 and below and above 1.4-1.5. For the ratios where DPOAEs were largest, mean DPOAE amplitude was larger for the young adults. Although many of the mean f2/f1 functions peaked at a ratio of 1.2-1.3, the difference in amplitude between young and older adults was often greatest at a ratio just above where the functions peaked. It appears that normal hearing thresholds in the frequency range of 8 kHz and below is insufficient proof of normal cochlear function in older human subjects.

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267 Expression of DNase I, an Endonuclease, in the Stria Vascularis

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The stria vascularis (SV) is a highly specialized tissue compartment responsible for the generation of the endocochlear potential (EP). A reduction in the EP associated with degeneration of the SV occurs with age as well as in mice suffering from a variety of genetic mutations. Analysis of gene expression in the lateral wall of W^v/W^v mutant mice, which lack intermediate cells and have a dysfunctional SV, and their wild-type (WT) background strain (C57BL/6J) has revealed differential expression of mRNA for a Ca^{2+}, Mg^{2+}-dependent endonuclease, DNase I. This endonuclease was originally identified as a secretory product involved in digestion, but more recently has been shown to play a role in apoptosis in both digestive and nondigestive tissues.

Here, the expression of DNase I was investigated immunohistochemically in the inner ear of various age groups of W^v/W^v mutant and WT mice. In young WT mice, immunoreactivity was restricted to vascular smooth muscle and strial marginal cells. Reaction product in the marginal cell occupied a perinuclear location and extended apically toward the scala media. In the W^v mutants, turns lacking intermediate cells failed to stain for DNase I. Enzyme expression varied in different turns of aged WT mice, apparently in relation to strial degeneration, being markedly reduced to absent in regions of severe atrophy.

Although DNase I has been shown to be involved in the digestive process and apoptosis, it likely serves a different role in normal marginal cells. DNase I is also known to bind monomeric actin, which inhibits polymerization as well as enzymatic activity. Since many ion transport proteins including pumps and channels are linked to and regulated by cytoskeletal components such as actin, it is probable that DNase I functions in this capacity in strial marginal cells, which are highly specialized for transporting ions.

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268 The effects of chronic furosemide on spontaneous rates of auditory-nerve fibers in young gerbils

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The activity of low-spontaneous rate (SR) fibers with high characteristic frequencies (CF) is reduced in quiet-aged gerbils compared to young controls (Schmiedt et al. J. Neurophysiol. 76:2799-03, 1996). Recently, we indirectly demonstrated a similar result in ears treated chronically with furosemide through the use of recovery functions of the compound action potential (Schmiedt et al., ARO Abstr. 23:80, 2000). In this study, the effects of furosemide on fiber populations were counted directly. Ten young gerbils were implanted with osmotic pumps containing 5 mg/ml furosemide for one week. The furosemide was applied directly to the round window (RW) via a cannula. The spontaneous activities of 188 auditory-nerve fibers were recorded along with CF and threshold information and compared to a group of 438 fibers obtained from 24 young controls. Low-SR fibers (SR $\leq$ 18 spikes/s) with CFs $\geq$ 6 kHz made up 47.5% and 17.6% of the control and furosemide-treated ears, respectively. The difference was significant ($p<0.01$). At CFs < 6 kHz, low-SR fibers consisted of 23.6% of the population in controls, and 16.9% in the treated ears;a difference that was not significant. Endocochlear potentials (EP) were measured in control and treated ears. Mean control EPs in the three cochlear turns (T1, T2, T3) were 95±8, 89±6, and 87±6 mV, respectively. Corresponding EPs in the furosemide treated ears were 56±8, 62±2, and 58±3 mV, demonstrating that the EP in the treated ears was reduced approximately 30 mV from that in the controls. These results show directly that chronic changes of the EP, similar to those

found in aged ears, can indeed differentially affect the activity patterns of primary auditory neurons according to spontaneous rate. Moreover, the data strongly support the modeling of metabolic presbyacusis in the gerbil with chronic furosemide application to the RW.

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269 Neurotrophin-3 and GDNF Transduction Protect the Aging Mouse Cochlea from Cisplatin Induced Damage

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Ototoxicity is one of the major dose-limiting side effects of cisplatin (DDP) chemotherapeutic treatments, which can cause destruction of both hair cells and neurons in the inner ear. Spiral ganglion neurons (SGN) and hair cells express neurotrophin receptors and may be protected from DDP by the localized expression of neurotrophins. We previously demonstrated that HSV-1 amplicon-mediated delivery of a neurotrophin-3 (NT-3)/myc chimera protects SGNs in murine cochleae from cisplatin-induced ototoxicity in vitro and in vivo. To extend these findings, an amplicon vector (HSVnt-3myc/SV40gdnf) that expresses a rat NT-3myc chimera and rat glial derived neurotrophic factor (GDNF) under separate transcriptional control was constructed. Helper virus-free amplicon stocks were assessed in vitro for their capacity to direct the expression of NT-3 and GDNF in cultured inner ear cells. HSVnt-3myc/SV40gdnf-mediated transduction of cultured cells at a MOI of 1 resulted in production of NT-3 up to 11.44 ng/ml, and GDNF up to 1.79 ng/ml in conditional culture over a 48-hr period. To determine whether NT-3 and GDNF overexpression in aging mice would abrogate DDP toxicity, three groups of aged mice (CBA) were injected with cisplatin (8mg/kg), following instillation of HSVnt-3myc/SV40gdnf, HSVnt-3myc/SV40lac or HSVSV40lac control vector. Cochleae transduced with HSVnt-3myc/SV40gdnf and HSVnt-3myc/SV40lac harbored significantly greater numbers of surviving SGNs, suggesting that delivery of both neurotrophin-3 and GDNF via an amplicon vector can protect the aging mouse cochlea from cisplatin-induced damage.

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270 Role of cochlear integrity in nucleus magnocellularis neuronal survival following cochlea removal in aging broiler chickens

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In the chicken auditory system, removal of the sensory cochlear hair cells results in the death of brainstem auditory neurons in nucleus magnocellularis (NM). Previous work has shown NM neuronal cell death following cochlea removal to be breed-dependent (Edmonds et al., Hearing Research 127, 62-76, 1999). Cochlea removal in both hatchling and adult egg-layer chickens results in neuronal cell death in NM, while only hatchling broiler chickens exhibit NM neuronal death after cochlea removal. In addition, it is known that absolute cell number does not decrease with age in the broiler chicken. Recently, it has been found that aging broiler chickens often display a large amount of progressive cochlear damage as well as age-related changes in neuronal cellular metabolism. This cochlear damage may play a role in the waning effect of cochlea removal on neuronal survival in aging birds. Here, we examined the effects of unilateral cochlea removal on broiler chickens of 2, 30, 39, and 52 weeks of age, a range that previously has included normal through severely damaged cochleae. Neuronal cell counts were performed in ipsilateral and contralateral NM in order to determine the age at which neuronal survival becomes insensitive to cochlea removal. The contralateral cochleae were examined for damage. Contrary to previous findings, the cochleae of these birds were found to be largely normal. Our results indicate that neuronal cell loss is apparent ipsilateral to the cochlea removal in every age group we examined. Thus, the status of the cochlea must play a role in the effect of deafferentation on NM neuronal survival. NM neuronal survival appears to be neither age- nor breed-dependent, but instead appears to be dependent upon cochlear integrity.

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271 Changes in Ganglion Cell Number During Age-Related Cochlear Damage in the Chicken Cochlea

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Recent studies have shown that untreated, commercially-raised broiler chickens (Cobb strain, Con Agra, Batesville, AR) show age-related cochlear damage (Jaeckel, et al. ARO 2000). Damage is first evident at 30 weeks in the basal high frequency region and increases in severity and extent with age. By 66 weeks all ears show extensive damage. Other concurrent studies have shown that brainstem cochlear nucleus neurons demonstrate a metabolic downregulation with age (Smittkamp, et. al., ARO 2001). The purpose of this study was to examine cochlear ganglion neurons to determine whether they exhibit age-related anatomical changes. Cochleae from chickens at two ages, 19 weeks and 66 weeks, were examined with scanning electron microscopy (SEM) to assess cochlear damage. Cochleae were then rehydrated and embedded in plastic and then sectioned in a plane orthogonal to the long axis of the ganglion. Sections were collected every 100 microns and stained with toluidine blue. Total counts were taken of all ganglion neurons with a visible nucleolus. Cochleae from 19 week old birds showed no damage, while all 66 week-old birds showed severe damage encompassing an average of 71% of the cochlear length. With the exception of one cochlea, 66-week-old birds showed a 50% decrease in ganglion cell number compared to 19-week-old birds. These data suggest that progressive cochlear damage results in ganglion cell death.

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272 An animal model of age-related hearing loss: correlation between loss of neuronal projections and decrease of distinct neurotrophins

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Age-dependent hearing loss concerns approx. 30% of the population (Cruickshanks et al. 1998, Wilson et al. 1999). In humans and animals a sensorineural hearing loss is associated with a loss of neuronal projections and spiral ganglia but not necessaryly associated with a loss of outer or inner hair cells (Felix, 2000). It is known, that the neurotrophins NT-3 and BDNF are responsible for the survival and the maintenance of inner ear neurons (Pirvola et al. 1994, Fritzsch et al. 1995). The BDNF gene consists of four transcription units in which the exons I - IV together with exon V generate eight splice variants (Timmusk et al., 1993). We cloned BDNF exon I -V and generated sense and antisense riboprobes from each individual exon. We analyzed in a first approach, hearing thresholds (click- and frequency-specific ABR, DPOAE), afferent and efferent neuronal projections (neurofilament 200, synaptophysin) and BDNF mRNA (exon I-V) in young (3 - 8 months) and old (22 - 39 months) Fischer 344 rats and gerbils. We noted an age dependent decrease of the hearing threshold which was determined by measurements of click- and frequency-

specific ABR and their decrease was especially pronounced between the 22nd and 28th month. Active cochlear mechanics also showed an increasing loss with age. We noticed a significant loss of afferent neurofilament - positive projections which is evident in more basal turns in Fisher 344 rats and gerbil, whereas the efferent synaptophysin - positive projections were less affected. A significant reduction of BDNF mRNA of exon IV and V could be detected in the spiral ganglia, whereas the amount of spiral ganglia was not reduced. The BDNF exon IV and V mRNA declines towards the apical turns. Our results point to a significant age-related loss of afferent cochlear projections pronounced in the basal turns. An age-dependent loss of neuronal afferent projections may thus be causally correlated with the loss of BDNF mRNA in spiral ganglia cells.

273 Alteration of auditory thresholds (ABR) in aged galectin-3 null mice

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Galectins, or galactoside-binding lectins, constitute a growing family of carbohydrate binding proteins. Among them, galectin-3 is a 30 kD protein that recognizes glycoproteins containing polylactosamines such as laminin (Hughes, Biochim Biophys Acta, 1999;1473:172). Galectin-3 has been localized in several tissues including epithelial cells. The tissue distribution of galectin-3 varies during development and according to the state of cellular activation, and during some pathological processes. Galectin-3 is required for cell-cell and cell-matrix interactions and thus plays a role in several physiological and pathological situations such as embryogenesis, oncogenesis, inflammation, or infection. In the inner ear, the distribution and function of galectin-3 has not yet been investigated. The aim of the present work was to study the putative role of galectin-3 in the mouse cochlea. To this purpose, galectin-3 null mutant mice (Gal3-/-) were studied. Animals of pure genetic background (129) were 6 or 13 month-old. Hearing thresholds (ABR, Tucker Davis) were determined with click stimulation. Cochlea were sampled and fixed for light microscopy examination. In 6 month-old animals, no difference in hearing thresholds was noticed between wild type (33 ± 3.5 dB, mean ± SEM, n=12) and Gal3-/- animals (32 ± 7.5 dB, n=12). At variance, 13 month-old Gal3-/- mice were deaf (83 ± 5.9 dB, n=14) compared to wild-type mice (33 ± 6.0 dB, n=6). Examination of cochlea did not show major difference between wild-type and mutant mice. Localisation of galectin-3 in cochlear cells is under investigation. In conclusion, aged galectin-3 null mutant mice present profound hearing loss. Further studies are needed to understand the mechanism of this hearing loss.

274 The changes of mitochondrial membrane potential of spiral ganglion neuron of aging rat

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Materials and Methods

Using specific fluorescent probe--JC-1 of mitochondrial membrane potential(MMP) to stain isolated spiral ganglion of adult and ageing rats.Laser scan confocal microscope (Leica company)was chosed to collect fluorescent data. we used the method to examine the characters of MMP in spiral ganglion cells of aging rat.

Results

1.Two-dimension and false three-dimension showed that in the base of peripherial and centeral process, green and red fluorecences is higher, especially in the base of peripherial processes.

2.The density of green and red fluorescences and their ratio in the isolated spiral ganglion cells from old and adult rat showed no significant difference.

Summary and conclusion:

1.JC-1,as a specific fluorescent probe of MMP,can credibly reflect the different energical statuses of mitochodria,especially for evaluation of two kind different energic status mitochondria;and can distinguish the heterogenity of mitochondria in same cells.

2.In the spiral ganglion cells of adult and old rats, the distributions of low and high energy of mitochondria have their specific characters. The low and high energy of mitochodria co-exist in the somas neurite processes of isolated spiral ganglion cells preloaded with JC-1, The highest density of low and high energy of mitochondria was located at the base of hillcock of peripherial processes ,The mitochondria of spiral ganglion cells have powerful compensatory abitity and Ca^{2+} pool.

3.The MMP of spiral gangolion cells between adult and aging rat did not show significant difference.The MMP of the surviving spiral ganglion cells can maintain normal level to support cell function because of their compensatory ability during aging process.

Key words: Spiral ganglion Mitochondrial Membrane Potential Aging

275 The effects of mtDNA deletion associated with abnormal expression in rat cochlea with presbyacusis

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Decreases in mitochondrial respiratory activity have been correlated with increasing age. Recently studies indicated that post-mitotic cells accumulate mutated mtDNAs. Deletions in mtDNA have been shown to be more prevalent in tissues from older people than in infants; however, the mtDNA deletions in inner ear tissues have not been well studied. To investigate the inner ear mtDNA deletions associated with presbycusis, we used a rat model of aging. The mtDNA 4834-bp deletion, which encodes the mitochondrial cytochrome c oxidase subunit I (COXI), was analyzed by PCR. Transcript level was measured by RT-PCR and cytochrome c oxidase (COX) activity was assessed by histochemical methods in rat cochlea tissues obtained from 2 month old and 24 months old rats. Our results show that mtDNA 4834-bp deletion occurred in all of the cochlear tissues from 24-month-old rats whereas the deletion was not detected in cochlear tissues from 2-month-old rats. Significantly, the age-related decrease in transcript level was associated with a decline of COX activity. These results suggest that the mtDNA 4834-bp deletion increases with age and is associated with a age-related decrease of COXI transcript level.

276 Apoptosis disturbance induced by retinoic acid in the mouse craniofacial embryogenesis

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Retinoic acid (RA) is a derivative of vitamin A with potent teratogenicity and it causes miscellaneous malformations when administered to mammals during pregnancy. In this study, we administered RA to a pregnant mouse in order to observe the apoptosis disturbance in the craniofacial region by way of TUNEL method. ICR mice were mated overnight and the morning of vaginal plug occurrence was designated as E0 (embryonic day 0). A pregnant mouse was injected a single dose of 12.5mg/kg all-trans-RA dissolved in soybean

oil intraperitoneally on E7. A control pregnant mouse, injected soybean oil without RA, was prepared as well. Mice were sacrificed on E9 and fetuses were obtained from the uteri and prepared for immunohistological examination. The specimen was sectioned at a thickness of 5 μm and mounted on glass. Every tenth section was stained with TUNEL method. TUNEL positive-cells were observed with particular attention to the otocysts, the first and second branchial arches. For the RA administered fetus, TUNEL positive cells were seen in ventral part of both otocysts. As for the right otocyst, TUNEL positive cells are seen in the dorsal side as well. Outside the otocyst, there was a cluster of TUNEL positive cells among the connective tissue cells. This corresponded to the site of future ganglion. The otocysts of the control fetus did not show TUNEL positive cells. TUNEL positive cells were seen along the surface of bilateral first branchial grooves of the RA administered fetus. The observation of first branchial arch of the control fetus disclosed TUNEL positive cells among the connective tissue. Its first branchial grooves did not present TUNEL positive cells. These results suggested that the malformations caused by RA might originate from the apoptosis disturbance early in the pregnancy.

277 Essential role of RAR signaling in inner ear development

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Retinoic acid (RA) exerts diverse biological effects through two families of nuclear receptors: the RA receptors (RAR) and the retinoid X receptors (RXR). Both receptor families include three subtypes (α, β, γ). To identify nuclear receptors that are involved in the formation and differentiation of the inner ear, we analyzed expression patterns of RARα, RARβ and RARγ transcripts and related them to phenotypes resulting from single or double mutation of these genes. All of the three RAR transcripts were expressed in the mouse otocyst as early as embryonic week two and the expression continued into adulthood. The distribution of these receptors, however, varied and was confined to largely non-overlapping regions: RARα in the presumptive sensory epithelium, RARβ in mesenchyme tissues and RARγ in the presumptive otic capsule. The RAR expression patterns remained complementary even in adulthood. Single RARα, β, and γ null mutant mice as well as double RARα/RARβ and RARβ/RARγ mutant mice did not exhibit any gross morphological abnormalities in the inner ear. In contrast, the RARα/RARγ null-mutant mouse displayed severe hypoplasia in the otocyst, which was already visible at embryonic day 10.5. The hypoplastic feature of the otocyst progressed with developmental stages and later became a failure in forming normal vestibular canals as well as a lack of the entire cochlear structure. Together, these results demonstrate that RARα and RARγ cooperate to play an essential role in the formation of the otocyst, while RARβ plays a minimum role in this process.

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278 Tbx1 gene mutation in mice affects the formation of the outer, middle and inner ear

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The chromosome 22q11 region is susceptible to rearrangements associated with multiple congenital anomaly disorders. To identify candidate genes, we generated bacterial artificial chromosome (BAC) transgenic embryos overexpressing four genes from this interval (TBX1, GP1BB, PNUTL1 and WDR14). The mice had a hyperactive circling behavior and hearing loss. We examined mutant embryos for morphological defects. The defect in the middle ear consisted of a malformation of the stapes. The inner ear had findings that were more significant. The mutant embryos had a Mondini type malformation that involved the cochlea and vestibular system. We propose that overexpression of one of the four transgenes was responsible for the phenotype. We examined the expression pattern of the endogenous mouse genes. Tbx1 was expressed in the otic vesicle, periotic mesenchyme and pharyngeal arches. Due to its pattern of expression in the developing ear, TBX1 was the likely candidate. We targeted the mouse Tbx1 gene for inactivation. Null mutants died in the perinatal period with multiple congenital anomalies including severe malformations of the ear. We examined the ear during embryological development. Dysmorphgenesis of the middle and inner ear was first evident in the E11.5 homozygotes. The normal extension of the first pharyngeal pouch that will form the middle ear cavity did not occur. The otocyst at E11.5 did not progress beyond the E10.5 stage. In contrast to the normal pattern of morphogenesis in wild type and heterozygous embryos at E13.5 through E17.5, there were no detectable pinna or external auditory meatus, middle ear ossicles, cochlea or vestibular system in the homozygotes. A cyst replaced the normal inner ear structures. These results demonstrate the global effect that inactivation of Tbx1 has on the development of the outer, middle and inner ear. Tbx1 is expressed in multiple interacting tissues during ear development. It is likely that it has fundamental roles in these tissues in patterning the ear. Studies are in progress to identify downstream targets of Tbx1 in the otic vesicle and surrounding mesenchyme.

279 Signalling of Otic Capsule Formation by Smads

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Growth factors of the transforming growth factor beta(TGFβ) and bone morphogenetic protein (BMP) families participate in regulation of otic capsule chondrogenesis in the developing mouse inner ear. Of central importance to understanding the mechanisms that underlie control of otic capsule formation by TGFβ and BMP during embryogenesis is understanding the signal transduction pathways through which these molecules elicit their biological effects. This study investigates the functional role of Smad proteins, i.e. downstream components of intracellular TGFβ/BMP signaling, in mechanisms of otic capsule formation by TGFβ and BMP. We demonstrate a pattern of expression of Smad2 in the developing mouse inner ear that is consistent with a function for Smads in mediating otic capsule formation. Using high density cultures of mouse periotic mesenchyme to model otic capsule chondrogenesis, we show a modulated expression of endogenous Smads in response to treatment of cultured periotic mesenchyme with exogenous TGFβ or BMP protein. Furthermore, we demonstrate that blocking of endogenous Smad3 signaling with Smad3-specific antisense oligonucleotides alters the chondrogenic response of cultured periotic mesenchyme. Our findings support a role for Smads in signaling of otic capsule chondrogenesis by members of the TGFβ superfamily in the developing capsule of the inner ear.

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280 Developmental Expression and Activity of DAN in Chick and Mouse Inner Ear Development

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Differential screening-selected gene aberrative in neuroblastoma (DAN) is a member of a recently described cystine knot protein family, including Cerberus and Gremlin. DAN is a secreted protein with tumor suppressor activity and exhibits weak BMP antagonist activity in vitro

and in vivo. Exposure to ectopic DAN protein in Xenopus animal cap assays induces anterior neural tissue and endoderm, suggesting a block in the BMP signaling cascade. Biochemical analysis has demonstrated that DAN binds directly to BMP2 and interferes with BMP4 activity, although weakly compared with other antagonists.

To determine DAN expression during chick inner ear development, sequences from the coding region of mouse DAN were used to isolate a chick homologue of DAN by RT-PCR and RACE. In situ hybridization performed on chick embryos using this chick DAN clone demonstrated DAN expression in the medial otic epithelium. DAN expression became restricted beginning at st. 23 to the endolymphatic duct and sac, the utriclar-saccular junction, and the lagena. In situ hybridization performed on embryonic mice between E8.5 and E14 demonstrated restricted DAN staining in the dorsal ectomesenchyme over the otocyst. CHO cells expressing V5-tagged mDAN were implanted into and around developing chick otocysts. Paint fill analysis of the resulting inner ears indicated that the outgrowth of the endolymphatic duct and sac were redirected toward the crux of the inner ear. Finally, DAN morpholinos were injected into the otocysts and the effects of blocking DAN translation in the otic epithelium examined.

These data suggest that DAN functions differently in mice and chickens. Specifically in the chick, the high levels of DAN expression first in the medial otic epithelium and subsequently in the endolymphatic duct and sac suggest a role for DAN in regulating and directing the outgrowth of these tissues.

281 Thyroxine Substitution Rescues Hearing in Pax 8 Mice Lacking Thyroid Hormones

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As a model for hearing deficits in congenital hypothyroidism we analyzed athyroid Pax 8 knockout mice (ko), devoid of thyroid hormone (TH). We measured hearing in ko and control mice and looked for the critical TH-sensitive period for hearing development by substituting thyroxine (T4) starting at postnatal days 1, 2, 3, 4, 6, 8 or 12. We compared auditory brainstem response (ABR) thresholds and the morphology of middle and inner ears of ko mice, T4-substituted, and control mice.

ABR thresholds were determined from averaged responses to Gauss–shaped tone pips with center frequencies of 1 to 64 kHz. One cochlea of each animal was prepared for light-, the other for scanning electron microscopy. ABR thresholds of untreated ko mice and littermate controls were determined at postnatal days 12-21. Substituted Pax8 -/- mice were treated with 20 ng/g T4 daily, initial dose 60 ng/g. Their ABR thresholds were measured at 6 weeks of age or older.

Control ABR thresholds reached adult values at postnatal day 20. Untreated athyroid mice were deaf and showed morphological signs of severely delayed development. They died within the first 3 weeks after birth, but could be rescued by T4 treatment without overt deficits, even when substitution started as late as postnatal day 12. ABR thresholds varied with the age at which T4 substitution started. Lowest thresholds were obtained with substitution from postnatal day 3 on. However, these were still 25 dB higher than control values. Malformations of tectorial membrane, IHC- and OHC-stereocilia were more pronounced in animals that received late T4 substitution. In addition, middle ear malformations that were observed, may contribute to the elevated thresholds in substituted animals.

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282 Eph receptor A4 provides repulsive signals to developing spiral ganglion neurites

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A central principle in the development of the nervous system is the guidance of axons from their origin to their target. Different molecules have been identified as being involved in this process, such as growth factors, netrins, semaphorins, slits and robos, as well as ephrins which interact with receptor thyrosine kinases of the Eph family. So far 14 different Eph receptors and 8 ephrin ligands are known to be expressed in the the nervous system and suspected to be involved in axonal guidance by repulsion. Both ephrins and Eph receptors fall into two classes, A and B. The extracellular A-class ephrins are bound to the membrane, while B-class ephrins are integral membrane proteins. Both provides bidirectional signaling when they bind to an Eph receptor. A-class ephrins appears to bind only to A-class Eph receptors and B-class ephrins only to B-class receptors, an unusual exception being the Eph A4 receptor which also binds B-class ephrins B2 and B3. Recently it has been demonstrated that EphA4 is expressed in the osseous spiral lamina of the cochlea, through which processes of spiral ganglion (SG) cells pass en route to the sensory epithelium, while ephrin B2 and B3 are expressed on SG cell dendrites (van Heumen et al. 2000).

To investigate the functional role of EphA4 in the guidance of inner ear neurons, SG explants of neonatal P4 rats were cultured for 72 hours on surfaces coated with stripes of EphA4. Control explants were cultured on stripes of FGF1 or NT3. Growth patterns of SG showed repulsion, with turning of the neurites at a distance of 30-50 mm from the EphA4 receptor stripes. In the case of FGF-1 and NT3, the neurites grew straight onto the stripes, without turning. It is concluded that EphA4 provides repulsive signals to developing SG neurites.

283 Support of Spiral Ganglion Neuron (SGN) Survival by Depolarization: Role of Ca^{2+}/Calmodulin-Dependent Protein Kinases (CaMKs) II and IV

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Using cultures of neonatal rat spiral ganglia, we have previously shown that depolarization promotes SGN survival by recruiting both CaMK and cyclic AMP prosurvival intracellular signaling pathways. Moreover, by transfecting constitutively-active truncated CaMKII(trunc) or CaMKIV(trunc) mutants into SGNs, we showed that activity of either CaMK isoform is sufficient to promote SGN survival. A remaining question is whether CaMKII, or CaMKIV, or both, are necessary for the prosurvival effect of depolarization. Here we further define the role of CaMK signaling, using reagents we have developed to specifically inhibit CaMKII and CaMKIV isoenzymes. CaMKII was inhibited by transfecting into SGNs a novel cDNA construct encoding a protein we term GFP-AIP. It consists of the autocamtide inhibitory peptide, which binds specifically and with high affinity to the CaMKII catalytic site, fused to the C-terminal of green fluorescent protein. Transfection of GFP-AIP strongly inhibited the ability of depolarization to promote SGN survival, showing that CaMKII is required for at least part of the prosurvival effect of depolarization. To specifically inhibit CaMKIV signaling, we constructed and transfected into SGNs a kinase-null mutant CaMKIV(K75E). This strongly inhibited the ability of depolarization to promote SGN survival, showing that CaMKIV is also required for at least part of the prosurvival effect of depolarization. SGNs express CaMKIIβ, which is largely cytoplasmic, and CaMKIV, which is largely nuclear. This nuclear localization suggested to us that CaMKIV promotes survival via a transcription-dependent mechanism.

Indeed, we found that co-transfection of a dominant-negative mutant of the transcription factor CREB inhibited the ability of CaMKIV to promote SGN survival, defining a pathway from membrane to nucleus for support of SGN survival by depolarization.

284 Support of Spiral Ganglion Neuron (SGN) Survival by Depolarization: Role of cAMP-Dependent Protein Kinase (PKA)

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Using cultures of neonatal rat spiral ganglia, we have shown that depolarization promotes SGN survival by recruiting both CaMK and cyclic AMP prosurvival intracellular signaling pathways, i.e., inhibition of any of these partially reduces SGN survival under depolarizing conditions. We had also shown that the permeant cAMP analog cpt-cAMP promotes SGN survival. To determine the molecular mechanism by which cAMP promotes survival, we constructed a cDNA encoding a green fluorescent protein (GFP)-tagged PKA catalytic subunit (GPKAc). Transfection of GPKAc into SGNs is sufficient to maintain their survival. PKA is typically activated in the cytoplasm but enters the nucleus after activation, allowing it to target both cytoplasmic and nuclear proteins. To determine whether it is the nuclear or cytoplasmic function of PKA that allows it to promote survival, we constructed variant forms of GPKAc that were either restricted to the nucleus by inclusion of a Nuclear Localization Signal sequence (GPKAc-nls) or restricted to the cytoplasm by inclusion of a Nuclear Export Signal sequence (GPKAc-nes). Assay of surviving and apoptotic transfected SGNs showed that cytoplasmic, but not nuclear, PKA maintained SGN survival (consistent with our previous observation that dominant-negative CREB mutants do not inhibit the prosurvival effect of cAMP). Therefore, we investigated the possibility that PKA promotes SGN survival via the cytoplasmic apoptotic regulatory protein Bad. We found that transfection of GPKAc or GPKAc-nes, but not GPKAc-nls, caused increased Bad phosphorylation in PC12 cells and that transfection of GPKAc or GPKAc-nes, but not GPKAc-nls, rescued PC12 cells and SGNs from apoptosis caused by Bad overexpression. Thus, depolarization promotes SGN survival via a nuclear CaMKIV-CREB pathway, and via cytoplasmic PKA and CaMKII signaling that, at least in part, involves Bad phosphorylation.

285 Interactive Roles of Growth Factors Define the Sequence of Migration, Process Outgrowth and Axonal Differentiation in the Cochlear Nucleus (CN) of the Mouse Embryo.

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Our previous work in the chicken embryo showed that the same growth factors can produce different actions in concert depending on the stage of development. We investigated this phenomenon in the mouse embryo by testing the interaction of FGF-2 and neurotrophins. Cochleo-vestibular anlage from the medulla of embryos (E 11,CBA/J males and C57BL/6J females) were explanted onto a Vitrogen substrate. Cultures were fed with FGF-2, BDNF and NT-3, alone or in combinations, on alternate days for 7 days. We measured the number of migrating neuroblasts and distances migrated, neurite outgrowth, fasciculation, and axon-like processes. Data, collected by taking computerized images of live cultures or after immunostaining with Tuj-1 or SV2 antibodies, were analyzed with NIH ImageJ software. The findings suggest interactive and sequential roles for the factors. Starting with neuroblast migration, FGF-2 alone had the most effect on increasing the number of migrating cells and distances migrated, but combining FGF-2 with NT-3 or BDNF inhibited this effect. Next, the initial signs of differentiation, i.e. neurite outgrowth and fasciculation (Tuj-1 stain), were differentially affected. FGF-2, BDNF, and NT-3 alone or in combinations increased neurite outgrowth compared to controls. FGF-2 combined with NT-3 produced the most robust, apparently synergistic increases in both neurite outgrowth and fasciculation, but there was no interaction between FGF-2 and BDNF. Finally, as the most differentiated feature, synaptic vesicle protein (SV2) was more abundant in NT-3 and BDNF treated cultures than in others.

Our working hypothesis is that FGF-2 alone plays a significant role in early migration, followed by a period in which BDNF and NT-3 stimulate neurite outgrowth, and NT-3, in combination with FGF-2, produces fasciculation and an increase in synaptic vesicle proteins.

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286 Substance P as a trophic factor for postnatal auditory neurons

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Although the presence of neuropeptides have been extensively studied in the cochlea, their physiological roles are currently poorly understood. Among them, enkephalins, CGRP, dynorphins, somatostatin, substance P and TRH were demonstrated in the lateral efferent nerve endings that contact the peripheral axonal projections of the type I spiral ganglion neurons. In this study, apart from the implication of these peptides in the neurotransmission of the inner ear, we have investigated other biological effects, such as trophic or co-trophic influences on auditory neurons.The expression of neuropeptide receptors was evaluated by RT-PCR of mRNA extracts prepared from postnatal day-3 (P3) spiral ganglia. Transcripts that encode for somatostatin, substance P, CGRP, TRH and opioids receptors were detected. Among these peptides, we focused our attention on the potential role of substance P on afferent auditory neurons. NK1 receptors were demonstrated on in vivo and cultured spiral ganglion neurons by immunocytochemistry. Using a fluo-3 based calcium imaging system, we showed that substance P induces an increase of the intracellular calcium concentration in spiral ganglion neurons in culture, arguing for functional NK1 receptors on these neurons. Finally, a survival-promoting effect of substance P was demonstrated by its ability to protect cultured spiral ganglion neurons from the neuronal cell death induced by serum deprivation, suggesting the implication of this peptide in the maturation of the auditory system.

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287 Beyond survival: neurotrophins are involved in directing afferents to specific endorgans

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Past research has demonstrated that survival of inner ear sensory neurons critically depends on neurotrophins. Absence of a given neurotrophin causes specific loss and some fiber reorganization. We have recently generated mice in which BDNF replaces NT3 (NT3tg BDNF). In these mice only BDNF is expressed in the ear and it is expressed both under control of its own as well NT3 promoter. Using these transgenic mice, we investigated whether the temporally delayed expression of BDNF in the basal hook of the cochlea is related to specific aspects of the hook region innervation.

We have analyzed both afferent and efferent projections of NT3 tgBDNF mice from embryonic day (ED) 13 to postnatal day 7. In each transgenic mouse we found variable numbers of afferent and efferent fibers which rerouted from the vestibular nerve branch to the posterior

crista to the basal turn of the cochlea. We do not yet know where these fibers end in the basal turn, as they disappear in the plexus of cochlear afferents.

The first afferent fibers to the posterior crista extend from the vestibular ganglion around ED 11 (Kim et al., 2001). LacZ reporter gene expression for BDNF does not show a positive signal in the basal turn hook region before ED 16 (Farinas et al., 2001). In contrast, NT-3 is prominently expressed in the hook region as early as ED11. Thus, the afferents to the posterior canal will not see any BDNF signal in control animals, but will do so in the NT3tgBDNF transgenic animals. We propose that the delayed upregulation of BDNF in the hook region provide a safety measure of ear development to prevent misinnervation from the posterior crista nerve. The evolutionary novel high frequency expansion of the cochlea in the basal turn has generated this unique proximity. This was resolved by delayed BDNF expression. A second neurotrophin, NT3, was upregulated in the basal turn of the cochlea of mammals.

288 Differential Phenotype Expression in Cell Lines from Mouse Otocyst

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Conditionally-immortalized cell lines from mouse otocyst (Immortomouse otocyst, IMO) express genes specific to the developing ear and are potential models of development. Previously, we reported simultaneous expression of mRNAs for multiple supporting cell (SC) markers, pendrin, and hair cell (HC) markers, in one clonal cell line. We have begun to test the hypothesis that this reflects, not the haphazard regulation of phenotype-specific genes, but rather the development of multiple distinct phenotypes within initially uniform cultures. Immunohistochemistry was performed on monolayers of mixed IMO and clonal IMO-2B1. Striking nonuniformity was observed in staining for Brn3.1, parvalbumin 3, myosin 7a, calretinin, and α tectorin, with only 5-20% of cells positive. SC-associated ephrin A1 and EphA5 also stained nonuniformly, but with a higher percentage of positive cells. By contrast, vimentin, which in vivo stains many auditory cell types, stained the monolayers uniformly and intensely. These data suggest that members of initially uniform IMO cell populations may be capable of differential phenotype expression in culture.

To broaden our understanding of IMO cells, we also profiled 8 other cell lines at 0d and 15d differentiation by RT-PCR. Most cell lines were similar in having abundant expression of BMP4, its receptors, and Notch-delta signaling genes jagged-1, jagged-2, HES-1, and Notch-1. Considerable differences, however, were seen among patterns for HC-specific transcription factors MATH-1 and Brn3.1, HC-associated α9 and –10 Ach receptor subunits, α-tectorin, the BMP antagonists, pendrin, and two zic genes. Of particular interest are clone 2D3, which shows considerable upregulation of both MATH-1 and Brn3.1 with differentiation, and 3A1, which downregulates α-10, Brn3.1, and MATH-1. This ongoing characterization effort will help in the selection of appropriate cell lines for future investigations in inner ear development.

289 Regulation of Integrin Expression is Associated with Neuroblast Delamination

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Development of the mammalian inner ear involves a complex series of cell-cell and cell-substratum interactions potentially mediated by adhesion molecules including the integrins. Once such event is the formation of the cochlear-vestibular ganglion (CVG). Neuronal precursors arising in the otic epithelium detach from neighbouring cells, migrate out of the epithelium and reaggregate to form the CVG. We have studied integrin expression by immunohistochemistry and flow cytometry in the murine otocyst during neuroblast delamination at embryonic day (E)10.5 and in conditionally immortal cell lines derived from the Immortomouse otocyst at E10.5. Otic epithelial cells expressed α3 and α6 integrins at E10.5. β4 was expressed at low levels at the epithelial-mesenchymal border. A proportion of cells within the developing CVG expressed α6 but delaminating neuroblasts, identified by NeuroD immunoreactivity, were α6-negative. Neither α3 nor β4 were expressed in the CVG at this stage. The cell lines E36 and N33 were derived from the ventral otocyst at E10.5 and potentially represent hair cell, support cell and neuronal precursors. Both lines expressed α3, α5, α6, αv and β1 integrins under both proliferating (33°C) and differentiating (39°C) conditions and at 33°C F-actin was predominantly organised into stress fibres. In E36 after 7 days at 39°C, F-actin was rearranged into sub-cortical structures. This was accompanied by a reduction in focal adhesions, redistribution of integrin subunits and onset of β4 integrin expression indicating an epithelial phenotype. In contrast, N33 remained predominantly β4-negative and the cytoskeleton remained rich in stress fibres indicating a less epithelial and potentially more motile phenotype. We hypothesise that integrin regulation is involved in neuroblast delamination and that N33 and E36 represent different cell phenotypes that undergo integrin and cytoskeletal rearrangements in line with migratory and non-migratory phenotypes.

290 Retinoic Acid Promotes Hair Cell Differentiation in an Inner Ear Epithelial Cell Line.

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Retinoic Acid (RA) is known to play an important role during different stages of development. In the ear, it is involved in controlling formation of gross morphological structures and it can also induce an increase in the number of hair cells and supporting cells in embryonic and neonatal explant cultures. In this work we have tested its effects on the differentiation of a conditionally immortal epithelial cell line, VOT-E36, which was derived from epithelial cells in the ventral part of the otocyst at embryonic day 10. VOT-E36 was selected for expression of the transcription factor GATA3 and it should retain the potential to differentiate into sensory hair cells, supporting cells and primary afferent neurons. It was immortalized by a temperature-sensitive variant of the T antigen (tsA58) and it can be induced to differentiate upon a temperature shift.

Cells were cultured and allowed to differentiate in a chemically defined medium (Ultraculture) supplemented with 10-5M RA. Control cultures were exposed only to the RA vehicle, dimethyl sulphoxide. Proliferation assays revealed that RA inhibited cell proliferation by about a third relative to controls. Cells were also assayed for hair cell markers by immunoblotting and immunofluorescence. In the RA-treated cultures myosin VIIa was upregulated by day 14 of differentiation but no protein was detected in the controls. The CP1 antigen, normally located within the cuticular plate during embryonic development, and SP1, normally located within the stereocilia during postnatal development, were also expressed. These results support the view that retinoic acid can induce new sensory epithelial cells in early development.

291 Inner ear sensory epithelial cells and ganglion cells have a common progenitor in vitro

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We established long-term cell culture system of fetal otocyst cells using by serum free media containing epidermal growth factor (EGF) (ARO, 2000). Here, by this culture system and limited dilution technique, several cell strains were established from embryonic day 12 (E12) otocyst cells. Progenies from one clone expressed several marker proteins of various cell types; neuronal marker neurofilament and MAPs, glial marker GFAP, epithelial markers cytokeratin and cadherin, and hair cell marker SP2. A part of them co-expressed neural and epithelial markers. These results indicate that EGF responsive fetal otocyst cells have multi-potencies to differentiate for ganglion cells and sensory epithelial cells in vitro. Inner ear sensory epithelial cells and ganglion cells may have a common progenitor in vivo.

292 Inhibition of protein kinase C induces supernumerary inner hair cells in the developing mammalian cochlea

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The determination of specific cellular phenotypes is a crucial step during the development of any organism. Precise specification of phenotype is particularly important within structures that are comprised of highly ordered cellular patterns. The sensory epithelium of the mammalian auditory organ, the cochlea, is one such example. A single row of inner hair cells and three rows of outer hair cells extend along the basal-to-apical axis of the cochlea. The intracellular signaling pathways that play a role in the development of these specific cellular phenotypes or that regulate the formation of this strict cellular pattern remain largely unknown. One factor playing crucial roles in cellular signal transduction and growth regulation is protein kinase C (PKC), a family of phospholipid-dependent serine/threonine kinases that phosphorylate a variety of target proteins. To determine whether PKC plays a role during the development of the cochlea, PKC was either inhibited or activated in embryonic mouse cochleae in vitro. PKC inhibition significantly increased the number of cells that developed as inner hair cells resulting in large regions of supernumerary inner hair cells (up to 8 rows instead of one row). To confirm that the additional cells were in fact hair cells, the level of expression of mRNA for the hair cell specific gene Math1 was quantified for control and PKC inhibited cultures by real-time quantitative PCR. Results indicated an increase in Math1 expression after 24 and 48 hrs of PKC inhibition. Furthermore, activation of PKC resulted in a regional decrease in the density of inner hair cells. These results suggest that PKC modulates the determination of inner hair cells in the cochlea, and demonstrates that the number of cells with the potential to develop as hair cells is significantly greater than the number of cells that normally develop with this fate.

293 Characterization of the Id Genes Expression in the Developing Cochlea of Rats

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Development of the hair cells involves a battery of growth factors, transcription factors, and dominant negative transcription factors. Our recent study demonstrated that the genes for inhibition of differentiation (Id, dominant negative helix-loop-helix transcription factors) are involved in this process. In this study, we characterized the expression of Id genes in the developing cochlea of rats using the techniques of molecular biology. Embryotic and postnatal rats on embryotic day 12 (E12), E14, E16, E18, and postnatal day 1 (P1), P3, P7, and P14 were used in this study for in situ hybridization (4 embryos or postnatals). Cochlear tissues of embryotic and postnatal rats were fixed in 4% paraformaldehyde, embedded in paraffin, and cut in 5-6 microns. Also, total RNA was isolated from the embryos and postnatals at the above time points, respectively, and Northern blot (3 embryos or 1 postnatal), and RT-PCR were performed. In addition, the entire otocyst on E12, E14, E16, E18, and P1 was surgically dissected, total RNA isolated, and Northern blot performed. The results demonstrated that the Id genes had two expression peaks during the development of the cochlea, one on E14 and the other on P1, which apparently correlated with the growth and differentiation of cochlear hair cells. Northern analysis on the cochlear and otocyst tissues demonstrated the same expression kinetics as that in the in situ hybridization study. The expression kinetics of the Id genes was consistent with the growth and differentiation of cochlear hair cells. We tentatively conclude that the Id genes play a role in the ontogenesis of the cochlear hair cells.

294 OCP 2 Immunoreactivity in the Human, Fetal Cochlea at Weeks 11, 17, 20; and 28

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As the two, most abundant proteins of the organ of Corti, OCP1 and OCP2 are acidic, cytosolic, low-molecular weight proteins diffusely distributed within the cytoplasm of supporting cells. Recent findings by Henzl (unpublished data) found first, that these two proteins colocalize with connexin 26 along the epithelial gap junction system and second, that OCP2 could participate with OCP1 in an OC specific SCF complex (Skp1, cul1in, and Fbp), a ubiquitin ligase complex. Previous study has also implicated OCP2 in the recycling and regulation of intracellular K+ efflux as well as pH homeostatic mechanisms. In the present study, we document the emergence and distribution features of OCP2 through various stages (weeks 11-28) of gestation in human, fetal cochleae. Four fetal cochleae, the cochleae of a normal, hearing human adult and a mature rat for positive control, were fixed in 4% formalin within two hours post mortem. Immunohistochemical studies were performed using a rabbit polyclonal antibody raised against a synthetic peptide and corresponding with amino acids 3-16. Specimens were mounted into paraffin sections. Results show that OCP2 immunoreactivity is evident at a prenatal age of 11 weeks, peaks in expression at the onset of cochlear function at 20 weeks and achieves adult-like patterns of distribution just prior to histological maturation at 28 weeks. Though this protein could be associated with the development, maturation, and electrochemical maintenance of the cochlear gap junction system, the nature of this protein's function in the developing and mature human cochlea remains unclear.

295 Regulation of Brn-3.1 gene expression

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A number of different cell-signaling pathways have been shown to influence the development of the inner ear. For example, the retinoid and thyroid receptors and signaling mediated by cyclic AMP (cAMP) have been implicated in proliferation and differentiation events in sensory epithelia. A possible mechanism for this activity is the regulation of genes involved in sensory epithelial development. Regulation of Brn-3.1, a POU-domain transcription factor that is expressed by hair cell precursors and hair cells, is required for their differentiation and survival. Using an upstream fragment from the Brn-3.1 gene driving a green fluorescent protein (GFP) reporter in transgenic mouse lines, we determined in previous studies that retinoic acid, thyroid hormone and cAMP can regulate expression of Brn-3.1.

Additional factors may also control the expression of this gene. For example, sequence analysis of the upstream fragment revealed the presence of glucocorticoid responsive elements (GRE). This suggests the possibility that Brn-3.1 activity may be modulated by steroid treatment. Environmental stimuli are another category of potential regulators. Long-term activation of hair cells by moderate levels of acoustic stimulation was found not to influence GFP expression in the transgenics. However, ototoxic stimuli such as gentamicin, which damage hair cells and eventually lead to cell death, reduces expression of GFP in transgenic hair cells. Since Brn-3.1 is required for hair cell survival, it is possible that down-regulation of Brn-3.1 plays a causative role in hair cell death.

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296 Cyclin E Expression is Restricted to Supporting Cells in the Organ of Corti of Young and Adult Mice

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Identification of supporting cell specific marker is critical to the study of supporting cell proliferation and hair cell regeneration. Previous studies reported that cytoskeletal proteins (e.g. acetylated α-tubilin, cytokeratin, & vimentin) as well as certain tyrosine kinase receptors (e.g. EphA5) could be used as supporting cell specific markers in the mammalian cochlea. Here, we report that cyclin E, a cell cycle regulatory protein, is exclusively expressed by supporting cells in the mouse organ of Corti (OC). Immunohistochemical double-staining with anti-cyclin E antibody and anti-calbindin (a hair cell marker) antibody was carried out on frozen sections of 4% paraformaldehyde-fixed cochleae from mice of the following ages: embryonic (E 18), postnatal day (P) 0, P7, P14, one month and two months. Analysis of double-stained sections was accomplished via fluorescence microscopy. A survey of cochlear frozen sections consistently demonstrated that when cyclin E is expressed in the OC, it is localized in the cytoplasm of supporting cells. No expression of cyclin E by hair cells was found. Furthermore, cyclin E immunostaining was shown to correlate with the age of the mouse. No cyclin E immunostaining was detected in OC from E18 and P0 mice. In P7 OC, cyclin E expression was observed in pillar cells, along with relatively weak staining of Deiter cells. Beginning at P14 and continuing into adulthood, cyclin E immunostaining was exhibited by the following supporting cell types: pillar cell, Deiter cell, Hensen cell and inner phalangeal cell. In comparison with untreated adult mouse OC, aminoglycoside-lesioned adult mouse OC, showed no change in the cyclin E immunostaining pattern. These data demonstrate that cyclin E is a supporting cell specific marker in the context of the organ of Corti. Consequently, this finding should prove useful in the study of supporting cell proliferation and differentiation in normal and damaged mouse organ of Corti.

297 A BAC-transgenic Mouse Model for Studying the Expression of the p27Kip1 Gene During Cochlear Development

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Previous studies from our laboratory have shown that regulation of p27Kip1, a cell cycle inhibitor, is involved in coordinating cell division and differentiation during development of the organ of Corti (Chen and Segil, 1999). p27Kip1 expression is induced in the primordial organ of Corti between E12 and E14, correlating with the time of cell cycle withdrawal of the progenitors of the hair cells and supporting cells. In the absence of p27Kip1, some of these progenitors fail to withdraw from the cell cycle in a timely way and supernumerary cells are produced.

How is the level of p27Kip1 regulated in the developing cochlea? In vitro studies have previously shown that p27Kip1 can be regulated at both transcriptional and post-transcriptional levels. To determine whether transcription plays a role in p27Kip1 regulation during development of the primordial sensory epithelium, we used homologous recombination and BAC (Bacterial Artificial Chromosome)-mediated transgenesis (Yang et al. 1997) to create a mouse harboring a GFP reporter under the control of p27Kip1 transcriptional regulatory sequences. GFP expression in histological sections of the developing inner ear of these transgenic mice was compared to the expression of endogenous p27Kip1 protein by immunolocalization. Starting at E13.5, GFP could be detected in the region of the cochlear duct corresponding to the sensory primordium and coinciding with the expression of endogenous p27Kip1protein. The results of in situ hybridization was also consistent with transcriptional control of the p27Kip1 gene. In addition, the normal down-regulation of p27Kip1 protein in differentiating hair cells was recapitulated by the p27Kip1/GFP transgene, indicating that this aspect of p27Kip1 regulation is also, at least partially, mediated at the transcriptional level. These results indicate that transcription of the p27Kip1 gene is developmentally regulated.

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298 Colocalization of KCNE1 and KCNQ1 in Gerbil and Mouse Stria Vascularis

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K^+ secretion by stria marginal cells (SMC) and vestibular dark cells (VDC) depends on the apical IsK channel that consists of two subunits, KCNQ1 (KvLQT1) and KCNE1 (IsK or minK). Knockout of either subunit abolishes K^+ secretion. The aim of this study was to identify and localize KCNE1 and KCNQ1 proteins in the inner ear of gerbils and mice (KCNE1 +/+, +/- and -/-). Crude membrane preparations of inner ear tissues and of heart were separated by SDS-PAGE and transferred onto nitrocellulose membranes for Western blotting. Cryosections were prepared from decalcified, paraformaldehyde perfused temporal bones and whole mounts of stria vascularis were prepared by microdissection and paraformaldehyde fixation. KCNE1 and KCNQ1 were detected by polyclonal rabbit and goat antibodies (ABs), respectively. Labeled proteins in Western blots were visualized by a HRP-conjugated secondary AB and luminol. Labeled proteins in cryosections and whole mounts were visualized by an Alexa-488-conjugated anti-rabbit AB and an Alexa-594-conjugated anti-goat AB. Specificity of labeling was evaluated by preabsorption of primary ABs with the peptide against which the AB was raised. Western immunoblotting of inner ear and heart tissues from gerbil and mice revealed a specific band for KCNE1 at ~10 kDa and for KCNQ1 at ~160 kDa. Confocal microscopy revealed specific apical staining of KCNE1 and KCNQ1 in SMC and VDC of gerbils and mice (+/+ and +/-). KCNE1 and KCNQ1 were colocalized in the apical membrane of nearly 100% of SMC. No specific apical staining was found for KCNE1 or KCNQ1 in SMC of -/- mice. These data demonstrate that SMC and VDC of gerbils and mice contain KCNE1 and KCNQ1 proteins in their apical membrane and that SMC are homogeneous with regard to the presence of these proteins.

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299 The Expression of KCC1 in the rat inner ear

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Introduction

K-Cl cotransporter (KCC), a member of the cation-chloride cotransporter family, mediates the electroneural, coupled transport of potassium and chloride. The KCC is also related to the potential pathways for recycling potassium used in the maintenance of inner ear elelctrochemical gradients. This study was designed to identify the expression of KCC1 in the rat inner ear by immunohistochemical staining and RT-PCR.

Methods

Reverse transcription-polymerase chain reaction (RT-PCR) was performed using rat cochlear. The forward primer was from rbKCC1 sequence, 5¡¯-ATGCCGCACTTCACCGTGGTG-3¡¯ and reverse primer was from clone ERB24, 5¡¯-TGGCTCCTAGGATATACATG-3¡¯. Paraffin embedded rat cochlear was immunohistochemically stained by polyclonal Antibody (Alpha international diagnositic Co.)

Results

In the RT-PCR, the signal was obtained in the 629 bp. KCC1 were expressed in the stria vascularis, spiral limbus, inner hair cell, outer hair cell, and the all the nerve endings), but not in the Reissner¡¯s membrane and spiral prominence.

Conclusion

This study identified the KCC1 is expressed in the rat inner ear by RT-PCR and immunohistochemical staining. This provides further evidence for their role in potassium recycling through hair cells into perilymph back to endolymph, as postulated in current models of inner ear ion homeostasis.

300 The functional anatomy of the human endolymphatic duct

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The human endolymphatic duct forms a pathway connecting the vestibule of the inner ear with the endolymphatic sac. This small epithelial tube is surrounded by a loose, richly vascularized connective tissue which seems to be important for the regulation of the inner ear fluid homeostasis. Due to the small size of the duct and the surrounding hard bone, it is difficult to perform physiological and biochemical investigations on this structure.

In the present study, a fine structual analysis using electron microscopy was made on human endolymphatic duct obtained during skull base surgery. In particular, we analysed the periductal connective tissue, including the fibroblasts and the extracellular matrix, and their connections with epithelium and blod vessels of the endolymphatic duct. We show that the fibroblasts, together with collagen fibres, form a cellular network. This network also makes direct contacts with the basal aspects of the ductal epithelium. In the periductal tissue, the network encloses glycosaminoglycans and presumably counteract their tendency to bind water and expand. It thus helps maintaining a low interstitial fluid pressure in order to make transepithelial outflow of endolymph possible.

In conclusion, considering modern concepts of connective tissue physiology and ultrastructure of the human endolymphatic duct, we suggest that this structure plays an important role for endolymph resorption. Pathological changes, such as inflammation, at this sight may play an important role for the development of endolymphatic hydrops and Ménière´s disease.

301 Epithelial Sodium Channels (ENaC) Mediate Cation Absorption By Semicircular Canal Duct Epithelium After Stimulation By Dexamethasone

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The epithelial cells of the semicircular canal duct (SCCD) form most of the boundary of the vestibular labyrinth. It was recently found that SCCD contributes to the homeostasis of vestibular endolymphatic ion composition by secretion of Cl under adrenergic control [Marcus, D.C., Lee, J.H. and Milhaud, P.G. (2001) Secretion of chloride by semicircular canal duct epithelium is stimulated by beta-adrenergic receptors. Experimental Biology 2001 Meeting, Orlando, FL]. We investigated here whether this epithelium is also capable of cation absorption in response to the glucocorticoid dexamethasone. SCCD cells were cultured to confluence on permeable supports and transepithelial voltage (VT) and resistance (RT) were measured in an Ussing chamber at 37°C. Dexamethasone (100 nM, 20 hours) caused a marked increase in VT that was reduced by blockers of the epithelial sodium channel (ENaC). Amiloride reduced VT with an IC50 of about 1 μM and benzamil was about 10 times more potent. Reduction of VT was accompanied by an increase of RT. These results are consistent with the absorption of Na via ENaC in the apical membrane of SCCD cells. It was further found that inhibition of ENaC in dexamethasone-treated epithelia (10 μM amiloride) did not prevent the usual stimulation of transepithelial current by forskolin, a stimulator of adenylyl cyclase. The results are therefore consistent with the conclusion that SCCD are capable of both cation absorption and anion secretion; the relative contributions to endolymph homeostasis of each process are under control of glucocorticoids and of adrenergic receptors coupled to intracellular cAMP.

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302 Acute ischemia causes 'dark cell' change of marginal cells in the gerbil stria vascularis

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The cochlear stria vascularis produces the endolymph and generates the endocochlear DC potential; both are indispensable for the normal function of hair cells. The marginal cell, one of the several cell types constituting the stria vascularis, is called the dark cell on the basis of its appearance by transmission electron microscopy (TEM). To clarify whether this commonly observed dark appearance is a normal characteristic of marginal cells as reported in the literature or an experimental artifact, we developed an in vivo fixation method for minimizing ischemic tissue damages. While under sustained systemic circulation with oxygenated blood, the stria vascularis of gerbils was chemically fixed by perilymphatic perfusion with a fixative, and the stria vascularis was observed by TEM. By contrast to a number of previous reports, the cytoplasm of marginal cells was not dark, and quantitative analysis showed that the difference between the cytoplasmic electron density of marginal cells and that of intermediate cells was not statistically significant. For comparison, gerbils were allowed to undergo 3 min of ischemia following decapitation. Under these conditions, marginal cells showed typical dark appearance as reported previously, and their cytoplasmic electron density was 1.7

times higher than that of the intermediate cells. In addition, the estimated volume of mitochondria in marginal cells that underwent 3-min ischemia was 1.3 times higher than that in specimens fixed in vivo.

We conclude that the widely recognized dark-cell appearance of marginal cells following conventional fixation procedures reflects cell injury due to ischemia, which is inherent in the standard fixation procedures.

303 Influence to cochlear blood flow in the topical application of prostaglandin E1

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Prostaglandin E1 (PGE1) has strong vasodilating and antiplatelet properties and is a medicine used for treatment such as spasmodic deafness or occulusive arteriosclerosis. But in case of systemic administration, there are problems to cause a fall of systemic blood pressure (BP) by the dosage speed, and it loses activity more than 60% when it passes lungs once. So we examined change of cochlea blood flow (CBF), BP and endocochleal potential (EP) by topical application of PGE1 onto the round window membrane in guinea pigs under general anesthesia. After tympanic bulla was opened by ventro-lateral approach, midle ear mucosa over the bony cochlea was removed. A laser-Doppler probe was attached to the basal turn of the cochlea to measure CBF, and a glass microelectrode was inserted into the endolymph through the round window to measure EP. In all cases, CBF began to rise within 10 minutes and reached a peak value of 150 % or more at about 60 minutes from the topical application. The peak continued about 30 minutes and declined slowly compared to the change of the rise time. This phenomenon depended on the density of PGE1. EP began to decrease within 10 minutes and reach the bottom of the value of 1/3 or less at about 60 minutes from the topical application. No significant change in BP was observed during the measurement time. Our result revealed that the topical application of PGE1 elevated CBF without significant change in BP but lowered EP.

304 Different membrane and vasomotion properties between the spiral modiolar artery and the mesenteric artery in guinea pigs

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The membrane potential of vascular smooth muscle cells (SMCs) is a major determinant of the cytosolic calcium concentration and thus the vascular tone. In small arteries, vascular tone may just be related to the degree of depolarization without involvement of action potentials [Nelson et al., 1990; Knot and Nelson, 1998]. The resting potential (RP) level varies a great deal (ranged -40 to -75 mV) among reports using different vessel preparations and recording methods [Hirst and Edwards, 1989; Nelson et al., 1990; Dietrich and Dacey, 1994; Faraci and Sobey, 1998; Welsh and Segal, 1998]. Using intracellular recording, nuclear labeling, and vessel diameter monitoring methods, we compared the membrane and vasomotion properties between guinea pig spiral modiolar (SMA) and small mesenteric (MA) arteries. We found that the recorded cells included both SMCs (n=28) and endothelial cells (ECs, n=9) in the SMA but only SMCs (n=9) in the MA. The membrane potentials of the SMCs and ECs in the SMA were not distinguishable, both or together showed a bimodal distribution peaked at -40 and -75 mV, called low and high RP, respectively [Jiang et al., 2001] but the RP in the MA displayed a single peak distribution around -70 mV. High K+ (10 mM) caused consistently a robust hyperpolarization (15-35 mV) in the low RP cells in the SMA while often a depolarization (~12 mV, n=12) and rarely a hyperpolarization (6 mV, n=1) in the MA cells which had a comparable low RP. Moreover, high K+ (10-20 mM) caused often a vasodilation in the SMA whereas a vasoconstriction in the MA. We conclude that the SMA has a unique membrane property compared to the MA, expressing a Kir channel highly sensitive to high K+, that renders the SMA a vasodilation to the elevation of interstitial potassium.

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305 The Role of Mannitol in Reducing Post-Ischemic Changes in Distortion-Production Otoacoustic Emissions (DPOAEs): A Rabbit Model

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The aim of this study was to evaluate the role of mannitol, administered topically at the round window (RW), in preventing ischemic changes in outer hair cell (OHC) function as measured by DPOAEs.

Methods: Ten 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) was measured using a laser-Doppler (LD) probe positioned at the RW niche. DPOAEs were measured at 4, 8, and 12 kHz geometric mean frequency (GMF) using 45 and 60 dB SPL primary tone stimuli. In five test ears, mannitol was administered topically at the RW for 60 minutes prior to the IAA compressions. In five control ears, the IAA compressions were undertaken without application of RW medication. Each ear underwent three 5-min IAA compressions with a 60 min rest period between compressions.

Results: In the control animals, it was observed that a progressive reduction in DPOAE amplitude followed each successive IAA compression at all 3 test frequencies. The reduction in DPOAE amplitudes was consistently greater at the higher test frequencies. In the test rabbits, the RW administration of mannitol resulted in significantly less reduction in DPOAE amplitude measures after repeated IAA compressions. For example, at 12 kHz GMF, DPOAE amplitudes in the control ears were reduced by 1.5, 4.5 and 12 dB, compared to 1, 3, and 4 dB in the mannito test ears.

Conclusions: Mannitol appears to exert a protective effect on OHC function after periods of ischemia. The RW appears to be an efficacious route for topical administration of mannitol into the inner ear.

306 The Role Of Papaverine in Prevention of Internal Auditory Artery Vasospasm

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The aim of this study was to evaluate the role of topically administered papaverine in prevention of vasospasm of the internal auditory artery (IAA) in young rabbits.

Methods: Six young (<3 months old) albino rabbits were used for this study. IAA vasospasm was induced by directly compressing the vessel. Cochlear blood flow (CBF) was measured using a laser-Doppler (LD) probe. On one side of each animal, papaverine was administered topically on the eighth nerve complex prior to IAA compressions. The contralateral side received a saline-soaked pledget. Each side underwent two IAA compressions, the first maintained for 3 min and the second for 5 min. with a 30 min rest period between compressions. In addition to LD CBF recordings, DPOAEs were measured at 4, 8, and 12 kHz GMF using 45 and 60 dB SPL primary tone stimulus.

Results: All of the saline-treated IAAs demonstrated compression-induced vasospasm based on LD CBF and DPOAE measures which did not return to baseline. Near complete recovery of CBF and DPOAEs was observed in papaverine-treated ears.

Conclusions: An animal model of IAA vasospasm was described. Mechanically-induced vasospasm of the IAA was prevented by topical pre-treatment with. These findings have clinical implications for surgical procedures such as acoustic neuroma removal.

307 Olivocochlear innervation of the mouse cochlea: immunocytochemical map and crossed vs. uncrossed contributions.

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The use of transgenic mice in auditory research has increased over the last decade. However, little is known about the cytochemical organization of the olivocochlear (OC) efferent innervation in the mouse. The purpose of the present study was to characterize the longitudinal and radial gradients of different cytochemical classes of OC terminals in the CBA/CaJ mouse, and to determine which populations arise from medial vs. lateral OC components. OC terminals in both inner (IHC) and outer hair cell (OHC) regions were quantitatively analyzed along the cochlear spiral via light-microscopic observation of cochlear whole-mounts immunostained with antibodies to GAD, VAT or CGRP. Further immunocytochemical characterization was performed in mice with chronic OC transection at the floor of the IVth ventricle in order to distinguish crossed vs. uncrossed contributions, and, indirectly, the contributions of lateral vs. medial components of the OC system. In control ears, in the OHC area, terminal distributions showed no radial gradient: mean areas were similar across all three rows for all three markers. VAT- and CGRP-positive terminals showed a similar longitudinal gradient, with a peak in roughly the 10 kHz region. GAD-positive terminals always peaked more apically (~6 kHz). In the IHC area, the distribution of terminals positive for VAT and GAD peaked near the 10 and 6 kHz regions, respectively; however, the peak of CGRP-positive terminals was more basal (20 kHz). In ears with successful midline section (3 analyzed to date), GAD-positive terminals were reduced by 2/3 in the OHC area, while the change re control in the IHC area was much smaller. These results suggest that the GABAergic innervation in the OHC area arises from the medial OC system.

308 Immunohistochemical Localization of GABA-A Alpha-1 and Alpha-3 Receptor Subunits in the Rat Cochlea

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In the mammalian cochlea, GABAergic efferent fibers of the olivocochlear bundle synapse on both the radial afferent dendrites and on the outer hair cells. Previously we obtained evidence for transcript expression of GABA-A receptor subunits in the mouse cochlea (Drescher et al., J. Neurochem. 61: 1167-1170, 1993). In the current investigation, GABA-A receptor subunits alpha-1 and alpha-3 were immunolocalized in the adult rat cochlea with polyclonal antibodies (Alomone Labs). Globus pallidus served as a positive control. For negative controls, primary antibodies were preabsorbed with the respective peptide antigens. Within the cochlea, immunoreactivity directed to the alpha-1 subunit was detected in cell bodies of type I spiral ganglion neurons in the apical and middle coils. Either very weak or no staining was detected for the alpha-3 subunit in the cell bodies of spiral ganglion neurons, although dendrites passing through the habenula perforata, en route to the inner hair cell, were immunoreactive. In the organ of Corti, staining was observed for both receptor subunits associated with predicted positions of afferent dendrites below the inner hair cell. In addition, weak immunoreactivity for alpha-1 and alpha-3 was detected in the inner hair cells. Outer hair cells exhibited immunoreactivity for the alpha-3 subunit. The presence of alpha-1 and alpha-3 subunits in the cochlea suggests the existence of benzodiazepine type I and type II pharmacology, respectively (Pritchett et al., Science 245: 1389-1392, 1989), potentially subserving lateral and medial GABAergic olivocochlear efferent pathways. The concentration of immunoreactivity for the alpha-1 subunit in spiral ganglion neurons of the apical turn, in particular, is consistent with post-synaptic receptor expression corresponding to the known pattern of GABAergic fiber density in the cochlea.

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309 Role of CGRP-Receptor Component Protein (RCP) in CGRP-Mediated Signal Transduction

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In the cochlea, CGRP receptors are found in the vasculature, where their activation causes vasodilatation. CGRP is also contained in cholinergic-containing fibers of the olivocochlear efferent system that synapse on the afferents of IHCs and onto OHCs. Co-release of CGRP with acetylcholine has been hypothesized to change the desensitization rate of the acetylcholine receptor.

We have discovered a novel protein required for signal transduction at receptors for calcitonin gene-related peptide (CGRP). This protein, named the CGRP-receptor component protein (RCP) is an intracellular peripheral membrane protein that coimmunoprecipitates with the calcitonin receptor-like receptor (CRLR) from both cell and cochlear lysates. CRLR functions as a CGRP receptor when coexpressed with an accessory protein named receptor activity modifying protein-1 (RAMP1), which affects intracellular trafficking and the pharmacologic specificity of CRLR.

RCP was originally discovered in a screen to clone the receptor for CGRP, and has no homology to other proteins in GeneBank beside itself (Luebke et al. 1996). We hypothesize that RCP is part of a complex of proteins that together constitute a functional CGRP receptor. To determine the role of RCP in cell culture, RCP was depleted from NIH3T3 cells using antisense strategy. No RCP protein was detected in three antisense cell lines, and the loss of RCP protein expression correlated with a loss of cAMP production by CGRP (Evans & Dickerson, 2000). In contrast, loss of RCP had no effect on CGRP-mediated binding; therefore RCP is not behaving as a chaperone. Instead, RCP is a novel signal transduction molecule that is required to couple CRLR to the cellular signal transduction machinery. RCP thus represents a prototype for a new class of signal transduction proteins that are required for regulation of G protein-coupled receptors.

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310 Cochlear projections of single medial olivocochlear (MOC) axons in the guinea pig.

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MOC neurons send axons from the brainstem to innervate outer hair cells of the cochlea. However, the cochlear extent and points of termination for individual MOC axons has only been determined previously for a few axons. These issues were investigated by intracellular labeling of single units with biocytin or HRP. The units were classified by their response to monaural sound: most responded only to ipsilateral sound (Ipsi units, n=21), some responded only to

contralateral sound (Contra units, n=7) and a few responded to sound in either ear (Either Ear units, n=2). Characteristic frequencies (CF) were determined from tuning curves (CF range 1.2 to 16.7 kHz).

Single labeled axons innervated from 14 to 69 outer hair cells, without clear differences in the number innervated by Ipsi, Contra, or Either Ear units. The high-CF units tended to have equal innervation of the three hair cell rows, and as CF declined, the innervation of row 3 and then row 2 outer hair cells decreased. The innervation of outer hair cells was in a patchy pattern separated by long segments of uninnervated outer hair cells along the cochlear length. The longitudinal span from the most basal to the most apical hair cell innervated showed considerable variability from axon to axon, ranging from 0.8 to 23.8% of total cochlear distance.

The midpoint of the innervation span was plotted against unit CF to form a cochlear frequency map for MOC axons. This mapping was in general agreement with the mapping for auditory-nerve fibers in the guinea pig (Tsuji and Liberman (1997) J. Comp. Neurol. 381: 188-202). Although there was some scatter in the data, somewhat more MOC axon midpoints were found apically of the auditory-nerve mapping than were found basally. The mapping was not obviously different for Ipsi, Contra, and Either Ear units.

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311 Effects of Electrical Stimulation of the Inferior Colliculus on Cochlear Mechanics in the Mustached Bat

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The auditory system of the mustached bat is sharply tuned and highly resonant to the dominant harmonic of the echolocation call at about 61 kHz. A well developed efferent system synapses on the outer hair cells with only one terminal on a single hair cell. Previous studies have shown that activity of that system, induced by contralateral sound presentation is able to alter the resonant properties of the bat's inner ear. Otoacoustic emissions (DPOAEs, DEOAEs and SFOAEs) are either suppressed or enhanced in dependence on the nature and intensity of the contralateral sonic stimulation. In the present study, cochlear microphonics (CM) were used to examine the influence of the inferior colliculus, activated by electrical stimulation, on cochlear mechanics. The CMs measured in the mustached bat have been evoked at the resonance frequency of the cochlea and show a long ringing after stimulus offset. A stimulation electrode was placed stereotacticly in the inferior colliculus, a recording electrode was placed near the cochlear aquaeduct. CM was evoked with acoustic stimuli with frequencies set within a window of 10 kHz around the individual resonance frequency and around the less prominent first and third harmonic (i.e. 30 and 90 kHz). Electrical stimulation of the IC resulted, in dependence on the position and depth of the stimulation electrode within the IC, in an enhancement of the amplitude and an upward frequency shift of the CM. These findings support previous results (Suga, N. et al, 2000 PNAS 22:11807-11814) and emphasize that in the mustached bat a strong (and maybe frequency specific) efferent control of cochlear mechanics does involve higher brain stem centers and may be crucial for echolocation tasks.

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312 Fast and Slow OlivoCochlear Efferent Effects on Basilar Membrane Motion Involve Different Mechanisms

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Inhibition of auditory nerve fiber responses by olivocochlear efferents occurs on two time scales, fast (tens of ms) and slow (tens of sec). A variety of evidence indicates that slow efferent effects are due to acetycholine-induced decreases in the somatic stiffnesses of outer hair cells (OHCs). It has been suggested that the same mechanism accounts for fast efferent effects.

To gain insight into the mechanisms involved, we measured basilar membrane (BM) motion in response to tones before, during and after bursts of efferent stimulation (e.g. 100 ms bursts of 300 shocks/s every 330ms) delivered over many tens of seconds. Efferent stimuli were applied via a bipolar electrode at the floor of the fourth ventricle in deeply anesthetized guinea-pigs (n=25). BM responses were monitored in the first cochlear turn using a displacement-sensitive laser interferometer.

For tones within ~0.5 octaves of the BM's best frequency, efferent stimulation produced effects on both fast and slow time scales. At low sound levels, both fast and slow changes involved decreases in the amplitude of basilar membrane motion. The slow effects were always accompanied by phase-lags, while the fast effects could involve either phase lags or leads, depending on the precise frequency and intensity of the tones. As a result, the phase changes associated with the fast and slow effects often occurred in opposite directions, especially for tones near and above the BM's best frequency.

The decrease in BM motion at low sound levels is consistent with the hypothesis that the gain of the cochlear amplifier is decreased during both fast and slow efferent effects. However, the differences in the direction of the induced phase changes show that different underlying mechanisms must be involved. If slow efferent effects are due to a decrease of OHC stiffness, then fast efferent effects must be due to something else.

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313 Protection From Acoustic Trauma is Not a Primary Function of the Medial Olivocochlear System

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The role played by the medial olivocochlear efferent (MOC) system in mammalian audition remains uncertain. It has been frequently suggested that the MOC system functions in a protective role by acting to reduce receptor damage during intense acoustic exposure. In this analysis, we review published reports describing the noise parameters necessary to demonstrate a role of the efferent system in protecting the cochlear hair cells from noise-induced trauma. We then survey the available literature describing the characteristics of ambient noise conditions from different natural acoustic environments. This approach allowed us to evaluate the hypothesis that naturally-occurring ambient noise may be sufficiently intense to result in permanent damage to the cochlea (a necessary condition to support the evolution of an MOC-based protective mechanism). Our survey of non-anthropogenic noise levels shows that moderate sustained abiotic (e.g., wind or water) sources of noise do occur naturally, but rarely exceed 80 dB SPL. The highest documented sustained ambient noise levels are biotic (e.g., animal choruses), and are all below ~92 dB SPL. This finding suggests

that even the most intense ambient SPLs are unlikely to cause permanent receptor damage, nor are they adequate to produce the reported MOC protection effect. Furthermore, these few, relatively intense noise environments are insufficiently distributed to account for the widespread presence of the MOC system across different mammalian species. If a protective function may be attributed to the MOC system in animals exposed to high-intensity anthropogenic noise, then this function represents an example of exaptation rather than adaptation. The near ubiquity of relatively low-level ambient noise is, however, supportive of the suggestion the MOC system functions principally as a mechanism for reducing the response of the cochlea to simultaneous "noise." This suggested role enjoys widespread experimental support.

314 The Tuning of Ipsilateral, Contralateral and Binaural Medial Efferent Reflexes in Humans

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One of the fundamental, but little studied, properties of the human olivocochlear efferent reflexes is their frequency tuning. Data are available describing tuning properties of the contralateral reflex, but there is almost no information about the tuning of the ipsilateral and binaural reflexes.

We studied the tuning of medial efferent reflexes using noise bands of various widths centered at frequencies below, at, or above the test frequency. Efferent effects were monitored in both ears simultaneously via the changes evoked in stimulus frequency otoacoustic emissions (SFOAEs). The SFOAEs were produced by a single 40 dB SPL tone in each ear. The SFOAE method for monitoring efferent activation has the advantage that the test tone elicits little or no efferent activity by itself.

Our preliminary results show that binaural stimulation was almost always the most effective elicitor of efferent activity no matter what bandwidth or center frequency was used. Whether ipsilateral or contralateral noise elicited a bigger change in the SFOAE varied with the band of noise used, the test frequency and the subject. Noise bands centered near the test frequency were generally more effective than distant bands, but the most effective band was not always centered at the test frequency and the tuning pattern was sometimes asymmetric. In some cases, noise bands centered more than two octaves above and/or below the test frequency elicited changes in the SFOAE. Thus, the tuning of efferent activation appears to be more broad band than is generally appreciated. The results provide a more complete picture of the sounds that activate medial-efferent reflexes in humans and help us to better understand the role of efferents in hearing.

315 Influence of Click-Evoked Otoacoustic Emission Amplitude and Linear vs. Non-Linear Mode on the Efferent Effect in Humans

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The aims of this study were: (a) to evaluate the influence of the initial TEOAE amplitude on the efferent effect, (b) to compare the contralateral acoustic stimulation (CS) effect in the left and right ear, and (c) to evaluate differences between efferent effects during linear and non-linear ipsilateral click stimulation. Methods: Twenty-two healthy adults were tested bilaterally (N=44). The TEOAEs were recorded and analyzed according to the method of Bray and Kemp (1987) using the Otodynamics ILO88 system. Linear and non-linear TEOAEs were recorded from both ears using click stimuli at 70 dB SPL without and with CS. The contralateral suppressor was a broadband noise (BBN) presented at 65 dB SPL. Statistical analyses of the group data were performed using Pearson correlation and Student's *t*-tests. The CS influence on TEOAE results was analyzed in various options. Initially, the TEOAE amplitudes (Response) were analyzed. Then, off-line analysis of the amplitudes (SNR) from 1 kHz-frequency bandwidth (WFB) centered at 1; 2; 3; 4; and 5 kHz were assessed. Also the half-octave (HOFB) analysis was performed. Results: No significant differences between contralateral effects in the left and right ears were found. Also *t*-test did not show significant differences of the efferent effect results obtained for linear and non-liner ipsilateral click stimuli. There was a significant positive correlation between TEOAE amplitude and suppression. The amount of the suppression effect increased with higher initial TEAOE amplitude. The strongest correlation was observed in the mid-frequency region for non-linear mode (1.0 WFB: 1 kHz r=0.64 *p=0.001*; 2 kHz r=0.44 *p=0.039*; HOFB: 1.5 kHz r=0.41 *p=0.01*; 2 kHz r=0.34 *p=0.04*). To conclude, the results of this study show significantly less suppression effect resulting from contralateral BBN in ears with weaker initial TEAOE amplitudes, especially in the mid-frequency region of the non-linear emission. Moreover, the presented findings suggest no lateralization of the efferent effect and no significant influence of the ipsilateral click stimuli (linear, non-linear) on the amount of the efferent effect.

316 Inter Subject Variability of Acoustic Emissions and its Relation to Noise-Induced Hearing Loss: Experiment and Model

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To study the correlation between noise-induced hearing loss and Transient Evoked Oto–Acoustic Emission (TEOAE), we have measured the audiogram and TEOAE of 50 combat soldiers during 2 years of service every 3-6 month. We have found a significant difference in the probability density functions (pdf) of TEAOE energy obtained prior the service of the soldiers who developed threshold shift (Group A) to those whose threshold were not effected (Group B). A Gaussian distribution for Group A, and a uniform distribution for Group B. For each TEAOE level a sensitivity index is defined as the ratio of the two density functions. Our results suggest that soldiers with low TEAOE levels are less sensitive to noise exposure than soldiers with moderate TEOAE levels. A cochlear model with embedded outer hair cells (OHC) was developed to explain the experimental results. The model is solved using numerical methods in the time and in the frequency domain. The frequency domain solution is compared to the WKB approximation. The model predicts the correlation between TEOAE levels and auditory thresholds.

317 Click-evoked otoacoustic emissions and click intensity: consequences of an unexpected phase shift

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The changes in level of the spectral components of click-evoked otoacoustic emissions (CEOAEs) against click stimulus are well-documented: their slope < 1 dB / dB allows the so-called nonlinear method of Kemp and Bray to be the most reliable one for separating genuine CEOAEs from stimulus artifacts. The phase dependence of CEOAEs with stimulus intensity is barely acknowledged. The present work used CEOAEs from 20 normal ears recorded in response to 50 to 86 dB p.eq.SPL clicks in 6 dB steps. We show that CEOAE phases varied much across intensity (from 30° to > 120°), mostly in a monotonic manner and in such a way that in a majority of ears, phase lagged with increasing click intensity. Synchronized spontaneous OAEs behaved the same when present. In a few instances, conspicuous frequency shifts of CEOAE spectral peaks were seen. In contrast to CEOAE phases, envelopes were almost intensity-invariant, and in rare instances when they changed, they led while the phases lagged. This

behavior is totally at odds with that of basilar membrane movements close to CF recently disclosed by Recio-Rhode, Lin-Guinan and Nuttall-de Boer, i.e. no phase shift and large envelope shift. Shera (2001) proved that it implies that whatever they do, outer hair cells cannot alter the resonance frequency of the cochlear partition. If one elaborates along this line of reasoning, the large phase shift of CEOAEs with click intensity implies that CEOAEs at frequency f cannot come from the place tuned to f and that instead, as suggested by Yates and colleagues, they could be intermodulation distortion products. Furthermore, the location of the source of spontaneous emissions at frequency f is questionable as well since their synchronized avatar behaves like CEOAEs and unlike the basilar membrane at the place tuned to f.

318 Multiple Sources of Electrically Evoked Otoacoustic Emissions

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It has been speculated that the energy of the electrically evoked otoacoustic emissions (EEOAEs) is generated at the site on basilar membrane (BM) near the stimulating electrode and has multiple components. From the original site, the energy propagates towards the oval window and gives rise to the short delay component (SDC); meanwhile, it propagates towards and from its characteristic frequency (CF) location, forming the long delay component (LDC). This hypothesis was tested by using an acoustical swept tone to modulate the EEOAEs generated by alternating electric current delivered to the round window niche in gerbils. When using a fixed EEOAE frequency, the swept acoustic tone induced a slight suppression on EEOAE near its emission frequency (f_e), a significant decrease around the frequency of *y* ($y=1.2f_e+3$, *kHz*), and an enhanced peak between the two depression valleys. When an acoustic tone and electric current were presented simultaneously and swept together with a constant ±1/16, ±1/8, and ±1/4 octave offset, the LDC was suppressed. The suppressive effect on the LDC was inversely related to the frequency separation between the acoustic and current stimuli, and proportional to the suppressor level. Since suppression results from overlap of traveling waves evoked by the acoustical and electrical stimuli, the suppression patterns observed in this study revealed that (1) the original generation sites of both the SDC and the LDC were from the cochlear partition near the stimulating electrode, (2) the LDC reflected from the CF place on the BM, and (3) the peak between f_e and *y* may be a result of the acoustically induced change in reflection patterns of electrically evoked traveling wave in the cochlea.

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319 Repeated Wideband Measurement of Otoacoustic Emissions in Gerbils

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Otoacoustic emissions are useful indicators of sensorineural hearing loss in both humans and laboratory animals. In studies of recovery from exposure to noise and other ototraumatic agents, it is desirable to record emissions repeatedly over days or weeks. We have developed a system for measuring otoacoustic emissions in Mongolian Gerbils, using a coupler-based acoustic calibration. A modified infant otoscope speculum is secured by a miniature gooseneck and positioned against the opening to the bony meatus of adult Mongolian gerbils lightly anesthetized with ketamine and xylazine. The animals are placed on a heated gel pad to help maintain normal body temperature. An Etymotic Research ER-10B+ emission probe is then secured into the speculum. Stimuli are delivered from two modified Radio Shack Super Tweeters (#1310B). Stimuli are generated and emissions measured using Emav (Neely and Liu (1993), Tech. Memo No. 17, BTNRH, Omaha). The stimulus levels are calibrated using a coupler incorporating the lateral wall of a gerbil temporal bone. A speculum similar to the one used in the experiments is sealed to the opening of the external auditory meatus and a B&K 1/8" condenser microphone is used to measure the sound levels at the former position of the eardrum and in a plane parallel to the tympanic ring. These coupler measurements of the driver responses are then used to calculate the voltages that will produce the desired eardrum stimulus levels. The frequency response of the ER-10B+ microphone is calibrated using the B&K 1/8" microphone to allow post-hoc correction of the measured stimulus and emission levels. When care is taken to position the speculum correctly, emissions may be measured repeatedly with stimulus frequencies as high as 40 kHz.

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320 Suppression of Stimulus Frequency Emissions by Tones

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Stimulus frequency otoacoustic emissions (SFOAE) measured in human subjects demonstrate highly irregular but reproducible levels as a function of frequency (Siegel, et al. (2001), ARO Abst.*24*:13). This observation led us to suggest that the emission is generated over a region of the cochlea with sources that interact with variable phase and magnitude. We tested this hypothesis by examining the suppression of SFOAE by a second tone. The emission was evoked by a probe tone at either 2, 4 or 8 kHz at 30 dB SPL. The suppressor tone was presented at 15-20 dB greater than the level of the probe tone and was varied in frequency increments of 21.5 Hz, starting just above the frequency of the probe tone. The residual was calculated by vector subtracting the response at the probe frequency for the probe presented alone from that of a second measurement in which the probe and suppressor were presented together. When the probe and suppressor frequencies were similar, the residual reached a plateau, indicating complete suppression of the SFOAE evoked by the probe tone. As the suppressor frequency was increased, the level of the residual decreased monotonically until it was indistinguishable from the noise floor.

We hypothesize that the suppression pattern represents (as the suppressor is varied from high to low frequency) the progressive removal of emission sources from the most basal part of the generation region to those located closer to the place of the probe frequency. If this is true, then the slope of the suppression level and phase curves (calculated point-to-point as a derivative) should be related directly to the spatial distribution of SFOAE sources. The slope of the level of the suppression curves showed highly irregular variation with suppressor frequency. The slope of the phase of the suppression curves showed large, nonmonotonic changes with changing suppressor frequency. This pattern is consistent with our hypothesis that stimulus frequency emissions result from a spatially distributed array of intracochlear generators that interact with a high degree of level and phase variation.

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321 BAPTA reduces the frequency of spontaneous otoacoustic emissions in lizards

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We have demonstrated that otoacoustic emissions in the Australian bobtail lizard are generated by an active mechanism integral to the hair-cell bundle (Manley, G.A., Kirk, D.L., Köppl, C., Yates, G.K., 2001, Proc. Nat. Acad. Sci. (USA) 98, 2826-2831). Several different mechanisms, each involving calcium, have been suggested as possible active processes driving hair-cell bundles (Hudspeth, A.J., 1997 Curr. Opin. Neurobiol. 7:480-486). Here, we have attempted to change the

level of calcium in the endolymph in vivo, while monitoring spontaneous otoacoustic emissions (SOAE). We iontophoresed the highly-specific calcium chelator BAPTA into the Scala media of anaesthetised Bobtail lizards. Since DC currents themselves affect SOAE directly (Manley and Kirk, JARO, in press), we compared the effects of control current applied through pipettes filled only with KCl.

Iontophoretic injection of BAPTA to an estimated peak level of 5mM in the endolymph resulted in a large and prolonged downward shift in the frequency of individual SOAE spectral peaks. Recovery of the SOAE spectra took more than one hour, presumably due to slow clearance of BAPTA from Scala media.

Since the availability of calcium ions would affect more than one potential active process in the same way (e.g. both Myosin-based motors and calcium binding and unbinding at transduction channels would be slowed down by reduction in calcium levels), these data do not select between these hypotheses. They do, however, implicate calcium ions in the generation of active motility in non-mammals.

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322 Effect Of Stereociliary Mechano-Channel Block on the Electrically Evoked Otoacoustic Emission

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We have hypothesized that the electrically evoked otoacoustic emission (EEOAE), from stimulation at the round window (RW), does not involve current flow through stereociliary mechanical channels (MC) (Nuttall and Ren, Hearing Res. 92, 1995, 170-177). To test this hypothesis d-tubocurarine (curare) was perfused into scala media (SM) to block MC and the change in the EEOAE was measured. Guinea pigs were anesthetized and a wire electode was placed on the round window to record cochlear compound action potentials (CAP) and cochlear microphonic (CM) and deliver electrical current. An Etymotic 10B+ microphone was use to measure the EEOAE. A glass pipette electrode with 9 μm tip dia. was held with a micomanipulator and advanced via the RW into the SM. Endocochlear potential was recorded from the pipette electrode. Artificial endolymph (AE) or AE containing curare (50-100 μM) was perfused from the pipette through the SM to an exit hole at the fourth turn. The EEOAE spectrum was recorded for current delivered to the RW (from the wire electrode) or into the scala media (SM) from the glass pipette electrode. The current level was approx. 35 μA and its frequency was swept from 0.4 to 40 kHz. Following curare perfusion we observed greatly reduced the CM and CAP consistent with significant MC block. Curare reduced the mean spectral level of the EEOAE from RW stimulation but for scala media current injections the EEOAE was little changed or increased. The effect of channel block on the EEOAE from RW stimulation could occur from two possibilities: 1) A decrease of applied current passing through MC and/or 2) A decrease of standing current from SM that modifies the outer hair cell (OHC) motility responsible for the EEOAE. The mechanism of the lack of effect of curare on SM evoked EEOAE will be discussed.

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323 Reflection emissions in the guinea pig

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A two-source interference model (Heitmann et al., 1998; Talmadge et al., 1998, 1999) has been used to explain quasi-periodic amplitude variation in distortion product otoacoustic emissions (DPOAEs) in humans, referred to as fine structure. Phase interactions of the energy from the two sources (f2 and CFdp) leads to amplitude and phase variation in the composite signal measured in the ear canal. Little attention has been paid to the origin of the DPOAE in rodents, although it has been reported that amplitude fine structure is evident in the chinchilla and the kangaroo rat (Long et al, 1999). There is however data that would not support two sources of DPOAE in the guinea pig (Withnell & Yates, 1998). This study investigated the extent to which the two-source interference model can be applied to the albino guinea pig using a variety of methods (suppression, input-output functions, and high resolution amplitude and phase data) to resolve the sources that underlie DPOAE production.

324 'Open-Canal' Measurements of Click-Evoked Otoacoustic Emissions

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We report here for the first time measurements of click-evoked otoacoustic emissions (CEOAEs) in human ears recorded with a physically open ear canal. This work was inspired by the report of Withnell, Kirk and Yates (1998), who described such recordings from the guinea pig ear and further reported responses with energy at much higher frequencies than are traditionally observed in CEOAEs. Our aims were to determine whether it is possible to measure human CEOAEs using an open-canal technique; to compare any such recordings with those obtained in the same ears using the traditional, closed-canal technique; and to determine whether significantly higher-frequency CEOAEs can be measured in humans with this technique. CEOAEs were recorded at click rates up to 5000/s, using the maximum length sequence technique, in order to improve the signal to noise ratio of the recordings and to provide a ready means of assessing the physiological origin of the signals.

Clear indication of CEOAEs in the open canal recordings were obtained in approximately half the 12 normal ears tested, with borderline/questionable CEOAEs in the others. The responses obtained from open canals had characteristics broadly similar to conventional closed-canal CEOAEs, albeit significantly lower amplitudes and higher noise levels. (This was the main reason why clear CEOAEs were not recorded in all normal ears.) However, the key difference between the recordings was the presence of significantly greater high-frequency energy in the open-canal recordings, as has been reported in the guinea pig.

This new development in recording techniques for human CEOAEs bears significant promise as a means for assessing cochlear function at frequencies above the ~ 4 kHz limit that typically applies to conventional closed-canal techniques.

325 The amount of audiogram fine-structure as a function of hearing threshold.

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Many normal ears exhibit a characteristic fine-structure in their audiograms related to otoacoustic emissions. The presence or absence of otoacoustic emissions (OAEs), and by implication of audiogram fine-structure, may be regarded as an indication of the state of cochlear functioning.

We investigated the amount of fine-structure in two groups of subjects: A group of 14 normal hearing subjects and a group of 21 patients with Meniere's disease. The patient data involved 39 ears with hearing thresholds varying from about 0 to 70 dB HL.

We measured the fine-structure in the frequency range from 500 to 3500 Hz. The hearing loss FI was expressed as the average loss at 0.5, 1, 2, and 3.5 kHz. The fine-structure was characterized by means of the number of sensitivity peaks Np, the average peak height Hp, and the accumulated peak height Sp=Np.Hp.

In the group of normal hearing subjects we found a variation from little fine-structure to strong fine-structure (Sp>200 dB). In the group of patients as a whole we also found strong spread of the amount of fine-structure. The present data indicate that at each degree of hearing loss a continuum of values for Sp can be found, ranging from almost no fine-strucure to an upper limit. The upper limit depends on the degree of hearing loss. In the range of 40 to 70 dB hearing loss, fine-structure was small (upper limit of 50 dB). For smaller hearing losses the upper limit for Sp increased from 50 dB at a hearing loss of 40 dB to Sp more than 200 dB for FI=0 dB.

All data taken together, we found a negative correlation between hearing loss and strength of fine-structure expressed as Sp, and to a lesser degree we found a negative correlation between hearing loss and the number of peaks Np and the average peak height Hp.

Also Np and Hp are correlated, i.e. the more peaks the higher the average peak height. Thus, strong fine-structure is expressed in the number of peaks as well as in the height of the peaks

326 Do spontaneous otoacoustic emissions alter tuning curves derived from ipsilateral suppression of transient-evoked otoacoustic emissions?

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The presence or absence of spontaneous OAE (SOAEs) may influence frequency selectivity characteristics of otoacoustic emission suppression tuning curves (OAE STC). Previous work from this lab has suggested that presence of SOAEs may determine the relationship between width of the tuning curve (Q10) and psychophysical threshold (Zettner, 1999). An adequate explanation for this result is lacking although we know that SOAEs can influence evoked OAE amplitude and spectrum in a given ear and that a relationship exists between threshold microstructure and the frequency location of SOAEs. A better understanding of factors that contribute to these measures is necessary in order to interpret the results of STCs with more confidence.

The purpose of this study was to compare OAE STCs from ears with SOAEs to ears without SOAEs. Toneburst-evoked OAEs at 4000 Hz were suppressed using puretones presented ipsilaterally. The suppressor level that reduced the OAE amplitude by -6 dB was plotted as a function of suppressor frequency. Q10 values for ears with SOAEs were compared to ears without measurable SOAEs as a function psychoacoustic threshold at 4000 Hz. Presence of SOAEs in general was not found to relate to tuning curve width as previously suggested (Zettner, 1999). However, significantly greater Q10 values were measured from ears with SOAEs within 100 Hz of 4000 Hz (n=5) compared to ears with SOAEs at other frequencies (n=37) as well as ears without SOAEs (n=36). The same relationship has been found between psychophysical tuning curves and SOAEs, (Bright, 1985; Michey and Collet, 1994). The current study lends support to a peripheral mechanism for this enhancement of frequency selectivity around SOAEs.

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327 Transient-Evoked Otoacoustic Emissions: Effects of Stimulus Level

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Several studies have determined the transient-evoked otoacoustic emission (TEOAE) response parameters that best separate ears with normal hearing from ears with hearing loss (Gorga et al., 1993; Prieve et al., 1993; Hurley and Musiek, 1994; Hussain et al., 1998; Harrison and Norton, 1999). With one exception (Harrison and Norton, 1999), data were obtained using a single stimulus level of 80 dB pSPL. Although now in common clinical use, there is a paucity of data to indicate that this stimulus level achieves the most accurate classification of normal-hearing versus hearing-impaired ears.

Click-evoked otoacoustic emission (CEOAE) input-output functions were obtained from 434 subjects at stimulus levels from 60-85 dB pSPL in 5-dB steps. CEAOE amplitudes and signal-to-noise ratios were parsed into 1/2-octave bands centered at 1000, 2000, 3000, and 4000 Hz and compared to an audiometric "gold standard". Clinical decision-theory analyses were used to construct relative operating characteristic (ROC) curves and to determine the area under these curves for each stimulus level at each 1/2-octave band.

328 Acoustic Modulation of Electrically Evoked Otoacoustic Emission in Chickens

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Electrically evoked otoacoustic emissions (EEOAE) can be elicited from the chicken inner ear [Chen et al., accepted]. Since lesion studies implicate hair cells as the source of avian EEOAEs, we hypothesized that acoustic stimuli would modulate EEOAE amplitude at cochlear locations where the acoustic and electrical stimuli overlap. To assess this interaction, EEOAEs were measured as the frequency and amplitude of the acoustic stimuli was varied. EEOAEs evoked by AC current (3-250 μA rms) delivered to round window had a broad bandpass response (1-6 kHz) with a peak between 3-4 kHz and maximum amplitude of 27 dB SPL. EEOAE suppression/enhancement tuning curves were measured at 2, 3, 4 and 6 kHz by varying the frequency of a 70 dB SPL tone and measuring the change in EEOAE amplitude. EEOAE tuning curves were characterized by a tip; a narrow range of frequencies where EEOAE amplitude was suppressed by as much as 5 dB, and by sidebands, a range of frequencies above and below the tip where EEOAE amplitude was enhanced by as much as 1.5 dB. The best suppression frequency, or characteristic frequency (CF), was close to the frequency of the EEOAE elicited by the 3 or 4 kHz electric stimulus. However, the CF was displaced toward higher frequencies for the 2 kHz electric stimulus and toward lower frequencies for the 6 kHz electric stimulus. EEOAE suppression increased approximately linearly with acoustic level. These results suggest that EEOAEs evoked by round window stimulation are predominantly generated by hair cells near the 3-4 kHz region of the cochlea.

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329 Retrieving Cochlear Phase Characteristics From High-Frequency Auditory Nerve Fibers

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Cochlear phase characteristics can be studied with mechanical measurements and, for low frequencies, via the phase-locked response of auditory nerve (AN) fibers. Due to the absence of phase-locking at high frequencies (HF, above 5 kHz) one cannot determine cochlear phase characteristics from AN responses to tones. A more sophisticated method like Reverse Correlation cannot be used for obtaining HF phase information either. Consequently, for frequencies > 5 kHz, phase data are only available from mechanical measurements. Such measurements are limited to the basal turn, and they are invasive to the cochlea and middle ear.

In an indirect way, however, cochlear phase and amplitude characteristics are revealed via the interaction of the different stimulus components. We developed a novel method which enables the extraction of cochlear phase and amplitude characteristics at high frequencies, based on an analysis of the envelope fluctuations of the response.

We obtained single-unit recordings from the AN of the cat and guinea pig and were able to measure, within the same animal, the phase characteristics of AN fibers of many different characteristic frequencies,

up to 25 kHz. The results show sharp tuning, latencies that grow with frequency, and frequency glides in the impulse response. We did not find any HF phase plateaus. These observations constitute the first data on cochlear phase at high frequencies, obtained from the intact cochlea, and suggest that auditory processing at the mid-frequency region of the cochlea does not differ markedly from that at the basal region.

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330 Frequency Selectivity of Auditory-Nerve Fibers Studied with Band-Reject Noise

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The notched-noise method (Patterson, J. Acoust Soc. Am. 59:640-654) is widely used for estimating auditory filters in humans and other species based on psychophysical masking data. To test the physiological validity of this technique, we recorded from auditory-nerve fibers in anesthetized cats using the same stimulus paradigms as in psychophysics. Neural auditory filters derived by the notched-noise method were compared with pure-tone tuning curves measured in the same fibers.

Stimuli were pure tones in band-reject noise, with rejection bands placed both symmetrically and asymmetrically around the tone frequency. The tone was always near the fiber's characteristic frequency (CF), 10-20 dB above threshold. For each notch width, we determined the threshold noise level which just masked the increment in average rate produced by the tone. Auditory filter models were fit to neural threshold curves by assuming that, for each fiber, threshold corresponds to a constant signal-to-masker ratio at the filter output.

Patterson's rounded exponential function gave good fits (0.5-4 dB rms error) to neural masked thresholds throughout the 0.2-23 kHz range of CFs. Models with a low-frequency tail produced the best fits for high-CF (> 3 kHz) fibers, while models without a tail sufficed for low-CF fibers. Both the center frequencies and the bandwidths of the fitted filters were, on the average, consistent with comparable measures for pure-tone tuning curves. However, for some fibers, the model filters were more symmetric than the tuning curves around their center frequency.

Our results indicate that the notched-noise method is applicable to auditory-nerve fibers, and gives estimates of frequency selectivity broadly consistent with pure-tone tuning curves. This quantitative description of auditory filters for individual fibers will allow us to devise neural population models for testing whether psychophysical auditory filters match the corresponding neural filters.

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331 A baseline characterization of activity growth in auditory-nerve fibers of normal-hearing cats

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While it is commonly assumed that loudness is related to the overall neural activity produced by a stimulus, the nature of this relationship in normal and impaired ears is still unclear. Physiological experiments were designed to characterize the population growth of activity with increasing sound level for several stimuli that are commonly used in psychophysical studies of loudness. These results from the auditory nerve (AN) of normal-hearing cats represent a baseline for comparison in future studies of activity growth in the AN and central nervous system of acoustically traumatized cats.

Rate-level functions (RLFs) were measured in 1-dB steps for seven stimuli for AN fibers with best frequencies (BFs) up to 10 kHz. Tones of 1 and 2 kHz represent frequencies at the corner of and within, respectively, the high-frequency hearing loss to be studied in future experiments. RLFs for broadband noise and a brief speech token (/besh/) characterize response growth for complex stimuli. RLFs for 1- and 2-kHz tones were also measured in the presence of a fixed-level, band-limited (1.8-3.0 kHz) noise masker, which was similar to noise maskers that have been used psychophysically to simulate hearing loss in normal-hearing subjects.

Response growth for tones was steeper for BFs above than below the tone frequency. The dynamic range for noise was generally greater than for BF tones, with the largest difference for medium-spontaneous-rate fibers for which BF-tone RLFs were steep and noise RLFs were shallow. RLFs for speech had wider dynamic ranges than for tones or noise and showed less evidence of high-level saturation. Decreasing RLFs were observed for the masked 1-kHz tone for BFs within the adjacent noise band due to suppression. A thorough characterization of peripheral activity growth in normal and impaired cats for simple and complex sounds will be useful for developing hearing-aid algorithms that overcome the effects of loudness recruitment.

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332 Responses to cochlear normalized speech stimuli in the auditory nerve of cat

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Previous studies of auditory nerve fiber (ANF) representation of vowels in cats and rodents have shown that, at typical values of conversational speech (60-70 dB), neuronal firing rate alone provides a poor representation of spectral prominences (formants) of speech sounds. Although it has been shown that in the cochlear nucleus, to which ANFs project, better rate-based representation by chopper neurons is possible, there are reasons to suspect that ANF rate representations may not be as inadequate as they appear. Here, we investigate whether some of the apparent inadequacy of ANF rate representations owes to the mismatch between animal cochlear characteristics and human speech sounds. For all animal models tested in earlier studies, basilar membrane length is shorter and encompasses a broader range of frequencies relative to humans. In this study, a customized speech synthesizer was used to create a rendition of the vowel [ɛ] with formant spacing and bandwidths that fit the cat cochlea in proportionately the same manner as would occur in the human cochlea. Recordings of cat ANFs in response to the cochlear normalized [ɛ] demonstrate that, at the level of AN, rate-based encoding of vowel sounds is possible even at 70-80 dB SPL. When appropriate adjustments are made to calibrate the speech signal to fit the animal cochlea as it would fit the human cochlea, rate encoding in AN appears more informative than was previously thought.

333 Comparisons of Rate-Place, ALSR, and ALIR Analyses of Auditory Nerve Responses to Naturally-Produced Normally-Voiced and Whispered Vowels

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The responses of auditory nerve fibers to synthesized, normally-voiced vowels have been shown to contain rate-place codes that correspond to the formant structures of the vowels at low intensities. The dynamic range of this type of frequency code has been shown to be limited. Synthesized vowel frequency content at higher intensities is better represented by a synchronization-place code, like the ALSR (Young

and Sachs, 1979). Whispered vowels lack much of the frequency information of normally-voiced vowels; most notably absent are the lack of the fundamental frequency produced by normal glottal pulsing and the prominent spectral peaks. Despite this absence of glottal pulsing as a source of excitation for the resonant frequencies of the vocal tract, whispered vowels are perceived with high rates of accuracy. An average localized interval rate (ALIR) analysis has been calculated from Fourier transforms of the auditory nerve fiber interval histograms (Voigt et al., 1982). The ALIR was shown to contain the spectral features of a synthesized whispered vowel, generated by exciting a vocal tract model with broadband noise.

This study examined how well these three analyses represented spectral features of naturally-produced vowel sounds. Single unit responses to spoken normally-voiced and whispered vowels were recorded from auditory nerve fibers in ketamine-anesthetized chinchillas. The spectral content of four examples of the vowel /a/ could be demonstrated by rate-place and synchronization-place responses of a population of auditory nerve fibers. Vowel formant structure was salient in rate-place codes, but synchronization-place responses (ALSR) encoded more details of the spectra. Spectral representations were present in both codes across 40 dB of vowel presentation. ALIR plots did not as accurately display spectral features or formant frequencies of the naturally-produced whispered or normally-voiced vowels.

334 Seismic and Auditory Sensitivity in the Bullfrog Auditory Nerve: Cross-modal Interactions

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The amphibian papilla (AP), an inner ear endorgan in the frog, subserves the detection of low-frequency airborne sound. The AP is also extremely sensitive to substrate vibration. Spike rate thresholds in AP fibers exhibit acceleration sensitivities as low as 0.01 cm/s2 in the Puerto Rican white-lipped frog (*Leptodactylus albilabris*) and 0.02 cm/s2 in the European grassfrog (*Rana temporaria*). Even greater seismic sensitivity is demonstrated if the synchronization of spikes in eighth nerve fibers is used as the threshold criterion: seismic thresholds as low as 0.001 cm/s2 have been shown in *R. catesbeiana*. AP fibers in all frogs recorded from to date exhibit comparable seismic sensitivities. Because of the sensitivity of these fibers to both airborne sound and substrate vibration we wondered if there would be interactions between these two modalities. We use white-noise analysis techniques to ask whether the neural encoding of airborne sound is affected by the presence of a seismic signal. In particular we use the second-order Wiener kernel to capture cross-modal interactions. Our results suggest that information about an airborne sound transmitted by AP fibers is modulated by the presence of substrate vibrations.

335 A Comparative Study of Avian Auditory Evoked Responses (AERs): Relationships with Phylogeny and Vocal Complexity, and Seasonal Effects

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Physiological measures of hearing were collected in six avian species differing in genetic relationship and vocal complexity. Auditory Brainstem Responses (ABRs) to broadband click stimuli and Cochlear Microphonic (CM) responses and Frequency-Following Responses (FFRs) to pure-tone stimuli were collected from anesthetized downy woodpeckers, Carolina chickadees, tufted titmice, white-breasted nuthatches, house sparrows, and European starlings. If AER patterns are influenced by phylogenetic relationship, we predicted that woodpeckers, sparrows, and starlings would be outliers relative to the other species. Woodpeckers are in a different Order (Piciformes) than the other five species (Passeriformes) and, within the Passeriformes, sparrows and starlings are in different Superfamilies than the nuthatches, chickadees, and titmice. However, nuthatches and woodpeckers have the simplest vocal repertoires at the lowest frequencies of these six species. Consequently, if AERs correlate with vocal complexity, we would predict nuthatches and woodpeckers to be outliers relative to the other four species. Our results indicate that AER measures in the spring broadly correlated with vocal complexity and, in some cases, phylogeny. However, these AER patterns shift from spring to winter due to species-specific seasonal changes. Whereas most species showed smaller amplitude responses in spring compared to winter months, nuthatches and woodpeckers showed greater winter responses, possibly suggesting an effect of vocal complexity. Comparative studies of auditory evoked responses may inform theories on vocal complexity and learning. We found significant seasonal changes at the auditory periphery, and possibly at the basilar membrane, which suggest perhaps even greater plasticity in avian auditory neurophysiology than might have been expected.

336 Amifostine and Hearing

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Amifostine is a new pharmaceutical agent intended for use as a protectant against toxicity induced by radiation therapy. Recently the agent has also been used to protect against toxicity from chemotherapeutic agents such as cisplatin. Several papers in the literature document the protective effect of amifostine against myelo- and renal toxicity, but auditory toxicity has been less well studied.

This project utilized auditory brainstem response (ABR) techniques in guinea pigs to determine whether amifostine is protective against oto- and neurotoxicity. Guinea pigs underwent ABR and blood tests before and one month after administration of amifostine (1000 mg/kg) alone, amifostine with cisplatin (15-30 mg/kg) or cisplatin alone.

ABR click latencies were consistently and statistically significantly prolonged after administration of amifostine even with no cisplatin. These findings indicate that amifostine may be associated with neurotoxicity. In addition, although myelo- and renal toxicity were protected, auditory toxicity from cisplatin was not reduced by amifostine.

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337 Phenotypic Presentation of Children With Non-Syndromic Sensorineural Hearing Loss (SNHL) to a Multidisciplinary Clinic

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This report describes the presentation of children with nonsyndromic sensorineural hearing loss (SNHL) to a multidisciplinary pediatric hearing loss clinic. Twenty-seven boys and 22 girls, from ages one year to 16 years old, underwent a comprehensive evaluation which included review of prenatal/antenatal risk factors, history of present illness, physical exam, metabolic testing, high resolution temporal bone imaging, psychoacoustic audiograms, tympanograms, DPOAEs and genetics evaluation and testing. Of these patients, 68% had bilateral SNHL, 23% had unilateral hearing loss and 9% were uncooperative for evaluation. Regarding severity, 5% of patients presented with *mild* hearing loss, 32% *moderate*, 36% *severe* and 27% *profound*. For duration, 76% of patients had hearing loss of greater than 2 years, 10% with hearing loss of 1-2 years and 14% with less than one year. Patients underwent CBC with differential, FTA, TFT and urinalysis. Reports

indicate 28% of the patients had increased platelet counts and 31% had elevated erythrocyte sedimentation rate. Temporal bone images demonstrated inner ear anomalies in 19% of patients tested. Of these patients, 41% had a positive family history. Full genetic workups were performed on all patients by the genetics staff and testing done for Connexin 26 gene analysis and A1555G mitochondrial mutational analysis. In conclusion, for children with nonsyndromic SNHL, 23% had unilateral hearing loss, 16% had progressive forms, 8% had fluctuating forms, 76% had stable forms, 8% had predisposing risk factors and 19% presented with inner ear anomalies. None of the patients with a positive family history for hearing loss (41%) tested positive for Connexin 26 or A1555G mitochondrial genetic mutations. This indicates that although 41% of patients had positive family histories, none demonstrated common hearing loss associated genes, suggesting that other previously unidentified genes may play a role in non-syndromic sensorineural hearing loss.

338 Proposed Mechanisms of Steroid Responsive Hearing Loss

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Glucocorticoids (prednisone) are given for various types of hearing loss, including autoimmune inner ear disease, sudden hearing loss, and idiopathic rapidly progressing hearing loss. However, we know little of these cochlear disease processes or how steroids counteract them. The glucocorticoids have several biological functions, including immune system suppression, anti-inflammation, and sodium reabsorption. We hypothesize that glucocorticoids restore hearing function through immune suppression mediated by the glucocorticoid receptor and sodium transport functions mediated by the mineralocorticoid receptor. An autoimmune mouse with immune related inner ear disease is used to test this hypothesis. Progression of its systemic autoimmune disease causes threshold elevations and cochlear autoantigens proposed in the literature (HSP70, collagen types II and IV, laminin, heparan sulfate proteoglycan, cardiolipin) are recognized by antibodies in its sera. This provides an excellent model in which to examine the hypothesized steroid effects.

Autoimmune mice were treated with either the glucocorticoid prednisolone or the mineralocorticoid aldosterone, the latter of which only enhances sodium transport. Both steroids improved cochlear function and the morphology of the stria vascularis, the presumed site of steroid mediated sodium transport mechanisms. Blockage of the mineralocorticoid receptor with spironolactone prevented both steroids from preserving cochlear function, while immune suppression (decreased systemic immunoglobulin) mediated through the glucocorticoid receptor was not affected. These results suggest that receptors for both steroids are involved in cochlear and systemic responses. Increased understanding of steroid responsive mechanisms in the ear may lead to improvement in steroid therapies for the different forms of hearing loss.

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339 Coating ELISA Plates with Peptides Coupled to BSA is a Highly Sensitive Method for Detecting Antibodies in Human Sera

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A 68 to 72 kDa antigen is commonly detected by antibodies in sera from a subset of patients with rapidly progressive sensorineural hearing loss. Screening assays for specific serum antibodies are complicated by the huge number of proteins in tissue extracts and similarly by the large number of antibodies in human sera. To resolve the question of antibody specificities we are developing ELISA assays to measure antibodies to potential inner ear antigen targets. Direct application of peptides to ELISA plates can detect antibody binding, but the sensitivity is very low. The objective of the study was to evaluate the use of BSA-coupled peptides as more sensitive screening tool for detection of anti-peptide antibodies. Using this method, we were able to greatly increase the sensitivity and specificity of peptide ELISA. Recent evidence indicates that the identity of the 68-72 kDa protein detected by the KHRI-3 antibody to the inner ear supporting cell antigen is CTL2, a member of the choline transporter-like family. Sera from 5 patients with rapidly progressive sensorineural hearing loss and a positive western blot for a 68-70 kDa band on a guinea pig inner ear extracts were tested on ELISA plates coated with BSA-conjugated to CTL2 peptide using glutaraldehyde and compared with the results from ELISA plates coated with CTL2 peptide. Results show that plates coated with CTL2 coupled to BSA were more sensitive in detecting CTL2 antibodies in patient sera by four to six fold. Therefore, the use of BSA-conjugated peptides to coat ELISA plates increases the sensitivity of detection of peptide specific antibodies and demonstrates its potential use as a highly sensitive screening tool in serodiagnosis of autoimmune sensorineural hearing loss.

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340 Somatic Modulation of Tinnitus III: Prevalence and Properties in Profoundly Deaf Subjects. A Functional Cochlea Is Not Necessary for Somatic Modulation of Tinnitus.

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Our previous work has shown that tinnitus is closely related to the somatosensory system. (1) Tinnitus can be induced by a somatic insult to the head or neck. (2) About 75% of tinnitus clinic patients can somatically modulate their percept. (3) About 75% of non-clinical subjects with ongoing tinnitus can somatically modulate their percept. (4) About 50% of non-clinical subjects without tinnitus, can elicit transient tinnitus with somatic testing. Furthermore these results can be interpreted in terms of the known anatomical and physiological interactions between the auditory and somatosensory systems. To understand the underlying mechanism responsible for changes in auditory perception with somatic testing, we studied 13 subjects with non-functioning inner ears. The subjects were postlingually profoundly deaf bilaterally. All had received a unilateral cochlear implant between 9 months and 16 years previously [mean= 8 years]. Six had tinnitus with their processor on or off, another four also had tinnitus only with their processor off. All subjects underwent somatic testing with their processor disconnected. While in a low-noise testing room subjects reported any changes in their auditory perceptions as 28 brief isometric but forceful cephalo-cervical or extremity contractions were performed. With somatic testing five of the ten subjects could modulate their tinnitus [3 increased, 2 decrease tinnitus loudness, 1 changed pitch]. Two of the three subjects without tinnitus could elicit transient tinnitus with somatic testing. We conclude that a functional cochlea is not necessary to (a) generate tinnitus de novo or (b) modulate ongoing tinnitus. These observations suggest that somatosensory-auditory neural interactions within the central nervous system can account for (i) the somatic tinnitus syndrome and (ii) somatic modulation of tinnitus.

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341 Torsional Eye Movements in A Case of Idiopathic Tullio Phenomenon

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Tullio (1929) first described vertigo and nystagmus on exposure to acoustic stimuli. Hennebert (1911) first described nystagmus provoked by pressure alteration in the external auditory canal in congenital syphilis. Nadol (1977) demonstrated the presence of Hennebert's sign in cases of Ménière's disease and presented temporal bone pathology suggesting the mechanism was due to fibrosis coupling the stapes footplate to the wall of the utricular otolith.

Videonystagmography (VNG) recordings were made at 5 and 8 months after onset of dizziness in a case matching Tullio's original description without evidence of Ménière's disease, middle ear disease or syphilis. Audiograms showed progression of a sensorineural hearing loss in the left ear. Vestibular function testing showed no evidence of a vestibular loss. High resolution computed tomography of the temporal bones was normal. Electrocochleography demonstrated a reversal of the SP/AP ratio in *both* ears. VNG showed paroxysmal counterclockwise torsional nystagmus in response to the Valsalva maneuver. Fistula tests for Hennebert's sign were negative. VNG at 8 months demonstrated rightward beating nystagmus in response to the Bárány noise apparatus presented to the right ear and a low frequency oscillating torsional nystagmus developed in response to the Bárány noise apparatus presented to the left ear.

These findings demonstrate the characteristics of the torsional nystagmus in a case of Tullio phenomenon and support the pathogenesis of vestibular fibrosis coupling middle ear structures with the utricular otolith. Mechanisms will be proposed to explain the bilateral electrocochleography changes as well as the differential nystagmus provoked by the Bárány noise apparatus.

342 Post-operative balance disorders in the elederly population. Preliminary report.

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Clinical features reveal in elderly population, symptoms of instability and vertigo after surgery. Our goal is to study the eventual changes in postural behavior after a surgical procedure with general anesthesia in this population.

The patients (65-81 y/o) were divided in two groups : A) Patients (N11) with central vestibular disorders (CVD) recorded by electronystagmography and clinically compensated in the preoperative state. B) Patients (N 14) without any pre-operative ENG alteration. A control group (N22) between 50 to 75 y/o. The three groups were studied before and after surgery. All of them underwent general anesthesic procedure. Through a balance platform, 95 % confidence ellipse (CE) of the center of pressure (COP) distribution and the sway velocity (SV) were measured pre and post surgery. The data were analized using time-frequency scalogram and velocity histogram.

Preliminary data showed that significant changes after surgery occured only in group A.

Although a bigger sample will be necessary, the fact that postural control after general anesthesia was affected in some of the patients who had compensated CVD previously the surgery, suggest the possibility that certain anaesthetic drugs could be involved impairing some of the recovered sensory motor processes.

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343 Virtual Temporal Bone Dissection Simulator

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We report on our continuing development of a temporal bone dissection simulation environment for training residents, the comparison of the system with traditional methods, and its efficacy in the curriculum.

We have integrated multimodal and multiresolution data sets into a single coherent simulation environment that provides stereoscopic display, haptic feedback, and aural simulation and presents a seamless experience for non-deterministic drilling of the temporal bone. The regional anatomy is presented in the surgical context. An intelligent tutor is combined with the simulator to provide identification of key structures as well as examples of expert practice.

The goal of this research is to limit the need for physical dissections, to mitigate the learning curve, and raise proficiency in surgical training. Through iterative development and evaluation, we will provide a valuable tool in training future otologic surgeons and an environment by which quantitative evaluation of surgical skill may some day be measured.

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344 ABR, NMR, and energy metabolism studies of the effects of long-term exposure to low doses of DEET in the rat.

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Despite investigations of the chemicals to which military personnel may have been exposed during the Gulf War, the role of these factors in the symptoms associated with the Gulf War syndrome is not clear. Effects of exposure to the insect repellent N,N diethyl-m-toluamide (DEET) were evaluated in the same animals by three different means: auditory brainstem response (ABR), nuclear magnetic resonance (NMR) and *in vitro* biochemical assays. ABR stimuli were frequency-specific tone bursts and 100μs clicks. Brain energy metabolites were assayed by monitoring the ratio of phosphocreatine to adenosine triphosphate by ^{31}P NMR. Relative levels of brain choline (Cho), creatine (CR), and N-acetylaspartate (NAA) were determined from proton NMR spectra. *In vitro* energy coupling ratios were assessed using succinate, pyruvate, and malate as substrates.

Male Sprague-Dawley rats were injected intraperitoneally once weekly for four consecutive weeks with either DEET (225 mg/kg in arachis oil) or vehicle (controls). ABRs and NMRs were done on anesthetized rats 24 hrs before the initial injection and at post-initial-injection times of 24 hrs (ABR), 1 week (ABR), 2 weeks (ABR, NMR), 3 weeks (ABR), and 4 weeks (ABR and NMR). After the final ABR and NMR measurements, rats were euthanized, mitochondria were isolated from brainstems, and respiratory chain function was assessed.

A repeated measures ANOVA ($p<0.05$) indicated no statistically significant changes in ABR thresholds with time for any of the acoustic stimuli used or in *in vivo* NMR measures of brain energy metabolites. Cho:Cr and NAA:Cr ratios were not significantly different (DEET-treated vs. control). A 15% inhibition of NADH dehydrogenase activity (DEET vs. control) was observed *in vitro*. No differences in respiratory control ratios, cytochrome oxidase activity, or succinate dehydrogenase activity were observed.

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345 Frequency and Localization of Congenital Anomalies of the Inner Ear

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The purpose of this study was to investigate histopathologically confirmed congenital anomalies of the inner ear, with particular attention to their localization and frequencies. One hundred ninety two human temporal bones from 136 individuals, whose age ranged from 1 day to 50 years old at time of death and each of whom had one of 35 congenital disorders, were used for this study. Among them, 107 bones from 71 individuals, who were involved with 25 congenital disorders, were found to reveal inner ear anomalies. Of these 25 congenital disorders, 24 revealed anomalies in the vestibular system, 16 in cochlea, and 15 in both vestibular system and cochlea. Among the vestibular anomalies, anomalies in the endolymphatic duct and sac, posterior semicircular canal, and lateral semicircular canal were encountered in a variety of disorders (13 of 24 disorders). Among the cochlear anomalies, general cochlear anomaly was associated with 13 disorders; the most common anomaly was shortened cochlea which was observed in 11 disorders. In this study, special attemtion was made to report on anomalies observed in cases of Kenny Linarelli Caffey syndrome (KLCS) and Ectrodactyly, Ectodarmal Dyplasia, and Cleft Lip/Palate syndrome (EEC), which have never been reported. Finally, the implications of these anomalies were discussed with regard to developmental and clinical issues to provide better diagnostic and therapeutic information.

346 Morphology and function of the tensor tympani muscle

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Introduction. The function of the tensor tympani muscle and its involvement in inflammatory processes of the middle ear still is subject of discussion. The aim of the present study was to investigate the age dependence of the muscle histology and its relation to inflammation.

Methods. Series of vertical temporal bone sections (10µm) of 67 individuals (0-89y) from the Hamburg Wittmaack collection were analyzed by digitizing light microscopy images. A subgroup of 20 individuals suffered from chronic middle ear infections. From this data diameters and relative portions of fat, connective tissue and muscle were determined.

Results. While the muscle diameter of the control group was found significantly increased (p=0.01) with age no trend was found in the otitis group (p=0.46). Relative muscle portion in the control group significantly decreased (p=0.01) by approx. 20% in 60 years, whereas the accompanying increase of fat was not significant (p=0.11). In the otitis group only the increase of connective tissue was significant (p=0.012).

Discussion. Fat portions were found from birth on. With increasing age a moderate atrophy of the tensor tympani muscle can be observed. In contrast to Abdelhamid et al. (1990) the age dependend fatty tissue degeneration is not significant. If the presence of fat tissue is an indicator of denervation (Howell, 1984) the functional importance of the tensor tympani muscle has to be questioned. This is supported by the different development of the stapedic muscle which is not influenced by age. Abdelhamid MM, Paparella MM, Schachern PA, Yoon TH (1990) Histopathology of the tensor tympani muscle in otitis media. Eur Arch Otorhinolaryngol. 248: 71-78

Howell P (1984) Are two muscles needed for the normal functioning of the mammalian middle ear ? Acta Otolaryngol (Stockh) 98: 204-207

347 Area Curve Ratios Significantly Improve Diagnostic Sensitivity of Electrocochleography in Early Meniere's Disease

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Electrocochleography (ECochG) is useful in supporting the diagnosis of Meniere's Disease (MD). Possible MD (early disease as defined by the 1995 Committee on Hearing and Equilibrium) is a readily treatable form of MD. We sought to identify whether ECochG SP/AP (summating potential/action potential) area curve measures were more sensitive than conventional SP/AP amplitude ratios in detecting Possible MD.

A retrospective chart review was conducted at our tertiary care institution to investigate this hypothesis. All MD patients seen over a three year period who had undergone tympanic ECochG were examined to identify Possible MD patients. Criteria for exclusion were incomplete work-up, ECochG performed using a prior system, cochlear microphonic spike obscuring ratio measurements, or prior otologic surgery. A control group of normal patients with ECochG were identified. SP/AP amplitude and area curve ratios for both groups were measured.

Of 138 MD patients reviewed, 20 (14%) had Possible MD, and 8 passed exclusion criteria. An audiologist blinded to subjects' diagnoses performed all measurements. The upper limit of normal for SP/AP amplitude and area curve ratios from the control group of ears (n = 13) (alpha=0.05) were similar to previously published results. In Possible MD, 4/8 (50%) patients had an abnormal SP/AP amplitude ratio, and 7/8 (88%) had an abnormal SP/AP area curve ratio; the difference between groups was statistically significant (p=0.03, chi-squared test).

The SP/AP area curve ratio significantly improves ECochG diagnostic sensitivity in Possible MD. This ECochG refinement will allow earlier intervention to preserve inner ear function in MD.

348 Blood flow measurements in the ears of patients receiving cochlear implants

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To measure cochlear blood flow (CBF), the tip of a laser-Doppler probe is placed on the bone over the cochlea. In this situation, the degree of contribution of the bone blood flow to the laser-Doppler output must be evaluated. The true component of CBF can thus be measured most accurately by laser-Doppler flowmetry in the ears during cochlear implantation procedures. The subjects for this investigation were 12 patients who received cochlear implants. The causes of deafness were unknown, except for one patient who had Waardenburg syndrome. CBF was measured using a laser-Doppler flowmeter (model ALF 21, Advance, Tokyo, Japan) under general anesthesia. The outer diameter of the probe was 0.8 mm. Blood flow was measured before, during, and after the cochlear bony wall had been opened. The laser Doppler output was largest when the tip of the probe was attached to the mucous membrane of the promontory. The laser-Doppler output was confirmed even after the tip of the probe was inserted into the perilymphatic space in all cases. A correlation was observed between the values of the laser-Doppler output measured when the tip of the probe was attached to the mucous membrane of the promontory and those measured when the tip of the probe was inserted

into the perilymph. We consider that the laser-Doppler output measured when the tip of the probe was inserted into the perilymphatic space reflected CBF without the mechanical or thermal effect of the drilling because the output reflected blood flow in the area illuminated by the laser light through the transparent perilymph, where it is distant to the drilled part. Laser-Doppler flowmetry is both safe and useful for measuring blood flow in the ears during cochlear implantation procedures. The measurement may be useful to investigate etiology of sensorineural hearing loss, the relationship between ossification and disturbance of blood flow and so on.

349 Rapid Screening of Patients' Sera for Inner Ear Antibodies

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Autoimmunity as a cause for sensorineural hearing loss has yet to be completely confirmed or characterized. However, the evidence of antibody to inner ear antigens in sera from patients with clinical diagnosis of autoimmune sensorineural hearing loss (AISHNL) and experimental models, strongly support the possibility. We have been screening patient's sera for antibodies against inner ear antigens using western blots. It was a slow process as we could test sera from only six patients at one time. Therefore we employed Biorad's multi screen apparatus to increase the number of sera we could test on a single blot. In this method instead of running protein (guinea pig inner ear lysate) in four wells in SDS PAGE, we ran the protein in one big well. After transferring the protein to a membrane, instead of cutting the membrane to strips for incubation, we used the multi screen apparatus to incubate primary and secondary antibodies along with a monoclonal HSP70 antibody to use as an internal mass standard. This allowed us to accurately score for 68KD and 72KD bands. With this method we screen thirty patients' sera at one time. We obtain results that are close to each other, making it was easier to compare individual patient's results and HSP70. There was no need to cut the strips to incubate and later put them back to compare to get the result. This avoids problems of orientation, realignment with mass markers and prevents mixing up the strips from different patients. This method is more efficient for screening patient's sera for antibodies against guinea pig inner ear antigens.

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350 Application of Enbrel in Experimental Immune-Mediated Sensorineural Hearing Loss

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The effect of blocking the pro-inflammatory cytokine, tumor necrosis factor a (TNFa) was investigated in a guinea pig (GP) model of immune-mediated sensorineural hearing loss. The model consists of a sterile labyrinthitis created by injection of 1 mg keyhole limpet hemocyanin (KLH) into the cochlea of GPs systemically sensitized to KLH. This induces a rapid infiltration of inflammatory cells. By 3 weeks the cochlear scalae are partially obstruction by fibrosis and new bone. Hearing loss is detectable 3-5 days after KLH challenge.

In order to determine whether the TNFa antagonist Enbrel (etanercept), a recombinant fusion protein containing human TNF-receptor linked to the Fc portion of human IgG1, would be effective in reducing the inflammation and hearing loss, we created labyrinthitis in both ears of 13 GPs, treating 6 with Enbrel. Enbrel has biological inhibitory activity in a broad range of animal arthritis models (Wooley, et al., J Immunol 1993; 151:6602). Bilateral hearing thresholds were measured by auditory evoked brainstem response (ABR) prior to cochlear challenge with KLH. The 6 treated animals received 5.6mg/kg Enbrel 30 min. before intracochlear KLH challenge and again 3 days later. Hearing tests were repeated 7 days after inner ear KLH challenge. Cochleas were fixed by cardiac perfusion (4% formaldehyde), decalcified, paraffin-embedded, sectioned and H&E-stained.

Hearing loss in the untreated animals averaged 71±6dB versus 42±9dB in the Enbrel treated GP (t-test, $p<0.05$). Inflammation seen in histological sections was scored on a scale of 0 to 5. There was significantly less inflammation in the cochleas from Enbrel-treated animals (3±0.5 in the KLH challenge group vs 2±0.5 in the Enbrel group (t-test, $p<0.05$). We conclude that prompt intervention with the anti-inflammatory drug Enbrel significantly reduced inflammation sufficient for substantive hearing preservation.

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351 In Vitro Permeability of the Round Window Membrane to Dexamethasone and Water

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Objective: The round window membrane (RWM) provides a route for drug delivery to the inner ear. This study used a novel in vitro perfusion chamber to establish quantitative data on the permeability of the RWM to ^{3}H-dexamethosone and ^{3}H-water.

Methods: The RWM of adult guinea pigs was mounted on a perfusion device, which clamped the RWM between two chambers. The upper chamber (middle ear side) allowed for a depot of drug and contained 50µL of tritiated dexamethasone or water. The lower chamber, the inner ear side of the RWM, was completely sealed except for inflow and outflow ports. This chamber, was continuously infused with balanced salt solution at a rate of 0.015 mL/min using a syringe pump connected to the inflow port. The outflow was collected every hour for 24 hours using a fraction collector. The entire apparatus was kept at 37°C using a circulating water bath. The amount of compound that had diffused through the RWM was measured in a liquid scintillation counter. The average permeability constant, K_{trans}, (cm/sec)over the 24 hour period was calculated for each compound as:

$$K_{trans} = (R_{total}/A \cdot t)\ (1/[D])$$

Where R_{total} equals the total moles through the RMW in time t, A = surface area of the exposed RMW (cm^2), t = time interval (sec), and D = concentration of original solution in donor chamber (mol/mL).

Results: K_{trans} for ^{3}H-dexamethasone was $1.5 \pm 0.4 \times 10^{-7}$ (mean ± SD, n = 4) and $3.1 \pm 0.8 \times 10^{-5}$ (n = 3) for ^{3}H-water.

Conclusions: This study showed that the RMW was permeable to ^{3}H-dexamethasone and ^{3}H-water, with the latter having s significantly higher permeability constant ($p<0.001$).

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352 Molecular Mechanisms Underlying Ectopic Expression of Otoconia-like Structures in Endolymphatic Sac (ES) of Embryonic Mammals

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In amphibia otoconia (OC) are strongly expressed in the extensive ES system, considered to be the main source of OC/otoliths for the gravity

receptor organs. This concept is supported by recent observations in newts exposed to microgravity (Wiederhold et al., 1997). Early effects were limited to the ES with a marked increase of OC mass. Several days later the new OC had shifted to the saccule, significantly increasing the mass of the saccular otolith. Well-developed OC are also present in the avian ES during embryonic life. Immoto et al. (1983) detected minute OC-like structures in the embryonic ES of guinea pig and concluded that the morphogenetic mechanism was analogous to that of amphibia, representing an evolutionary vestige. The recent cloning of otoconin 90 (OC90), the principal mammalian otoconial matrix protein, provided the tools to elucidate morphogenetic mechanisms of OC-like structures in the ES. In the mouse embryo OC90 was first detected by immunocytochemistry in the ES on E15. By E16 the lumen was filled with copious amounts of OC90, soon coalescing into OC-like aggregates. OC90 was also strongly expressed on the surface and in the cytoplasm of the ES. At this point it seemed obvious that luminal OC90 was due to local secretion. However in situ hybridization studies indicated complete absence of OC90 mRNA in the ES epithelia and endolymphatic duct (ED), while mRNA expression was massive in the vestibular periphery. We conclude that the luminal protein originated in the periphery and is transported into the ES via the ED. Accumulation of OC90 in the epithelium can only be ascribed to absorption of luminal material. These experiments demonstrate that OC-like structures of the ES of mammals are not due to local secretion, and that morphogenesis is fundamentally different from that of lower vertebrates.

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353 Expression Pattern of OC-22 Provides Insight into the Formation of Amphibian Otoconia (OC)

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In Xenopus laevis (X. laevis) the saccule contains aragonitic, and the utricle calcitic OC. Pote et al. (1993) determined on the basis of the amino acid sequence, that the aragonitic matrix protein otoconin-22 (OC22) of X. laevis is a homolog of phospholipase A2 (PLA2). Cloning of otoconin-90 (OC90), the calcitic matrix protein in mouse, revealed five domains, two of which are homologs of PLA2. The similarities of the respective functional domains suggest that both calcification systems rely on similar mechanisms of formation. The observed differences in the respective molecules, however, may be the reason that each leads to formation of a different crystal polymorph. As a prelude to test this hypothesis, we cloned OC22 of X. laevis. In situ hybridization of OC22 mRNA in late premetamorphic tadpoles indicated massive expression of mRNA in the endolymphatic sac (ES), but the vestibular periphery was entirely devoid of mRNA. These data relate directly to recent observations in newts exposed to microgravity, where early changes were limited to the ES, demonstrating a marked increase of OC mass (Wiederhold et al., 1997). Several days later the newly formed OC had shifted to the saccule, thereby increasing saccular otolith mass. These data suggest that increased formation of high density particles in the ES represents an attempt to compensate for the loss of gravity. However, the intended increase of sensitivity of the gravity receptor organ per se evidently is delayed, due to the spatial separation of the site of synthesis from the site of otoconial deposition. The exclusive expression of OC22 mRNA in the ES of X. laevis observed in our study supports this concept on the molecular level.

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354 Characterization of mammalian homologues of the sunfish saccular collagen

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Our aim is to identify mammalian homologue(s) of the saccular collagen (SC) that we previously identified in the bluegill sunfish, lepomis macrochirus. The SC protein appeared to be inner ear-specific and it is possible that a mammalian SC-related protein may exist and may also be inner ear-specific.

Southern evidence was presented previously showing that a single band was detected when the SC-specific probe was hybridized to BamHI digested sunfish, mouse, and human genomic DNAs. This provided evidence for the existence of mammalian homologues of the SC gene. Degenerate RT-PCR efforts have been employed using mouse whole inner ear and rat utricle-derived cDNAs without success yet. This may be due to the difficulty in knowing which regions of the SC protein are most conserved (which can complicate primer selection) or simply to the significant evolutionary distance between teleosts and rodents. Alternatively, the SC may not be utilized in mammalian otolithic organs at all or it may be present in small quantities so the presence of transcripts are too limited in number or are not expressed at the timepoints from which we chose to prepare template cDNAs.

Therefore, a new strategy to identify SC-related proteins has begun in which the sunfish SC sequence (both the nucleotide and amino acid) is being used to screen the EST and genome databases. Candidate SC-related proteins have been identified in the mouse and human and are being further evaluated to determine the whether any of these is the authentic mammalian homologue of the sunfish SC protein. Analysis of these sequences will be presented and discussed.

355 Do Gravitational Effects on Otolith Growth Require Functional Hair Cells?

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The marine mollusk, *Aplysia,* or fish larvae raised on a centrifuge had fewer statoconia or smaller otoliths than 1-g controls, suggesting a mechanism to "normalize" the weight of the test mass. Otoliths of late-stage larval swordtail fish developed in space were larger that those of ground-reared controls. However, for early-stage larvae, this effect was not seen. Here we report features of the early development of the otolith and sensory epithelium of zebra fish (*Danio raria*) which may explain the critical period for gravitational effects on otolith growth.

By 16 hours after zebra fish fertilization (at 28.5 ^{o}C), precursors of the otoliths are seen in the forming otic vesicle but no sign of a sensory epithelium is present. The first putative hair cells are seen at 24 h after fertilization but only a few stereocilia are seen. At 36 h, a kinocilium and stereocilia are seen but the stereocilia appear separated from one another. At 36 h, multiple otolith precursor particles are joined to form a solid otolith and flocculent material is seen connecting the kinocilium to the otolith. An identifiable sensory epithelium, with distinguishable hair cells and supporting cells can be seen by 48 h. By 72 h, adult-appearing hair cells, with a compact bundle of stereocilia, are first seen. At this stage, afferent and efferent synapses are also seen at the base of the hair cells. The individual particles forming the otolith can no longer be distinguished at 72 h. Thus, from morphological criteria, the hair cells do not appear capable of mechanotransduction until between 48 and 72 hours after fertilization, whereas the otoliths are forming as early as 16 hours post fertilization. If the fish use their hair cells to assess otolith weight in a regulatory role, one would not expect this to be possible before the hair cells become functional. Experiments to test this hypothesis will be described.

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356 Topographic Localization of $G\alpha_{i2(vest)}$ Expression in the Rat Vestibular Periphery

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The present study investigated the regional distribution of $G\alpha_{i2(vest)}$ expression in the rat vestibular end organs. $G\alpha_{i2(vest)}$ is a novel G-protein α-subunit previously identified in the rat vestibular periphery (PA Wackym et al., Neuroscience Letters **280**:159, 2000).

The response of vestibular afferent neurons to efferent modulation is regionally variable within the sensory neuroepithelium. These regional differences in modulation, however, cannot be explained by the pattern of efferent innervation since the number of bouton terminals on individual calices and type II hair cells is uniform throughout the vestibular epithelium. Alternatively, the complex afferent response has been proposed to arise from the differential distribution of neurotransmitter receptors and their associated intracellular second message signaling machinery. The hypothesis of this study is that differences in the expression of the $G\alpha_{i2(vest)}$ subunit within the vestibular periphery contributes to the regionally dependent effects of efferent activation.

Messenger RNA for $G\alpha_{i2(vest)}$ was detected by *in situ* hybridization on 10μm cryostat sections of Scarpa's ganglia and cristae ampullares using complimentary RNA probes labeled with ^{35}S or digoxigenin. Radioactive labeling was detected with photographic emulsion and visualized with darkfield light microscopy. Digoxigenin labeled preparations were treated with a secondary antibody and color reaction kit, and visualized with light microscopy.

Strong staining for $G\alpha_{i2(vest)}$ was detected in the somata of vestibular afferent neurons and throughout the crista ampullaris. These findings indicate $G\alpha_{i2(vest)}$ is ubiquitously expressed in the vestibular periphery and suggest that it is not responsible for the topographic difference in vestibular afferent response to efferent stimulation.

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357 Expression Of M2 And M4 Muscarinic Receptors In The Crista Ampullaris Of The Rat

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The present study investigated the expression of the muscarinic acetyl choline receptor subtypes M2 and M4 in the rat crista ampullaris sensory epithelia. The primary efferent vestibular neurons release the neurotransmitter acetyl choline (ACh). Five muscarinic acetyl choline receptor (mAChR) subtypes (M1-M5) have been cloned. The subtypes M2 and M4 are coupled to intracellular inhibitory proteins. The expression of mRNA for both receptor subtypes has been demonstrated in the rat vestibular periphery using polymerase chain reaction techniques. However, their cellular localization remains unclear. Rats were anesthetized and perfused with 4% paraformaldehyde for light microscopy (LM) studies, or with 4% paraformaldehyde and 0.5 % glutaraldehyde for transmission electron microscopy (TEM) studies. The temporal bones were post-fixed overnight in the same solution. The cristae ampullares were embedded in agarose, sectioned at 50mm thickness, and processed by immunocytochemistry for M2 and M4 receptors. In LM studies, the primary antibody was visualized with the peroxidase-diaminobenzidine technique, and preparations were embedded in epoxy resin, sectioned at 2 mm thickness and analyzed under bright field microscopy. In TEM studies the primary antibody was reacted with gold-coupled secondary antibodies, and preparations were embedded in epoxy resin, sectioned at 100 nm thickness, counterstained and studied under a transmission electron microscope. In LM preparations positive immunoreactivity for both M2 and M4 was found in the calyces surrounding type I hair cells. In TEM preparations strong reaction for M2 and M4 was confined to the lateral cell membrane of afferent calyces, often adjacent to efferent bouton type nerve endings, and weak reaction was observed inside the calyx. In conclusion, M2 and M4 mAChRs may play an important role in the modulation of primary vestibular afferent neurons by efferent nerve cells.

358 Intracochlear administration of ATP rapidly corrects vestibular imbalance in a guinea pig model of unilateral peripheral vestibular disorder

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Systemic administration of adenosine triphosphate (ATP) is common in the treatment of vertigo. But there is no study about topical treatment using ATP. The effect of inner ear administration of ATP on vestibular function was investigated in the present study. For the vestibular disorder, the right lateral semicircular canal in guinea pigs was cut surgically. Animals were then treated with saline, 5 mM ATP, 50 mM ATP, or 50 mM ATP + 10 mM pyridoxal-phosphate-6-azophenyl-2Õ, 4Õ-disulfonic acid (PPADS), a P2X receptor antagonist, administered directly into the scala tympani by osmotic pump. Before treatment, and at 3, 5 and 7 days after treatment, trapezoid rotation tests were performed on all animals, and the post-rotatory nystagmus (PRN) ratio (number of nystagmus beats after counterclockwise rotation/number of nystagmus beats after clockwise rotation) was calculated and compared between groups. The PRN ratio was statistically greater at 5 days after treatment in the 50 mM ATP group than in the saline group. A statistical difference was also observed in animals treated with 50 mM ATP + 10 mM PPADS. Our results indicate that ATP may play an important role in the vestibular periphery to correct vestibular imbalance and that this action may not occur via P2X receptors.

359 Connexin 43 Expression in the Tangential Vestibular Nucleus of the Hatchling Chicken

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Located in the lateral medulla oblongata, the chick tangential nucleus contains vestibular nuclei neurons that receive their major input from primary vestibular fibers. Most of these neurons are principal cells that process signals in the vestibulo-ocular and vestibulocollic reflexes. After vestibular nerve lesion, vestibular compensation is characterized by neuron and glial changes in the vestibular nuclei. The principal cells lose their vestibular inputs without degenerating, which makes them good candidates for studies on vestibular compensation (Aldrich and Peusner, 2001). Accordingly, preliminary to vestibular compensation studies, here we have focused on defining the distribution of Connexin 43 (Cx 43), a main component of gap junctions between glial cells in the brain.

Immunocytochemistry and confocal microscopy were applied to vibratome sections from the medulla of 1 and 9 day old hatchling chicks. The primary vestibular fibers were well labeled by placing biocytin crystals on the vestibular nerve. Sections were incubated in rabbit antibodies specific for Cx 43 and GFAP, either alone or in combination with mouse antibodies specific for neurons (MAP2), oligodendrocytes (anti-myelin) or Cx 43. Cx 43 was strongly expressed in the tangential nucleus neuropil at 1day, but was strikingly weaker at 9 days. The combination of MAP2 with Cx43 revealed that Cx 43 was not expressed by principal cells, but was localized in their vicinity. Combination of Cx 43 with biocytin staining showed that Cx 43 was concentrated on the surface of the vestibular fibers. Finally, the combination of Cx 43 and markers for GFAP and myelin revealed a

strong colocalization with both markers at 1 day that was much weaker at 9 days. These observations suggest that Cx 43 is an important component of the gap junctional complex formed at astrocytes and oligodendrocytes in 1 day tangential nucleus, but may undergo a down-regulation later on.

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360 Expression and distribution of gap junction proteins in the developing mouse vestibular labyrinth

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Till now, at least 14 different connexin (Cx) genes are known in mammals. In these Cxs, three kinds of Cxs involved in human non-syndromic deafness were identified. Mutations in the GJB2 gene encoding for gap junction protein Cx26 and mutations in the GJB3 gene encoding for gap junction protein Cx31 can cause both recessive and dominant forms of deafness. Mutations in the GJB6 gene encoding for Cx30, a third gap junction protein, can lead to dominant forms of non-syndromic deafness. However, no disorder of the vestibular labyrinth was identified in these cases. In the present study, by using immunohistochemical and molecular biological methods, the expression and distribution of Cx26, Cx30 and Cx31 in the developing mouse vestibular labyrinth were systematically investigated in order to evaluate the possible role of gap junction communication.

In this study, it is found that Cx26-like immunoreactivity was sparsely distributed among the supporting cells and transitional epithelium, and was densely expressed among the fibrocytes on 6 days after birth (DAB). The immunoreactivity increased rapidly among fibrocytes within vestibular connective tissue on 12 DAB and reached the adult pattern on 15 DAB. Moreover, the distribution and expression of Cx30 were found to be nearly the same as those of Cx26. However, Cx31-like immunoreactivity was weakly distributed among fibrocytes beneath the sensory epithelium of utricle and saccule in the adult mice only. The mRNAs expression and distribution of Cx26, Cx30 and Cx31 detected by in situ hybridization were corresponding with those detected by immunostaining in the adult mouse vestibular labyrinth. The possible role of these gap junction proteins in the developing mouse vestibular labyrinth was discussed and was compared with that of developing mouse cochlea.

361 Expression of type 1 vanilloid receptor (VR1) and 5-lipoxygenase (5-LO) by rat vestibular and auditory ganglion cells.

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VR1 is a non-specific cation channel activated by capsaicin, LO products, heat and acid. VR1 expression by dorsal root and trigeminal ganglion cells is necessary for the burn produced by hot peppers. This study demonstrates VR1 and 5-LO expression by inner ear ganglion cells. A PCR product (210 bp) was amplified from both oligo(dT)- and random primer-generated cDNAs of rat spiral ganglion cells using VR1 gene-specific primers constructed from the 3' non-homologous region. This PCR product shared 100% sequence homology to a rat VR1 cDNA (GenBank accession no. AF029310) and a rat vanilloid receptor splice variant mRNA (GenBank accession no. AF158248). Frozen sections of PLP-fixed, decalcified (1% formic acid) Long-Evans and Sprague-Dawley rat temporal bones were stained immunohistochemically for VR1 (affinity purified rabbit anti-VR1, Alpha Diagnostic) using standard ABC methods. Neurons and some satellite cells in both the vestibular and spiral ganglia were VR1-immunopositive. Neurons and supporting cells in adjacent sections of these ganglia were immunopositive for 5-LO (mouse anti-79kDa 5-LO, Transduction Laboratories). These findings raise the hypothesis that activation of VR1 by endogenous ligands may contribute to hypersensitivity of the eighth nerve to hair cell inputs in a variety of pathologic conditions, such as tinnitus, Meniere's disease and migraine. In particular, these data suggest that LO activation during inflammatory processes or during cyclooxygenase inhibition (e.g., by aspirin) is a potential intrinsic source of VR1 activation in inner ear ganglia.

362 Comparison of human vestibular and spiral ganglion cells

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The present study investigates the human vestibular ganglion cells ultrastructurally and is focused on an analysis of cytoskeletal proteins, the presence of neutopeptides, calcium-binding proteins, and the marker protein neuron-specific enolase for determination of morphological differences in the neuronal population. Compared with the model of human spiral ganglion cell differentiation, it was to be investigated for different human vestibular ganglion cells associated with different immunohistochemical staining patterns.Our study supported the hypothesis for presence of two cell subpopulations in the human vestibular ganglia. The two maxima of ganglion cell diameters could present a corresponding division. The several protein markers showed a specific distribution pattern in most findings. Thus, the protein markers are helpful for the classification of vestibular ganglion cells, but significant differences in immunostaining distribution could not be observed. Also the localizations of immunoreactivities for different neuropeptides could not simply divide the ganglion cells into two subpopulations.

363 Vestibular mGluRs: Signal Transduction

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The response of the frog *(R. pipiens)* semicircular canal (SCC) to the group I mGluR-selective agonist DHPG (300uM)--a facilitation of multiunit afferent discharge rate--is dose-dependently antagonized by the phospholipase C inhibitor U-73122 (1-100uM; IC_{50}: 33uM). The protein kinase C inhibitor bisindolylmaleimide I-HCl (10-100uM) has no effect on this response. The response of the SCC to DHPG is reduced (63.5+/-12.9%; n=4) by the smooth endoplasmic reticulum Ca^{++} ATPase inhibitor thapsigargin (1uM), and eliminated by ryanodine (1mM), an antagonist of the intracellular receptor which mediates Ca^{++}-induced Ca^{++} release, nimodipine (10uM), an L-type voltage-dependent Ca^{++} channel (VDCC)-selective blocker, Co^{++}(2mM), a non-selective VDCC blocker, and xestospongin C (1uM), an IP_3 receptor antagonist. Caffeine, at a concentration (5mM) which sensitizes ryanodine receptors to Ca^{++}, increases the amplitude (34.6+/-13.4%) and duration (453+/-169.8%; n=4) of the response of the SCC to DHPG, while depletion of the ryanodine/caffeine-sensitive intracellular Ca^{++} store with 50mM caffeine eliminates this response. The cyclic-ADP ribose antagonist 8-Br-cyclic-ADP ribose (1-10uM) has no effect on the response of the SCC to DHPG. Blockade of transmitter release with low Ca^{++}, high Mg^{++} artificial perilymph eliminates the response of the SCC to DHPG, while the afferent response to the iGluR agonist AMPA (30uM) is unchanged. Under low Ca^{++}, high Mg^{++} conditions, pre-application of DHPG has no effect on the amplitude of the afferent response to AMPA, suggesting that afferent endings do not express group I mGluRs. **Conclusion**: release of Ca^{++} from IP_3-sensitive intracellular stores, augmented by subsequent release from

ryanodine/caffeine-sensitive stores, may be associated with the increase in transmitter release mediated by activation of group I mGluRs on VHCs.

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364 Visual fixation suppression of caloric nystagmus in mutant mice deficient in delta 2 glutamate receptor subunit

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It is well known that the fixating mechanism of visual system influences nystagmus of vestibular origin. Nystagmus induced by caloric stimulation in dark is reduced in light (visual suppression). And it is generally assumed that floccular Purkinje cells encodes retinal slip and controls the gain of the horizontal vestibulo-ocular reflex. Previous reports showed that lesions in the flocculus abolish visual suppression. However the precise mechanism of the visual suppression is not completely understood. In the present study, we recorded the visual suppressions in mutant mice deficient in the delta 2 subunit of the ionotrphhic glutamate receptor (GluR delta-2) in Purkinje cells to investigate the role of glutaminergic system on the control of VOR. Wild type and homozygous mutant mice GluR delta-2 (-/-) 6-10 week old were used. The left eye was illuminated by an infrared LED and monitored by an infrared CCD camera. Eye movements were captured and analyzed by a computer. The pupil was fitted as an ellipse and the horizontal eye angular movement was calculated using the shape and direction of ellipse (Iwashita et al 2001). Caloric stimulation was made by injection of 5-ml ice cold water into the left external ear canal in dark and light. In wild type the slow phase velocity in dark was reduced about 90 % in light. In GluR delta-2 (-/-) mice the velocity was reduced very little (10 %), no change or even increased in light. These results suggest that the parallel fiber input to floccular Purkinje cells mediated by ionotrophic glutamate receptor play important roles in the visual suppression of the vestibular nystagmus.

365 Neurotoxic effect of glutamate in the vestibular endorgans during ischemia

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It was suggested that excessive glutamate released from intracellular stores caused neurotoxic effect through the glutamate receptors. Our previous studies revealed that the glutamate concentration in the perilymph was increased and the source of the glutamate was inner hair cells and supporting cells following ischemia (Haruta et al. 1998, Matsubara et al. 1998). Although neuronal damage of the organ of Corti has also been described (Tabuchi et al. 1998, Hakuba et al. 2000), that of vestibular endorgans is still unclear. In the present study, we investigated by means of post-embedding immunoelectron microscopic analysis whether neuronal damage in the vestibular endorgans is associated with the change of cellular glutamate concentration during ischemia. Transient local anoxia (10min, 30min) of guinea pig inner ear was induced by pressing the left labyrinthine artery. The right sides were used as controls. The morphological changes of the vestibular endorgans and the areal gold particle densities representing glutamate were compared between the ischemia side and the control side. In the ischemia side of the 30min anoxia, nerve chalices surrounding type I hair cells and afferent dendrites in contact with type II hair cells were swollen. The particle densities in type I and type II hair cells of the ischemia side were significantly lower than those of the control side. However, there were no significant differences of glutamate concentration in supporting cells between the two sides. The distribution of AMPA receptors in the nerve chalices and afferent dendrites has been already described (Matsubara et al. 1999). The present results indicate that excessive glutamate released from both type I and type II hair cells of the vestibular endorgans caused the swelling of both nerve chalices and afferent dendrites through glutamate receptors.

366 Mutation analysis of the Cx26 gene in simplex and multiplex non-syndromic deafness in Chinese population

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Mutations in the GJB2 gene are responsible for the commonest form of non-syndromic recessive deafness in many populations. Although more than 50 mutations have been described, one particular variant, 35delG, accounts for up to 70% of the pathologic alleles in European as well as North American populations. It has been recently reported that the 35delG mutation is exceptionally low in Japanese and Korean populations, but another deletion, 235delC, is relatively frequent. So far there have been no reports regarding the prevalence of Cx26 mutations in Chinese population. Whether Cx26 mutations are an important etiology of deafness and whether 235delC is also the most common mutation in Chinese are of importance. We conducted mutation screening for the Cx26 gene in 118 deaf Chinese probands, including 60 from simplex and 58 from multiplex families. The coding region of GJB2 was amplified by PCR. The PCR products were sequenced. Four mutations, including 235delC, 299-300delAT, V37I, and 35delG, were found in the patients. 39% of probands from simplex or multiplex families had mutations in GJB2. Of the 118 probands, 19 carried both mutations that were definitely pathogenic. The 235delC mutation was the most prevalent mutation (22% of alleles), accounting for 81% of the pathologic alleles in simplex cases and 67% in multiplex cases. The 35delG mutation was only noted as a heterozygous change in two simplex cases (1.2% of alleles). These results indicated that mutations in the GJB2 gene are a major cause of inherited and sporadic congenital deafness in Chinese population. The 235delC mutation, not 35delG, is most common mutation found in Chinese deaf population. Our data support the view that specific combinations of GJB2 mutation exist in different populations.

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367 Mutations in the Connexin 26 Gene among Bangladeshi and Americans with Nonsyndromic Hearing Impairment (NSHI)

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We report a comparison of the genotypic and phenotypic characteristics of a hearing impaired population from the Midwestern US and Bangladesh. In our Midwestern hearing loss group, 25.8% (16/62) were homozygous for mutations in Cx26. Fourteen of 16 of the DFNB1 group were Caucasian. The most common mutant allele was 35delG with an allelic prevalence of 16.7% (19/124), although only 20% of DFNB1 patients were 35delG homozygotes. The next most common allele was M34T in 2 patients with mild hearing loss and a 35delG/M34T genotype. Three novel missense mutations were identified (K15T, L90V, and K224N) that were not present in 96

control chromosomes. In 48 normal hearing control subjects, single allele mutations were found for 35delG, V27I and two patients with M34T. Overall, 33% (11/33) of subjects with severe to profound hearing loss had a diagnosis of DFNB1, compared to only 1 of 9 (11.1%) with moderate hearing loss and 3 of 20 (15%) with mild hearing loss. In patients with DFNB1, 73.3% (11/15) had severe to profound hearing loss, compared to only 44.7% (21/47) of the non-DFNB1 group. In the Bangladeshi group, 37 of 74 (50%) had idiopathic severe to profound hearing loss, while 11 had moderate loss, 7 had mild loss and 19 had no sensorineural loss. No disease causing Cx26 mutations were identified in any alleles in the Bangladesh population, although several polymorphisms were detected (V27I, E114G). The preliminary results of this pilot study indicate the need for further study of the genetic factors of NSHI in Bangladesh. Interestingly, our Midwestern population exhibits a lower 35delG mutation rate than previously described and demonstrates three novel deafness related sequence variants.

368 Connexin 26 and Pediatric Sensorineural Hearing Loss: Type of Mutation and Degree of Loss

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Mutations in the Connexin 26 (Cx26) gene are responsible for up to half of all recessive non-syndromic sensorineural hearing losses (SNHL), with 2 mutations (35delG and 167delT) being found most commonly. An infant or young child with a CX26 mutation(s) and SNHL may present with either congenital bilateral profound SNHL or with different degrees of hearing impairment. We looked at 42 children aged 1 week to 16 years who had SNHL and were found to have either homozygous or compound heterozygous CX26 mutations. 23 children were congenitally deaf; of these, 18/23 (78%) were either homozygous or compound heterozygous for 35delG or 167delT mutations. In contrast, of the 19 hearing-impaired (HI) children, 3 (16%) were homozygous for 35delG, none for 167delT, and there were no compound heterozygotes of either. Audiologic follow-up ranged from 4 months to 17 years. In the hearing-impaired group, two children progressed to profound bilateral SNHL, and two others had very mild decreases in their hearing. None were homozygous for 35delG.

In summary, children with Cx26 mutations and congenital bilateral profound SNHL are much more likely to have homozygous/compound heterozygous 35delG/167delT Cx26 mutations than hearing-impaired children. The two children who progressed to profound were V84L/V84L and 35delG/novel mutation, while the 3 with 35delG/35delG had stable bilateral moderate to severe losses. There was a 21% audiologic progression rate in the hearing impaired group, and the type of mutations did not predict the probability of progression. Therefore, although the "n" is small, patients need to be counselled about the possibility of decreasing hearing if Cx26 mutations are associated with the hearing loss.

369 Non-Syndromic Recessive Auditory Neuropathy Due to Mutations in Otoferlin (OTOF)

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Auditory neuropathy (AN) is characterized by absent or abnormal auditory brainstem response with normal outer hair cell function. Individuals with AN generally have poor speech reception out of proportion to the degree of hearing loss. They also tend to have disappointing results with hearing aids, however cochlear implants have been shown to help in many cases including some of our patients. AN is frequently associated with syndromic types of peripheral neuropathy, however there is a less common non-syndromic AN that is inherited as an autosomal recessive.

A genome screen was initiated and linkage analysis was performed on a group of non-syndromic recessive AN (NSRAN) families using LINKAGE program version 5.1. A critical region on 2p23 was identified that included the OTOF locus. The entire OTOF coding sequence was screened revealing pathologic mutations in our NSRAN families. OTOF is expressed primarily in the inner hair cells and is believed to be involved in synaptic vesicle membrane fusion. Previously, mutations in this gene have been associated with a non-syndromic recessive hearing loss, DFNB9. Since approaches to treatment and remediation differ between AN and non-AN groups, these findings have important implications for diagnosis, newborn screening, and prognostication.

370 Genetic features of hearing loss associated with ear anomalies: PDS and EYA1 mutation analysis

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The PDS gene was originally reported to be responsible for Pendred syndrome, but later was demonstrated to also cover the non-syndromic hearing loss associated with the enlarged vestibular aqueduct (EVA) anomaly. The EYA1 gene is responsible for Branchio-Oto-Renal (BOR) syndrome, which is well known to be accompanied by inner, middle, or outer ear anomaly.

Mutation analysis was performed in 24 hearing loss patients with various ear anomalies to clarify the spectrum of middle/inner ear malformations covered by the PDS gene and the EYA1 gene. PDS mutations were found only in the patients associated with EVA and EYA1 mutations were detected only in the patients with ear pits and cervical fistula, indicating that these two genes are each associated with specific middle/inner ear malformation. therefore genetic backgrounds should be considered when diagnosing ear malformations.

371 Genomic Structure of Protocadherin 15, the Gene Associated with Usher Syndrome Type 1F and the Mouse Mutation Ames Waltzer

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Usher syndrome type 1(USH1) is the most common cause of the dual sensory impairments of deafness and blindness. Affected persons are born with hearing loss and develop progressive pigmentary retinopathy leading to blindness in the 2nd-4th decades of life. Clinically subdivided into types 1-3 based on the degree of deafness and the presence of vestibular dysfunction, USH1 is the most severe. Approximately 70% segregate for mutations in myosin 7A (USH1B); the second largest contribution to the USH1 genetic load is at the USH1D-USH1F region on chromosome 10. The mouse Ames waltzer (*av*) causes inner ear dysfunction associated with degeneration of the neuroepithelia. The gene that harbors the *av* mutation was identified to be a protocadherin, *Pcdh15*. Mutations in the human homologue of *Pcdh15* were identified in two USH1F families. Here we report the ongoing analysis of intron-exon structure and the genomic sequence of the mouse and human Pcdh15 genes. They both have 32 exons with high homology in exons 2-31, which codes for the extracellular (EC) domain of the encoded protein. The human gene spans ~700 Kb region on chromosome 10q21.1; the mouse gene is expected to be of similar size. The 5'-UTR region of *PCDH15* shows key features such as the presence of CpG islands upstream of the putative transcription start site. Compared to other protocadherins, Pcdh15 has a unique genomic structure with one large exon encoding the cytoplasmic domain and 30 exons encoding the EC domain. Based on the analysis we have done so far it appears that the genomic structures of the mouse and human Pcdh15 genes are highly conserved and it is likely that they are derived from a common ancestral gene.

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372 Missense mutations in USH1C and CDH23 cause non-syndromic autosomal recessive deafness

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Truncated mutations in the USH1C gene (USH1C) and in the USH1D gene (CDH23) have been shown to underlie type 1 Usher phenotype, the most severe form of USH manifested by profound deafness, retinitis pigmentosa, and vestibular dysfunction. USH1C Encodes a PDZ-containing protein, while CDH23 encodes a cell adhesion protein with multiple cadherin-like domains. The localized expression of some USH1C transcripts in the inner ear along with a form of non-syndromic deafness (DFNB18) which maps to the same chromosomal location suggests that USH1C is a candidate for DFNB18. Missense mutations in CDH23 were found to lead to a form of non-syndromic deafness (DFNB12). To investigate whether USH1C could be involved in DFNB18 and whether missense mutations in CDH23 result in deafness alone, we analyzed 28 exons of USH1C and 69 exons of CDH23 by SSCP and direct sequencing in DNA samples from 300 ethnically diverse probands with non-syndromic deafness. We identified four different USH1C mutations in four probands in alternative exons B and D that are not expressed in eye. One was homozygous for Pro632Arg substitution which was heterozygous in a normal hearing sister of this proband, while the others were heterozygous for Ala455Thr, Arg660Cys, and Arg644Leu substitutions. In CDH23, we have only identified several novel missense mutations and polymorphisms in first panel (96) of these probands. Screening of the remaining probands is underway. The present study has demonstrated that missense mutations in alternative exons in USH1C can cause non-syndromic deafness. Identification of novel missense mutations in CDH23 may define the nature and extent of genotype-phenotype correlations.

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373 Audiologic Findings in 167 Subjects with Usher Syndrome Type 2 (Non-2A) and 2A

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Usher syndrome is an autosomal recessive condition affecting hearing and vision. Three clinical types are recognized: Usher syndromes type I, II, and III. Usher syndrome type II is distinguished by moderate to severe sensorineural hearing loss with normal vestibular function and retinitis pigmentosa. There are three Usher type II genes (2A-2C). The Usher 2A gene has been identified.

We have examined 853 audiograms of 167 persons with Usher syndrome type II with scatter plot and decade audiograms. A subset of 109 persons with 3 or more serial audiograms over 5 or more years was examined for progression of hearing loss using two distinct methods: method 1 used low PTA (0.5, 1, 2 KHz), and high PTA (4, 6, 8 KHz. Method 2 used PTA 4(0.5, 1, 2, 4 KHz). We defined four levels of progression: Mild is equal to or greater than 1 dB PTA/year with minimum 10 dB change over 5-10 years; Moderate is equal to or greater than 1.5 dB PTA/year with minimum 15 dB change over 5-10 years; Severe is equal to or greater than 2.5 dB PTA/year with minimum 25 dB change over 5-10 years and Rapid is equal to or greater than 3.5 dB PTA/year with minimum 35 dB change over 5-10 years.

Patients were grouped into two genetic classes:

1. Usher 2 but not 2A (55 patients)
2. Usher 2A (54 patients)

Results- Bilateral progression was seen in: 13/55 (23.6%) group 1 and 5/54 (9%) group 2 when progression criteria was PTA4. The difference between these groups is significant (P=.05).

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374 USH2A Mutations Associated with Phenotypic Variation in Usher Syndrome and Retinitis Pigmentosa Patients

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Usher syndrome is an autosomal recessive disorder characterized by sensorineural hearing loss and retinitis pigmentosa (RP). Mutations in the USH2A gene cause hearing and vision loss in about 20,000 people and vision loss in at least 7000 people in the USA. Usherin, a 171.5 kD protein, contains four extracellular matrix domains: thrombospondin (TS), laminin type VI (LN), laminin epidermal growth factor (LE), and fibronectin type-III (FN). Mutation searches of Usher II probands revealed 33 distinct mutations in 15 of the 21 exons, with 2299delG (16%) as the most frequent. Variation in the severity of the hearing loss has been observed. Significantly, one missense mutation (C759F), in the

LE domain, was reported in 10 RP patients with normal hearing (Rivolta et al., 2000 Am J Hum Genet 66:1975).

Our purpose was to determine the distribution of USH2A mutations, and clarify the association of specific mutations with phenotypic variation. Usher II and RP probands were screened for the 2299delG mutation by an amplification refractory mutation detection system, and additional mutations were detected by heteroduplex, DHPLC, sequence and restriction endonuclease analyses. Novel mutations were identified in both groups. The RP associated mutation (C759F) was detected in 3 atypical Usher families, and 5/41 RP families. At least 35% of RP families carried USH2A mutation(s). Identification of USH2A mutations as a common cause of RP may reveal additional protein segments required in the retina, but dispensable in the cochlea. Information about functional effects of mutations will be a rationale for developing treatments for inherited hearing loss.

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375 Auditory Characteristics in Carriers of Genes Related to Usher Syndrome

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Usher syndrome is a recessively inherited disorder characterized by sensorineural hearing loss and retinitis pigmentosa. Parents of offspring with Usher syndrome are obligate carriers since they have one abnormal copy of the gene related to that trait. While carriers display no apparent clinical hearing deficit, there is interest in determining whether subtle changes in auditory function may exist. We studied parents and carrier siblings using physiological measures of auditory function including spontaneous, transient, and distortion product otoacoustic emissions (OAEs), suppression of OAEs, auditory brainstem responses (ABR), and ABR binaural interaction. Most of the parents and all siblings of offspring with the Acadian Usher syndrome, type 1c were confirmed as carriers by molecular genetic testing. Experimental subjects were compared to age and gender matched control subjects. All subjects had normal pure-tone thresholds bilaterally. Results show lower amplitude for transient and distortion product OAEs for the experimental group compared to the control group. Decreased amplitude and increased latency of ABR components were observed in experimental subjects that may be related to peripheral differences. The results found with these physiological measures of auditory function support the hypothesis that carriers of genes related to Usher syndrome display subtle changes in auditory function and provide a refinement to earlier studies which reported differences in pure-tone and middle-ear muscle reflex thresholds in carriers.

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376 Development of a Mouse Cochlea Database

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Both the National Science Foundation and the National Institutes of Health have reported that future scientific information will arise not only by laboratory experimentation but also by discovery based upon information contained in community-accessible databases. The purpose of this research is to develop a collection of databases, called the Mouse Cochlea Database (MCD) that will contain anatomical images and complete bibliographic information of the mouse cochlea.

The MCD will consist of four, linked databases that will provide a comprehensive collection of information on the anatomy of the mouse cochlea. The four, Web-accessible databases are: bibliographic, image, cytocochleogram, and Magnetic Resonance Microscopy (MRM). The bibliographic database presently contains 972 citations, spanning a period of 1965-2001, that were obtained from Pub Med and other peer-reviewed sources. These records are searchable online and also can be downloaded. In addition, this database contains summary statistics of the literature including: mouse strain frequency data, scientific focus, author productivity, and a citation analysis. The second, image database will consist of anatomical data from normal cochleas using light microscopy and immunohistochemical methods for the purpose of understanding the molecular anatomy of the mouse cochlea. The third database is a digital cytocochleogram in which Web-based users can interactively view and measure structures of the organ of Corti along the complete length of the basilar membrane. The fourth database will contain individual MRM cochlear sections and 3D renderings of the cochlea using two mouse strains: CBA and C57. The MCD has received preliminary funding for its development; however, much work remains to be done to make it a useful community resource.

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377 Anatomy of the Human Temporal Bone Using High Resolution Magnetic Resonance (MR) Imaging

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OBJECTIVES: Very few reports have looked at high-resolution MR imaging of the human cochlea. MR imaging is advantageous over other imaging modalities in that it has the potential for high-resolution visualization of cochlear membranous ultrastructure, in a non-invasive manner, without exposing patients to ionizing radiation. In this study, we attempted to define the anatomy of the human temporal bone using a 9.4 Tesla MR imager. This scanner at this time has the most powerful MR magnet available in the world.

METHODS: A 9.4 Tesla MR scanner was used to image normal cadaveric temporal bones in different planes.

RESULTS: Detailed anatomy of the modiolus, utricle, saccule, semicicular canals and facial nerve were seen on the images. Identifiable structures within the cochlea included, the osseous spiral lamina, Reissner's membrane, membranous spiral lamina, and spiral ligament among others.

CONCLUSIONS: The data obtained from the imaging of the cochlea, facial nerve, and vestibular complex using this technique provides a baseline for developing protocols for testing temporal bones and eventually patients with inner ear pathology.

378 New applications of the microdissection technique of the human inner ear.

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The objective of the present study was to apply the techniques of immunohistochemistry to the microdisected inner ear in human using surgical specimens and temporal bones acquired post-mortem. The whole endorgans were microdisected from the temporal bone as follow: the tympanic membrane and the ossicular chain are detached, the remaining bone is carefully removed to expose the membranous labyrinth, the vestibular endorgans and cochlea are separated individually. Microwave irradiation of the isolated tissue was performed to unmask antigens. Immunohistochemical staining was performed in whole endorgans using antibodies against calmodulin to identify hair cells cytoplasm, phalloidin Oregon green to identify hair

cells stereocilia, neurofilament antibodies and the lipophilic dye DiI to stain nerve fibers and terminals. Cell nuclei were identified using the fluorescent dye DAPI. The immunohistochemical reaction was visualized using HRP or fluorescent-conjugated secondary antibodies. Whole mount preparations were examined using light and fluorescent microscopy. In the vestibular sensory epithelia and organ of Corti calmodulin like-immunoreactivity was found in the hair cell cytoplasm, their stereocilia was specifically stained with phalloidin. Neurofilament immunoreactivity was found in nerve fibers running throughout the stroma crista and fibers the running throughout the osseous spiral lamina. The lipophilic dye DiI labeled nerve fibers and terminals. In conclusion different cellular components in the human inner ear can be identified with the use of specific cellular markers. Pathological changes in the sensory epithelia can be documented with the use of immunohistochemical techniques.

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379 Guinea Pig Cochlea Transfected by an Antisense Oligonucleotide in Presence of ExGen 500 *in vivo*

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Studies of gene delivery to the inner ear have employed two main vector systems: 1) viral vector, & 2) liposomal vector. Each of these vector systems has certain inherent limitations in the context of efficient gene delivery to the cochlea. This study introduces a new gene delivery method employing the "proton sponge" reagent, ExGen 500 (polyethylenimine), to transfect antisense oligonucleotide (AON) into cochleae *in vivo*. Pigmented guinea pig (300-400 g, 10-12 weeks old) received a mixture of digoxigenin labeled $p27^{kip1}$ (dig-$p27^{kip1}$) AON (20 μM) and ExGen 500 (at 6 equivalents of DNA) in 5% glucose by perfusion directly into cochlea using a mini-osmotic pump and microcatheters for one week. Control animals received either dig-$p27^{kip1}$ AON only or ExGen 500 alone by cochlear perfusion. Immunohistochemistry with anti-digoxigenin antibody was performed on whole mount and frozen section samples. Immunoreactivity to digoxigenin, as detected by DAB staining, was present in the cochlea infused with dig-$p27^{kip1}$ AON and ExGen 500. The anti-digoxigenin signal exhibited a gradient of intensity from base to apex. Moreover, the signal appeared to be highly concentrated in the nuclei of transfected cells. A survey of the organ of Corti revealed that the transfected cells include inner hair cell, outer hair cell, pillar cell, Deiter cell, inner phalangeal cell, and Hensen cell. Spiral ganglion neurons were clearly transfected. In addition, some of the cell in Reissner's membrane, underneath the basal membrane and in spiral ligament was also transfected. No positive signal was detected for either of the controls. These findings show that ExGen 500 enhances the transfection efficiency of AON in the guinea pig cochlea.

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380 Microchannels in the Modiolar Wall of the Human Scala Tympani.

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In the human cochlea, the peripheral processes of the acoustic nerve are organized into bundles between the spiral ganglion and the osseous spiral lamina (OSL). The bundles traverse within hollow bony column-like structures in the modiolar wall of the scala tympani, and are most prominent in the first and second turns of the cochlea. Between the columns and just below the projection of the OSL on the modiolar wall, are regularly spaced fenestrations in the bony matrix of the modiolus, covered with a thin layer of soft tissue that lines the scala tympani. These fenestrations open into a cloister-like space in the modiolar wall that follows a spiral course medial to the OSL. A study of modiolar wall anatomy by orthogonal-plane optical sectioning (OPFOS) microscopy revealed two types of channels emanating from the spiral cloister, both types running roughly parallel and between the bony columns. One type of channel runs immediately beneath the tissue layer of the modiolar wall, containing venules that connect to the spiral modiolar vein. The second type of channel courses from the spiral cloister through a cribriform maze and into Rosenthal's canal via regularly spaced perforations. These channels may also facilitate modiolar vasculature to the region of the spiral ganglion. Both types of channels may represent low-impedance pathways for electric fields and the regular spacing may have design implications for cochlear implants, particularly those designed to fit against the modiolar wall of the scala tympani. Images of these channels are presented, as are data pertaining to their dimensions and spacing.

381 Three-Dimensional Computer Reconstruction and Quantitative Analysis of the Basal Region of the Scala Tympani in the Human Cochlea.

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Orthogonal-plane fluorescence optical sectioning (OPFOS) microscopy was used to examine a human cochlea in a region extending from the round window niche to the interior of the basal region of the scala tympani. A series of images of parallel optical sections separated by 50 microns was used to create a 3D computer reconstruction of the RW niche, RW membrane, scala tympani, basilar membrane, cochlear aqueduct and spiral modiolar vein. The images and 3D reconstruction are presented, as are the intrinsic and relative dimensions of anatomical features in this region of the cochlea. The immediate reason for undertaking this study was to aid in the design of a catheter-mounted ultrasound transducer to be inserted into the RW niche and aimed at the RW membrane in order to assess blood flow in the spiral modiolar vein. However, these data may also be useful to other areas of work such as cochlear implants, drug delivery and gene therapy.

382 Localization of Hsp32 (heme oxygenase 1) in the rat cochlea

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Cells respond to traumatic stress through a variety of pathways, some leading to protection, repair and recovery, and others to cell death. One of the best-characterized protective pathways involves the heat shock proteins (Hsps). In response to stress, Heat Shock Transcription Factor 1 (Hsf1) is activated, which in turn upregulates the expression of several Hsps resulting in enhanced cell survival. We showed expression of Hsf1 in the mouse and rat cochlea, with immunolocalization in hair cells and stria vascularis. We then showed its activation following stress. One target of Hsf1 is Hsp70. We have previously shown upregulation of Hsp70 in the rat cochlea following noise overstimulation (Lim, et al., 1993) or ischemia (Myers, et al., 1992) with immunolocalization in outer hair cells. Dechesne et al. (1992) and Yoshida et al. (1999) have shown Hsp70 upregulation in the rat and mouse cochlea, respectively, following heat stress. In the present study we have examined expression and localization of another Hsf1 target, Hsp32 (Heme Oxygenase 1), which degrades heme to the antioxidants biliverdin and bilirubin, providing protection against free radical damage. Semi-quantitative RT-PCR showed constitutive expression of Hsp32 in the normal rat cochlea. It was more highly expressed in the sensorineuralepithelial and lateral wall subfraction of the cochlea than in the modiolus. Western blot showed constitutive Hsp32 expression in extracts from whole cochlea. Immunocytochemistry in surface preparations from rat cochlea showed constitutive Hsp32-like immunoreactive staining in the three rows of outer hair cells. This localization correlates well with Hsf1 as well as Hsp70 induction and

suggests a potential role in protecting these cells against oxidative stress. We will next determine if Hsp32 levels change in response to heat or noise stress.

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383 Messages from the Bottom of the Atlantic Ocean: Comparative Studies of Anatomy and Ultrastructure of the Inner Ears of Several Gadiform Deep-Sea Fishes

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Deep-sea fish live in an environment with little or no natural light. We hypothesize that they have evolved acoustic communication and good hearing. Since deep-sea fish rarely can be taken alive, the only way to study their hearing is to extrapolate from anatomical studies. In this study, we examined the ears of five Gadiformes species collected from a depth of 1500-4000 m.

Four grendiers (*Coryphaenoides rupestris, C. mediterraneus, C. leptolepis* and *C. guentheri*) have similarities, as well as substantial divisity, in their saccules. All saccules are relatively large. The saccular maculae are shaped like two wings connected by a narrow band. The orientation of the hair cell ciliary bundles in these species is similar to that found in the Atlantic cod (*Gadus morhua*) (Dale, 1976, Norw. J. Zool, 24: 85-128), with a bi-directional vertical pattern in the middle, and a gradual shift to a bi-directional horizontal pattern at both ends. The inner ear of another bathypelagic gadiform fish, *Antimora rostrata*, has a number of unique features in its morphology and ultrastructure. The sacs of the three end organs are rigid and partially sclerotic, with tight attachment to all of the surrounding bones. The saccule has a very large otolith. The saccular macula, which is exceptionally thin and long, is in three segments. The rostral end does not have clear boundaries of differently orientated bundle groups, but instead, it has ciliary bundles that gradually change in direction. The middle part of the macula has ciliary bundles oriented ventrally, while the caudal part is horizontally bi-directional.

These results suggest that deep-sea gadiform fishes may have evolved specializations for hearing that are unique to this group. Further studies will examine the other end organs, and also correlate ear structure and the relationship to peripheral sound detecting structures, such as the swim bladder.

384 Structure-Function Relations In The Ear Of Silver Perch: The Story Of A Hearing Specialist.

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Silver perch (Bairdiella chrysoura) is a member of the teleost family Sciaenidae. Members of this family show substantial diversity in inner ear structure, as well as in the relationship between the swim bladder and the ear. In all sciaenids, the saccule is enlarged, and in silver perch, the lagena also shows expansion. Additional specializations in the ear of this species include the shape and orientation of the saccular sensory epithelium or macula, and the intimate relationship between the anterior swim bladder chamber and the inner ear. A close association between the swim bladder and the ear can allow for enhanced audition. This is because gas bladders respond to acoustic pressure signals and re-radiate the energy as acoustic particle motion, which may then stimulate the ear.

In this study, the auditory brainstem response (ABR) was used to determine frequency range of detection, as well as auditory sensitivity in silver perch. It was found that this species detects frequencies at least up to 2 kHz, with best sensitivity at around 400 Hz. Other sciaenids investigated with ABR include spot and kingfish, and it was found that these species detect up to 600 Hz, with best frequency at about 200 Hz. In spot and kingfish, the anterior region of the swim bladder is not in close proximity to the ear, and hence is unlikely to be contributing to audition. Silver perch is therefore a hearing specialist, and its auditory abilities correlate well with its unique ear morphology.

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385 Ultrastructural evaluation of calcitonin gene-related peptide immunoreactivity in the human cochlea and vestibular endorgans

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Calcitonin gene-related peptide (CGRP) is a neuropeptide widely distributed in the peripheral and central nervous system. Demonstrated in the efferent systems of the mammalian cochlea and vestibule, immunoreactive patterns of CGRP may vary by species. There is however, no information in the literature investigating CGRP localization in the human cochlea. In the present study, the ultrastructural localization of CGRP immunoreactivity was evaluated in the human inner ear with immunoelectron microscopy. It was found that in human cochlea, CGRP immunoreactivity was located in unmyelinated nerve fibers of the spiral lamina, inner spiral fibers beneath inner hair cells, tunnel spiral fibers, tunnel crossing fibers and outer radial fibers. In endorgans of human vestibule, CGRP immunoreactivity was located in vesiculated nerve fibers and bouton-type nerve terminals which were seen to contact afferent nerve chalices surrounding type I sensory cells and afferent nerve fibers, or to form an 'en passant' contact with afferent dendrites. CGRP immunoreactivity appeared to be confined to efferent systems in all cases. This study presents evidence that CGRP could serve a role in neurotransmission or neuroregulation in both cochlear and vestibular efferent systems of human.

386 Wdr1 Expression in the Developing and Adult Mammalian Cochlea.

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WDR1 encodes the vertebrate homologue of yeast actin interacting protein 1 (AIP1) (Adler et al., Genomics 56, 59-69, 1999). WDR1 interacts with actin depolymerization factor (ADF)/cofilin to enhance the disassembly of actin filaments. In the chick, WDR1 is expressed in two regions of the developing cochlea that will form homogene and cuboidal cells, cells that have high levels of F-actin filaments. In the hatchling chick, WDR1 was also found in hair cells in the auditory sensory epithelium and in the tegmentum vasculosum, and auditory ganglion cells (S.H Oh, unpublished data). In this study we examined expression of Wdr1 during mouse development, and in normal and noise exposed C57BL/6 mice. Northern blot analysis identified three Wdr1 mRNAs: 2.0, 2.7, and 3 kb. In situ hybridization was performed to examine the pattern of expression of Wdr1 in the mammalian cochlea. The probe used detected only the 3-kb mRNA. At embryonic day 18, Wdr1 mRNA was detected in the sensorineural epithelium of the cochlea, in spiral ganglion cells and in the stria vascularis. For the noise exposure studies, mice with normal hearing were exposed to noise overstimulation (104 dB BBN, 2-20 kHz) for 2 hours. Cochleae were removed and analyzed by in situ hybridization at various times after the noise exposure: 1, 6, 24, 72 hours, and 7 days. Sections from the cochleae of control, unexposed mice or mice exposed to noise were collected on the same slide and used for in situ hybridization. The 3.0-kb Wdr1 mRNA was not detected in the normal cochlea, but was seen in Deiter's cells at both 72 hours and 7 days after noise exposure. Supporting cells, including Deiter's cells, are responsible for the

structural integrity of the reticular lamina in the mammalian cochlea. Wdr1 may be involved in the response of the mammalian cochlea to noise overstimulation.

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387 Estrogene and hearing - is there a correlation?

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Estrogen - the female sex hormone - is classically known to influence growth, differentiation and function of the reproductive tract in both females and males. In recent years it has also been established that estrogens also are important in the maintenance of the skeleton and in the cardiovascular system, where estrogens have certain protective effects. In the brain estrogens affects both the activity and the connectivity of specific neuronal populations. There are indications that estrogens may have an effect on the ear and hearing, but the relationship is not fully investigated. When comparing the hearing of elderly males and females of the normal population, there is a gender difference with a generally poorer hearing of the males than that of the females. There are also well-documented differences in auditory brainstem responses, with shorter latencies in women. Women with Turners Syndrome,missing one X-chromosome resulting in loss of estrogene, also have poor hearing. Estrogen receptors exist in an alfa and a beta form, and both have been shown to be present in the inner ear of normal mice and rat.

In this study immunohistochemistry was used to visualize estrogen receptor alfa and beta in human inner ear (adult and fetal) and in Turners Syndrome. The morphology of an estrogen receptor beta knock-out mouse and a Turner mouse was also investigated. Estrogen receptor alfa was present in the spiral ganglion and estrogen receptor beta was present in the stria vascularis in the human specimens. The possible influence of estrogen on the hearing will be discussed.

388 Expression of MACF2 During Development of Inner Ear

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Plakins, a group of crosslinking proteins that connect cytoskeletal elements to cellular junctions and the extracellular matrix, are primarily responsible for the mechanical properties of cells and tissues. They include desmoplakin, envoplakin, plectin, dystonin/BPAG1 and Kakapo. Mutations in plakins cause severe skin, muscular and neurological disorders. We assume that the normal functions of the organ of Corti are highly dependent on structural proteins connecting different cytoskeletal elements and cellular junctions. We identified a novel gene MACF2 on human Chr 6p12 that encodes a structural protein (KIAA0728) with a Gar22/Gas2 domain, similar to Drosophila Kakapo and human MACF1 proteins. These proteins connect intermediate filaments and actin filaments to cellular junctions and to the extracellular matrix. The protein structure deduced from the partial cDNA (KIAA0728) demonstrated the presence of seven spectrin-like repeats, and the Gar22/Gas2 domain. Northern blot analysis of mouse RNA with a gene-specific probe identified three large transcripts, 9 kb, 11 kb and 15-16 kb, that were present in ovary-uterus, brain, skeletal muscle and heart, respectively. In situ hybridization demonstrated the expression of KIAA0728 gene in sensory and motoneurons of central and peripheral nervous KIAA0728 gene is expressed in neurotissues, in epithelial and mesenchimal cells We complete the study of expression of MACF2 during mouse ear development using mouse embryo from 7 embyonic day throw newborn mice. Expression of MACF2 gene in auditory and vestibule sensory epithelium beginning at embryonic day 12 and continues in the neonatal mouse. The localization of the MACF2 on protein level in progress. We propose that this novel structural protein may play an important role in maintenance of the cytoarchitecture of the inner ear.

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389 The Membrane Potential of Type I Fibrocytes is Controlled by Ca^{2+}-dependent K+ Conductance.

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Spiral ligament fibrocytes are connected to each other by gap junctions (Kikuchi et al, 1995) and possess a variety of ion transport mediators. One proposed functional role for these cells is to recycle K+ from perilymph to endolymph (Spicer & Schulte, 1996). If ture, regulation of their membrane potential (Em) would be critical to maintaining the electrochemical gradient needed to move K+. Defining the mechanisms whereby different types of spiral ligament fibrocytes regulate their Em is of importance to understanding their biological roles.

The Em was measured in cultured type I fibrocytes by conventional and perforated whole cell current clamp with 150 mM KCl in the pipette and 4 mM in the bath. The Em of type I fibrocytes measured at the breaking-in point ranged from –10 to –90 mV and averaged –47.1 ± 1.7 mV (n=111). The Em gradually shifted to an average of –80.6 ± 1.2 mV (n=9) after 3 mins of dialysis and maintained stable thereafter. Changing the bath [K+] from 4 to 150 mM shifted the Em from –80 to 0 mV (n=9), indicating that the Em of these cells is controlled largely by K+ conductance.

Chemical compounds know to block K+ conductance significantly reduced the Em. Bath application of 1 mM BaCl2 promoted a 20 to 50% reduction of the Em (n=4). Application of 10 mM TEA generated comparable inhibitory effects on the Em (~35 %, n=3).

The Em also is highly dependent on the availability of free [Ca^{2+}]. Depletion of Ca^{2+} from the bath solution gradually shifted the Em from –80 to –40 mV (n=3). Adding caffeine (10 mM) to the Ca2+free bath, which depletes the intracellular Ca^{2+} stores, produced a biphasic effects. The Em first returned to –80 mV, then shifted back to a more depolarized value of –30 mV (n=3).

These data demonstrate that the Em of type I fibrocytes is controlled mainly by a Ca^{2+}-dependent K+ conductance.

390 Demonstration of Type II Collagen in the Basilar Membrane of New Zealand White Rabbits Using Pre-embedding Immunoelectron Microscopy

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In a previous investigation, post-embedding immunoelectron microscopy was used to demonstrate the presence of collagen II and its arrangement in the fibrous component of the basilar membrane (BM) (Dreiling et al., 1999 Midwinter Research Meeting of ARO, Abstr. 303). The current study amplified and extended those results using pre-embedding immunoelectron microscopy. Scanning and transmission electron micrographs showed the arrangement of collagen II in the fibrous sheet of the pars tecta and in the two fibrous layers of the pars pectinata; isolated individual 10-12nm fibrils were immunolabeled. Since much of the ground substance surrounding these fibrils had been removed, these results reduce the likelihood that the collagen II antibody was reacting with some unknown molecule and giving a false positive. The likelihood of false positives was also reduced by use of a negative control and a primary antibody with extensive crossreactivity testing. Despite disruption of the BM to expose the epitopes, recognizable ultrastructure was maintained through the use of a gentle crosslink breakage technique, microscopic observations to regulate

epitope exposure, and a tissue preservation method that employed little or no fixation. The presence of collagen II in the fibrous layers of the basilar membrane places constraints on the biomechanical properties of this important structure.

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391 Characterization of Cultured Spiral Ganglion Cells

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Presbycusis, gradual loss of hearing with age, is a common disorder affecting as many as 50% of adults 75 years and older. Given its far-reaching consequences, there is an enormous amount of on-going research on presbycusis. Although cochlear cell culturing is an increasingly utilized technique, there have been minimal efforts to characterize the various cells found in these cultures. In spiral ganglion explant cultures, it is clear that there are many cells in addition to auditory neurons, including fibroblasts, epithelial cells, glial cells and occasional vascular elements.

Spiral ganglia were dissected from non- to partially-calcified cochleas from postnatal day 3-13 Sprague-Dawley rats and maintained in tissue culture for 4-10 days. After fixation with 4% paraformaldehyde, identification of neurons was accomplished by staining with OSO4/cresyl violet, or labeling by immunohistochemistry with monoclonal anti-neurofilament antibody, either anti-200kD (Sigma Aldrich) or clone NE-14 (BioGenex, Inc). Specimens were evaluated by light microscopy and photographed with the Spot 2 camera.

Minimal growth was observed in cultures grown for either 4 or 10 days, reflecting an insufficient or excessive number of days in culture respectively. Explants cultured for 7 days, on the other hand, demonstrated maximal cell dispersion and neurite growth. Despite trials of various dilutions, there was a significant amount of non-specific staining using either of the anti-neurofilament antibodies. Neurons were identified by the presence of a visible nucleus within a round soma, approximately 15x20µm. In contrast, fibroblasts were distinguished by their characteristic fusiform cell bodies, measuring 8x40µm. Moreover, cultures contained cells with numerous processes measuring 13x18µm, most likely glial cells. Furthermore, all cultures invariably contained large, 25x45µm, squamous accessory cells, yet to be identified.

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392 Afferent Innervation of Inner Hair Cells in the C57BL/6J Mouse

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Pathology at the synaptic interface between the inner hair cell and its afferent innervation may explain the sometimes poor correlation between measures of auditory function and cell loss in the organ of Corti and Spiral ganglion. Acute swelling and distortion of auditory nerve endings at IHCs are known to follow acoustic overexposure (Goulios, H. and Robertson, D. Hear Res. 11:327,1983), and denervation of the IHC by intact auditory nerve fibers has been demonstrated in Meniere's disease (Nadol, J.B. and Thornton, A.R. Ann Otol Rhinol Laryngol. 96:449, 1987). When normal innervation and afferent synaptic structure of IHCs are disturbed, signaling in the auditory nerve is also likely to be impaired. We present a structural analysis of IHC innervation in a young C57BL/6J mouse with normal hearing.

Serial section electron micrographs were taken through the afferent endings of four IHCs in the 8kHz region of a 2-month-old mouse, and analyzed using 3-D reconstruction methods. Two morphologically distinct types of endings are observed. Bouton-shaped afferents are small (1.50±1.16 μm^3), tend to be located at the base of the IHC, and are not contacted by efferent terminals. Folded endings are larger (4.87±2.38 μm^3), consist of two leaf-like components in close apposition with each other, and terminate higher on the IHC. The outer leaf receives an efferent synapse, whereas the inner leaf forms an afferent synapse with the IHC. One membrane feature likely to herald afferent synapses is a large electron-dense plaque that usually accompanies a presynaptic body. This structure is found in nearly all folded endings but only about half of the bouton endings. These preliminary data at 8kHz in a C57BL/6J mouse reveals some differences in the structure of IHC innervation compared to cats and humans, but the functional significance remains unclear.

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393 The Differentiation of Reciprocal and Spinous Synapses in the Mouse Organ of Corti

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The reciprocal synapse is the "simplest microcircuit," whereas the synaptic spine synapse is a "multifunctional integrative unit" for exchange of excitatory or inhibitory impulses in the visual and olfactory systems (Shepherd, 1996). The auditory organ, due to its seemingly simplistic innervation, is perceived only as a means for transfer of auditory signals to the brain. However, in the developing cochlea, complex synaptic networks (triadic, serial, converging) differentiate, involving inner hair cells, their afferent dendrites and olivocochlear terminals (Sobkowicz, et al., 1997). This work - in progress - was done on 9 postnatal day (PN) to 2 month cochleas. Reciprocal synapses differentiate between inner hair cells and afferent dendrites from 9 PN up. They were seen on the hair cell soma and spines that intussuscept the terminals in conjunction with the ribbon synapse. Inner hair cell spines: Olivocochlear synapses on inner hair cell spines are integral components of the triadic synapse in conjunction with the ribbon synapse. Around 14 PN, this synaptic input is translocated from the inner hair cell soma to its spinous processes and made at an increasing distance from the hair cell body. Synaptic spines of afferent dendrites: a) Axodendritic - in contact with efferent terminals. These postsynaptic spines occur in convergence with a ribbon synapse. They were identified in the adult; b) Somato-dendritic - in contact with inner hair cells. Mushroom-like protrusions covered by a distinct postsynaptic density and surrounded by a crown of presynaptic vesicles were observed in 14-16 PN cochleas. Spinous processes of olivocochlear terminals: They are formed as specialized spines, derive from synaptic terminals, and synapse on adjacent afferent dendrites, thus integrating their action. Reciprocal and spinous synapses in the cochlea provide a morphological substrate for local processing of auditory signals.

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394 Acoustic Trauma in Animals With and Without Outer Hair Cells

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The effect of acoustic trauma, known to produce hearing loss in normal animals, varies across subjects. The reason for this variability is unknown but some candidates are likely contributors. The outer hair cell (OHC), through active mechanisms, provides the exquisite threshold sensitivity. The response of the OHC to extreme suprathreshold sounds is unclear but they are likely targets of the damaging effects of noise trauma. The OHCs also receive innervation from the medial efferent system. Activation of both the crossed and uncrossed components of this system is known to produce protective

effects. The strength of the uncrossed efferent reflex is inversely correlated with the degree of threshold shift. To further study the effects of acoustic trauma we exposed two groups of guinea pigs to noise (2-20kHz) at 116 dB SPL for two hrs. One group was exposed to the noise only; the 2nd group was noise exposed after 14 daily injections of gentamicin (160 mg/kg). ABR thresholds, at 2, 8, and 16 kHz, were obtained at baseline, at the end of gentamicin dosing, 7 days post dosing, immediately after and 14 days after noise exposure. At the end of the study animals were euthanized and phalloidin-labeled whole-mounts of the organ or Corti were prepared. Gentamicin produced the predicted 40-50 dB threshold shift associated with OHC loss. At 14 days post noise exposure the threshold shifts in the gentamicin plus noise group were not significantly different from the noise alone group. The noise alone group, however, had much greater variability. Standard deviation values ranged from 15.7 to 9.2 dB across frequency in the noise alone group, the gentamicin plus noise group ranged from 9.7 to 3.4 dB. These results suggest that the OHC or related efferent system is a likely cause of the variability. Preliminary analysis of the morphological data shows results consistent with the physiology.

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395 Susceptibility to impulse noise trauma is different among species

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Noise-induced hearing loss is a common inner ear disorders, and individual variability of susceptibility to noise trauma is great. In addition, different species have different susceptibility to noise trauma. The most common sources of impulse noise trauma are firearms and industrial equipment, which generate high intensity noise levels. Counter et al (1993) found that impulse noise (160 dB SPL, 50 times) caused significant permanent hearing loss in rabbits. The present study further investigates sensitivity of different species including rat, mouse and guinea pig to impulse noise trauma. When rats were exposed to impulse noise at 160 dB SPL for 50 times the temporary threshold shifts (TTS) were from 40 to 70 dB at different frequencies immediately after impulse noise trauma (N=8), and there were 20 dB permanent threshold shifts (PTS) (4 kHz-40 kHz) at 4 weeks after impulse noise trauma. When mice were exposed to impulse noise at the same level and exposure time TTS were from 35 to 50 dB (6.3 kHz-40 kHz) immediately after impulse noise trauma (N=8), and PTS were 25 to 30 dB. In contrast, when guinea pig was exposed to 160 dB impulse noise for 50 times no significant TTS developed (N=5). Still no significant TTS (N=5) occurred after impulse noise at 160 dB 100 times. When exposure was increased to 200 times there was about 15 to 25 dB TTS immediately after impulse noise trauma but thresholds returned to normal values of pre-exposure at 24 hours after impulse noise trauma. Finally the impulse noise exposure was increased to 400 times at 160 dB SPLwhich resulted in a 20 to 50 dB TTS immediately after noise trauma, the extended impulse noise trauma caused PTS. Morphology of the cochleae was also analysed by surface preparation. Thus, susceptibility to impulse noise trauma differs between different rodent species and the guinea pig.

396 Influence of Pre-Existing Hearing Loss on Susceptibility to Subsequent Noise-Induced Hearing Loss

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The influence of a pre-existing hearing loss on susceptibility to subsequent noise-induced hearing loss (NIHL) is controversial due to the variety of etiologies of pre-existing hearing loss, the difficulty in determining the portion of a subsequent hearing loss that is strictly due to noise exposure, and the ethics of exposing human subjects to high noise levels. Thus, the goal of this experiment was to determine how the permanent NIHL from two successive noise exposures interact in an animal model of sensorineural hearing loss, the Mongolian gerbil. Several outcomes might be predicted from multiple exposures: (a) the hearing loss from one exposure might be an asymptote so that further exposure will not cause greater hearing loss or (b) the hearing loss from two exposures is greater than that from one exposure. In the latter case, a number of outcomes might occur, including an additive hearing loss in dB, an additive loss in intensity, or a less predictable loss. To examine the interaction of two noise exposures, gerbils were exposed monaurally at ages 6-8 months and 12-14 months to a 3.5 kHz tone at 113 dB SPL for 1 hour. Thresholds were determined at octave intervals from 1-16 kHz using auditory brainstem response audiometry. Significant permanent threshold shifts (PTS) occurred after the first exposure at 8 and 16 kHz, but no further PTS was measured after the second exposure. In contrast, no PTS was measured at 2 kHz after the first exposure but significant PTS was observed following the second exposure. PTS was measured at 4 kHz after the first exposure and further PTS was observed after the second exposure. No PTS was observed at 1 kHz following either exposure. Possible mechanisms underlying the growth of PTS with multiple exposures will be discussed.

397 Prenatal Exposure to High Levels of Glucocorticoids Increases Cochlear Susceptibility to Noise Exposure

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There is growing concern that prenatal exposure to excessive glucocorticoids (GC) may have deleterious effects on the development of various organs, including the nervous system. Exposure to high levels of GC in utero increases the susceptibility of rat cerebellar granule cells to oxidative stress and alters mitochondrial function and catalase activity. These findings suggest that prenatal exposure to GC may have long-term effects on the nervous system. To test the relevance of this hypothesis, an in vivo experimental model was designed where adult rats that had been exposed to GC in utero underwent acute noise exposure (6-12 kHz tone at 110 dB SPL for 4 hours), a stimulus inducing a moderate cochlear injury via an oxidative stress mediated mechanism. The auditory brain response (ABR) was measured before, immediately, 48 hrs and 4 weeks after the exposure. A quantification of hair cell loss was performed 4 weeks after exposure. The acute effects of noise trauma were the same in the rats exposed to prenatal GC and the control rats, as shown by equally increased ABR thresholds. However, the chronic effects (4 weeks after noise exposure) demonstrated significant differences where the rats exposed to GC showed minimal recovery, while the controls showed near complete recovery. In addition, there was massive outer hair cell (OHC) loss in the GC rats compared to minimal OHC loss in the controls. Administration of the antioxidant agent PBN during and after noise exposure minimised cochlear damage in the GC rats. These data support the hypothesis that alterations in the intrauterine environment may modify the developmental programme of fetal organs, inducing dysfunction later in life. Excessive prenatal exposure to GC increases the susceptibility of the cochlea to oxidative stress induced by noise overstimulation.

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398 The Effects of Underwater Low Frequency Sound on the Inner Ear

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Guinea pigs were exposed to underwater (UW), low frequency sound (LFS) using an Acoustic Traveling Wave Tube to determine inner ear damage response thresholds. Animals were subjected to 5 minutes of pulse wave stimuli at either 746, 1250 or 2500 Hz delivered at 3 intensities (160, 180 & 190 dB 1[mu]Pa)for each frequency. Auditory brainstem response (ABR), horizontal vestibuo-ocular (HVOR) gain and off-vertical axis rotation (OVAR) modulation sensitivity were measured before and 48 hrs post-UW noise. A separate group of animals, which were anesthetized and UW-exposed but received no sound, served as controls. Sound exposures at all 3 frequencies produced a dose-dependent response pattern across the 3 intensities with the 190 dB SPL exposure eliciting the largest decrease in VOR and the greatest auditory threshold shifts (TS). Post-exposure TS, HVOR and OVAR responses after 160 dB SPL were not significantly different than control values. At 746 Hz, TS for most ABR test frequencies were significantly elevated 10-12 dB at 48 hrs post-UW 190 dB SPL, whereas the 180 dB UW exposure caused significant TS only at the lowest ABR test frequencies. Post-UW TS were less after the 1250 Hz exposure and only minimally increased after 2500 Hz exposure. At 746 Hz, 190 dB casued substantial decreases in OVAR modulation sensitivity and HVOR gain. The effects on VOR responses for 180 dB SPL at 746 HZ were somewhat lower than the 190 dB exposure, and there was very little effect on VOR for animals exposed to 160 dB SPL. While the 1250 Hz exposure produced large redcutions in OVAR sensitivity for the higher sound intensities (190 and 180 dB), it was primarily for the 3 highest rotational table velocities. Litte effect was noted for the 160 dB and no sound controls. The 2500 Hz sound exposure may be less damaging. These data suggest that LFS delivered at the 3 test frequencies produced a dose-dependent effect on auditory and vestibular responses with 190>180>160 dB SPL.

399 Acute endolymphatic hydrops is induced by non-traumatizing low frequency stimulation.

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Prior studies have shown that low frequency sounds presented at high but non-traumatizing levels (90 to 105 dB SPL) induce temporary hyperacusis in humans and animals (Kirk and Patuzzi, Hear. Res. 1997; 112, 49). One explanation for this finding is that the basilar membrane operating point is disturbed by an endolymphatic volume change. This possibility was investigated through measurements made in the second cochlear turn of guinea pigs. Volume and flow markers tetramethylammonium or arsenic hexafluoride were iontophoresed into endolymph and marker concentrations were measured with ion-selective microelectrodes placed apically or basally to the site of iontophoresis. Changes of endolymph cross-sectional area and flow were derived from the measured time course data. Stimulation with 200 Hz at 115 dB SPL for 3 min was found to elicit substantial changes in recorded marker ion levels, consistent with a transient, pronounced increase in endolymphatic area (mean increase 32.6%, SD 19.7, n=11) and a sustained basally-directed displacement of endolymph (mean 0.33 mm at 3 mins post-exposure, SD 0.12, n=11). A number of controls were performed to exclude other interpretations of the observed marker concentration changes. Sound-induced changes of marker concentration, endocochlear potential and action potential (AP) thresholds to tones at 2, 4 and 8 kHz were compared over a range of exposure frequencies and levels. For 200 Hz stimulation, hypersensitivity of the AP was found to occur at stimulus levels below those inducing endolymph volume disturbances. These data confirm that non-traumatizing, low frequency stimulation can induce endolymphatic hydrops. However, the relationship to sound level suggests that endolymph volume changes may be the result, rather than the cause, of changes in transducer operating point.

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400 Lateral Wall Histopathology and Endocochlear Potential in the Noise-Damaged Mouse Cochlea

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The contribution of endocochlear potential (EP) changes to noise-induced hearing loss has not been well studied, although histopathology has been noted in the lateral wall. The present aim was to assess acute and chronic ultrastructural changes in the lateral wall and to correlate histopathology with EP magnitude and threshold shift as a function of post-exposure time in both temporary (TTS) and permanent threshold shift (PTS).

CBA/CaJ mice were exposed to octave band (8-16kHz) noise for 2 hrs at intensities from 94 to 116 dB SPL and evaluated 0 hrs to 8 wks post exposure. Functional tests included tone-pip evoked ABRs and EP measures in apical and basal turns. Cochleas were plastic-embedded and thick-sectioned for light-microscopic evaluation followed by ultrastructural analysis.

EPs in controls averaged 86 mV and 100 mV in apical and basal turns, respectively. 94 dB exposures caused 40 dB TTS at 24 hrs, without change in EP. After 112 and 116 dB, no ABRs were measurable at 0 hrs, and EP was significantly decreased: 21 mV and 27 mV in apical and basal turns after 116 dB. By 1 wk, EP in all groups returned to control values, although ABR thresholds were still elevated by at least 60 dB for 112-116 dB groups.

Acute morphologic changes at 112-116 dB included vacuolization and degeneration of Type II fibrocytes of the spiral ligament and intrastrial edema, with swelling of marginal cells, shrinkage of intermediate cells. Strial changes peaked at 24 hrs, when significant EP recovery had taken place. In the chronic state, 112-116 dB groups showed massive loss of type II fibrocytes and degeneration of strial intermediate and marginal cells, with drastic reduction in membrane surface area.

The results suggest that EP shifts do not contribute to TTS nor to PTS magnitude in the steady state. EP recovery in the face of significant strial degeneration may reflect decreased transduction current due to hair cell damage.

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401 Influences of hypoxia on noise-induced hearing loss

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Noise-induced oxygen level decrease in the cochlea is one of the mechanisms underlying noise-induced hearing loss (NIHL). During noise exposure the partial pressure of oxygen (pO2) in the cochlear perilymph could drop as much as 50%. It has also been reported that severe hypoxia alone (the arterial pO2 lower than 30 mm Hg) caused hearing loss. Our previous studies have showed that CO interacted with noise to cause hearing loss exceeding the summated loss caused by noise and CO alone. Though it is known that CO causes tissue hypoxia, it is still unclear about the influence of hypoxia on NIHL directly. This is important since hypoxia can happen under many conditions where noise pollution may also exist. It is known that heavy smokers may have arterial pO2 level lower than 90 mm Hg. Patients with obstructive lung disease may have arterial pO2 level lower than 60 mm Hg. Even in normal subjects, the arterial saturation of hemoglobin with oxygen has

been reported to drop to about 85% after an ascent from 1300 to 3500 meters. While this oxygen drop alone may not cause hearing loss, it may interact with noise to cause more auditory impairment.

In this experiment, rats were exposed to 14-kHz octave-band noise (105-115 dB SPL) for 4 hrs with normal air or low-oxygen air mixture. The lowest oxygen level in the air mixture was 10% that is the half of the normal level and is comparable to the oxygen supply at a height of 5500 meters. Auditory impairments were determined 4 weeks after the exposure. The hypoxic exposure alone at an oxygen level as low as 10% did not cause significant hearing loss. However, the combined exposure to noise and low-oxygen air mixture caused significantly more hearing loss than the noise alone. The results indicate that hypoxia can potentiate NIHL.

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402 Expression of hypoxia-inducible factor-1[alpha] in the noise-exposed 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.

We recently demonstrated that transcription factor, Activator protein-1(AP-1), is activated in response to the intense noise exposure in the guinea pig cochlea. We have suggested the direct involvement of ROS in the stimulation of AP-1/DNA binding.

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. One of transcription factor, hypoxia-inducible factor-1 alpha (HIF-1alpha) is known to respond to the redox state. This characteristics is similar to AP-1.

We observed the change of HIF-1alpha expression by noise-exposed guinea pig cochlea. The expression of HIF-1alpha significantly increased after intense noise exposure in the cochlear sensory epithelia and lateral wall tissues. These data suggested that HIF-1alpha is involved in the molecular mechanisms mediating noise-induced cochlear damage.

403 Expression of inducible nitric oxide synthase during noise stress and inhibition of mitochrondrial respiration by nitric oxide.

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Our previous work has revealed increased nitric oxide (NO) production in the cochlear perilymph following noise stress (Shi et al., ARO. 2001). Although it has been reported that inducible nitric oxide synthase (iNOS) can dramatically increase the amount of NO present in tissues following injury (Kubes, GUT. 47, 2000, 6-9) and that high levels of NO can result in cell injury by inhibition mitochondrial respiration (Brown GC, Biochimica et Biophysica Acta. 1504, 2001, 46-57), it is not clear if iNOS plays a significant role in the increased levels of NO observed following noise induced hearing loss and whether it causes hair cell damage. In this study, iNOS immunoreactivity in the lateral wall and organ of Corti was examined by confocal microscopy in normal mice and guinea pigs and compared with similar animals exposed to 110 dBA broadband noise, 3 hours/day, for three consecutive days. In the normal animals, weak iNOS expression was observed in the vascular endothelium, marginal cells, spiral ligament, nerve fibers, hair cell stereocillia and supporting cells of the organ of corti. More intense iNOS fluorescence signals were observed in cochlear tissues (particularly in hair cells and marginal cells) of the noise exposed animals. In a related study, we observed, a time course of reduction of mitochondrial membrance potential in isolated outer hair cells following exposure to various concentrations of the NO donor (NOC-7). These results provide further evidence suggesting that excessive NO production secondary to enhanced iNOS activity may be involved in the pathology of noise-induced hearing loss, particularly in the pathologically reduced OHC mitochondrial energy status.

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404 The effects of NMDA on the cochlear potentials

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The purpose of this study was to investigate the effect of NMDA on cochlear potentials, and to figure out the action of NMDA receptors on afferent dendrites in cochlea of guinea pigs. After basal compound action potentials (CAP) and cochlear microphonics (CM) were recorded by round window electrode, guinea pigs (n=10) were treated with Hanks applied to the round window membrane (RWM) for 20 min as control. Then 100 mmol/L NMDA was applied to the RWM for another 20-min. The result showed that Hanks produced no obvious changes in CAP threshold, CAP N1 and CM latency and amplitude. CAP thresholds at all frequencies were significantly elevated by application of NMDA, Threshold shifts ranged from 13-27 dB. NMDA significantly reduced the CAP N1 amplitudes at all intensities of stimulation. 50-75% CAP amplitudes were suppressed. NMDA also significantly increased the CAP latency, the latency of CAP evoked by 6 kHz tone-burst at intensity of –90 dB was (1.9±0.06) ms after Hanks treatment and (2.76±0.21) ms after NMDA treatment ($p<0.05$).NMDA did not significantly change the CM latency and amplitude evoked by all intensities of tone-burst. The result suggests that NMDA induced a neurotoxic effect on cochlea function and indicates that NMDA receptor expressed by the spiral ganglion neurons is involved in glutamate excitotoxicity.

405 Role for NMDA Receptors in Noise-induced Hearing Loss

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It has been suggested that N-methyl-D-aspartate (NMDA) receptors are involved in noise-induced hearing loss. We reported earlier that the non-specific NMDA receptor blocker MK-801 attenuates noise-induced cochlear lipid peroxidation and threshold shifts. We now assess the effect of the antagonist PD 174494 which is selective for the NR1/2B subunit of the NMDA receptor. (+)-MK-801 (1 mg/kg) or PD 174494 (10 mg/kg) were administered ip to guinea pigs 30 min before and 2 hr after the start of noise exposure (4 kHz octave band, 115 dB SPL, 5 hr). Levels of 8-isoprostane were measured in the cochlea immediately after noise exposure as a marker of lipid peroxidation. Permanent threshold

shifts (PTS) and hair cell loss were assessed 10 d following noise exposure.

PD 174494 as well as MK-801 attenuated the noise-induced increase of 8-isoprostane in the organ of Corti and modiolus but had differential actions on PTS and morphology. MK-801 protected against noise-induced PTS more effectively than PD 174494 at high frequencies. Interestingly, MK-801 reduced the loss of both outer and inner hair cells while PD 174474 protected inner but not outer hair cells.

These findings support a general involvement of NMDA receptors including the NR1/2B subtype in noise-induced lipid peroxidation and hearing loss. However, different subtypes of the receptor may contribute to different aspects of the pathology depending on their cellular localization or the toxic mechanisms they mediate.

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406 ROS as mediators of noise-induced hair cell apoptosis

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There is an increasing body of evidence implicating reactive oxygen species (ROS) in hair cell (HC) pathology. However, most of this evidence is indirect and not specific for any single species of ROS. We have attempted to develop approaches that provide a more direct assessment of ROS and to determine the role of ROS in apoptosis. In order to address this question we utilized the herbicide paraquat, whose mechanism of activation is well characterized. Paraquat (PQ) is reduced by endogenous NADPH to generate the PQ+ radical, which in turn, reacts with molecular oxygen to generate the superoxide radical. The superoxide driven Fenton reaction can then result in cellular toxicity through the formation of the highly reactive hydroxyl radical. We have incorporated the use of dichlorofluorescein (DCF) to measure total peroxide content, which reacts with hydrogen peroxide, lipid hydroperoxides as well as peroxynitrite to yield a fluorescent response. Apoptosis was assessed by the combination of propidium iodide incorporation and caspase-3 activation. Our results demonstrate that both continuous and impulse noise results in the dramatic increase in DCF fluorescence that continues over for at least four days. Secondarily, Paraquat was shown to mimic the effects of noise in that both treatments resulted in the induction of apoptosis as indicated by the incorporation of propidium iodide and caspase-3 activation. The addition of glutathione monoethyl ester prevented the DCF fluorescence and dramatically inhibited the apoptotic responses of HCs. We can conclude that superoxide may be the biologically relevant species generated which initiates the oxidative cascade of events leading to the formation of highly reactive forms of ROS and lipid peroxidation. Lipid peroxidation products such as 4-hydroxy-nonenal are known inducers of apoptosis and may provide the long-term basis for HC loss.

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407 Activation of multiple caspases in noise-induced apoptosis

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We have previously reported that intense noise exposure causes outer hair cell (OHC) death primarily through apoptotic pathway. Here we investigate the intracellular signal pathways associated with apoptotic OHC death. Chinchillas were exposed to a 4 kHz narrow band noise at 106 dB SPL for 1 hour. After the noise exposure, the cochleas were stained for the activation of either caspases-3, -8 or -9 with carboxyfluorescein labeled fluoromethyl ketone (FMK)-peptide inhibitors of these caspases. Some cochleas were also stained for detection of cytochrome c release from mitochondria to cytosol by immunohistology. The results showed that the noise exposure triggered activation of caspase-3, an important mediator of apoptosis. The noise exposure also caused the activation of caspase-8 and caspase-9, each of which is associated with a distinct signal transduction pathway that leads to activation of caspase-3. The activation of these caspases occurred only in the apoptotic OHCs, and not in the necrotic OHCs, suggesting the involvement of the caspase pathway in noise-induced apoptosis. The results also suggest that multiple caspase-3 activation pathways exist in the apoptotic OHCs. In addition to caspase activation, the noise exposure caused release of cytochrome c from mitochondria to cytosol, forming punctate fluorescence in the cytosol. In contrast to the activation of the caspases, the release of cytochrome c took place in both apoptotic and necrotic OHCs. Moreover, the release of cytochrome c also occurred in certain OHCs in the early phase of cell damage, suggesting existence of a common pathway shared by the apoptotic and necrotic cells before entering the phase of necrosis or apoptosis.

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408 Impulse noise induces a rapid hair cell death through apoptosis

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We have previously reported that the high level of continuous noise induces hair cell (HC) death primarily through the apoptotic pathway. Here we report that exposure to an impulse noise also cause apoptotic HC death in a similar fashion produced by the exposure to a continuous noise. Chinchillas were exposure to 50 pairs of impulses at 155 dB SPL. The cochleas were stained for examination of HC nuclei with propidium iodide either immediately or one hour after the noise exposure. Cochleas were also double stained for either activation of caspase-3 or release of cytochrome c from mitochondria to cytosol. The results showed that, immediately after the impulse exposure, damaged HCs showed early signs of apoptotic nuclei, such as slight nuclear shrinkage with increased PI staining. These apoptotic cells were focused in either a small section or scattered over the basal part of the cochlea. In contrast, nuclear swelling, which is a morphological feature of necrosis, was barely seen in the cochleas collected immediately after the noise exposure. One hour after the impulse exposure, the damaged area expanded toward both apical and basal parts of the initial damaged area. Swollen nuclei appeared in the damaged section of the cochlea. The detection of cytochrome c by an immunohistochemical method showed translocation of cytochrome c from mitochondria to the cytosol in the cochlea prepared immediately after the impulse exposure. Examination of caspase-3 activity showed activated caspase-3 in the apoptotic HCs. These results clearly indicate the apoptotic pathway is the primary driving force leading to HC demise at the early stage of the pathologic development of the impulse-induced cochlear lesion.

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409 Determinants of Response Latency in Spiral Ganglion Neurons

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Postnatal spiral ganglion neurons show a range of action potential latencies that vary with cochlear location. Neurons isolated from the apical cochlea display significantly longer latencies than those from the basal cochlea. To examine this relationship, we carried out sequential whole-cell voltage- and current-clamp recordings from the same neuron

and directly compared voltage responses with the underlying outward currents.

Spiral ganglia were dissected from 4-6 day old CBA mice and maintained for an average of 8 days in tissue culture. For statistical comparisons, recordings from 85 cells were divided into 3 similar size groups according to action potential latency. Mean values for brief, medium and long latency categories were 7.4 ± 0.4 ms, 11.6 ± 0.2 ms, and 19.3 ± 1.0 ms. Outward current magnitude and activation time course correlated with action potential latency; the time course of current inactivation, however, did not. Peak current amplitudes at -40 mV showed significant differences between the brief (0.46 ± 0.05 nA), medium (0.27 ± 0.02 nA), and long (0.11 ± 0.01 nA) latency categories ($P<0.01$). By fitting the current onset, we also found significant differences between the taus for the brief (0.74 ± 0.04 ms), medium (0.95 ± 0.05 ms), and long (1.31 ± 0.08 ms) latency categories ($P<0.01$). Although outward current inactivation showed significant heterogeneity within the population of spiral ganglion neurons, this parameter did not correlate with latency (brief = 259.4 ± 33 ms; medium = 200.47 ± 26.9 ms; long = 227.9 ± 50.7 ms).

Our findings suggest that the magnitude and activation rate of outward currents may produce the differences in response latency. Pharmacological experiments indicate that a cadmium sensitive, Charybdotoxin-insensitive current may be responsible for the rapid onset and inactivating components of the outward currents.

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410 Differential Distribution of Membrane-Associated Guanylate Kinases (MAGUKs) in Type I and Type II Spiral Ganglion Neurons

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The spiral ganglion is composed of type I and type II neurons that can be distinguished by their unique peripheral innervation patterns. Type I neurons compose the majority of the ganglion and form one-to-one synaptic connections with inner hair cells. Type II neurons make up a small percentage of the ganglion yet innervate many outer hair cells. These two cell types have also been shown to differ in cell size, shape, myelination and central innervation patterns. It is not surprising, therefore, that molecular markers have revealed additional distinguishing features of type I and type II spiral ganglion neurons. For example, specific neurofilament proteins, such as peripherin, are more abundant in type II neurons.

In order to identify additional, physiologically relevant markers that distinguish type I from type II spiral ganglion neurons, we utilized antibodies against MAGUKs that are responsible for targeting proteins to defined cellular regions. By clustering ion channels within restricted regions, these proteins are instrumental in establishing the basic polarity of a cell and forming specialized regions. We chose to investigate the role of one particular MAGUK protein, SAP102, because it binds specific ion channel proteins, such as Kv1.4, that could have a profound effect on neuronal firing features. We found that SAP102, like peripherin, was enriched in neurons that innervated the outer hair cells. This pattern of labeling did not differ substantially in cultures exposed to either brain-derived neurotrophic factor or neurotrophin-3, two neurotrophins present in the cochlea during development.

Our findings are consistent with the idea that distinct molecular markers can be utilized to distinguish type I from type II spiral ganglion neurons, and, furthermore, may provide insight into their functional differences.

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411 Characterization of Peripherin-Positive Spiral Ganglion Neurons

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The electrophysiological characteristics of type II spiral ganglion neurons are largely unknown because the small diameter of their axons makes in vivo recordings difficult to obtain. To elucidate the firing features of this class of neuron, we utilized an in vitro method to record from neurons identified with a marker that distinguishes type I from type II neurons.

Whole-cell current clamp recordings were made from mouse postnatal spiral ganglion neurons (P4-P6) maintained in culture for an average of 6 days. Lucifer yellow was included in our internal solution and only one recording was made in each culture dish as to ensure that the filled neuron was identified unequivocally. The culture was then fixed and stained with anti-peripherin, a marker that labels type II spiral ganglion neurons preferentially. Out of 24 recordings from apical neurons and 21 recordings from basal neurons, only two neurons, one from each category, were peripherin positive. These neurons were similar to one other when their firing patterns were compared. This is different from type I neurons which show firing features that vary with cochlear location. Basal neurons typically display brief latencies and rapid adaptation whereas apical neurons show larger latencies and slower adaptation. The putative apical type II neuron had a latency and adaptation rate (19.7 ms and 2spikes/240 ms depolarization) comparable to type I apical neurons. The peripherin-positive basal neuron also had a latency and adaptation rate (20.6 ms and 2 spikes/240 ms depolarization) similar to apical neurons and unlike its basal counterparts.

Although the sample size is too small to draw any conclusions at present, our working hypothesis is that type II neurons do not show the topological variation in electrophysiological response properties. Future studies are aimed at increasing the number of recordings from peripherin stained neurons to strengthen these conclusions.

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412 Hypoplasia of spiral and Scarpa's ganglion in GABAA receptor beta 3 subunit knockout mice

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This study documents morphologic alterations in the spiral ganglion and Scarpa's ganglion from GABAA receptor beta 3 subunit null mutant mice. The ganglion cells of the mutant mice were hypoplastic in H&E sections. Hypoplasia was observed at every location of the spiral ganglion and Scarpa's ganglion except the apical cochlear turn. Calretinin immunostaining demonstrated a selective hypoplasia of calretinin-negative cells at every location of spiral and Scarpa's ganglion cells, while the soma area of calretinin-positive cells was not affected by the gene deletion. Meanwhile, in the spiral ganglion of both wild type and knockout mice, there were apical to basal gradients in the soma size and the proportion of calretinin-positive cells. The absence of statistically significant hypoplasia in H&E sections through the apical turn of the cochlea can be explained by the relatively higher proportion of calretinin-positive ganglion cells, which were unaffected by the gene deletion. These findings suggest that GABAA receptor isoforms containing the beta 3 subunit may play an important role in the development and differentiation of non-calyceal terminals of Scarpa's ganglion cells and type II and smaller type I spiral ganglion cells.

413 Substance P depolarizes and mobilizes intracellular Calcium via Tachykinin (NK) receptors in rat spiral ganglion neurons

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Although ACh and GABA are supposed to be the principal neurotransmitters of the LSO system, efferent regulation of spiral ganglion neuron (SGN) excitability still remains largely unexplored, including other possible neurotransmitters involved. We have recently demonstrated that ATP and ACh activate, via a similar intracellular pathway, depolarizing non-selective cation conductance in SGNs (ARO2001, IFOS2001). Although substance P (SP) and other tachykinins have been shown to act as neurotransmitters in various neurons in peripheral and central nervous systems of mammals, the presence of functional NK receptors in SGNs has not yet been elucidated. The present study characterizes the effects of SP on isolated rat SGNs, using indo-1 spectrofluorometry and whole-cell patch-clamp recordings.

Under voltage clamp, brief application of SP (10 μM for 1 s) evoked a large depolarizing inward current in 56% of tested SGNs (n=57), averaging 611±434 pA at Vh = -50 mV. The I-V relationship displayed inward rectification at negative potentials and a reversal potential near 0 mV, indicative of a nonselective cation conductance. The SP-evoked current developed with a significant delay of 1.8±1.2 s, suggesting the involvement of metabotropic processes rather than direct activation of an ionotropic current. This response was not blocked by removing extracellular calcium, or with 10 mM intracellular BAPTA, a potent calcium-chelating agent, or by the application of U-73122, a potent blocker of PLC pathway. Interestingly, SP was also able to release calcium from intracellular store in a dose dependent manner (EC50 = 14.7 μM), which was sensitive U-73122, suggesting that the SP-current was not activated by the elevation of intracellular calcium. We conclude that SP can play a role of neuromodulator in SGNs, via calcium signals or via cation non-selective channels, possibly in a similar way as ACh and ATP.

414 Substance P Suppresses Potassium and Calcium Currents in Spiral Ganglion Neurons of Cochlea

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Substance P (SP), a member of the tachykinin family of neurotransmitters, may be a neurotransmitter/neuromodulator in the inner ear. SP agonists increase the amplitude of the compound action potential suggesting it may modulate the activity of type I SGNs through neurokinin I (NK1) receptors; however, the cellular mechanisms underlying this effect are unknown. To address this issue, whole-cell patch clamp recordings were obtained from SGN in cochlear cultures from P0-5, B10J mice. SGNs, identified from their anatomical position and round phase-bright soma, had a mean resting potential of -54±6 mV (n=65). Local application of SP (25 μM) induced a constant outward current in voltage clamp (-70 mV, n=10). Current amplitude increased when the cell was hyperpolarized and decreased when it was depolarized. High doses of SP (25 μM) appeared to affect voltage-gated K+ and Ca^{2+} channels. SP reversibly blocked voltage-gated K+ by up to 50 % (n=6) and Ca^{2+} currents by up to 25 % (3 of 7), but had no effect on Na+ currents (n=3). Under current clamp, application of SP caused a slight reduction in action potential (AP) amplitude elicited by a train of current pulses (200 μA, 0.8 ms) and a slight hyperpolarization (0.8 mV, n=2). Using RT-PCR, the mRNA for the NK1 receptor, which preferentially binds SP, was detected from whole cochlear samples from 3 and 8 week old mice. To determine the location of the receptor, an antibody against the NK1 receptor was used to identify the location of the receptors on SGNs. Thus, SP appears to act on NK-1 receptors on SGNs neurons where it modulates activity through voltage gated K+ and Ca^{2+} channels.

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415 BDNF promotes neurite extension *in vitro* in dissociated spiral ganglion cells of developmentally mature mice.

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Several neurotrophic and/or growth factors have been reported to promote neurite outgrowth from spiral ganglion cells *in vitro*. Most previous studies, however, have utilized neurons from developing or very early postnatal animals. We have investigated the influence of neurotrophic factors on neurite extension in spiral ganglion cells of mature mice. Dissociated mouse spiral ganglion cells (taken from C57 mice at P28-30) were cultured in Medium-199/10% Fetal Bovine Serum (M199/10%FBS), either alone or with 1 ng/ml, 10 ng/ml, or 100 ng/ml human recombinant brain derived neurotrophic factor (BDNF) or neurotrophin-3 (NT-3). Following 7 days in culture, neurons were fixed and immunocytochemically labeled with TUJ1. Images of labeled neurons were digitized, and the lengths of the neurites were quantified using Image J (NIH Software). Spiral ganglion cells that were cultured in M199/10%FBS alone had a mean neurite length of 710mm±285mm (±S.D.). Culture with 10 and 100 ng/ml BDNF resulted in increased neurite length. The mean neurite length in cultures treated with 10ng/ml BDNF was 1070mm±275mm ($p<0.005$), while cultures treated with 100ng/ml BDNF had neurite lengths of 1010mm±350mm ($p<0.05$). In contrast, 1 ng/ml BDNF and NT-3 at all concentrations did not affect neurite length. In other experiments, reverse transcriptase polymerase chain reaction (RT-PCR) was used to demonstrate that the secreted diffusible axon guidance molecules Netrin 1 and Semaphorin 3A are expressed in mouse organ of Corti, and that their respective receptors DCC and Neuropilin 2 are expressed in mouse spiral ganglion cells. We are currently investigating whether the direction of extending spiral ganglion cell neurites *in vitro* can be modified by treatment with these diffusible axon guidance molecules.

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416 Spiral ganglion neurons are protected from degeneration by GDNF gene therapy and electrical stimulation

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Perceptual benefits from the cochlear prosthesis are related to the quantity and quality of the patient's auditory neurons. Electrical stimulation (ES) of denervated auditory neurons has been shown to enhance survival of these cells. Glial cell line-derived neurotrophic factor (GDNF), has also been shown to increase survival of deafferented inner ear auditory neurons. In this study, we tested the cumulative effect of the GDNF transgene delivered by adenoviral vectors (Ad-GDNF) and ES on the spiral ganglion cells (SGCs) after aminoglycoside/diuretic-induced ototoxicity that eliminated the inner hair cells. Ad-GDNF group animals were inoculated with 5 μl (10^{10}pfu/ml) of a replication-deficient adenovirus with a human GDNF cassette in their left ear four days after the deafening. ES group animals were implanted with cochlear implant electrode in the left ear and stimulated (250Hz, biphasic, 100μS/ph, charge-balanced 100μA current pulses, for 36 days). Ad-GDNF+ES group animals received both Ad-GDNF inoculations plus ES identical to the other groups. Electrically evoked auditory brain stem responses were recorded from ES animals at

the start and end of the stimulation period to assure implant function. All animals were sacrificed 43 days after deafening and their inner ears prepared for evaluation of hair cell survival and SGC counts. In all three group all but a few apical inner hair cells were ototoxically eliminated. The left ears exhibited significantly higher SGC density than the non-treated (right) ears. GDNF and ES provided significantly better preservation of SGC density than either single treatment. The protective effects of Ad-GDNF + ES on denervated auditory neurons were additive, and indicate that the mechanism of GDNF protection is, at least in part, independent of the mechanism of protection afforded by ES.

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417 In Vitro Growth of Human Auditory Neurons

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The purpose of this study was to develop techniques for culturing human spiral ganglion cells (SGCs) in vitro and to assess the influence of neurotrophins on their survival and growth. SGCs were obtained during petro-clival meningioma surgery. With immunohistological studies, we also assessed the expression of neurotrophin receptors in human SGCs. This may lead to treatments for better cochlear neuronal reserve following deafness and improvements in the design of cochlear implants.

SGCs from human or rodent tissue were placed in 0.25 % Trypsin. Cell pellets were dissolved in medium containing neurobasal, B27, gentamycin and L-glutamine. Cells were incubated on Poly-DL-ornnithine coated culture plates. Various combinations of nerve growth factors, including BDNF, GDNF, NT 3, FGF1, and CNTF, were also added. In addition, human SGCs were fixed in 4% paraformaldehyde and immunolabeled with antibodies to Trk B or C, the high affinity receptors for BDNF and NT-3 respectively.

Cultured guinea pig SGCs showed growth of auditory neurons with stellate-like branching and usually with a distal cone-like terminal. The cells generally died after 11 days; terminals showed immunostaining for calbindin. The human SGCs also grew but were smaller than the cultured guinea pig auditory neurons and their growth arrested earlier. Human SGC survival and growth were positively influenced by neurotrophins and also expressed Trk B and Trk C.

This study shows that it is possible to grow isolated human auditory neurons in vitro, that human SGCs express receptors to neurotrophins, and that they are sensitive to these factors.

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418 Signaling by metabotropic glutamate receptors in cultured spiral ganglion neurons

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Besides activating ionotropic glutamate-receptors in neurons, glutamate also activate a variety of second messenger systems via G-proteins coupled metabotropic glutamate-receptors (mGluRs). Activation of mGluRs generates either postsynaptic potentials or intracellular second-messenger molecules. The excitability of postsynaptic neurons thus is directly or indirectly modified. Evidences have accumulated over the years supporting glutamate as the primary neurotransmitter released by hair cells. However, few experiments have examined the role of mGluR played in cochlear neurotransmission. In this work, we investigated the presence of various subtypes of mGluRs in the cochlea and studied their signaling roles in spiral ganglion neurons in cultures.

Eight major subtypes of mGluRs have been cloned so far, we first examined their presence in the cochlea by RT-PCR. All but mGluR2 were detected from cochlear total RNA, while all eight subtypes were amplified above detection level from the brain total RNA using the same PCR protocol. We next used antibodies against mGluRs to find out their cellular distributions in the cochlea. Our preliminary immunolabeling work localized mGluR8 and mGluR5 in a subset of spiral ganglion (SG) neurons, at cell membrane and nerve terminals respectively. Cultured SG neurons obtained from postnatal mice were used to examine the signaling roles of mGluR by monitoring the intracellular Ca++ concentrations ([Ca++]i) using the fura-2 ratio imaging technique. While kainic acid (300 μM) induced positive responses in all neurons tested, activating group I mGluRs by specific agonists DHPG (100 μM) or ACPD (100 μM) increased [Ca++]i in cultured SG neurons (n=16) isolated from P7 mice, but not from P0-P2 mice (n>20).

Our results suggested that mGluRs participate in cochlear neurotransmission. In another companion abstract, we will present data on roles of mGluRs in cochlear neurotransmission tested in vivo.

419 The different concentration of glutamate effect the mitochondrial membrane potential of spiral ganglion neuron of guinea pig

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Materials and Methods:Using specific fluorescent probe--JC-1 of mitochondrial membrane potential(MMP) to stain isolated spiral ganglion of guinea pig.Laser scan confocal microscope (Leica company)was chosed to collect fluorescent data. The changes of MMP resulted from foreign different Glutamate were examined with LSCM,using a 10¡Á20 object lense and linear mean.

Results:1. 10¡Á40 LSCM:Two-dimension and false three-dimension showed that in the base of peripherial and centeral process, green and red fluorecences is higher, especially in the base of peripherial processes of guinea pig.2. 10¡Á100 LSCM demonstrated that in somas of spiral ganglion cell of guinea, A number of green and red fluorescentes concentrated as groups or clusters.3.Different concentration of Glutamate did not influence the green and red density in isolated spiral ganglion cells of Guinea Pig preloaded with JC-1.

Summary and conclusion: 1.JC-1,as a specific fluorescent probe of MMP,can credibly reflect the changes of mitochodria under different condition;and can distinguish the heterogenity of mitochondria in same cells.2.In the spiral ganglion neuron of neurons, the distributions of low and high energy of mitochondria have their specific characters as SGN of rat. The mitochondria of SGN have powerful compensatory abitity and Ca^{2+} pool. 3.Different concentrations of Glutamate did not influence the MMP of spiral ganglion cells of Guinea pig. Neurotoxity of Glutamate mainly through NMDA. Mitochondria of spiral ganglion cells has powerful buffer ability to Ca^{2+} changes induced by Glutamate.

Key words: Spiral ganglion Glutamate receptor

Mitochondrial Membrane Potential

420 Can adaptation processes account for the responses of inferior colliculus neurons to dynamic interaural phase-modulated stimuli? Predictions based on phenomenological models.

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Many low frequency inferior colliculus (IC) neurons are sensitive to the interaural phase disparities (IPDs) of low-frequency sounds, including the dynamic IPD cues of interaural phase modulation (IPM). Neuronal responses to IPM are shaped by the immediate response history of the neuron, such that they are sensitive to the direction of the IPM. One explanation for this is that IC neurons are subject to adaptation-of-excitation. Previously, we described a method by which binaural adaptation in IC neurons can be assessed. Here we use this technique to provide empirical observations in order to generate phenomenological models of binaural adaptation. Responses of IC single neurons were recorded to dichotic tones presented through a sealed sound system. A 3s continuous dichotic tone containing variable IPDs was used to assess the time course of binaural adaptation. Tone IPD, fixed at worst IPD for 1s, was ramped rapidly (1-10ms) to a range of more favourable IPD values for 1s, and then ramped rapidly back to worst IPD. Neural responses to the epoch at each favourable IPD were fitted with exponential decay functions. The initial and steady state firing rates and the time constant of the decay functions were determined as a function of IPD. These were used in basic mathematical models to test the hypothesis that differential sensitivity of IC cells to IPM stimuli is due to adaptation-of-excitation. Two forms of adaptation model were implemented. An “additive” model derived from the assumption that adaptation results from post-synaptic membrane channels, and a “multiplicative” model derived from the assumption that the adaptation results from pre-synaptic depression. The multiplicative model accounted for a greater percentage of the variance of the data, and fitted well the responses obtained with IPM. The additive model fitted the data only poorly. These data suggest that the response to IPM is predictable from the response to static IPDs plus a mechanism of adaptation.

421 Responses of Neurons in Cat Inferior Colliculus to Bird Calls in Natural Noise

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This study concerns responses to bird chirps in a noise background consisting of broadband noise and echoes of the chirp. In auditory cortex, responses evoked by the noise components presented alone were sometimes more similar to the responses to the natural sounds than were the responses to the isolated main chirp (Nelken and Bar-Yosef ARO Abst. 1999). This was so even though the chirp was near BF and the noise had less energy. This result implies that some cortical neurons are monitoring the background and not the foreground signal. In contrast, in the ventral cochlear nucleus responses to the natural sound and the main chirp are more similar to each other than to the noise responses, consistent with the expectation from the units' tuning curves. In this poster, we describe responses to the same stimuli in the inferior colliculus (IC). Four natural bird chirps were separated into a main chirp and the accompanying noise; the noise was then further divided into echoes and wideband backgrounds. Neurons were tested with the whole stimulus (natural), main chirp, noise, echo, background, and combinations of these. The stimuli were presented at their nominal-sampling rate and at rates sufficient to present the chirp at the BF of the unit. When the chirp was located away from BF, responses evoked by the noise components were more similar to the responses to the natural sounds than were the responses to the chirp. However, when presented at the neurons' BF, the response to the main chirp was more similar to the natural sound. These results suggest that IC neurons responses are determined by the stimulus components within their response maps, without the background responses seen in the cortex.

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422 Response Properties of Neurons in the Superior Paraolivary Nucleus of the Rat

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The superior paraolivary nucleus (SPON) of the rat contains 2,500 GABAergic neurons that project to the inferior colliculus. This nucleus receives its major inputs from the contralateral posteroventral cochlear nucleus and the (contralaterally driven) ipsilateral medial nucleus of the trapezoid body (MNTB). To date, only fragmentary information is available about the physiological properties of rat SPON neurons.

We are investigating the monaural or binaural activation of rat SPON neurons and their responses to broad band noise, pure tones and amplitude modulated tones. We present here data from 68 units histologically localized to the SPON by lesions or deposits of biocytin. Best frequencies (BFs) of localized units were topographically organized within the nucleus (high BFs medially; low BFs laterally) and spanned virtually the entire hearing range of the rat (1-37kHz). The vast majority of units were monaural (66 of 68) and responded only to contralateral stimuli. Nearly all units were inhibited during a broad band noise or pure tone stimulus and responded only at the sound offset (65 of 68). SPON neurons had offset responses throughout their response maps, however occassional onset spikes were observed in response to high intensity, low frequency combinations. The latency of the offset response was variable, but there was a trend for shorter latencies following longer tones. Thus, the main response of SPON neurons is inhibition during sound stimulation followed by a rebound from inhibition. In contrast, SPON neurons phase-locked strongly to amplitude modulated BF tones up to 200Hz (mean r = 0.77). We presume the inhibition during sound stimulation arises from the glycinergic neurons of the MNTB. To explore this possibility, we are beginning to record from SPON units using multi-barrel electrodes in the presence of strychnine, a glycine receptor antagonist.

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423 Periaqueductal Gray and the Paralemniscal Region control echolocation calls and communication calls differentially in the bat Phyllostomus discolor.

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The periaqueductal gray and the paralemniscal region in the brain of the bat Phyllostomus discolor (Wagner, 1843), seem to differ in their functional involvement of vocal control. While electrical and pharmacological microstimulation in the periaqueductal gray as part of the vocal pathway trigger both echolocation calls and communication calls, the paralemniscal region seems not to be involved in the control of communication calls, but rather represents an exclusive specialization towards the control of echolocation calls. Control of respiration is similarly influenced by microstimulation with kainic acid in periaqueductal gray and paralemniscal region, pointing to a convergence of both areas on the final common pathway for respiration.

Combined experiments with electrical microstimulations, electrically induced lesions and iontophoretically applied kynurenic acid indicate that the paralemniscal region might be an important relay station for echolocation calls only, triggered in the periaqueductal gray.

Possible differences in pathways, responsible for a separate control of echolocation calls and communication calls will be discussed on the basis of our data.

424 In vivo intracellular recording from owl's laminaris neurons using coaxial glass electrodes.

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The owl's nucleus laminaris (NL) neurons detect interaural time differences (ITDs) with an accuracy of few tens of microseconds by acting as coincidence detectors of binaural inputs. However, the biophysical mechanisms involved in this process still remain to be elucidated. In vivo intracellular recording in this nucleus is notoriously difficult. To overcome some of the difficulties, we developed a coaxial glass electrode that consisted of an inner sharp electrode (resistance of 70-100Mohm) for recording and an outer patch-shaped electrode as a guide. We recorded 28 binaural and ITD sensitive cells. Some of these were outgoing axons of NL neurons, because injection of positive or negative current did not change their excitability. In 15 out of the 28 cells, current injection from the recording electrode changed the cell's excitability. Some cells showed outward rectification against step current pulses around resting membrane potential. This outward rectification diminished when the cell was hyperpolarized with a negative current. Resting membrane potentials of NL neurons ranged from –40 to –65 mV and spike height ranged from 15mV to 6mV. The width of spikes at half height was about 400-500us. In some cells, favorable ITDs and not unfavorable ITDs induced small oscillatory potentials whose frequency was similar to the stimulus. This oscillatory component is likely to be postsynaptic potentials. These lines of evidence indicate that we have achieved in vivo intracellular recording in the owl's nucleus laminaris.

425 Morphological Analysis of Hindbrain Time Coding Nuclei in Three Species of Birds

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Bird species with complex vocalization systems are widely used as models to study auditory computation. Auditory psychophysical tests of small birds such as budgerigars, zebra finches, and canaries show that they share a high degree of both spectral and temporal resolving ability. Birds appear to be almost three times more sensitive than humans to changes in temporal fine structure. In sound localization experiments canaries have similar localization abilities as budgerigars, but zebra finches are about three times worse than both of the other species. Temporal coding precision must have its substrate in the central auditory system of these birds. While complex auditory processing should take place in higher neuronal centers, the temporal features of the stimulus must be faithfully preserved throughout the pathway to ensure precise computation. We have taken a bottom up approach to this problem by analyzing the neurons and synaptic organization of the auditory hindbrain in zebra finches canaries and budgerigars.

The overall organization of the cochlear nuclei was comparable in these three species. We found, however, differences in the expression of calcium binding proteins. Both zebra finches and canaries, but not budgerigars, show high expression of calretinin in all auditory nuclei. Budgerigars express parvalbumin in all three auditory nuclei and calbindin in nucleus laminaris. In addition, we found differences in the morphology of cells in nucleus laminaris. While the overall organization of the auditory circuit is fundamentally similar, small differences in the components may contribute to the precision of temporal coding along the brainstem temporal axis.

426 Organization of Nucleus Laminaris in Different Species of Birds

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427 Bilaterally Projecting Cochlear Efferent Neurones in the Barn Owl's Brainstem

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The efferent innervation of the inner ear is assumed to play an important role in many physiological and psychoacoustical phenomena in vertebrate hearing. While most of the studies of the auditory efferent system deal with mammals, birds offer a promising model for comparative studies. Schwarz et al. (1992) showed that there are bilaterally projecting efferent neurones in the chicken. However, other studies using similar techniques (e.g. Cole & Gummer, 1990) did not find any bilateral cells, nor were different species of birds tested. In our experiments, two potent retrograde tracers were injected through the round window into the scala tympani of anaesthetised barn owls and chickens. Choleratoxin was applied to one, Fluorogold to the other ear. The injection site was sealed with gelatine. Five to 7 days later the birds were sacrificed with an overdose of pentobarbital and perfused with 4% paraformaldehyde in phosphate buffer. Cross-sections of the brainstem were cut on a cryostat and processed imunochemically to visualise the tracers. Cells labelled with both tracers, indicating bilateral projections, were observed in both barn owls and chickens. Preliminary analysis suggests no significant differences in the relative contributions of bilaterally projecting cells to the auditory efferent systems in the two species. An important finding was that all the double-labelled cells were located in the ventrolateral group of efferents. This group most likely represents exclusively auditory efferent neurones supplying the basilar papilla (and not the vestibular lagenar macula).

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428 Innervation of Cat MSO.

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The cat medial superior olive (MSO) receives substantial bilateral excitatory innervation from spherical bushy cells of the cochlear nucleus (CN). It is also contacted by inhibitory neurons of the medial and lateral trapezoid nuclei, which convey bilateral inhibitory inputs (NB Cant, Chapt. 5 in Neurobiology of Hearing, 1992). Our goal is to quantify convergence and divergence of neural inputs to MSO neurons. To this end, we have determined the number and location of cells making these projections by applying small quantities of tract tracer to the MSO and the trapezoid nuclei. Also, we measured the density of MSO neurons along its tonotopic axis by using unbiased stereology procedures.

The cat MSO contained approximately 6700 neurons, 4400 of which were located in isofrequency laminae below 4kHz. Click-evoked potentials were used to locate the longitudinal axis of the MSO. Focal injections of biotinylated dextran amine (BDA) in two animals were confined entirely to the cell body layer below 4kHz and labeled a small number of spherical bushy cells in each CN (up to 70). In one case the injection was centered just medial to the MSO axis and labeled cells primarily in the contralateral CN. Neurons in the MNTB, main LNTB and posteroventral (pv)LNTB were retrogradely labeled. MNTB cells were located as far as 500um rostral or caudal to the injection; LNTB cells as far as 1.5mm, including pvLNTB cells at the caudal pole of the superior olive. Anterogradely labeled MNTB cell axons extend over 600um in the MSO, consistent with retrograde labeling. Therefore, these cells may set up inhibitory delay lines that match excitatory innervation.

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429 Pharmacological Modulation of Superior Olivary Complex Ionic Conductance

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Temporal and binaural processing of sound information in the central auditory system is expected to be shaped by ionic conductances, many of which can be modulated by second messenger systems. Deficits in temporal processing ability in the auditory system may underlie the pathogenesis of presbycusis and language learning deficits in children. Pharmacological manipulation of cyclic AMP cascade and ion channels was examined by pressure ejection of pharmacological agents from piggy-back electrodes. Forskolin, an adenylyl cyclase activator, produced a dose-dependent (10 uM and 50 um) increase in the acoustically evoked responses and spontaneous firing rate of SOC neurons, possibly due to cAMP induced direct or indirect modulation of I_h and I_t. Application of ZD7288 (50uM and 0.1mM), a specific blocker of I_h, decreased the rate of neuronal firing and decreased the acoustically evoked responses of SOC neurons. During sequential characteristic frequency tonal stimuli, ZD7288 produced a greater reduction of responses to the second tone, when presented at longer compared to shorter intertone intervals. The excitability and temporal processing of central auditory neurons are affected by cyclic AMP-dependent mechanisms, and through ion channels such as I_h, may be important in modulating excitability and temporal processing in auditory neurons of SOC. Greater effect of ZD7288 on second tone was on one with greater interval in the trial of inter-tone interval dependent suppression of responses to the second of sequential characteristic frequency tonal stimuli.

430 Synaptic Response Pattern and Pharmacology in Neurons of the Ventral Nucleus of the Lateral Lemniscus

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Our previous studies have shown that neurons in the ventral nucleus of the lateral lemniscus (VNLL) can be classified as onset, regular, onset-pause, adaptation and burst types by their firing patterns in response to depolarizing current injection (Wu, 1999; Zhao and Wu, 2001). Electrical stimulation of the lateral lemniscus (LL) can elicit excitatory and/or inhibitory postsynaptic potentials (EPSPs and/or IPSPs) in all these types of neurons. To further understand the physiological role of different VNLL neuron types, in this study we investigated whether distinct types of neurons display certain patterns of synaptic responses. We also studied putative neurotransmitters and receptors in the VNLL. Brain slices of 400 microns were taken in frontal plane through the VNLL of young rats (14-27 days old). Intracellular recordings were made from 40 VNLL neurons. The LL just ventral or medial to the VNLL was stimulated. Onset neurons (n=8) showed brief IPSPs (10-15 ms), some of which truncated the following EPSPs. Regular neurons (n= 9) showed mostly EPSPs with a wide range of duration (10-60 ms). Synaptic responses of adaptation neurons (n=15) were characterized by long duration EPSPs (30-80 ms). Some of these neurons also had an early brief IPSP (10-15 ms). Most pauser neurons (n=6) had IPSPs with duration of 10-30 ms. Bursting neurons (n=2) showed EPSPs with a long duration (30-90 ms). These results indicate that different VNLL neuron types are associated with different patterns and time course of synaptic responses. Also, 5/40 cells (one regular and four adaptation neurons) displayed double-peaked EPSPs with an early fast component followed by a late slow one, suggesting possible presence of both AMPA and NMDA receptor mediated synaptic excitation in VNLL neurons. IPSPs in one adaptation and two onset neurons tested were all blocked by strychnine, indicating that glycine acts as an inhibitory neurotransmitter in the VNLL.

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431 Origin of Pontine Noradrenergic Projections to Cochlear Nucleus

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The origin of noradrenergic neurons in the pontine tegmentum that project to the cochlear nucleus was determined with retrograde tract-tracing combined with neurotransmitter immunohistochemistry in cat. Double-labeled neurons were observed in all noradrenergic cell groups, in both the dorsolateral and ventrolateral tegmentum. Half of the double-labeled cells were located in the locus coeruleus complex. Most of these were situated in its ventral division. Most other double-labeled cells were located in peri-brachial regions, especially lateral to the brachium conjunctivum. Relatively few double-labeled cells were observed in both the A4 and A5 cell groups, 2% and 0.4%, respectively, of the total. Except for neurons in A5, which projected only contralaterally, the projections were bilateral with an ipsilateral preponderance. The results indicate that neurons located in the ipsilateral dorsolateral tegmentum, namely in the locus coeruleus complex and the peri-brachial region, are the primary source of pontine noradrenergic afferents to the cochlear nucleus of the cat.

432 Do Cortical Projections Contact Both Ascending and Descending Brainstem Auditory Pathways?

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Cortical projections to the inferior colliculus (IC) are in a position to modulate a variety of brainstem auditory circuits. The preceding abstract (Coomes and Schofield, '02; ARO Abstr.) demonstrates that cortical axons are likely to make direct contacts with the IC cells that project to the thalamus. The IC also projects to lower auditory nuclei, including the cochlear nuclei (CN) and the superior olivary complex (SOC). These descending projections arise from different cells than those that project to the thalamus (Schofield, '00, ARO Abstr. 23:35). The present study examines whether the cortical axons also contact IC cells that give rise to descending projections in guinea pigs.

We labeled cells that project to the ipsilateral or contralateral CN, or to the ipsilateral SOC, by injecting various fluorescent tracers (FluoroRuby, Fast Blue, or red or green fluorescent microspheres) into these targets. In the same animals, we injected a different tracer (fluorescein dextran or FluoroRuby) into temporal cortex to label corticocollicular axons. We then examined the IC ipsilateral to the cortical injection for apparent contacts between labeled cortical axons and labeled IC cells. We observed apparent contacts between cortical axons and IC cells that project to each of the 3 lower centers: ipsilateral CN, contralateral CN, or ipsilateral SOC. The contacts were observed frequently in both the ICd and ICx.

While the ascending and descending projections from the IC originate from different populations of cells, our results indicate that cells in both populations probably receive direct synaptic inputs from cortical axons. We conclude that cortical projections are in a position to modulate both ascending and descending pathways from the IC, and thus to play a major role in brainstem auditory processing.

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433 Cortical Projections to the Inferior Colliculus in Guinea Pigs: Do They Contact Ascending Pathways?

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The inferior colliculus (IC) is the target of a large descending projection from auditory cortex. The IC is also a source of projections to many brainstem nuclei. An important question to address is which of these projections are contacted by cortical axons. Physiological studies (Mitani, et al., '83, Neurosci. Lett. 42:185-189) suggest that cortical axons contact IC cells that project to the medial geniculate body (MG). The present study uses anterograde and retrograde tracing techniques to examine the corticollicular projections and to determine whether the cortical axons are likely to contact colliculo-geniculate cells.

Fluorescein dextran was injected into temporal cortex to label corticocollicular axons and FluoroRuby or Fast Blue was injected into the MG to label colliculo-geniculate cells. The corticollicular projection terminates bilaterally. Ipsilaterally, there are dense terminations in the dorsal (ICd) and external (ICx) cortices, and light terminations in the central nucleus. Contralateral projections are much more sparse and appear to be restricted to the ICd and ICx.

We examined the IC ipsilateral to the cortical injection for apparent contacts between labeled cortical axons and colliculo-geniculate cells. We observed many apparent contacts with IC cells that project to the ipsilateral MG. With less frequency, we also observed axons that appeared to contact IC cells that project to the contralateral MG. In all cases, the contacts were observed in either the ICd or ICx.

The results indicate, first, that corticocollicular projections in guinea pigs are similar to those described in other species. Further, the cortical projections are likely to modulate the ascending auditory pathways via direct synaptic contacts onto IC cells that project to the thalamus. It will be important to determine whether cortical projections contact other IC projections or selectively target the ascending auditory pathway.

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434 Analysis Of Spatial Patterns In The Ongoing Activity After Cortical Microstimulation

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In the development of neuroprosthetic devices, electrical microstimulation in the depth of sensory neocortex might provide a promising alternative to peripheral nerve stimulation (Breindl 2000, Norman 1999). Spatial patterns in the ongoing activity of auditory cortex have been shown to reflect perceptual relevance in acoustic learning parardigms (Ohl 2001).

Here we study patterns of ongoing activity induced by direct electrical microstimulation in the depth of auditory cortex in the Mongolian gerbil.Using multivariate classification of multichannel EEG maps, we demonstrate the existence of spatial patterns seconds after stimulus presentation, which reflect an animal's stimulus discrimination as it develops with the history of learning. It is these patterns a neuroprosthetic device has to interact with in order to yield perceptually relevant stimulation (Deliano et al. 2001).

Breindl (2000) Dissertation, O.-v.-Guericke-University of Magdeburg, Germany.

Deliano et al. (2001) ARO Abstract #22023.

Normann et al. (1999) Vis. Res. 39:2577.

Ohl et al. (2001) Nature 412: 733-736.

435 The role of gerbil auditory cortex in discrimination learning of amplitude modulations

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Periodic amplitude modulations (AM) are characteristic for animal communication sounds, speech sounds and music, and within an appropriate frequency range elicit the perception of periodicity pitch. It has been shown for a number of species that whereas bilateral ablation of auditory cortex (AC) seems to have little effect on the discrimination of simple stimuli like pure tones, it does impair the discrimination of more complex stimuli (like frequency modulations (FM), cf. Ohl et al. 1999, Learn & Memory 6:347-362). Furthermore, it was shown by unilateral AC ablation that right but not the left AC is essential for FM directional discrimination in gerbils (Wetzel et al. 1998, Neurosci Lett 252:115-118). Here we examine the role of auditory cortex in discrimination learning of AM tones. Learning behavior and performance were studied in a footshock-motivated shuttle-box avoidance GO/NO GO paradigm. Because of the known difference in discrimination learning performance (Schulze & Scheich 1999, Neurosci Lett 261:13-16) and cortical processing (Schulze & Langner 1997, JCPA 181:651-663) of AM tones with low (< 100 Hz) or high (> 100 Hz) modulation frequency (fm), discrimination was investigated using two sets of AM tones (20 vs. 40 or 160 vs. 320 Hz fm; carrier: 2 kHz). Bilateral ablation of AC had severe effects on AM discrimination learning: Lesions in both naive and pre-trained animals led to almost identical CR+ and CR- rates, that is, in contrast to sham-lesioned animals, animals with bilateral cortex lesion were not able to discriminate AM tones. In most cases this was due to an increased CR- rate without effect on the CR+ rate, indicating that animals were still able to detect the stimuli but not to discriminate them. Unilateral ablation experiments in naive animals showed that neither left nor right

AC lesion impairs discrimination of AM tones with low fm, whereas discrimination of AM tones with high fm was selectively impaired by left but not right AC lesion.

436 Neurodynamics In Auditory Cortex During Category Learning

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We investigated cortical neurodynamics as a potential basis for auditory category learning (concept learning). During category learning equivalence classes of meaning (categories) are established over stimuli that enable an organism to adequately respond even to novel, previously unfamiliar, stimuli that it encounters.

Using a recently developed experimental paradigm we trained Mongolian gerbils (Meriones unguiculatus) to discriminate frequency-modulated (FM) tones with respect to their modulation direction (i.e. 'rising' vs. 'falling'). The training can be conducted such that the animals form the respective concepts of 'rising' and 'falling', respectively, and transfer the concept to novel, previously unheard, FM tone stimuli (Wetzel et al. 1998, *Behav. Brain Res.* **91**, 29-39) after a behavioral state transition.

Since the FM tone discrimination learning has been demonstrated to be dependent on auditory cortex (Ohl et al. 1999, *Learn. & Memory* **6**, 347-362) we have studied the potential neural correlates of the behavioral state transition using multichannel recording of epidural electrocorticograms. State space analysis of spatio-temporal activity patterns revealed that the initial response components to FM tones were predominantly determined by physical stimulus features and anatomical connectivity patterns (Ohl et al. 2000, *J. Neurophysiol.* **83**, 3123-3132). Later response components showed transient epochs (several 10s of ms) of clustering into particular subregions of the state space after discrimination learning. Moreover, after the transition to categorization behavior the state space representation of these clusters reflected the perceptual scaling exhibited by the animals in their behavioral category seletion (Ohl et al. 2001, *Nature* **412**, 733-736).

437 New opportunities for zoological and auditory research provided by an outbred strain (Ugoe:MU95) of wild Mongolian gerbils (*Meriones unguiculatus*, Milne-Edwards 1867).

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Mongolian gerbils are one of the mammalian species best suitable for auditory research. Offspring of 20 pairs trapped in 1935 and distributed world-wide by Tumblebrook Farm Ltd (strain Tum:MON, referred to as "laboratory gerbils") underwent genetic bottlenecks, e.g., 9 specimen in 1954. Domestication is indicated by 17-18 % lower brain weights compared to wild specimen trapped in 1995 (Stuermer et al. 1997, Soc. Neurosc. Abst. 23, 2067). A new breeding line of defined geographic origin and high genetic diversity was established by wild gerbils shipped to Germany. Here, we compile basic data on the new strain Ugoe:MU95 ("wild gerbils") now available for auditory and zoological research.

Founder animals were collected near 47° N, 105,5° E. Out of 72 gerbils mated, 30 pairs gave birth to litters. Offspring were weaned after 1 month and mated as F-1 (F-2, F-n) following an outbred scheme designed by the Aachen University of Technology. In contrast to the low microsatellite variation in laboratory gerbils, genetic diversity in Ugoe:MU95 fall in the range of other wild rodent populations (Neumann et al. 2001, J. Hered. 92, 71-74).

Breeding of the wild strain as well as auditory and neurophysiological experiments did not demand special requirements. Stimulus-induced activity in the auditory cortex was mapped in Ugoe:MU95 using Fluoro-2-Desoxyglucose, and animals performed well in Shuttle-Box and Open-Field tests. Intratesticular testosteron concentration in Ugoe:MU95 was dramatically reduced (Blottner et al. 2000, J. Zool. 250, 461-466). Histology revealed microlesions in the cochlear nucleus (CN), but domestication affected the size of CN subdivisions (Gleich et al. 2000, JCN 428, 609-615). Seizures frequently seen in laboratory gerbils were absent in the wild gerbils (F-0) and rare in their offspring. Life spans of 3 to 5 years in Ugoe:MU95 provide additional opportunities in aging research.

438 Intermediate Responses between Auditory N1 and Mismatch Field Activated by Pitch Glides

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Pitch glide of a continuous tone activates auditory N1-like responses. We presented five types of continous tones which intermittently glide thier pitches (to subject's both ears) and measured their magnetic responses (Neuromag-122). N1m, P2m-like responses were elicited after onsets of all rapid pitch glides. However, as the rate of pitch glide decreases, the latency of the responses became as long as those of mismatch field (MMF), source locations of them shifted anteriorly from the regions of true N1m up to those of MMF. These results strongly suggest that the response continuously varies the characteristics from true N1m to MMF as a function of the pitch glide rate, and there exist intermediate responses between auditory N1m and MMF.

439 Representational Plasticity of Marmoset and Human Vocalizations in Rat Auditory Cortex

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We have recently reported that differential sensory experience with elemental tonal stimuli can substantially alter spatial and temporal responses in primary auditory cortex (A1) using a single paradigm that eliminates task-specific variables (Kilgard et al, 2001). A primary goal of our ongoing research is to extend these studies by documenting how experience with complex stimuli directs plasticity in A1. To accomplish this, we continue to employ activation of the basal forebrain to engage neural plasticity mechanisms.

To extend our previous studies with simple stimuli, we are beginning to investigate how long-term experience with different spatiotemporal input patterns alters distributed cortical responses in rat A1. A marmoset twitter call (n=5 rats) and the human vocalization 'sash' (n=7 rats) were repeatedly paired with electrical activation of the basal forebrain ~300 times a day for one month and were compared to naïve controls (n=6 rats). The cortical representation of tones, modulated noise bursts, and vocalizations was obtained by mapping multiple unit responses from microelectrode penetrations in each animal during an acute experiment using barbiturate anesthesia. For each recording site (40-80 sites/rat), we determined the excitatory frequency-response area, repetition rate transfer functions, and the response to vocalizations. After extensive experience with vocalizations, population analysis suggests a temporal sharpening of the cortical responses. Our results

support a learning hypothesis in which intensive and focused exposure to complex acoustic signals can increase the fidelity of neuronal representations and generate a more temporally coordinated distributed cortical neuronal network response.

440 Auditory Enrichment Enhances Evoked Potential Amplitude in Rat Auditory Cortex

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Prior research has demonstrated that environmental conditions influence cortical development. In this study, we characterize the effects of enrichment on evoked potentials in rat auditory cortex and document the time course of these changes.

After weaning at one month of age, eight rats were chronically implanted with a ball electrode over AI and a ground screw over the cerebellum. Each rat was randomly assigned to either an enriched acoustic environment or standard housing conditions. The enriched environment housed 4-7 rats and consisted of a large cage with many sound sources, including motion detectors, bells, hanging chains, and a running wheel. A CD played 74 random tracks, including seven which were paired with a food reward to encourage attention to the sounds. The standard condition (n=4) consisted of single housing in a hanging wire cage.

Middle latency auditory evoked responses (0-150ms) were recorded once a week from each animal for six weeks. Animals were tested with alternating noise bursts (68dB, 100ms) and tones (70dB, 9kHz, 25ms) delivered 125 times in random order (10s ISI). The amplitude of the evoked response to tones was greater (+60%) in animals raised in the enriched environment compared to the standard condition. Most of this enhancement was observed after only two weeks of exposure to the enriched environment, though the amplitude of the response continued to grow through week six. The evoked potential amplitude in response to the noise burst did not differ between the two groups. Our prior results from unit recordings in anesthetized animals indicate that enrichment lowers tone thresholds, decreases response latency, and increases frequency selectivity. It appears that the net result of these changes is to enhance the amplitude of the evoked response to tones, but not to the noise burst. These results support the hypothesis that environmental enrichment has an important influence on the development of cortical response selectivity.

441 Characterization of Response Properties in Rat Posterior Auditory Cortex

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In the visual modality, spatial and temporal selectivity is more complex in higher cortical fields compared to primary visual cortex. In this study, we contrasted both spatial and temporal response properties of neurons in the rat posterior auditory cortical field (PAF) with those of the primary auditory field (A1). Frequency-intensity tuning curves were derived for each of ~50 cortical sites per animal (n=10). Response parameters derived from the tuning curves included best frequency (BF), threshold, bandwidth (BW) at 10,20,30&40 dB above threshold, and latency.

Both minimum latency and bandwidth were significantly greater in PAF neurons ($p < .01$). The mean latency was 14.79 msec (SD=8.81) for A1 and 32.82 msec (SD=15.22) for PAF. The mean BW20 was 1.82 octaves (SD=0.77) for A1 and 2.93 octaves (SD=0.90) for PAF. The BF gradients in A1 and PAF form a mirror image with low frequency neurons at their border. Unlike A1, high frequency PAF neurons tend to have greater bandwidths and longer latencies than low frequency neurons. BF range and minimum threshold are similar in the two fields. These findings indicate that A1 and PAF may operate in conjunction to represent complex auditory stimuli. FM sweeps, tone burst trains, and noise burst trains were also presented to better characterize the response of PAF neurons. PAF and A1 neurons were equally selective for FM direction in response to fast sweeps, but PAF neurons were less selective for direction in response to slower sweeps. Preliminary analysis suggests that PAF has a slower maximum following rate for tone trains than A1. Further documentation of spatial and temporal response properties in non-primary cortical fields will be useful for future plasticity studies.

442 Effect of dopamine on glutamatergic transmission in the rat auditory cortex.

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Dopamine is a neuromodulator involved in cortical plasticity in the auditory cortex (Bao et al., Nature 412, 79, 2001). The cellular mechanisms leading to dopamine induced cortical plasticity are still unknown. We wanted to investigate whether the presence of dopamine affected glutamatergic synaptic currents in the rat's auditory cortex. To such purpose we measured AMPA-receptor mediated spontaneous excitatory postsynaptic currents (sEPSCs) in layer II/III neurons of rat brain slices. We used voltage-clamp recordings at a holding potential of –60 mV in the presence of the GABA receptor blocker bicuculline (10 uM). Spontaneous EPSCs had a mean frequency of 0.33± 0.11 Hz, amplitude of 8.9±1.4 pA, a rise time of 2.6±0.5 ms and a decay time of 7.1±1.1 ms. Application of dopamine (300 uM) induced an increase of 71±20 % of sEPSCs frequency leaving unaltered amplitude and rise and decay times, suggesting an action at the presynaptic neurons(s). Such increase in sEPSCs frequency could be due to an increase in the presynaptic neuron firing rate or to an increase in glutamate release or both. In order to dissect out the contribution due to presynaptic spiking, we measured miniature EPSCs (mEPSCs) in the presence of the Na-channel blocker TTX (1 uM), beside bicuculline. Dopamine application increased mEPSCs frequency (41±6 %) without changing mEPSC amplitude nor rise or decay times, proving that the effect of dopamine is at least partially independent on neuron spiking.

We conclude that dopamine increases the probability of release by acting directly at presynaptic glutamatergic terminals. Such effect represents a possible cellular mechanism for the modulation and induction of synaptic plasticity in the auditory cortex.

443 Changes In Cat Primary Auditory Cortex After Minor Noise Induced Hearing Loss.

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Twelve cats were exposed for 2 hours to a 115 dB SPL pure 6 kHz tone at different ages: 36 days (4 cats), 56 days (5 cats) and 118 days (3 cats). Recordings were done from the right hand cortex in cat primary auditory cortex (AI) between 2 and 16 weeks after exposure and compared to 7 age matched controls. The right ear canal was filled with an ear mold substance. Peripheral hearing sensitivity was determined by auditory brainstem response (ABR): the 56 and 118 days exposure groups showed at most 20 dB hearing loss for frequencies above 6 kHz, whereas thresholds in the 36 day old group were not significantly different from controls. Frequency tuning curves were equated with the iso-rate contour at 25% of maximum response. We investigated the effect on the tonotopic map organization for characteristic frequency (CF) and best frequency at 45 dB SPL. In addition, the threshold at CF, the excitatory- and inhibitory-tuning curve bandwidth at 20 dB above CF threshold, were determined. Firing rates and cross-correlation functions were obtained for 15 minute long spontaneous activity recordings. For the 56 day and 118 day exposure groups, reorganization of the tonotopic maps was observed, but not in the 36 day exposure group. Frequency tuning curves in normal and reorganized cortical areas were of normal shapes with near normal thresholds. The control

group CF-thresholds were 27 ± 20 dB SPL. The average CF thresholds in the 56 and 118 day exposure groups were at most 6 dB higher than the control group. The CF thresholds did not change in the 2-16 weeks after exposure, suggesting that any changes take place in the first two weeks. Excitatory and inhibitory tuning curve bandwidth were not significantly different from controls, and no effect of time after exposure was found. Spontaneous activity was unchanged in the three experimental groups compared to controls. Peak cross-correlation strength was about 50% higher in the 56 and 118 day exposure groups compared to controls.

444 Longitudinal fMRI studies in cochlear implanted congenitally deaf cats (CDCs)

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To study activity in the auditory cortex of cochlear implanted CDCs by functional imaging (fMRI) we developed a new stimulation system and a volume optimized coil for the cat head [Thierfelder et al., ARO 2000, 813].

The maturation of auditory cortical activity was investigated in chronically stimulated CDCs. Mean spatial deviation of the center of activation was 2.4+/-0.2 (SD) mm in the horizontal plane and 4. $^{2+}$/-2.8 (SD) mm in the vertical plane. Given an area of 10mmx20mm, the auditory cortex of CDCs can unambiguously be identified. As a correlate of the electrically evoked, auditory activation, the number of active voxels was determined. Contralaterality index (CL=contralateral activity/[contralateral+ipsilateral activity]) allows the relative evaluation of activity. Similar to results from acoustical stimulation in humans [Hart et al., ARO 2001, 655], also electrical stimulation in cats shows significant increase in CL with intensity levels.

Response amplitudes and surface areas of field potentials in CDC primary auditory cortex increase with the duration of chronic, electrical cochlear stimulation [Klinke et al., Science 285, 1729]. This increase is the larger, the earlier stimulation was initiated [Kral et al. ARO 2001, 455].

To study these effects of maturation, we evaluate CL in a young (3.5mo) and an adult (6mo) implanted cat. First naïve measurements in these animals demonstrate very small CL-values (0.0 and 0.3, resp.). With duration of chronic stimulation (58d and 76d, resp.), in the young cat CL clearly increased (1.0), whereas no changes were found in the adult (0.15). Assuming that the contralateral auditory cortex becomes the dominant side for the encoding of auditory information during maturation, the results suggest for an early implantation.

445 No evidence for cross-modal reorganization of AI field in congenitally deaf cats

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Congenitally deaf cats (CDC) have no specific input to the central auditory system.Activity evoked in area AI by stimulation through a cochlear implant is rudimentary (Klinke et al,Science 285:1729, Kral et al,Cereb Cortex 10:714).Is therefore area AI, deprived of its normal input, taken over by other sensory systems?Visually-evoked local field potentials (LFP) were recorded in AI of CDCs (Rebillard et al,Brain Res 129:162).Yet, this finding remains controversial (Hartmann et al,Hear Res 112:115).The question was re-addressed using multi-unit (MU) recordings and calculating current-source-densities (CSDs) with visual stimuli on CDCs and hearing cats.The refraction of the eyes was corrected using contact lenses.Recordings were performed with microelectrodes at a raster of 16 positions (distance 1-2 mm in AI) from surface down to a depth of 4200 µm (300 µm steps).MU responses were manually searched for in the whole visual field for 100-200 MUs per animal.In addition,visual stimulation was performed with flashed stimuli and phase-reversal gratings of 0.1-1.6 cycles/° (3-8 different orientations) activating both the magnocellular and parvocellular subsystems.Somatosensory stimuli were applied using cotton pads on face,head,both pinnae,back,forepaws,hindpaws and part of the abdomen.Visually evoked MU responses were quantified using post-stimulus time histograms.For somatosensory stimulation,MU activity was evaluated audio-visually.Averaged LFPs were used for CSD calculations.There were large LFPs in area 17 (~400µV) and LFPs of moderate amplitudes (~40µV) in AI of CDCs and hearing cats;however,CSDs did not reveal any current sources in AI.No MU responses could be evoked in AI by visual or somatosensory stimuli.Thus,LFPs in AI of CDCs result from passive volume conduction from other cortical areas. Secondary auditory areas were not investigated.

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446 Correlation of Hemodynamic Based Neuro-imaging with Single Unit Activity in Auditory Cortex

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Neuro-imaging techniques based on the detection of local hemodynamic changes (e.g. fMRI, optical imaging of intrinsic signals) have become powerful tools for exploring brain function. These imaging techniques have been able to reflect some aspects of neuronal function quite adequately. For example, in previous studies with intrinsic signal imaging, we have been able to determine tonotopic maps in both the primary and secondary auditory cortex in the chinchilla. It is clear however that the coupling of hemodynamic events (including BOLD effects) with neuronal activity is an indirect one, which means that using imaging to resolve temporal features of neural activity is problematic. However, we present here a possible temporal correlation.

In the present study we have recorded the long term (over 4s) adaptation properties of neurons in primary auditory cortex (AI) and in non primary areas (e.g. AII). We have used standard electrophysiological recording techniques. We have found that AI has a higher proportion on "onset response" cells compared with those outside AI, and that the adaptation rates of AI neuron responses have shorter time constants. We have also studied the temporal properties of blood flow change during the presentation of long (4s) stimuli in both AI and AII, and found that hemodynamic changes are more sustained in AII compared with more "phasic" changes associated with AI.

We suggest that this more sustained hemodymamic profile may be linked to the sustained activity of "tonic" neurons in non primary auditory cortex.

447 Development of Intrinsic Horizontal Connections in Primary Auditory Cortex

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A common feature of intracortical circuitry throughout the mammalian neocortex is the organization of intrinsic connections into a horizontal network of patches or clusters. The topographic organization of these patchy intracortical connections has been correlated with cortical function, and is altered by sensory deprivation during development (White et al., 2001). In the primary auditory cortex, this patchy network of intrinsic horizontal connections is distributed in a pattern related to the cortical map of sound frequency (Matsubara and Phillips, 1988; Wallace and Bajwa, 1991). The purpose of this study was to assess the development of clustered intrinsic horizontal connections in the primary auditory cortex of the neonatal ferret (Mustela putorius furo). Glass

micropipettes coated with DiI or DiA (Molecular Probes) were placed in the middle ectosylvian gyrus of the fixed brain. At 2.5-3 postnatal weeks of age, a diffuse distribution of labeled cells and axons was found surrounding the injection site. However, by four weeks of age indications of the onset of clustering could be discerned with the emergence of patches of labeled cells. These results are similar to those reported for the development of clustered horizontal connections within area 17 of the ferret visual cortex (Ruthazer and Stryker, 1996).

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448 History of Recent Past Affects Neural Responses in Auditory Cortex of Awake Primates

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Auditory perception depends upon delineating sequences of sounds. How are neural responses to sounds delivered at one time affected by sounds delivered earlier? We investigated this question in the auditory cortex of awake monkeys. Acoustic stimuli consisted of broadband noise or a 4000 Hz tone (duration 200 ms) delivered under free field conditions. Inter-stimulus intervals ranged from 200 to 5000 ms. Monkeys maintained visual fixation before and during sound presentation, but were not trained to respond to the sounds. Standard techniques for single-unit recording were employed.

We found that neural responses to a given stimulus were suppressed by about 15% when preceded by the same stimulus within the previous 1000 ms. At interstimulus intervals of 3000 ms or longer, this suppression was no longer evident. The time course and magnitude of response suppression were similar for tone pairs and for broadband noise pairs. Suppression also occurred when pairs of sounds were delivered from different locations in space. On individual trials, neurons tended to respond more vigorously to the second sound if they had also responded strongly to the first sound. This positive correlation between the two responses occurred at all inter-stimulus intervals tested.

Previous single-unit studies in auditory cortex of anesthetized cats have demonstrated strong suppression of responses for inter-stimulus intervals of 400 ms or less (Reale and Brugge, J Neurophysiol 84: 435-450, 2000). Our results confirm that response suppression is also evident in the alert animal. Most surprising, though, is the long time course over which suppression occurs. These findings suggest that neural responses to sounds in auditory cortex are a complex synthesis of the acoustic present and past.

449 Contralateral and Ipsilateral Responses to Pure-tone Bursts in the Primary Auditory Cortex of the Squirrel Monkey

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The relationship of receptive field properties in squirrel monkey primary auditory cortex (AI) elicited by tone stimulation of contralateral (contra) and ipsilateral (ipsi) ears was assessed in 4 animals. Earlier observations had suggested that the characteristic frequency (CF) of low frequency (0.5-5kHz) cortical neurons was nearly identical for both ears. This study quantified the extent to which CF, minimum threshold, lat20 (latency 20 dB above threshold at CF) and sharpness of tuning (Q10 factor) overlapped and diverged under monaural stimulation of each ear.

Tone bursts that varied in frequency and intensity were presented to each ear and extracellular responses were recorded at 158-190 cortical sites. At each site that responded to sound from both ears (528 total) response parameters were compared. When comparing contra to ipsi stimulation, contra CF was usually higher (342/528 sites, difference μ/σ=0.07/0.24 oct), Q10 was lower (311/528, 0.11/0.49), and lat20 was shorter (369/528, 1.2/2.4 ms). Minimum threshold was negatively correlated with CF and Q10 was positively correlated with CF for both ears. Lat20 was negatively correlated with CF for the contra ear, but positively correlated with CF for the ipsi ear.

Spatial organization of response parameters was compared after removing CF-dependency using a local regression (parameter vs CF) technique. Following this decorrelation, residuals were modeled by 2-dimensional local regression (parameter vs spatial coordinates) to reveal spatial distributions of values. Residual threshold and lat20 topographic maps for contra and ipsi ear stimulation were highly correlated in 3 of 4 animals. Residual low- and high-frequency band edges were also similar for the two ears in all four monkeys. This suggests that response maps for tonal stimulation of contra and ipsi ears are highly correlated when CF-dependence has been accounted for.

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450 Neurophysiological Correspondence in Monkey Primary Auditory Cortex to Human M100 Responses to F1-Cutback Vowels

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We investigated temporal processing of vowel stimuli, in which a first formant (F1) onset asynchrony led to a change in elicited percept (from /a/ to /u/), by formant substitution. The stimulus elements (/a/ or /u/) were associated with distinctly different neuromagnetic evoked response latencies for the M100 component, detectable by MEG. Nine subjects underwent MEG recording. F1 onset asynchrony varied from 0ms to 200ms. For F1 onset asynchrony up to ~30ms, evoked response latency was constant corresponding to the latency of an isolated /u/. At onset asynchronies from 30 to ~80ms, a progressively increasing M100 latency was observed suggesting that the M100 response component was influenced by the delayed onset of the second token of the stimulus (/u/). For asynchronies longer than 100ms, the M100 latency reverted to a shorter latency corresponding to that of the isolated vowel (/a/).

A quantitatively similar response behavior that mirrored human MEG latency and latency changes was observed in neuronal responses in AI of anesthetized squirrel monkeys. A subset of recording locations revealed an oscillatory response at ~100-150ms after stimulus onset and the elicited short-latency response. For a subset of cortical locations, the timing of the first oscillatory period underwent changes with onset asynchrony that closely matched those seen with MEG in humans. The close temporal correspondence between late monkey AI activity and human M100 indicates that AI contributes to the generation of M100.

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451 Representation of stimulus relations by correlated firing in the auditory cortex of the monkey

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Previous studies in cat primary auditory cortex (AI) have shown that neurons with similar response properties are more likely to fire synchronous spikes. The goal of the present study was to further examine the potential role of neural synchrony for representations of auditory objects. We recorded, with an array of 7 electrodes, multiunit activity from AI and the field CM in 7 anesthetized monkeys. For each unit, we determined its frequency response curve by presenting pure tones of different frequencies. Neural synchrony was assessed by calculating the cross-correlation of the spontaneous firing of pairs of units. In AI, 272/926 pairs (29.4 %) were significantly correlated; in CM 214/905 pairs (23.7 %). Cross-correlations were approximately symmetrical around the origin of the correlogram and had half-height widths between 6 and 386 ms (medians 178 and 182 ms in AI and CM, respectively). Correlation strength was low or moderate (AI: 0.045-0.250 [0.076]; CM: 0.040-0.180 [0.078]). In contrast to previous findings, neural synchrony fluctuated considerably with the ratio of the best frequency of pairs of units. In AI, the percentage of correlated pairs was highest for units with similar BF. It decreased irregularly with increasing BF ratio and reached another peak around 1 octave. In CM, the percentage of synchronized pairs was high only for very specific BF ratios. In AI, the percentage of synchronized units was the higher the more their tuning curves overlapped, whereas in CM, neural synchrony varied only weakly with overlap of tuning curves. Yet frequency response curves were similar in the two fields. Our findings suggest that the two auditory fields are functionally specialized to what neurons tend to synchronize with each other rather than to the spectral features represented by the field's neurons.

452 Processing of Temporal Coherence Across Frequency by Neurons in Primary Auditory Cortex

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Most natural sounds contain energy at many frequencies and are therefore likely to overlap spectrally. Despite the obvious difficulty in separating sounds by their spectral content alone, the auditory system is able to perform this segregation even in quite noisy environments (e.g., the cocktail party effect). Because different sounds have unique temporal characteristics, the temporal incoherence between sounds provides a potential cue for segregation. Using extracellular electrophysiological recording techniques, we analyzed well-isolated single units in the primary auditory cortex (A1) of awake marmoset monkeys (*Callithrix jacchus jacchus*) for sensitivity to the temporal coherence of two modulated tones. We presented two sinusoidal amplitude or frequency modulated tones at different carrier frequencies but the same modulation frequency and manipulated the temporal coherence between these tones by varying their relative modulation phase. The most common response was a release from some flanking inhibition in the maximally incoherent condition, although a much smaller population showed the opposite effect. Only a portion of the flanking inhibition was generally involved, indicating that only some off-CF inputs show temporal dynamics similar to that of the CF input. Temporal coherence sensitivity could not be predicted from the properties of one- or two-tone frequency response areas. From these results we conclude that a large proportion of A1 neurons receive multiple inputs that operate on similar time scales and interact largely under temporally incoherent stimulus conditions, such as when two different sound sources are present in the acoustic environment. The collective activity of these neurons may serve as a basis for the auditory system to segregate sounds into separate perceptual objects.

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453 Emergence of Sustained Discharges in Auditory Cortex under Awake Condition

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Neurons in the anesthetized auditory cortex generally display phasic responses to sustained acoustic stimulation. A prominent characteristic of cortical responses under the awake condition is the appearance of sustained discharges that often span the entire stimulus duration. In general, neurons are more likely to fire in a sustained manner when stimulated by a nearly optimal stimulus, whereas they tend to display just onset responses when stimulated by non-optimal stimuli. Functionally, neurons often show greater selectivity to particular stimulus parameters in their sustained discharges than in their onset discharges. For example, temporal selectivity is greater in many neurons when measured by sustained discharges than by onset discharges (Lu et al. J. Neurophysiol. 85, 2001). Rapid sequences of successive sounds resulted in sustained discharges in a substantial population of cortical neurons whereas these stimuli produced onset or no discharges under anesthetized conditions (Lu et al. Nat Neurosci. 4, 2001). Because sustained discharges generally have longer latencies than onset discharges, they are more likely to reflect properties resulting from or enhanced by cortico-cortical processing both within and across cortical areas. The increase in sustained activities suggests increased excitability of cortical neurons under the awake condition. At the same time, our data also indicate stronger context-dependent inhibition in unanesthetized auditory cortex. Inhibition appears to limit the range of stimuli to which a neuron may respond and contribute to a greater degree of non-monotonicity with regard to sound level. The combination of stronger and more specific excitation and inhibition may underlie increased stimulus selectivity in the auditory cortex under the awake condition.

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454 Long-lasting Inhibition Following Stimulus Transitions in the Auditory Cortex of an Awake Primate

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The ability to track changes in the acoustic stream is critical for sound segregation and recognition. Previous studies of cortical neurons have shown that short-duration stimuli generate an inhibitory period lasting up to 200 ms after stimulus offset. Since many natural sounds have relatively long durations, we investigated the effects of a long test stimulus on the responses of a subsequent long probe stimulus in well-isolated single units in the auditory cortex of awake marmosets. A striking observation was that responses to the probe stimuli were often significantly inhibited for durations much longer than have been previously reported. In most neurons, we observed >50% reduction in firing rate that often persisted for ~ 1 s and could last as long as 3 s. Test and probe stimuli are composed of unmodulated or modulated tones or noises (0.5-3.5 s duration). Carrier and modulation parameters of the two stimuli were manipulated to dissociate their contributions. In some neurons, maximal inhibition occurred when the carrier frequencies of the test and probe stimuli matched. In contrast, a number of neurons were inhibited when the modulation frequencies of the test and probe stimuli were mismatched. Some probe responses were also broadly and strongly inhibited by test stimuli of a different stimulus class, such as bandpassed noise inhibiting modulated tone responses. Our preliminary findings indicate that specific long-duration stimuli can

induce inhibition that persists well beyond stimulus offset. This enduring inhibition may play a role in the cortical coding of stimulus transitions occurring over seconds-long time scales or in maintaining a sensory memory.

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455 Virtual Vocalization Stimuli for Systematic Investigation of Cortical Coding of Vocal Communication Sounds

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The majority of work on the neural basis of the perception of vocal communication sounds has involved studying neuronal responses to recorded species-specific vocalizations. These studies, while interesting and informative, do not allow for a systematic dissection of which acoustical properties of the vocalization are essential for neuronal response selectivity, and do not fully reveal the neural codes employed in the discrimination of vocalizations which differ acoustically along multiple parameter dimensions. In this work, we take a novel approach to the study of vocalization processing by creating synthetic vocalizations which are defined by a parametric model which captures the most essential acoustic properties of species-specific vocalizations of the common marmoset, our experimental animal. In order to determine the parameter space which yields acoustically realistic vocalizations, we employ numerical optimization procedures to derive parameters from time-frequency and envelope traces extracted from a database containing a large number of actual marmoset vocalizations. This allows us to build probability distributions from which parameter values for synthetic model calls can be derived. Using these probability distributions, we can probe acoustically realistic and unrealistic regions of "vocalization space". The accuracy of our model is quantified by comparing the spectral-temporal properties of the synthetic vocalizations to those measured from actual marmoset vocalizations. These virtual vocalization stimuli will serve as a basis for quantitative and systematic investigation of cortical coding of species-specific communication sounds.

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456 A Behavioral Investigation of "Separate Processing Streams" Within Macaque Auditory Cortex

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Recent anatomical and electrophysiological evidence suggests that the superior temporal gyrus (STG) of macaques may be organized into two separate processing streams. The first is a rostral stream believed to be involved in the processing of complex sounds including frequency sweeps and species-specific vocalizations, whereas the second is a caudal stream primarily involved in the processing of auditory spatial information. The purpose of this study was to determine whether macaques with lesions restricted to the rostral or caudal portions of the STG would be differentially affected in their ability to perform a relatively complex auditory discrimination. Specifically, the animals were required to discriminate frequency sweeps from steady tones that spanned the same frequency range—a discrimination known to be abolished by the complete bilateral removal of the STG. Three Japanese macaques (Macaca fuscata) were tested using a conditioned suppression/avoidance procedure. A large lesion involving all but the extreme rostral tip of the STG in one hemisphere was followed by a smaller lesion in the other hemisphere that was aimed at removing either the rostral STG, the caudal STG, or the auditory core on the supratemporal plane. The results indicated that the ability to perform the discrimination was lost following restricted lesions of the rostral STG or the auditory core, whereas a lesion of the caudal STG had no effect. These findings are consistent with the suggestion that the rostral, as opposed to the caudal STG is primarily involved in the processing of complex sounds. The next step is to determine whether the ability to perform a sound-localization discrimination would also be differentially affected by these lesions.

457 Evidence for Dual Processing Streams in the Auditory Cortex of Primates.

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The auditory system serves a dual function of identifying "sound objects" and localizing sounds in space. A central tenet of neuropsychological theory is that conscious perception in mammals is bound to the cerebral cortex. Where, then, in the cerebral cortex are the dual functions of hearing to be found? Clearly, an analysis of functional specialization would start with primary auditory cortex (A1). Neurons have been found in A1 that respond to tones of a single frequency but also to more complex sounds. A1 neurons may also be tuned to sound location. Thus, A1 seems to contain all ingredients to fulfill the functional requirements of hearing completely by itself. On the other hand, several additional auditory cortical areas have been found recently outside of A1 both physiologically and anatomically. What is their role if A1 already does everything? It turns out that certain specialized response properties are encountered at a much higher incidence in some of these surrounding belt areas than in others (or in A1). This is one of the arguments on which the claim for functional specialization in any cortical system is based. Another argument is that the anatomical projections of some of these areas target farther remote regions of the brain, such as parietal and prefrontal cortex, that are known to subserve specific functions. Parietal cortex, for instance, is known to be involved in spatial analysis. Different areas of prefrontal cortex are involved in the processing of space and object information. In humans, functional imaging has made specialized processing streams especially evident by lighting up cortical areas that are jointly activated during a specific task. We will review recent evidence from both human and nonhuman primates that supports the existence of specialized processing streams in the auditory cortex.

458 Spectral Ripple Resolution in Cochlear Implant and Normal-Hearing Listeners: The Effect of the Number of Channels

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The ability to resolve spectral peaks is important in the successful discrimination of speech. The first experiment assessed the differences in spectral resolution abilities between cochlear implant (CI) and normal-hearing (NH) listeners, and among CI users having a range of speech perception abilities. The stimuli were rippled noise signals with ripple densities ranging from 1 to 500 peaks between 0 and 8 kHz, and were presented to 18 CI subjects using a 12-channel CIS processing strategy, and to 10 NH subjects via a 12-channel CI simulation. An adaptive procedure was used to determine the highest resolvable ripple density (the ripple density at which an interchange in peak and trough positions in the rippled spectrum could be discriminated) for each listener. In addition to the broadband condition, ripple resolution was assessed in the frequency regions where spectral resolution is of primary importance, using 3 kHz low-pass filtered stimuli. The results showed poorer spectral resolution in CI compared to NH listeners (when listening with the same number of channels), and a significant relationship between spectral resolution and vowel recognition. This time-efficient, non-linguistic, yet speech relevant, test is now being applied to the assessment of the effect of different speech processing parameters on spectral resolution. In the second experiment, ripple resolution was assessed in both CI and NH listeners as a function of the

number of channels (ranging from 1 to 16). The results showed a decrease in ripple resolution ability as the number of channels decreased. Differences between CI and NH listeners in the effect of the number of channels on ripple resolution, as well as the relationship between speech perception ability and the effect of the number of channels on spectral resolution in CI listeners, will be discussed.

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459 TMTFs in Cochlear Implant Users: The Role of Loudness Cues

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The temporal modulation transfer function (TMTF) has been widely used to characterize auditory temporal resolution in acoustic and electric hearing. For continuous broadband carriers, the TMTF for normal-hearing listeners has a lowpass characteristic with poorest sensitivity to amplitude modulation at high modulation frequencies. TMTFs in cochlear implant listeners may have a lowpass, bandpass or relatively flat shape [Shannon 1992, JASA 91:2156; Busby et al. 1993, JASA 94:124; Cazals et al. 1994, JASA 96:2048]. Interpretation of differences in TMTF shapes across cochlear implant listeners is problematic because experimental paradigms have not controlled for loudness cues that may occur with intensity increases due to modulation. In acoustic hearing, a simple correction is used to equate the loudness of modulated and unmodulated stimuli; however, this correction is based on a proportional relationship between RMS intensity and loudness that does not hold in electric hearing [Zhang & Zeng 1997, JASA 102:2925].

In the present study, level-roving was used to eliminate loudness cues to modulation detection and to determine whether such cues affected TMTF shapes in Nucleus-22 users. Stimuli were 500-ms trains of 80 us/ph, 500-Hz biphasic pulses, either unmodulated or square-wave modulated at frequencies ranging from 4 to 400 Hz. Carrier levels for unmodulated stimuli were presented with or without level roving. In the no-rove condition, most subjects reported using a loudness cue to detect modulation at the highest modulation frequencies (200 and 400 Hz). Consistent with this, sensitivity to modulation was reduced by level-roving at these modulation rates. In a few cases, changes were substantial and resulted in a different TMTF shape. Findings suggest that previously published TMTFs may have overestimated sensitivity to high frequency modulation in cochlear implant listeners.

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460 Modulation Detection In Noise By Cochlear Implant Listeners: Across-Channel Vs. Within-Channel Effects

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We report on continued experiments on the effects of noise on modulation sensitivity in Nucleus-22 cochlear implant listeners. Stimuli are periodic pulse trains presented in interleaved sequence to masker and signal channels. Masker pulse train envelopes are of two kinds: i) noise or ii) steady-state at the peak of the noise fluctuation (SSpeak). The ratio ρ between modulation thresholds obtained with the noise masker and the SSpeak masker yields a conservative estimate of the temporal component of the interaction. When signal modulation frequency is 50 Hz, ρ ranges from 1.5 to 3.5 (the noise masker is more effective than the SSpeak masker). For a 20 Hz modulation frequency, ρ is reduced. When the task is intensity discrimination (0 Hz modulation), ρ is close to 1.0 (detection thresholds are similar for SSpeak and noise maskers). This pattern of results would suggest that the greater masking effect of the noise is specific to the dynamic nature of the task. The ratio ρ is not monotonically related to the tonotopic distance between the masker and the signal, suggesting that the interaction between the two envelopes occurs at a relatively central stage of processing.

In a second experiment, we introduced independent noise into the signal envelope to study the interactions between "signal" noise (within-channel noise) and "masker" noise (across-channel noise). We find that when the across-channel masker has a strong masking effect, optimal within-channel noise can improve modulation thresholds and provide a release from this masking. The non-monotonic effect of the within-channel noise is consistent with "stochastic resonance"-type effects observed in our earlier studies of single-channel modulation detection.

461 Stochastic Resonance in Cochlear Implant Patients

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An array of computational simulations, electrophysiologic studies in experimental animals and psychophysical studies in humans have suggested that stochastic resonance occurs in human hearing both with electric stimulation of the deaf ear and acoustic stimulation of the hearing ear. Until now, psychophysical studies have revealed a real but limited effect of great theoretical interest, but of little practical importance. Using the Clarion C-II cochlear implant and its associated DSP-based research interface, we have demonstrated stochastic resoanance effects as great as 10 dB. Using a 202 Hz sinusoid as a test stimulus and 5000 pps biphasic pulse train as a "conditioner" or "desynchronizer", significant enhancements in dynamic range have been obtained in five human subjects. A subset of these, demonstrate a clear resonance peak for the conditioner level consistent with predictions from a computational model of the auditory nerve. The results demonstrate both the existence of substantial stochastic resonance effects and support the concept of using conditioning stimuli in analog speech processors to increase dynamic range and decrease the need for compression. Because the models also predict that desynchronization will increase temporal resolution, the results are promising for the improvement of coding temporal fine structure with cochlear implants.

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462 Temporal Pitch Perception in Acoustic and Electric Hearing

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We investigated how cochlear implant users derive a pitch from the rate of pulses applied to a single channel of their implant. Different theories state that pitch is determined from the intervals between each pulse and every other pulse ("autocorrelation"), from only those intervals between each pulse and the next ("1st-order intervals"), or simply from the total number of pulses per unit time ("mean rate"). We describe two experiments to distinguish between these theories, using both acoustic and electric stimuli. The acoustic stimuli were filtered pulse trains presented to normal-hearing listeners against a continuous noise background. Electric stimuli were biphasic pulse trains applied to one electrode pair of a LAURA or Cochlear CI24 implant. In experiment 1 subjects compared the pitch of a "standard," containing inter-pulse intervals that alternated between 4 and 6 ms, to that of a range of isochronous "signals" having inter-pulse intervals of 3,4,5,6, or 7 ms. Both groups of listeners judged the pitch of the standard to be approximately equal to the longer of its two intervals. In experiment 2

the irregular pulse train consisted of a mixture of two regular-rate pulse trains (F1 and F2 pps, F2/F1=1.29). 7 of 8 normal-hearing listeners and 3 of 4 implant users perceived the pitch of the mixture as close to that of an F2 regular rate signal. The results suggest that the pitch of pulse trains is determined primarily by the longest 1st-order inter-pulse interval and demonstrate that filtered acoustic pulse trains provide a useful analog of electric hearing.

463 Spectral and Temporal Cues to Pitch in Noise-Excited Vocoder Simulations of Cochlear Implants

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Acoustic simulations were used to investigate spectral and temporal cues to pitch in the output of a cochlear implant speech processor. Four-band and single-band noise-excited vocoders were employed, in which noise carriers were modulated by amplitude envelopes extracted by 1/2 wave rectification and low-pass filtering at 32 or 400 Hz. The four-band, but not the single-band processors, may preserve spectral correlates of fundamental frequency. Processors with 400 Hz envelope smoothing preserve temporal correlates of fundamental frequency, while these are eliminated with 32 Hz smoothing.

The inputs to the processors were sawtooth wave frequency glides, in which spectral variation is completely determined by F0, and synthetic diphthongal vowel glides, whose spectral shape is dominated by varying formant resonances. Glide F0 was varied about center frequencies of 146, 208, and 292 Hz. Normal listeners labelled the direction of pitch movement of the processed stimuli.

For processed sawtooth waves, purely temporal cues led to decreasing performance with increasing F0 that approached chance around 292 Hz. With purely spectral cues, performance was above chance despite the limited spectral resolution of the processors.

For processed diphthongs, performance with purely spectral cues was at chance, showing that spectral envelope changes due to formant movement obscured any spectral cues to F0. Performance with purely temporal cues was poorer than for processed sawtooth signals and showed very limited discrimination at higher F0.

We conclude that for speech signals through a typical cochlear implant processor, spectral cues to pitch will have little utility and further that temporal envelope cues are useful only at low F0.

464 Variation in Thresholds and Comfort Levels Across Cochlear Implant Stimulation Sites: Effects of Electrode Configuration and Stimulus Level

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In cochlear implants, variation across stimulation sites in psychophysical detection thresholds (T-levels) and maximum comfortable loudness levels (C-levels) is believed to be inversely related to the spatial extent of activating currents. In theory, greater current spread will result in more consistent activation of the most sensitive neurons and/or integration across larger overlapping populations of neurons, both of which would lead to reduced across-site variation. In this study we examined effects on across-site variation of two variables known to affect current spread: electrode configuration and stimulus level. T-levels and C-levels were measured in 13 subjects with Nucleus C124M prostheses. Consistent with the hypothesized relationship between current spread and across-site variation, the variances of T- and C- levels across the total electrode array for the BP configuration were, on average 10 times larger than those for the MP configuration. However, contrary to the hypothesis, no significant differences were found between variances for T-levels and variances for C-levels for either the BP or the MP configuration, though there was a trend toward smaller variance for C-levels in cases where T-level variance was very large. Control studies suggested that these results were not dependent on psychophysical procedure, absolute stimulus level, method of quantifying across-site variation, or on differences in inherent variability of the dependent variables. These results suggest that the effects of electrode configuration on current spread and its functional consequences are much more significant than the effects of stimulus level within the subject's dynamic range.

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465 Intensity Discrimination in Clarion Cochlear-Implant Users: Effects of Pulse Rate and Electrode Type.

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In recent years, cochlear implants have incorporated increasingly higher rates of pulsatile stimulation and new peri-modiolar electrodes. These changes have facilitated improved speech recognition; however, relatively little is known about their effects on basic psychoacoustic phenomena. This study examined the effects of pulse rate on gated intensity discrimination in Clarion users with the spiral electrode (SPRL) or the HiFocus electrode with electrode positioning system (HF+EPS). We previously demonstrated that operating range decreased with pulse rate in both groups, that SPRL and HF+EPS subjects have similar electrical thresholds, and that HF+EPS subjects have significantly smaller electrical dynamic ranges (Kreft et al., CIAP 2001).

Gated intensity DLs were measured for 200-ms trains of 77-us/ph biphasic pulses, at five levels across the dynamic range, for pulse rates of 200, 1625 and 6500 pps. Intensity DLs were expressed as Weber fractions in decibels (10 log $\Delta I/I$) and as a percentage of dynamic range ($\Delta I_{dB}/DR_{dB}$ x 100). Average DLs decreased systematically with increasing current amplitude, both within and across pulse rates. The smallest DLs were obtained for 200 pps stimuli, since operating ranges for these stimuli extended to the highest current levels. Similarly, average DLs were smaller for SPRL subjects than for HF+EPS subjects because SPRL subjects had higher operating ranges. Smaller dynamic ranges and larger intensity DLs combined to produce larger normalized intensity DLs in HF+EPS subjects (13%DR) than in SPRL subjects (4%DR). There was no obvious relation between intensity resolution and speech recognition.

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466 Forward-Masked Spatial Tuning Curves in Cochlear Implant Subjects

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Stimulation of multichannel intracochlear electrodes can give rise to peripheral and central channel interactions. Detailed knowledge of such interactions may prove useful for improving stimulation schemes and for understanding individual differences in speech recognition ability among cochlear implant users. In this study, a forward masking paradigm employed a fixed-level probe and variable-level maskers to estimate current spread and characteristics of neural channel selectivity for bipolar electrical stimulation. A "spatial tuning curve" (STC) was generated by determining masked thresholds for a number of masker electrodes surrounding a probe electrode. STCs were measured at several probe levels for an electrode in the middle of each subject's array. Current spread was characterized by STC slopes in dB of masker amplitude per mm of masker-probe electrode distance. These slopes varied across subjects from 0.6 to 5.3 dB/mm, corresponding to space constants of 18.8 to 2.2 mm. Channel selectivity was characterized by STC response slopes in percent dynamic range per mm, and by STC width at 50% of masker dynamic range. Channel slopes varied from 2.5 to 50% /mm.

Channel widths varied from 2.6 to 16.2 mm. Implications for speech processing strategies are discussed.

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467 Cochlear Implant Speech Recognition with Speech Maskers

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Speech recognition with cochlear implants has improved significantly over the past decade, with scores averaging 70-80% for sentences in quiet. Although promising, high levels of performance decline sharply with background noise. Natural environments contain many types of noise that fluctuate in frequency and intensity over time (e.g. speech) or maintain a relatively constant level and frequency (e.g. the low hum of an air conditioner). In this study, IEEE sentence recognition was measured in normal-hearing (NH) and cochlear implant (CI) listeners in the presence of one of three noise maskers: steady-state speech-spectrum-shaped noise, a competing sentence spoken by the same talker, or by different talkers. Scores were compared at several signal-to-noise ratios. NH listeners also listened to sentences in noise as a function of the number of noise bands modulated by the speech envelope. Stimuli were presented monaurally through headphones in NH listeners and with a direct connection in CI listeners. Preliminary results from NH listeners showed improved performance in noise with a greater number of spectral channels. Most importantly, sentence recognition scores were lower with a competing sentence spoken by the same talker than with speech-shaped noise. This difference became more prominent with reduced spectral information. These results indicate that the ability to segregate individual speech sources ("cocktail party effect") may be more difficult than steady-state noise when only temporal envelope information is extracted.

468 Speech Recognition under Conditions of Frequency-Place Compression and Expansion

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In normal acoustic hearing the mapping of acoustic frequency information onto the appropriate cochlear place is a natural biological function, but in prosthetic devices like cochlear implants it is determined by the programming of the speech processor. In cochlear implants, the cochlear tonotopic range is determined by the length and insertion depth of the electrode array. However, most cochlear implant speech processors map the entire acoustic frequency range from 100 Hz to 10,000 Hz onto the electrodes, resulting in a compression of the tonotopic pattern of speech information delivered to the brain. The present study measured the effects of such frequency-place compression on speech perception, as well as the effects of an expansion, which results in an expanded representation of speech in the cochlea. Speech recognition was measured by presenting the conditions to normal-hearing listeners using a noise band vocoder. For each condition, the result was compared to that of the perfect tonotopic match, where the analysis and carrier bands were perfectly matched. Speech recognition with either expansion or compression was generally equal to or poorer than the matched condition. Frequency-place compression or expansion on the apical end was more disruptive to speech recognition because more critical spectral speech information is concentrated in this region. A similar pattern of results was observed at different signal-to-noise ratios, indicating no interaction between SNR and frequency-place distortion. Compression and expansion were also combined with spectral shifting. Compression combined with an apical shift produced better performance than a matched condition, indicating that compression and shifting are interacting variables and should be both taken into account for an optimal fitting.

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469 Speech Perception and Production in Cochlear Implants: Short-Term Adaptation Effects for Spectrally Shifted Speech

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In the present study, speech perception and production of Nucleus-22 cochlear implant users were measured over a 2-week period, during which subjects continuously wore an experimental speech processor that was purposely mismatched in terms of the frequency-to-electrode assignment (lowered by 1 octave). Speech recognition measurements included phoneme and sentence recognition while speech production measurements included F1 and F2 of vowels for words-in-isolation. Speech recognition and production were measured using each subject's clinically assigned speech processor just prior to implementation of the experimental processor, and were measured again at the end of the 2-week period. Speech recognition and production with the experimental processor was measured daily during the two-week period. Results showed that speech recognition with the experimental processor was significantly lower immediately following implementation while speech production was unchanged (in terms of F1 and F2 values). Over the two-week test period, speech recognition with the experimental processors gradually improved along with a significant change in speech production. Speech recognition with the clinically assigned processor immediately after its re-implementation was largely unchanged when compared to the baseline data. However, the acoustic measurements of F1 and F2 values with the re-implemented clinical processor were significantly different from the baseline data. The results suggest that, when presented with an altered acoustic input, cochlear implant users may slowly "re-calibrate" their speech production patterns, depending on the degree of mismatch in the auditory feedback. The results also suggest that "internal" representations of frequency may not be reshaped by a relatively brief exposure to new patterns of stimulation, despite the gradual increase in performance and a significant change in speech production.

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470 Speech Perception Effects of Short Automatic Gain Control Release Time Constants for Users of Cochlear Implants

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Cochlear implants are sensory aids for the profoundly deaf. Improved speech processing designs allow implant users to achieve increasing levels of speech perception. Automatic gain control (AGC) is one of the design features often used to help maintain an appropriate volume level, which may result in improved speech perception. One important AGC parameter is release time, which guides the time over which gain is increased when the input signal is softer than threshold. In general, hearing aids and cochlear implants use release times in the order of a few hundred milliseconds. However, a recent report by Stöbich, et al. [1999, Ear and Hearing] found that a fast-acting AGC component can improve performance in the face of intense transients. Slow-acting AGC settings can improve listening levels, particularly when coupled with advanced methods of handling transients. Furthermore, Eddington et al. [1983, Fourth Quarterly Progress Report] have shown improved speech perception in three implant users with AGC release times set to a

few tens of milliseconds. Although promising, it is unclear if these results can be replicated in more listeners, and in particular in users of stimulation strategies other than the "compressed analog" scheme used by Eddington's subjects. This study examines the effect of fast AGC release times for several listeners using pulsatile strategies.

Several users of the Nucleus devices were tested using a laboratory-based speech processor and the subjects own speech processor. Speech perception scores for monosyllabic words and for medial consonants were obtained using release times of 5, 20, and 250 ms. The remaining processor parameters were adjusted to approximate the subjects usual speech processor settings. Preliminary data suggest that the effect of release time varies between individuals, but no universal benefit from fast AGC release time has been demonstrated.

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471 Speech Perception In Noise As A Function Of Stimulation Level And Rate, And Frequency Response For Adult Cochlear Implant Users: Loudness, Quality And Discrimination.

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Speech perception by cochlear implant users can be reduced for presentation levels emulating soft and conversational speech (Skinner et al., 1997, JASA 101, 3766-3782). The input sensitivity characteristics and the range of electrical stimulation levels, representing psychophysical threshold (T) and maximum comfortable levels (C), may be manipulated to enhance the audibility of speech (Skinner et al., 1999, JSLHR, 42, 814-828). The effects on the loudness and intelligibility of speech of adjusting the T and C levels in the amplitude coding process were studied. T and C levels were reduced by a fixed proportion of the subjects' psychophysical ranges for each channel. When using the full input range to amplitude coding, some subjects were able to maintain good open-set sentence perception for shifts of 100%DR. However, these subjects reported the speech was too soft for comfortable listening for shifts greater than 25%DR. Further experiments explored the perception and loudness of speech presented in various SNRs. In experiment II, all subjects judged the quality of speech in noise to be greatest with no T and C shift (0%DR). Lowering T and C levels did not reduce the loudness of noise alone, but reduced the loudness and intelligibility of speech in noise. In experiment III, de-emphasis reduced speech quality and loudness for some subjects and reduced speech discrimination for all subjects. In experiment IV, two of four subjects judged the quality of speech higher with 900pps than for the 250pps. For all four subjects speech discrimination was better with 900pps, particularly at 0dB SNR. These results have implications for the design of speech processing, amplitude coding, and choice of stimulation parameters in the fitting of cochlear implant devices. Funded by the Commonwealth Government of Australia via the Cooperative Research Centre for Cochlear Implant and Hearing Aid Innovation.

472 Talker Discrimination and Spoken Word Recognition 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 listeners suggests that encoding talker-specific speech information is important for interpreting the linguistic content. Cochlear implant recipients' word and sentence recognition skills have been studied extensively. However, little is know about their ability to extract talker-specific information from speech. This investigation assessed talker discrimination by adults with cochlear implants and the relationship between talker discrimination and their spoken word recognition performance.

Participants were presented with pairs of words or sentences produced by 10 different talkers. In the A-A condition, the linguistic content of each stimulus pair was identical (e.g, cat-cat); in the A-B condition, the linguistic content of each pair differed (e.g., cat-dog). The talker was the same in half the pairs and differed in the remaining pairs. Participants indicated whether the pairs were produced by the same or different talkers. Participants also were administered standard word and sentence recognition tests. Results demonstrated that talker discrimination was significantly more difficult when the linguistic content varied. Spoken word recognition was significantly correlated with talker discrimination in the A-B condition but not in the A-A condition. Like normal hearing listeners, cochlear implant recipients cannot completely ignore linguistic information when attending to indexical speech cues. In addition, the correlation between talker discrimination in the presence of linguistic variability and word recognition performance suggests that the same perceptual processes are involved in both extracting fine-grained details in speech and interpreting the linguistic content.

473 Can we Model Word Recognition by Cochlear Implant Users from Psychophysical Performance?

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Understanding the mechanisms employed by cochlear implant (CI) users to recognize spoken words has both theoretical and clinical implications. In the present study, we provide a quantitative description of open-set word recognition by CI users through a two-step process: (1) use the Multidimensional Phoneme Identification (MPI) model (Svirsky & Meyer, 1998) to generate phoneme confusion matrices from performance on psychophysical tasks, and (2) use the Neighborhood Activation Model (NAM) (Luce & Pisoni, 1998) to the predict and generate word recognition from the phoneme confusion matrices. The MPI model produces a confusion matrix from an individual listener's JND's along perceptual dimensions hypothesized to be important to phoneme perception. We postulate that the dimensions most important to vowel recognition are the places of maximal stimulation along the cochlea. For consonants, two additional dimensions are hypothesized to be important: the length of silent periods, and the ratio of the low-to-high-frequency energy in the stimuli. JND's along each of these dimensions are estimated from performance on specific psychophysical tasks. The confusion matrices generated by the MPI model are used as input to a second model , NAM, to predict and generate open-set word recognition. NAM postulates that words are recognized in relation to similar-sounding words (a neighborhood). NAM also takes into account word usage frequency and neighborhood density - the number of similar-sounding words that may be confused with the stimulus word. The probability of correctly identifying the stimulus word is based on the probabilities of perceiving the individual phonemes in the target word correctly weighted by the frequency of occurrence of the target word in relation to the frequencies of the neighbors. Results from information transmission analysis as well as benefits and shortcomings of this approach will be discussed.

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474 Mechanosensory Transduction in Drosophila

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Our diverse mechanosensory system encompass the salient senses of hearing, balance, touch, and proprioception, as well as less conscious senses like the detection of blood pressure and gut stretch. The mechanosensory cells that mediate these senses are structurally and functionally dissimilar, yet share a central feature: mechanically gated transduction channels. Unlike other sensory signaling modalities, which use second messengers to relay sensory information, mechanosensation occurs through the direct opening of mechanically gated ion channels by applied forces. To understand mechanotransduction, we take advantage of the ease and elegance of Drosophila research. Fruit flies make an ideal organism for research on mechanotransduction for several reasons: renowned molecular-genetic tools, the ability to electrically record from mechanosensory bristles, and surprising similarities between the development and physiology of fly mechanosensory neurons and that of vertebrate hair cells. Using this complement of tools, we identified a gene, no mechanoreceptor potential C (nompC), that encodes a mechanosensory transduction channel. Mutations in nompC abolish about 90% of the mechanosensory transduction current in mechanosensory bristles. Because they receive virtually no mechanosensory feedback from the periphery, nompC mutant flies are profoundly uncoordinated. The NompC protein is a member of the TRP family of cationic channels, but has an unusual N&#64979;terminus comprised of 29 ankyrin repeats. Because ankyrin repeats are common protein interaction domains, their abundance in NompC suggests a strong interaction with other members of a transduction complex. We are now searching for the molecules with which NompC interacts. NompC's critical role in mechanosensory signaling gives us an important toehold into the transduction machinery that will facilitate identification of the remainder of the molecules.

475 Myosin-X is a MyTH4-FERM myosin that undergoes a novel form of motility in filopodia

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We recently reported the discovery and initial characterization of myosin-X (Myo10), an unconventional myosin that is widely expressed in vertebrate tissues. Like the "deafness myosins" Myo7a and Myo15, Myo10 is a member of a large group of unconventional myosins whose tail domains contain MyTH4 and FERM domains. Myo10 is unique among myosins in that its tail also contains three PH domains, suggesting that it binds to PI 3-kinase products such as PIP3. We report that Myo10 exhibits a striking localization to the tips of filopodia, which are slender cellular extensions containing a core of actin filaments. Consistent with a role in filopodial function, overexpressing full length GFP-Myo10 in COS-7 cells leads to a doubling of filopodial length and a fourfold increase in filopodial number. Live cell imaging in HeLa cells reveals that GFP-Myo10 is present at the tips of virtually all filopodia during both extension and retraction. GFP-Myo10 also undergoes remarkable forward and rearward movements within filopodia. The rearward movements occur at ~15 nm/s, consistent with retrograde actin flow, while the forward movements are more rapid (~80 nm/s at ~25 C) and may be due to Myo10 motor activity. Although the identities of Myo10's cargos are not yet known, the movements of large puncta of Myo10 correspond virtually perfectly with the movements of dark structures visible by phase microscopy. Together these data suggest the existence of a novel actin-based transport system in filopodia. These results also raise the question of whether other MyTH4-FERM myosins exhibit analogous movements in other actin-based structures such as cytonemes, microvilli, or stereocilia. Current research is directed at characterizing Myo10's interactions with putative cargos such as integrins and at investigating the role of Myo10 in processes such as filopodial extension, phagocytosis, and nerve growth.

476 Ras Signaling and Growth Control

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Ras proteins are highly conserved membrane-bound nucleotide binding proteins. They are essential for the transduction of diverse extracellular signals that control cell growth, and abnormal activation of Ras proteins is implicated in the development of several types of human cancers. It is now well established that Ras proteins control cell growth through the activation of multiple effector pathways. These pathways from a signaling network that enables the cell to interpret biological inputs in a context-dependent manner. I will describe our efforts to identify Ras-dependent signaling events that control normal cell proliferation and how this control is disrupted in cancer cells. Our recent studies on the regulation of the signaling activities of Ras by dosage effects and by spatial and temporal cues will be also discussed.

477 The Use of Fluorescence Resonance Energy Transfer and Luminescence to Study Protein: Protein Interactions in Drug Discovery

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Cell signalling events are often mediated by the co-ordinated aggregation of transient protein complexes within cellular compartments and the movement of key messenger proteins between them. This presents a challenge in drug discovery to develop methods to detect the effect of compounds on such processes. However, a number of techniques are emerging that may allow the visualisation of protein interactions in intact cells and in real time. Fluorescence Resonance Energy Transfer (FRET) between mutants of green

fluorescent protein has been demonstrated when they have been cloned as fusion proteins linked together by peptide linkers. Many such protein constructs have been reported in the literature and form the basis of biosensors used to detect cell-signalling events including caspase activation and changes in cell calcium concentration. We have investigated the use of FRET to measure protein interactions in Rho family GTP binding protein signalling cascades. The insertion of amino acids 75-118 of PAK between GFP mutants allows significant FRET between the GFPs and retains the ability to bind to activated Rac/Cdc42 with a binding affinity similar to the uncomplexed PAK fragment. Furthermore on binding to activated Rac/Cdc42 a marked change in FRET takes place. This can be used as a method for measuring the binding of activated Rac/Cdc42 to their effector proteins with the potential for use in live cells. Nuclear factor kappaB (NF-kB) proteins are dimeric transcription factors responsible for the control of many genes in response to cell stimulation. In resting cells NF-kB activity is suppressed by binding to the inhibitory protein IkBa in the cytoplasm which masks its nuclear localisation sequence. On cell activation IkBa is phosphorylated by upstream kinases which targets IkBa for ubiquitination by ubiquitin ligases and subsequently directs its proteosomal degradation. This results in the release of free NFkB, which translocates into the nucleus initiating gene transcription. We have developed an IkB degradation reporter assay, which allows us to measure this process by luminescence. Stimulation of cells expressing a fusion protein comprising IkBa fused to Renilla luciferase results in the rapid phosphorylation and degradation of the fusion protein. Degradation can be measured as a decrease in luciferase activity and provides a rapid measure of NFkB activation.

478 Chemical Genetics: Controlling Biology with Chemical Probes

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Each year, many promising natural products are identified as being biologically active in cell culture assays. Despite the proven in vitro efficacies of these compounds, development of these 'drug candidates' into clinically useful therapeutic agents is an arduous procedure, often due to issues unrelated to the compound's mechanism of action (e.g. poor pharmacokinetics, unfavorable side effects, etc.). While many of these compounds have limited therapeutic potential, investigation of their mechanism of action can provide new information about complex intracellular signaling pathways. In addition, these studies can serve as the starting point for the rapid development of additional efficacious compounds having more favorable pharmacological profiles. Starting with the antiangiogenic agent fumagillin, the antitumor natural product epoxomicin and anti-inflammatory parthenolide, we has identified and validated new targets for antitumor and anti-inflammatory therapeutic intervention. Recently, we have also developed a new chemical genetic strategy for the identification of key components of signaling cascades.

479 Age-related change in the number of neurons in the human vestibular ganglion

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Dysequilibrium of aging in humans has been speculated to arise from progressive deterioration within anatomical components of the vestibular system. An integral part of this system is vestibular ganglions, which are bipolar neurons that relay peripheral vestibular information to the central nervous system. To assess the effect of aging on the number of human vestibular ganglion neurons, assumption-free stereology in the form of the optical fractionator was used on 20 serially sectioned archival human temporal bone specimens. Donors had no history of vestibular pathology and ranged in age from 2 to 88 years. An average of 25812 (CV = 0.13) vestibular ganglion neurons was found throughout this age range, a significant departure from the results of past studies. Logistics based regression analysis pointed to a non-linear pattern of decline in the neuronal population: the number of cells remained roughly constant at about 28,952 cells in youth, then declined gradually between 30 to 60 years of age before leveling off at approximately 23,349 cells in older individuals. This study confirmed the existence of an age-related decline in the primary neurons of the human vestibular system, thus providing one anatomical basis for the increased incidence of imbalance seen with age.

480 The Pharmacology of the non–α9/α10 Nicotinic Receptor of Hair Cells: Clues as to Subunit Composition

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The presence of the mRNA of nicotinic subunits other than α9 and α10 has been reported in several inner ear preparations (Elgoyhen et al,1994,2001;Hiel et al,1996;Anderson et al,1997). This suggests that functional nicotinic receptors other than α9/α10-containing might also exist in the inner ear. The present research examined the pharmacology of one such receptor called the DMPP receptor (R-DMPP)after the agonist 1,1dimethyl-4-phenyl piperazinium. Examination of the mRNA complement of individual nicotinic subunits of frog semicircular canal hair cells exhibiting R-DMPP-responsiveness found: α 4, 6, 7, 9, β4 and epsilon. This newly uncovered receptor can be distinguished from the α9/α10-containing receptor by: its high sensitivity to ACh (EC_{50} 67nM) in isolated hair cells; its insensitivity to strychnine and α–bungarotoxin (μM range) and its high sensitivity to α– conotoxin–M II (IC_{50} 30nM) in whole organ studies. Other antagonists studied were methyllycaconitine (IC_{50} 9μM) and dihydro-β-erythroidine (IC_{50} 50nM). The insensitivity to strychnine, methyllycaconitine and α–bungarotoxin (μM range) would tend to eliminate the α7 subunit from consideration. The following drugs usually thought of as agonists produce a persistent inactivation: nicotine, cytisine and epibatidine. The last-mentioned is extraordinarily potent (EC_{50} 1.5nM) but is a partial agonist of low efficacy producing a maximal effect only about 10% of that of DMPP. Of the subunit mRNA's found in the DMPP-reactive cells, the heteropentamer that best fits the pharmacological profile is one composed of the α6 and β4 subunits. Stay tuned.

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481 Expression of hyperpolarization-activated, cyclic nucleotide-gated ion channel (HCN) isoform transcript in a hair cell layer from the trout saccule

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Ih, a slowly developing inward cation current activated in response to hyperpolarization, is encoded by the HCN gene family. Ih for excitable cells in the CNS is involved in the regulation of the cell membrane potential, and consequently, spontaneous activity and synaptic output. For the inner ear, inwardly rectifying conductances with the electrophysiological characteristics of Ih have been detected in saccular hair cells of the goldfish (Sugihara and Furukawa, J. Physiol. 495:665-679, 1996) and leopard frog (Holt and Eatock, J. Neurophysiol. 73: 1484-1502, 1995). In the present investigation, we have utilized degenerate primers targeting sequence conserved across HCN isoforms with RT-PCR and 5' and 3' RACE to determine HCN isoform expression in a model hair cell preparation from the trout saccule. Cloning of the PCR products obtained with degenerate primers indicated relative abundance of sequences to be 7:2:1 (HCN1:2:4) for the trout saccular hair cell layer compared to 1:1:7 for trout brain. Specific primers were designed for teleost HCN1 allowing full length sequence determination for a HCN1-like isoform expressed in trout saccular hair cell cDNA. It is 3053 bp in length, with a 2817 bp open reading frame and 93% amino acid identity in S1-S6 membrane spanning domains, pore region and cyclic nucleotide binding domain compared to mouse HCN1. The N- and C-terminals display 51% and 43% amino acid identity, respectively. Although HCN1 appears to represent the primary HCN isoform expressed in trout saccule hair cells, smaller representations of sequence from HCN2 and HCN4 among the cloned products may indicate co-expression of HCN1 with other HCN isoforms, or alternatively, a differential hair cell expression of HCN isoforms. We hypothesize that HCN isoform expression, and in particular the expression of the HCN1-like isoform, dictates molecular aspects of spontaneous release of transmitter from trout saccular hair cells.

482 Gravity Receptor Function and Balance Behaviors in Inbred and 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). Small numbers ($n \geq 4$) of the following strains have been screened (mean age in days): inbred strains BUB/BnJ (84d), C3H/HeSnJ (120d), C57BL/6J (35d, 190d, 389d); mutations *Pldn^{pa}* (30d), *jc* (40d), *Cdh23^{v-2J}* (46d), *qk*

(45d), *Myo7a*^{sh1} (26d), *tlt* (51d), *qv*$^{lnd\text{-}2J}$ (25d). VsEP thresholds, latencies and amplitudes were quantified for each animal and descriptive statistics generated for each strain. Swimming behavior and drop reflexes were also recorded. Average values for normal VsEP response parameters at +6 dBre:1.0g/ms: P1=1.3ms, P2=2.2ms, P3=2.8ms; P1/N1=2μV; P2/N2=1.6μV. Thresholds averaged –8 dBre:1.0g/ms. B6 (35d, 190d), C3H/HeSnJ and *jc*/+ mice had VsEPs with values comparable to normal for all VsEP parameters. Strains that had one or more parameters outside of normal ranges included elderly B6 (high thresholds), BUB/BnJ (high thresholds, reduced amplitudes), *qk*/+ (high thresholds, reduced amplitudes), *qk/qk* (prolonged latencies, increased amplitudes), *pa*/+ (slightly reduced amplitudes), *qv*/+ (high thresholds, prolonged latencies). Despite these abnormalities, these animals demonstrated normal swimming and drop reflexes. These results suggest deficits in vestibular function that were not evident with behavior. Homozygotes for *sh1*, *jc*, *v*^{2J}, and *qv* mutations had no vestibular responses at the maximum stimulus intensity. 3 of 4 *pa/pa* mice and 7 of 8 *tlt/tlt* mice also had no discernible VsEPs. s*h1, jc* and *qv* mutants did demonstrate some swimming ability and normal drop reflexes. *v*^{2J}, *pa* and *tlt* mutants with absent VsEPs were unable to swim. These results demonstrate profound otolithic deficits for some strains; deficits that may have been overlooked based on behavioral screening alone.

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483 VEMPs: A Clinical Review

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Vestibular Evoked Myogenic Potentials provide the opportunity to assess the status of the saccular organ on each side individually. At the Midwinter ARO meeting of 1999 we presented normative data across age for latency and amplitude of this potential. Reported here is the use of this tool in a series of 53 patients referred to the Balance Center for evaluation. Following completion of a written and signed consent form, click stimuli at 95 dB nHL were presented unilaterally while recording the activity from the ipsilateral SCM muscle referenced to the manubrium of the sternum with the forehead as a common. Patients activated the SCM by turning the head against resistance to produce a raw EMG signal with p-to-p amplitude of 100 microvolts. The P1-N1 response was analyzed for latency and amplitude and assessed for its threshold level for click stimulation. A variety of diagnostic entities were evaluated including 17 Meniere's patients (8 of whom were tested multiple times prior to their ongoing Gentamicin treatments) and 5 bilateral paresis patients. All 53 patients were contacted by phone following completion of their treatments to establish any predictive ability of the P1-N1 response for prognosis of vestibular rehabilitation therapy or chemical ablation treatments.

The results indicate complete independence of this response from the caloric irrigation response or earth vertical axis rotational chair findings. The P1-N1 appears as an all-or-none response with minimal gradation change with treatment as the caloric response progressively would decrease in response. Specific indications for clinical use based on the response's ability to correlate with outcomes of treatments and a suggestion for ratio of the P1-N1 response to the contraction strength of the SCM as a reporting mechanism will be presented.

484 Migraine Related Vertigo: Quality of Life and Outcomes Analysis

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Migraine is one of the most common causes of vertigo. Its management may include: lifestyle management, including avoidance of triggers and good sleep and exercise habits, symptomatic and prophylactic pharmaceutical treatment and vestibular rehabilitation. Recent studies of migraine headache patients suggest they have poor quality of life including reduced functioning and reduced well-being, particularly associated with depression. We wished to examine the quality of life of migraine vertigo patients and determine if the patient's knowledge of their disorder influenced their outcome. Methods. We enrolled 11 female and 1 male subjects, excluded other causes of vertigo and ensured that they met the International Headache Society criteria for the diagnosis of migraine. They had to have at least one migraine vertigo attack per month for the last 6 months. Measures were applied at intake and at follow-up at 3 months. Measures included: Center for Epidemiologic Studies – Depression Scale (CES-D), the Satisfaction with Life Areas Scale (SLA), and a quiz on migraine-vertigo. Quality of Well-Being Scale (QWB) was done via telephone interview. Results. In all scores there was no significant change in score from the intake to the follow-up measures. The depression scale suggested that 78% of the subjects were depressed. The SLA showed satisfaction somewhere between neutral and "somewhat good". Quiz scores were unchanged. QWB scores were very poor, averaging 0.64, a level that approximates the score reported for a group of AIDS patients at baseline prior to starting AZT. Correlation analysis demonstrated that satisfaction with life and quality of well-being was correlated with depression levels. Discussion. The lack of improvement may have been related to the brief duration of follow-up (3 months) or ineffective therapy. The poor quality of life and the relation to depression suggests that depression management should be an integral component of migraine vertigo management.

485 The Sidelying Maneuver as an Alternative to the Dix-Hallpike Maneuver to Evaluate the Posterior Semicircular Canal

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The Dix-Hallpike maneuver is the standard test for benign paroxysmal positional vertigo of the posterior semicircular canal. It easily performed when the patient has normal range of motion, and a normal musculoskeletal system. In patients with musculoskeletal limitations, however, this test is difficult to use and may yield inaccurate results. Another way to test the posterior canal is the sidelying maneuver. We tested patients on both maneuvers, to determine if the sidelying and Dix-Hallpike maneuvers yielded comparable results in subjects without musculoskeletal impairments. Subjects were patients referred for diagnostic testing and suspected of having benign paroxysmal positional vertigo by history. Eye movements were recorded with electronystagmography, with eyes closed for 1 minute. To do the Dix-Hallpike maneuver a technician tilted the subject backward rapidly with the head rotated 45 deg toward the ear being tested. To do sidelying one technician applied force through the shoulders to lie the subject on one side, as another technician held the head turned 45 deg away from the side being tested, so that in the test position the head was positioned roughly nose upward.

Velocity of nystagmus was measured. The responses from each ear were compared for each test. Horizontal and vertical responses were compared separately. Wilcoxon matched pairs signed ranks tests showed no significant difference between the two tests. Chi-square tests indicated that significantly more subjects had zero-velocity responses than minimal or stronger responses, despite their complaints of having vertigo elicited by pitch rotations of the head. These data suggest that the sidelying maneuver is a valid alternative test to the Dix-Hallpike maneuver. Thus it may be used when giving the Dix-Hallpike maneuver is not practical.

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486 Exercise Induced Dizziness: Recogniton and Treatment

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Exercise induced dizziness is a newly recognized disorder. Individuals present with a variety of symptoms including headache, nausea, vertigo, and disequilibrium, which occur after exercise. The symptoms generally worsen with time and can significantly impact patients' daily routines since, often, only minimal exertion produces symptoms. We present a cohort of fifteen patients with exercise-induced dizziness. We detail the diagnostic work-up in this group of individuals including differentiating this disorder from common motion sickness and other vestibular pathologies. All of the individuals responded to customized vestibular rehabilitation including vigorous physical exertion with head motion. Since this disorder is poorly recognized and responds well to treatment we hope to provide guidelines that will allow practitioners to manage this disorder.

487 Recovery Of Dynamic Visual Acuity (DVA) In Patients With Vestibular Loss: Effect Of Exercise.

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Introduction: People with vestibular loss often complain of visual blurring with head movement. This occurs because of the decrease in gain of the vestibulo-ocular reflex. We hypothesized that patients performing exercises based on principles of vestibular adaptation would have improved visual acuity during head movement (DVA) compared to patients performing exercises that are "vestibular neutral". Purpose: to examine the effect of exercise on DVA in patients with unilateral vestibular loss (UVL).Methods: Patients with UVL were enrolled in this study after informed consent was obtained. Subjects were randomly assigned to either the experimental (n=12) or control (n=5) group. Visual acuity was measured during predictable (P) and unpredictable (UP) head movements before and after the six-week study using a computerized system in which the target letter (E) is displayed only when head velocity is between 120 and 180 degs/sec. Data included subject age and time from onset. Student's t-test was used to compare age, time from onset, initial DVA between groups. Paired t-test was used to examine change in DVA in each group. Level of significance was p=0.05.Results: There was no difference in age, time from onset or initial DVA between groups. There was a significant difference in DVA with intervention in the experimental group for predictable (pre = 0.395 + 0.136; post = 0.208 + 0.097) and unpredictable (pre = 0.415 + 0.156; post = 0.301 + 0.114) head movements (p=0.005). There was no change in DVA in the control group.Conclusions: These preliminary results suggest that the use of exercises based on vestibular adaptation results in improved visual acuity during head movement in patients with UVL.

488 Control of Sway in Vestibulopathic Subjects using Vibrotactile Display of Body Tilt

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We evaluated the effectiveness of a vibrotactile balance prosthesis precursor in maintaining postural stability during distorted sensory input. balance on dynamic posturography. Thirteen subjects, with vestibular deficits, as determined by electronystagmography and vertical axis rotation, were studied were studied using Equitest® computerized dynamic posturography (CDP). Their anterior-posterior (A/P) motion at the waist was measured with a micromechanical rate gyroscope and linear accelometer. These signals were processed by a portable computer and the resulting tilt estimate was displayed to the subject by an array of tactile vibrators (Audiological Engineering) attached to the torso. Both magnitude (three levels) and direction were coded. A modified Balance Master training task was used to familiarize and train the subjects to use this information prior to testing. Training time was typically 20 - 40 minutes.

The balance prosthesis precursor reduced the subjects' A/P root mean square tilt (RMS Tilt) and improved their balance. The subjects' RMS Tilt without the balance prosthesis during CDP sensory organization test (SOT) 5 and 6 were compared to RMS Tilt with the prosthesis. Average RMS Tilt decreased significantly ($p < 0.05$) when vibrotactile feedback was used as compared to the no-balance-aid condition. This finding was true for both SOT 5 and 6 conditions. Furthermore, several subjects who fell during SOT5 with no balance aid were able to stand when vibrotactile display of body tilt was provided.

489 Postural Sway in a Virtual Environment in Patients with Unilateral Peripheral Vestibular Lesions.

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Vestibular compensation adjusts for abnormalities of the vestibulo-ocular reflex (VOR) and postural instability seen acutely following unilateral peripheral vestibular lesions (UPVL). The goal of this study was to assess the visual motion sensitivity of patients with chronic UPVL's.

Seven patients who had undergone a vestibular nerve section at least 10 months prior to testing (3F/4M, ages 31-65) and seven gender and age-matched controls participated in the experiment. A visual stimulus of an infinitely long tunnel with checkered walls was displayed in the BNAVE, a virtual environment display facility. Subjects stood barefoot for 80 seconds while viewing sinusoidal movements of the virtual tunnel. Sixty seconds of movement were preceded and followed by 10 seconds of quiet standing. Scene movement occurred forward and back along the long axis of the tunnel at sinusoidal frequencies of 0.1 or 0.25 Hz. The field of view (FOV) conditions consisted of 1) full FOV, 2) central 30 degrees FOV, and 3) peripheral 30 degrees FOV. Head movement and center-of-pressure (COP) was measured using a magnetic tracking device and a force plate. Repeated measures ANOVA was used to test for the effects of subject group, movement frequency, and FOV condition.

There was no difference in the amount of sway elicited in the patients versus controls. However, the amount of sway was significantly affected by the FOV: full FOV and peripheral FOV elicited sway that exceeded the sway elicited by central FOV conditions. This effect was seen for both patients and controls and did not depend on the frequency of scene movement. We conclude that FOV significantly influences visual motion-induced sway in normal subjects and in patients with chronic UPVL's.

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490 The Effect of Age on Vestibular Rehabilitation Outcomes

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The purpose of this retrospective chart review was to compare vestibular treatment outcomes in young versus older adults. Persons with vestibular disorders who were either 20-40 (n=23; mean age 33) or 60-80 years of age (n=23; mean age 71) were matched by vestibular diagnosis and gender. Seven males and 16 females were included in each group and the diagnoses included: BPPV, unilateral vestibular hypofunction, bilateral vestibular hypofunction, head trauma, anxiety disorder, central dysfunction, labyrinthine concussion, migraine-related vestibulopathy, neuronitis, and unspecified dizziness. Vestibular test results were matched as closely as possible. The Wilcoxn Sign test was used to determine if there was a difference in scores between the age groups at the beginning and end of physical therapy using a p value of .05. There was no statistical difference between the younger and older age groups in treatment duration, number of visits, ocular motor testing, calorics, positional testing, and rotational testing. Older adults reported greater space and motion discomfort, more impairment on a 0-100 scale, and more impairment on the Dizziness Handicap Inventory (DHI) physical component. Younger adults had more impaired Timed "Up & Go" and Dynamic Gait Index (DGI) scores. Total DHI, the Activities-specific balance confidence scale (ABC), and number of reported falls were not different. At discharge, there were no statistical differences between the two groups on their DHI , the Timed "Up & Go", the DGI, reported symptom scale, or number of falls. The ABC was different with the older adults having better scores at discharge. We conclude that both older and younger adults improve with vestibular rehabilitation.

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491 The Contribution Of Central And Peripheral Vision To The Postural Sway Response Elicited By Moving Visual Environments In Healthy Children Aged 8-12

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The development of sensory integration for balance has not been examined in great detail. Central and peripheral vision may perform different roles in the visual control of posture. The goal of this study was to assess how central and peripheral vision contribute to the visual control of balance in children aged 8-12.

Ten healthy subjects (5 female, 5 male, ages 8-12) participated in the experiment. A visual stimulus of an infinitely long tunnel was displayed in the BNAVE, a virtual reality facility that displays a contiguous image across 3 back-projected screens that encompass approximately 75° vertical and 180° horizontal field of view (FOV). The walls of the tunnel had a texture of alternating black and white squares. Subjects stood barefoot on a force platform for 80 seconds while viewing sinusoidal movements of the virtual tunnel. Sixty seconds of movement were preceded and followed by 10 seconds of quiet standing. Scene movement occurred forward and back along the long axis of the tunnel at frequencies of 0.1 or 0.25 Hz. The FOV of the subjects was altered by software controls to consist of 1) full FOV, 2) central 30° FOV, or 3) peripheral 30° FOV (i.e. blocking out the central 30°). The RMS center of pressure during the tunnel movement was computed from the force platform data. Repeated measures ANOVA was used to test for the effects of movement frequency and FOV condition. The amount of sway was significantly affected by the FOV: full FOV and peripheral FOV elicited sway that exceeded the sway elicited by central FOV conditions. This effect did not depend on frequency of the scene movement. We conclude that peripheral vision plays an integral role in the control of posture in children aged 8-12.

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492 Sub-threshold Monopolar Galvanic Vestibular Stimulation Improves Postural Stability

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Galvanic vestibular stimulation (GVS) is a technique in which small currents are delivered transcutaneously to the afferent nerve endings of the vestibular system through electrodes placed over the mastoid bones. The current alters the firing dynamics of the afferents, thereby impacting vestibular perception and postural control. In this study, we examined the effects of sub-threshold monopolar binaural GVS on a subject's stability during perturbed stance. With monopolar binaural GVS, the basal firing rates of the vestibular afferents can be *clamped* to a higher (cathodal current) or lower (anodal current) level of activity. The goal of this study was to test the hypothesis that increased vestibular activity corresponds to increased relative stability in a subject's response to a perturbation.

To test this hypothesis, a movable platform was used to perturb the stance of subjects; the relative stability of their responses was compared between trials with a sustained cathodal monopolar GVS (0.05 or 0.10 mA) and no GVS. Sub-threshold GVS was used to ensure that the stimulus did not induce any forward-backward sway, which is typical of higher levels of monopolar GVS. A second-order transfer function model based on an inverted-pendulum model of human balance control was fit to each subject's responses, so that the relative stability parameter could be measured as the real part of the system poles.

Analysis of the transfer-function models showed that all subjects had an increased relative stability, on average, during trials with GVS (0.05 or 0.10 mA), compared to trials without GVS. This result supports the hypothesis that increased vestibular activity induced by sub-threshold cathodal monopolar binaural GVS can lead to improved postural stability.

493 Molecular Otopathology - Past, Present and Future

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Over the past several decades, the light microscopic study of human temporal bones has influenced profoundly our understanding of many hearing, facial nerve, and balance disorders; however, significant gaps do remain in our understanding of most of these disorders. These gaps may result from the limitation in the number of well-characterized disorders with documented auditory and/or vestibular test data available for study or a methodologic limitation imposed by the traditional histopathologic approach. Adaptation of the techniques of immunohistochemistry, immunoelectron microscopy, PCR, RT-PCR, laser capture microdissection, proteomics, and in situ hybridization histochemistry to the study of the temporal bone have afforded investigators the tools necessary to study the normal function and pathology of the inner ear and facial nerve. With these tools, phenotype-genotype comparisons can be made, as well identification of viral DNA or RNA that may be associated with the development of hearing, facial nerve, or balance disorders. The development of these applications, as well as examples of how these tools are being used will be presented. Opportunities for future research will also be highlighted.

494 Laser Capture Microdissection and Proteomics

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Proteomics based approaches are beginning to be utilized to study the natural history and treatment of a variety of diseases, particularly cancer. Two-dimensional gel electrophoresis is still the foundation of most proteomics studies, and we have used this approach to look for molecular changes that accompany early breast cancer progression. Newer technologies such as laser capture microdissection and highly sensitive mass spectrometry methods are currently being used together to identify greater numbers of lower abundance proteins which are differentially expressed between defined cell populations. Novel technologies, some still in developmental phases, will enable identification of validated targets in small biopsy specimens, including high-density protein arrays, antibody arrays, lysate arrays and surface enhanced laser desorption/ionization time of flight analysis. Such technologies are expected to supplement mRNA based assays, and provide critical information on protein levels and post-translational modifications.

495 Molecular biology of the spiral ligament

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There is growing evidence that the spiral ligament plays critical roles in cochlear function. There are two major cell classes within the ligament, type I and type II fibrocytes. Type II fibrocytes appear to be the principal site of uptake of K+ ions from the perilymphatic space for recycling to the stria vascularis. Less is known about the function of the more abundant type I fibrocytes, but these cells clearly play a larger role than merely serving as a pathway for movement of K+ ions to the stria. In the hydropic guinea pig model, type I fibrocytes show immediate post surgical cytochemical changes that precede significant development of endolymphatic hydrops by weeks. These cells continue to deteriorate as the hydropic condition progresses, which suggests that their deterioration may lead to the hydrops. In contrast to type II fibrocytes, type I fibrocytes have far more abundant content of molecular constituents of pathways that control cellular stress responses. Two families of transcription factors that control cellular stress responses, NFkappaB and AP1, as well as numerous related compounds are well represented in type I fibrocytes but not in type II fibrocytes. In response to a systemically administered inflammatory agent, LPS, type I fibrocytes show NFkappaB activation, usually with a marked bilateral asymmetry, which is reminiscent of hearing loss asymmetries seen in sudden hearing loss and immune mediated hearing loss. In contrast, exposure to noise induces symmetric activation of NFkappaB in type I fibrocytes as well as marked changes in the cytochemistry of type II fibrocytes. Cells of the organ of Corti rarely show NFkappaB activation to either LPS or noise exposure. An overview of the stress response pathways within type I fibrocytes will be presented as a context for consideration of responsiveness of these cells to systemic and local stresses that may account for previously poorly understood cochlear pathologies.

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496 Analysis of Archival Temporal Bone DNA - A Valuable Technique

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During the last decade, there has been growing interest in the development of molecular biological techniques that can be applied to the pathologic investigation of archival human temporal bones. The impetus for the development of these techniques is in large part related to the fact that the temporal bone collections within the United States and Europe represent a tremendous resource of available pathological material which has been compiled over decades and which is otherwise usually not available from living patients. Although light microscopy, electron microscopy and immunohistochemical studies have all led to the advancement of our understanding of many otologic conditions, there is a void in our comprehension of the molecular pathogenesis of many disorders. Over the past several years, we have been engaged in molecular studies of otopathology using archival human temporal bones. Our initial interest arose during our investigation into the possible viral etiology of otosclerosis and from a need to demonstrate viral nucleic acids in archival temporal bones. Capitalizing on that prior experience we have explored the use of the techniques in temporal bone research to investigate several other genetic diseases in archival tissue. The results of these investigations have made apparent several problems with the current technology, which have posed a significant potential barrier to both current and future investigations

497 Molecular pathology of otosclerosis

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Otosclerosis is a chronic inflammatory disease restricted to the human temporal bone. The disease is a frequent cause of deafness in adults of the United States (about 10%). Morphologic investigations including immunohistochemistry and electronmicroscopy have shown, that otosclerosis is a chronic three-step process with all characteristics of an chronic inflammation. The cause of this bone disease, morphologically very similar to Paget`s disease, is still not clear. Actually the major hypothesis discussed are an (auto-)immunologic, a genetic and viral etiopathogenesis of otosclerosis. The application of antibodies against collagen II in mice have been shown to induce lesions in the temporal bone which were morphologically very similar to otosclerotic foci. Mutations in the collagen gene Col 1A1 have been shown in a family with high incidence of otosclerosis. In three further families loci designated as Otoscleoris 1, 2 and 3 have been identified by linkage dysequilibrium mapping. Investigations of temporal bone specimens from otosclerotic focus have shown the presence of measles virus RNA related sequences by RT-PCR. These results are in good agreement with the electronmicroscopic and immunohistochemic findings of the past. Further biochemic investigations on the perilymph support the results of a measles virus presence within the otosclerotic tissue. Very recently we have employed on paraffin-embedded and decalcified temporal bone sections in situ RT-PCR which combines the morphology with the high sensitivity of DNA amplification. By the use of this technique we were able to localize measles virus related sequences within the otosclerotic tissue. Furthermore, analyzing by in situ RT-PCR primary preosteoblast cell culture we have found in one case measles virus RNA related sequences. In conclusion by the use of highly sensitive and specific molecular biologic methods advances especially in genetics and virus association of otosclerosis have been made.

498 Temporal Bone Findings Associated with GJB2 Deafness

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Mutations in gap junction protein ß-2 (GJB2), which encodes connexin 26 (Cx26), account for one-half of autosomal recessive non-syndromic moderate-to-profound inherited congenital sensorineural deafness. A single mutation (35delG) that produces a truncated protein is found in 97% of persons with Cx26-related deafness. Cx26 molecules oligomerize to form hexameric connexons that are embedded in the plasma membrane and allow for intercellular transport of electrolytes and second messengers. Cx26 is believed to be essential for potassium recycling in the inner ear.

Our understanding of inner ear histopathology associated with Cx26 deafness has been limited by the inability to produce a viable Cx26 deficient rodent model. We therefore sought to identify examples of Cx26 deafness in temporal bones from the Universities of Iowa and Chicago Archival Temporal Bone Libraries. We selected for screening temporal bones from persons who had congenital severe-to-profound non-syndromic deafness attributed to unknown or genetic causes.

Eleven temporal bone specimens were analyzed. Three celloidin embedded 20 - 25 µm sections were used for DNA processing and five mid-modiolar hematoxylin and eosin permanent slides from each temporal bone were reviewed for histopathologic changes by light microscopy in a blinded fashion. Each temporal bone sample was screened by PCR amplification with primer pairs that span the 35delG mutation. If the 35delG mutation was found, the remainder of the gene was screened for other GJB2 deafness-causing mutations.

Two temporal bones were identified that carried Cx26 mutations. One individual was homozygous for the 35delG mutation; the other individual was a compound heterozygote carrying the 35delG mutation and an E101G missense mutation. We review the histologic findings associated with these temporal bones and discuss the limitations of this type of study.

499 Mitochondrial mutations affecting the temporal bone

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The majority of cases of MELAS (mitochondrial encephalopathy, lactic acidosis and stroke-like episodes) are linked to a mitochondrial DNA (mtDNA) mutation at nucleotide 3,243. In MELAS, normal and mutant mtDNAs co-exist in a heteroplasmic manner. Hearing loss is common in MELAS and clinical studies have suggested that the SNHL is often bilateral, generally symmetric and progressive, with a down-sloping or flat audiometric pattern. However, temporal bone histopathology and distribution of mutant mtDNA in the inner ear have not been reported. I present the otopathology from a patient with MELAS and quantitative mtDNA analysis in the inner ear. Histopathological examination of the temporal bones revealed severe degeneration of the stria vascularis and mild to moderate loss of spiral ganglion cells. Marked loss of the ventral cochlear neurons was also demonstrated. Both the stria vascularis and spiral ganglion cells are rich in mitochondria and their function is presumably disturbed by the mutant mtDNA. Quantitative analysis of mutant mtDNA was performed on the right ear. The membranous part of the inner ear was dissected under the microscope and total DNAs were obtained by En-Zap kit. The mtDNA was amplified using PCR and a sense primer and an antisense primer. The percentage of mutant/total mtDNA was measured by the ratio of fluorescent intensity of the mutant fragment. The quantitative DNA studies showed that the amount of mutant mtDNA was; organ of Corti 85%, stria vascularis 78%, lateral semicircular canal 89%, facial nerve 82%, and brain 74%. The proportion of mutant to wild-type DNA was similar in both clinically affected and unaffected tissues within the inner ear. These findings suggest that dysfunction of the stria vascularis and spiral ganglion cells within the inner ear as well as lesions in the central auditory pathway results in sensorineural hearing loss in MELAS.

500 Relevance of Standard Light Microscopy in Studies of Genetic Deafness

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The standard light microscopy method of studying human temporal bones involves formalin fixation, EDTA decalcification, celloidin embedding, serial sectioning at 20 micron thickness and staining of every tenth section. Quantitative assessments of sensory and neural structures in the cochlea are performed, which can then be correlated with audiometric data. There are many reasons why standard light microscopy of human temporal bones is relevant in studies of genetic deafness. Each reason will be illustrated with a representative case study.

1. Very little is known of the histopathology of genetic hearing loss in the human. Less than 10 cases of otopathology where the precise mutation was known have been reported worldwide. A review of these few cases has shown that many different mechanisms can cause genetic hearing loss. Light microscopy can highlight those structures of the inner ear that are most affected in each syndrome; these affected areas are potential therapeutic targets for any future pharmacologic or gene therapy.

2. Standard light microscopy can help to pinpoint an area of potential pathologic change which can then be studied in more detail by electron microscopy, immunostaining or PCR-based molecular techniques. Such study can be performed on unstained, archivally stored sections.

3. Human and animal otopathology can provide valuable complementary information. The validity of animal models can be verified by comparison with human otopathology. Studying human bones can also generate hypotheses regarding possible mechanisms of hearing loss, which can then be tested in a suitable animal model. Also, studying human specimens becomes important in many syndromes for which no animal models currently exist.

4. Human otopathologic studies can sometimes lead to identification of new loci and genes responsible for genetic deafness.

(Supported by NIDCD)

501 The Professional Life of Graeme K. Yates (1944-2000)

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Graeme K. Yates was born in Australia in 1944. After obtaining a physics B.Sc. (with Honors) in 1970 from the University of Western Australia, he began graduate work there in the laboratory of Prof. Brian Johnstone. After earning his Ph.D. degree in Physiology, Dr. Yates did 3 years of post-doctoral work in Nottingham, England. He then returned to the University of Western Australia for the remainder of his distinguished research career. Concentrating his efforts on the vertebrate cochlea, Dr. Yates made important contributions to a surprisingly wide range of topics, including: basilar membrane vibrations; patterns of auditory nerve fiber discharges; cochlear electrical potentials; functional effects of cochlear toxins; coupling of cochlear vibrations to hair cells; endolymphatic flow; the workings of lizard and emu ears; the effects of efferent neurons; oto-acoustic emissions; and electrically evoked cochlear emissions. He was working on that last topic at his tragic death in 2000. Dr. Yates' work in some of these areas will be reviewed by those of his former friends and colleagues who will speak in this Symposium. My own work with Dr. Yates was undertaken in Perth during the summer of 1988, when he, Dr. Robert Patuzzi, and I together studied the two-tone suppression of cochlear transduction potentials. Our results strongly supported a seminal OHC-feedback-amplification theory then being pioneered by the Perth group. I found Dr. Yates to be a wonderful colleague, true gentleman, and treasured friend.

502 The Role of Research on Lizards in Understanding Peripheral Hearing Mechanisms

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Lizard ears show highly frequency selective and sensitive responses to sound. In collaborations with Graeme Yates over a period of 12 years, we studied a number of stages of peripheral auditory processing that lead to the excitation of hair cells in the Australian Bobtail lizard Tiliqua rugosa (Trachydosaurus rugosus). At the beginning, recording from primary auditory nerve fibres and studying the vibration of the basilar membrane in the same ears, we demonstrated that the vibration

of the basilar membrane of this species is not especially frequency selective, but that its movement rather simply reflects the middle-ear transfer function. Instead, our studies showed that the high frequency selectivity of primary auditory afferent fibres derived from the micromechanics of local hair-cell groups.

These groups of coupled hair cells, that consist of roughly equal numbers of hair cells with oppositely-oriented bundles, also show spontaneous otoacoustic emissions (SOAE). Using single-neural recordings and studying the suppression of SOAE, we showed that small groups of hair cells that are coupled by pieces of tectorial material (sallets) form highly sensitive and frequency-selective response units. Using electrical activation of hair-cell activity in vivo and measuring the emitted sounds (EEOAE), we were later able to demonstrate that in these lizards, the active process in these hair cells is located in the stereovillar bundle.

Supported by grants from the Australian NH&MRC to Graeme Yates and from the German DFG to Geoff Manley.

503 Mechanical Pre-processing of Sound in the Base and Apex of the Cochlea

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Auditory-nerve fibers reflect information about the mechanical pre-processing of sound that occurs in the cochlea. In the late 1980's, Graeme Yates developed a technique which allowed the characteristics of this pre-processing to be derived from the responses of individual auditory nerve fibers to sounds of different frequencies. In the early 1990's, Graeme and I used this technique to show that the pre-processing characteristics differed markedly between fibers innervating the two halves, i.e. the base and apex, of the cochlea. Fibers with characteristic frequencies above ~4kHz, which innervate the basal half of the cochlea, reflected a classical type of pre-processing whereby responses to near-CF tones grew almost linearly at low sound levels, but at highly compressive rates (e.g. <0.2 dB/dB) at higher levels. Fibers with characteristic frequencies below ~4kHz, which innervate the apical half of the cochlea, appeared to reflect less nonlinear pre-processing, with responses to near-CF tones growing at rates of around 0.5 dB/dB over a wide range of levels. Graeme encouraged me to continue my own more direct mechanical investigations of the apical cochlea in order to work out what the real differences between apical and basal pre-processing were. The most recent of these investigations show the mechanics of the apical cochlea to be nonlinear in a manner which is qualitatively similar to, but quantitatively weaker than that observed in the basal cochlea. The weaker apical nonlinearities are not as sharply tuned in the frequency domain as the basal turn nonlinearities are. In fact, one form of mechanical nonlinearity appears to both generate and operate at sub-sonic frequencies in the apex of the cochlea. All of these observations are perfectly compatible with the predictions of classical positive-feedback models, the type of model which Graeme was so successful at understanding and promoting.

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504 Waves In and Under Water

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Guided by a set of notes, the main cochlear mechanics program of Graeme K. Yates has been analyzed. Potentially, the program is very flexible and allows for many, many variations and extensions; only very few of these have been explored. The main problems addressed in the program are: a) intensity-dependent nonlinearity, and b) properties of electrically-evoked otoacoustic emissions. Salient features of the results will be presented, along with a description of the means by which these have been attained.

505 A mathematical model of stria vascularis

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Normal mammalian hearing requires the endocochlear potential (EP) above hair cells to be maintained at about +90mV. The generation of this unusual extracellular potential and the maintenance of the K+ rich endolymph of the cochlea is accomplished by the multilayered pumping epithelium of stria vascularis. Stria vascularis is comprised of basal cells, marginal cells and intermediate cells, with a rich supply of blood vessels. It accomplishes its tasks of current generation, ion exchange, Cl- scavenging and fluid secretion in at least a two stage process: voltage generation followed by ion exchange. The roughly three layers of basal cells, which are not particularly rich in mitochondria, are bathed in a perilymph-like solution rich in Na+, and appear capable of generating a large positive potential (more than +100mV) within the intrastrial space, which is also rich in Na+. This positive intrastrial potential is clear evidence that the basal cells are capable of pumping ions electrogenically (either K+ and Na+ towards endolymph, and/or Cl- away from endolymph). The generation of the K+ rich endolymph, on the other hand, is clearly performed by the marginal cells that separate the basal cells from the endolymph, are rich in mitochondria, and exchange Na+ and K+ (Na+ from endolymph and K+ into endolymph). It is also clear that the third role of stria vascularis is the re-uptake Cl-, which moves passively into the endolymph of scala media down its electrical gradient. Ultimately these ion movements are not perfectly balanced osmotically, and stria vascularis delivers a net amount of salt into endolymph, creating a small osmotic gradient. This gradient produces a net water and salt flux into endolymph that must be removed by bulk flow along the cochlear duct, to be reabsorbed by the endolymphatic sac. Just what the role of the intermediate cells is in this complex epithelium is not completely clear, but they are certainly essential: their absence leads to a failure in EP generation, and deafness. While much good research is under way on the molecular biology of the membrane proteins responsible for this salt transfer, and some suggestions have been made concerning the mechanisms of ion transport, there has been a lack of rigor in the analysis of the ionic exchange, with little acknowledgement or discussion of the need for the proposed schemes to obey the rules of epithelial transport. A mathematical model of strial function will be presented, which obeys all known rules of epithelial transport (voltage, ionic and osmotic steady state; bulk charge neurtrality), and is consistent with the available experimental evidence (the location, type and electrical properties of the various membrane transporters on the surfaces of the marginal and basal cells, and the known potential and ionic profiles within the epithelium). The model is a minimal model, including only basal cells and marginal cells. It accomplishes the voltage generation, ion exchange and Cl-scavenging roles of the stria without intermediate cells, suggesting that they may not be required for active ionic transport per se, but may simply serve a crucial isolation role, separating the actively pumping basal and marginal cells from the blood vessels. Without such “electrically insulating cells”, the good work of the basal and marginal cells would be squandered because the electrical and ionic gradients they create would be “short circuited” to blood.

506 Mechanical and chemical modulation of electrically-evoked oto-acoustic

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Since the first descriptions of electrically evoked oto-acoustic emissions (EEOAEs) by Hubbard and Mountain (Science, 1983, 222, 510-512) interest in these, sometimes enigmatic phenomena has grown, and other laboratories around the world are now stimulating the cochlea with alternating current. EEOAEs hold promise as an in-vivo alternative, complementing the isolated outer hair cell in the search for the "cochlear amplifier". Our lab began looking at EEOAEs in 1993. Early attempts were frustrating because the techniques are fraught with

pitfalls, even for enthusiastic beginners. An amplifier we were using to monitor the current strength produced our first "emission". But we soon learned the ropes and thanks largely to Graeme's talents in software development and electronics progress was made. This talk will cover work done over the past two years, focusing specifically on interactions between the chemical and mechanical modulation of what we assume to be the EEOAE generators.

507 Auditory-nerve rate-intensity functions as a window on cochlear micromechanics

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A highlight of Graeme Yates' work was his detailed model of the relationship between the micromechanical movement of the basilar membrane and the spike output of auditory-nerve fibres. The combination of a square-law function representing synaptic behaviour, and a nonlinear, compressive function representing the known mechanical behaviour at the basilar-membrane level explained crucial features of auditory-nerve rate-intensity (RI) functions. For example, different relative thresholds of the two processes were able to account for the known types of RI-function. Also, the compressive nonlinear component was similar for nerve fibres of similar CF in the same individual, reflecting the common basilar-membrane input to hair cells of a restricted cochlear region.

Together with Graeme Yates, we studied RI-functions in two species of birds, the emu and the barn owl, since comparative studies had suggested an independently-evolved tandem of two functionally-different hair-cell types in the bird cochlea. However, little is known about the micromechanics of the system. We showed that auditory-nerve RI-functions in birds also display evidence of an underlying mechanical nonlinearity. They were well fit by the mammalian model and the compressive nonlinear component was restricted to RI-functions at frequencies close to CF. This provided further evidence for an amplification mechanism increasing sensitivity at low sound levels in birds. In striking contrast to mammalian data, however, the compressive nonlinearity in birds was not the same for fibres of closely-similar CF in the same individual, but varied with fibre sensitivity. This suggested an individual, localised amplification effect instead of a global feedback loop uniformly driving all hair cells within a narrow range of CFs.

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508 Coupling of electromechanical force into the organ of Corti

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The mechanism by which the outer hair cell (OHC) force is coupled into the organ of Corti to produce frequency tuning of the travelling wave on the basilar membrane (BM) at low sound pressure levels remains unknown. In order to elucidate coupling mechanisms, vibration measurements are required from the apical surface of the hair cells and the tectorial membrane (TM). However, to date these structures still remain optically inaccessible in the high-frequency region of the cochlea, where tuning is most pronounced. As a first step in addressing the coupling question, we made vibration measurements in three orthogonal directions from the apical region of the in-vitro cochlea, where these structures are optically accessible [Gummer et al., 1996; Hemmert et al., 2000]. Evidence was found that the TM is tuned in the radial direction and that the resonant frequency is about 0.5 oct below that for the BM. According to this experimental data, the 90° phase lead of OHC force relative to passive BM displacement, required for active "amplification", derives from the 180° phase lag of TM displacement relative to BM displacement near the BM resonant frequency, together with the 90° phase lag of OHC force relative to stereocilia displacement, which in turn results from the time constant of the basolateral cell membrane. This time constant also causes the somatic mechanical response of the OHC to be attenuated, estimated to be up to 40 dB. It is still not understood how the attenuation problem has been resolved. In order to address this question, the force produced by OHCs in response to extracellular electrical stimulation in a cochlear explant was measured with a high-impedance cantilever. The force was independent of frequency up to at least the characteristic frequency and was a factor of ten larger than the force produced by isolated OHCs. Further experiments are being conducted to measure the extra- and intracellular potentials.

509 Vestibular evoked myogenic potentials in audiogenic seizure prone mice.

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Background. Following early reports by Bickford, et al. (1964), myogenic activity evoked by vestibular stimulation has had widespread research interest. Recording vestibular evoked myogenic potentials (VEMPs) using a high amplitude acoustic signal suggests possible generator initiation site at the saccule, a structure credited with both vestibular and acoustic capabilities. Studying the VEMP in deaf mice which are audiogenic seizure prone suggests a possible source of the seizure activity not of cochlear origin, but rather vestibulogenic and therefore likely a saccular origin. To this end microphthalmic white mice were studied using auditory brainstem response instrumentation to evoke ABRs and VEMPs. Also, cochlear histological examination of inner and outer hair cells was performed.

Results. Hair cells and stereocilia of microphthalmic white mice were photographed using scanning electron microscopy. Results show no evidence of normal development of cochlear hair cell stereocilia in homozygous mutant (miwh/miwh) mice. Auditory brainstem responses indicate deafness except during a narrow age span between 18 and 36 days. Audiogenic seizure activity occurs regardless of measured hearing responses. In heterozygotes with defective hair cells, damage patterns include fractured tips, fusion, disarray and, notably in inner hair cell stereocilia, complete loss of vertical alignment. ABRs are abnormal in all heterozygote mice and audiogenic seizure activity is sporadic. Normal littermates (+/+) have normal hearing and normal hair cells and stereocilia and also are prone to seizures. VEMPs are evident inconsistently in mutant audiogenic seizure prone mice suggesting the possibility of a saccular origin to the seizure activity.

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510 Sound Evoked Myogenic Response in Mice: Analysis of Saccule Function with Aging

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Function of the mouse saccule was investigated using sound evoked myogenic responses. CBA and C57Bl/6 mice were sedated and secured in a neck extended position. The sternocleidomastoid muscle was exposed and needle electrodes placed into the body of the muscle. Sound evoked myogenic responses were recorded with 250 and 500 Hz tone bursts. The robustness of the response decreased with increasing sedation of the animal. 250 Hz tone bursts produced a robust signal at 15-25 ms that was abolished by paralyzing the sternocleidomastoid muscle. C57Bl/6 mice showed an age related decline in the presence and robustness of the waveform. Sound evoked myogenic response remained normal in young and aged CBA mice.

511 Evaluation and Prevention of Gentamicin induced Vestibular and Cochlear Ototoxicity

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This study investigates the ototoxicity of gentamycin (GM) on the functions of semicircular canals and otolithic organs in rabbit, and to compare the results with cochlear ototoxicity. The preventive effects of nitric oxide (NO) inhibitor (L-NAME, MK-801), iron chelator (deferoxamine), and neurotrophic factors (GDNF, BDNF) against GM induced vestibular and cochlear ototoxicity are also investigated.

Animal rotation system is designed to rotate the animal sinusoidally or in velocity step rotation, and record the eye movement and analyze it automatically. Off-vertical rotation is performed with tilting angle of 30 degrees to evaluate the otolithic function. Evaluation of cochlear ototoxicity is performed by DPOAE, ECoG and ABR. GMs are administered intraperitoneally or topically to cochleostomy site using osmotic minipumps. Microscopic structural changes of the semicircular canals, otolithic organs and cochlea of the animal are examined under scanning electron microscopy.

GMs induce vestibular toxicity including otolith organ confirmed by low gain, bias and modulation. Deferoxamine, MK-801 and BDNF have protective effect for the cochlear and vestibular toxicity, but not in GDNF. In SEM study, GMs induce cochlear hair cell damages in baseal turn, but not evident in utricular macule.

In summary, GMs induce cochlear and vestibular ototoxicity including functional otolith organ damage, and these damage are prevented using Deferoxamine, MK-801 or BDNFs.

512 Trophic factors induce partial restoration of otolith and canal function after gentamicin ototoxicity in the guinea pig

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This study examined the recovery of guinea pigs' vestibular function using a combination of trophic factors after either unilateral or bilateral gentamicin instilled locally into the middle ear. Vestibular tests were performed using a scleral search coil system and rate table adapted for sinusoidal and off-vertical axis rotation (OVAR). Horizontal vestibular ocular reflexes (HVOR) were tested at sinusoidal frequencies from 0.02 to 2.0 Hz, and macular ocular reflexes (MOR) were assessed using OVAR constant tilt table velocities in the counterclockwise or clockwise direction. HVOR and OVAR tests were performed 87-103 days after gentamicin injection (20 mg/ml, 0.20 ml bolus). For HVOR, gain and phase were acquired. For OVAR, modulation sensitivity (peak eye modulation/exposure to gravity) and bias velocity were used. Two groups of animals received a mixture containing artificial perilymph, retinoic acid, insulin-like growth factor-1, and transforming growth factor-[alpha], delivered to the inner ear though a catheter and mini-osmotic pump, over a course of 28 days (Durect Corp., model 2002). One of these groups received unilateral gentamicin, and the other bilateral gentamicin, seven days before start of trophic factor administration. There were three control groups in which animals received either gentamicin bilaterally, unilaterally, or no gentamicin at all. For control groups, artificial perilymph alone was infused for 28 days. Both HVOR and OVAR control values did not differ significantly from those acquired previously from this lab. Animals that received the trophic factor mixture showed noticeable increases in both HVOR and OVAR function. Hence, for canal and otolith function, the growth factors appeared to enhance recovery from gentamicin intoxication.

513 Vestibular Nerve Afferent Responses after Intratympanic Gentamicin

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Intratympanic gentamicin is effective in controlling vertigo in Meniere's disease. In order to elucidate the effect of gentamicin on the labyrinth, we performed extracellular recordings from vestibular afferents in 17 chinchillas either 2 weeks (n=9; group A) or 3 months (n=8; group B) after a single unilateral intratympanic gentamicin injection (26.7 mg/ml, 0.2 - 0.6 ml). Comparisons were made to responses on the untreated side. In group A, 185 units were recorded from the treated side and 69 from the untreated side. In group B, the number of afferents studied was 163 and 79, respectively.

The spontaneous firing rate of the afferents and their sensitivity to pitch tilt, roll tilt, or sinusoidal rotation were examined. The spontaneous firing rate (± 95% confidence interval) was lower ($p<0.01$) on the treated side (47.0±3.7 spikes/s in group A and 36.1±3.4 spikes/s in group B) than on the untreated side (56.7±5.5 and 51.1±5.4 spikes/s, respectively). Only 18% of the afferents on the treated side in group A and 27% of the afferents on the treated side in group B responded to rotational or tilt stimuli. Tilt sensitivity of the otolith afferents was lower on the treated than on the untreated side ($p<0.001$, 10.4±2.0 (n=21) vs. 44.1±11.7 (n=25) in group A, and 10.7±3.3 (n=12) vs. 4.0±6.2 spikes$\cdot s^{-1} \cdot g^{-1}$ (n=26) in group B). For canal afferents, the rotational sensitivity of regular units was decreased on the treated side when compared to the untreated side ($p<0.001$, 0.04±0.04 (n=9) vs. 0.34±0.15 (n=24) in group A; 0.03±0.01 (n=18) vs.0.28±0.07 spikes$\cdot s^{-1}$/deg$\cdot s^{-1}$ (n=32) in group B).

Intratympanic gentamicin ablates or markedly reduces the responses of vestibular-nerve afferents to motion stimuli. The spontaneous firing rate is also lowered, but to a lesser extent. These changes in the responses of afferents are present for at least 3 months after the gentamicin treatment.

514 Linear and nonlinear components of the pitch VOR evoked by high-frequency, high-acceleration rotations in the squirrel monkey

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We have identified linear and nonlinear 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 nonlinear pathway has a gain that rises with velocity at higher frequencies and is more modifiable than is the linear pathway following vestibular lesions and spectacle-induced adaptation. We sought to determine if these pathways were also evident in the VOR evoked by pitch rotations.

In 3 animals, we analyzed the 3-dimensional VOR evoked by steps of acceleration (500 - 6000°/s^2) and by sinusoidal rotations (0.5 - 15 Hz, 20 - 100°/s) delivered about the earth-vertical axis while each animal was in the right-ear-down position. Linear and nonlinear components were identified in the responses. The gain during the acceleration step (G_A) was greater than that during the velocity plateau in each of the animals ($p < 0.001$). This difference between G_A and G_V was 9 ± 16%. After adaptation to 2.2X magnifying lenses, G_A was 27 ± 15% greater than G_V. An increase in gain measuring 10 ± 7% was noted in the

responses for 4 Hz rotations at 100°/s in comparison to the responses for 4 Hz rotations at 20°/s in 2 of the animals. The findings are similar to those we have previously reported for the horizontal VOR.

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515 Vergence-Mediated Modulation of the Human Horizontal Angular VOR Provides Evidence of Pathway-Specific Changes in VOR Dynamics

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We recorded the horizontal angular VOR (scleral search coil) evoked by high-acceleration, passive, head-on-body rotations (head thrusts) in 5 subjects with normal vestibular function and in 1 subject with unilateral vestibular hypofunction. Head thrusts were delivered while subjects were fixating a near (15 cm) or far (124 cm) target. The initial eye velocity after the onset of head movement was similar for both viewing conditions. The vergence-mediated increase in VOR gain occurred after a latency of 20.8 ± 4.2 ms.

We modeled these responses with 2 pathways. The first pathway is represented by a constant gain term. The second pathway is composed of a gain term (specified by the vergence angle), a first-order lead term with a break frequency of 2-4 Hz, and a time delay of 10 ms. This pathway has directional specificity and is rectified. Simulations of this model predict the dynamics of the VOR for the 2 vergence conditions.

This model also accounts for the observation in the subject with unilateral hypofunction that vergence-mediated changes in VOR gain are absent for responses to ipsilesional rotations. Our representation of the second pathway is similar to the nonlinear pathway we have described in the squirrel monkey VOR.

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516 Spectral Analysis of Eye Movements Induced by Pulsatile Electrical Stimulation

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Our neural vestibular prosthesis offers the opportunity to stimulate the nerve innervating a single sensory organ (e.g., lateral canal) with electrical pulses. We used this opportunity to investigate the frequency response of the angular VOR at high frequencies (1 Hz to above 500 Hz) using signal waveforms and frequencies impractical to achieve via motion.

Stimulation consisted of two waveforms multiplied together: a biphasic pulse train (pulse rates between 50 and 5000 Hz) and a set of modulating square waves (1 to 319 Hz, 50% duty cycle). We hypothesized that VOR responses would be measured at frequencies exceeding 100 Hz. Results showed that the angular VOR is constant within a factor of 3 from 1 Hz to 159 Hz.

We also hypothesized that we would be able to observe spectral responses at frequencies corresponding to our input frequencies, as well as at sidebands predicted by pulse rate modulation theory. Spectral results showed eye responses at frequencies exceeding 500 Hz. Furthermore, the eye movements included (1) responses at odd harmonics of the modulating square wave, (2) responses at the frequency of the biphasic pulse train and integer harmonics of that frequency, and (3) responses at those frequencies plus and minus harmonic frequencies of the modulating square wave.

It appears that the nervous system passes signal components at frequencies up to and exceeding 500 Hz, since responses at these frequencies were found in the eye movements. Furthermore, the eye movement spectra support theoretical predictions for pulse rate modulation.

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517 The Early Kinetics of Gentamicin Uptake into the Inner Ear

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Transtympanic gentamicin administration has become a popular modality for the treatment of Meniere'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 into the inner ear after delivering the medicine into the middle ear using a variety of different techniques and sustained release modalities. In our previous work we reported an early peak perilymph concentration and the presence of intracellular gentamicin at the four-hour time point. We have also demonstrated the activation of inner ear apoptotic pathways at this early time point as well. In this report we examine the kinetics and effects of gentamicin at very early time points at one to four hours after administration. Healthy adult Chinchilla's undergo implantation of two different middle ear sustained release devices (one in each ear) containing gentamicin. The animals are then maintained in a neutral position and undergo perilymph sampling at predetermined time points. This technique allows us to accurately assess very early time point inner ear gentamicin kinetics as well as to compare the activity of different sustained release devices by using each animal as its own control. 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.

518 Effect of Intense Gravitational Stimuli on Cerebellar Purkinje Cells

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The cause of motion sickness is generally considered to be related to asymmetries within the vestibular system or to a mismatch of vestibular and visual sensations. However, the neural pathways that manifest this dysfunction are poorly understood. Most, if not all, incidences of motion sickness are correlated with exposures to rapid, acute accelerations. The purpose of these experiments was to examine the effect of exposing mice to brief (8.5 min), intense changes in gravity (rapidly changing rates of linear and angular acceleration) on long-term behavioral changes and on the morphology of vestibulo-cerebellar neurons. Mice exposed to accelerations that varied between 2-4 G/sec for 8.5 min exhibited decreased activity levels as much as 20% below control mice for 12 hrs following stimulation. In addition, Purkinje cells in the cerebellar vermis underwent morphological changes in response to this stimulation. Within 15 min of stimulation the cell cytoplasm of some Purkinje cells contained swollen, laminated endoplasmic reticulum (ER). Some mitochondria were dense while others appeared normal. Within the dendrites more clathrin-coated vesicles were apparent, and ER appeared swollen. Within 2 hrs the cytoplasm darkened, contained numerous free ribosomes, and the ER was swollen. In the dendrites the swelling in the ER increased and clathrin-coated vesicles within the dendrites were still apparent. The topography of affected Purkinje cells corresponds to the location of primary vestibular mossy fiber projections to granule cells in the cerebellar vermis.

Current models of motion sickness do not explain these observations. A novel model of vestibular information processing will be presented to explain these changes in terms of motion sickness.

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519 Vestibular Compensation is Retarded in Delta2 Glutamate Receptor Subunit Knock-out Mice

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Vestibulocerebellum plays an important role in plasticity of the vestibulo-ocular reflex (VOR). Mutant mice deficient in the delta2 subunit of the ionotrophic glutamate receptor (GluR delta-2), which is specifically expressed in the cerebellar Purkinje cell, show defects in motor learning including long-term depression. A previous study on this strain of mice showed that they exhibited delay in compensation of asymmetry of vestibulospinal reflex after unilateral vestibular deafferentation (UVD) (Funabiki et al, 1995). Another study demonstrated that beats of spontaneous nystagmus after UVD was larger than wild-type (Kitahara et al, 1998). However, compensation of dynamic aspects of VOR after UVD in GluR delta-2 knock-out mice has not been investigated. GluR delta-2 (-/-) mice and wild type (+/+) mice were anesthesized by intraperitoneal injection of xylazine hydrochloride and ketaral, and UVD was performed by means of injection of pure ethanol into the left lateral semicircular canal and posterior semicircular canal. Before and after UVD, horizontal vetibulo-ocular reflex (HVOR) during sinusoidal rotation with a frequency between 0.8 and 3.2 Hz and a maximal head velocity of 150 degree/second was recorded with an infrared system (Iwashita et al, 2001). Gain of HVOR was analyzed with a VOR analyzing software for clinical use (IRN-2, Morita mfg, Japan). The ratio of HVOR gain after UVD to HVOR gain before UVD was smaller in GluR delta-2 (-/-) mice than in wild-type (+/+) mice all through the recording period for seven days. These results suggest that GluR delta-2-mediated pararrel fiber-Purkinje synaptic neurotransmission in the vestibulocerebellum has a certain role in vestibular compensation after UVD.

520 Potential Pathways Relaying Vestibular Inputs to Head Direction Cells in the Rat

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The activity of cells in the anterodorsal thalamic nucleus (ADN) of freely behaving rats corresponds to the current head direction of the animal. These head direction (HD) cells seem to provide a "robust neurophysiological correlate for a sense of direction, which is necessary for any spatial navigation" (see J.S. Taube, Prog Neurobio, 55:225, 1998, for review). It is not currently known how the HD signal is generated, or how necessary sensory information reaches the ADN. There is no definitive anatomical evidence for the contribution of vestibular information to HD cell circuitry, although current models of the HD system postulate a strong influence of the vestibular system in the generation of HD cell activity. The goal of this pilot study is to determine the nature of input from the vestibular nuclei to the ADN of the rat. Our laboratory uses unilateral injections of a retrograde viral tracer (pseudorabies virus) in the rat ADN. This tracer allows allowing us to determine the input to the ADN over several synapses. The results of this study will complement the growing electrophysiological HD cell literature by anatomically determining the extent of the putative vestibular component of the HD signal. HD related activity has been found in other areas of the rat brain including the postsubiculum, lateral dorsal thalamic nucleus, retrosplenial cortex, striatum, lateral mammillary nucleus (LMN), and dorsal tegmental nucleus (DTN). Lesion and electrophysiology studies suggest that information necessary for the HD cell activity in the ADN relies on input from LMN, which receivs input from DTN. The signal prior to the DTN may originate in the vestibular nuclei receiving input from the semicircular canals. Results from this study will serve as a foundation for further electrophysiological and behavioral investigation of the relationship between the vestibular and head direction systems.

521 Eye Movements Evoked By The Selective Utricular Nerve Stimulation In Cats

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Eye movements evoked by selective utricular nerve stimulation were investigated with aids of either video recording or electrooculography (EOG) in decerebrated cats with or without light anesthesia (halothane-nitrous oxide mixture). Electrical stimulation of utricular (UT) nerve was applied with implanted acupuncture needles. In the ipsilateral eye abduction was recorded following UT nerve stimulation at a stimulus intensity of 1.9 ± 0.5 x N1T in cats without any anesthesia, whereas facilitation of the abduction evoked by contralateral horizontal canal nerve stimulation was recorded with light anesthesia. On the other hands in the contralateral eye even smaller intorsion was recorded with corresponding stimulus intensity. Other types of eye movements like pure upward or diagonal to upper medical direction were observed in both eyes with current more than 6.2 ± 1.2 x N1T (with anesthesia) and 8.1 ± 3.2 x N1T (without anesthesia), with which the current spread to the adjacent nerve could not be ruled out.

The selectivity of the UT nerve stimulation was further confirmed by the electromyogram (EMG) from the neck muscle. Observed contribution of the utricular input to the horizontal eye movement is quite reasonable when we consider the macula of the utricle lies roughly in the horizontal plane while the head is held upright. These results also confirm the known monosynaptic and disynaptic connections from utricular primary afferents to the ipsilateral abducens (AB) nucleus neurons.

522 Tilt and translation eye movement and motion perception responses during off-vertical axis rotation

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Constant velocity off-vertical axis rotation (OVAR) provides dynamic linear acceleration stimuli that can be used to assess otolith function. Fourteen healthy subjects were rotated in darkness about their longitudinal axis 20 deg off-vertical at low (0.125 Hz) and high (0.5 Hz) frequencies. Oculomotor responses were recorded using infrared videography, and perceived motion was evaluated using verbal reports and a joystick with four degrees of freedom (pitch and roll tilt, front-back and lateral translation). The modulation of torsion was greater at the low frequency while the modulation of horizontal slow phase velocity was greater at the high frequency. During low frequency OVAR, subjects reported the perception of progressing along the edge of a cone, with the modulation of roll tilt recorded from the joystick in phase with right and left ear down orientations. During high frequency OVAR, subjects reported the perception of progressing along the edge of an upright cylinder, with the modulation of lateral translation approximately in phase with right and left ear down orientations. These results indicate that low-frequency OVAR generates tilt otolith-induced responses (modulation of torsion with perceived conical motion path) whereas high-frequency OVAR generates translational otolith-induced responses (modulation of horizontal slow phase velocity with perceived cylindrical motion path). This crossover of tilt and translation ocular responses and perceptual reports during OVAR support the hypothesis that both frequency segregation and multisensory integration are used to resolve ambiguous linear acceleration information. The clear

correspondence between eye movements and motion perception data suggests that subjective reports can be used for evaluating both tilt and translational OVAR responses when no eye movement recording system is available, or for evaluating tilt responses during low frequency OVAR when only horizontal eye position is available.

523 Aminoglycoside Clearance in the Avian Vestibular System

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Auditory and vestibular hair cell damage in humans is believed to be irreversible. Avian hair cells recover from damage over a period of weeks. Aminoglycoside antibiotics can create this damage, but systemic toxicity occurs in high dosages. Therefore, a model of unilateral vestibular hair cell damage has been created in the chicken to study the hair cell's regenerative capacity and central nervous system compensatory changes. Application of aminoglycoside-soaked pledgets results in significant hair cell damage, limited systemic concentrations, and allows for comparison with the contralateral ear as a control. This current study is designed to study the time course of clearance of aminoglycoside from chicken vestibular perilymph.

94 broiler chicks (Cobb x Ross; age 9 – 21days) underwent anesthesia and microsurgical removal of the columella and cochlea. A pledget soaked in gentamicin (500mg/ml; N=74) or streptomycin (500mg/ml; N=20) was placed in the cochlear duct for three hours. The animals were sacrificed at either 4, 12, 24, or 120 hours and perilymph was immediately collected with a Hamilton syringe. Aminoglycoside levels were measured and compared between groups. Mean gentamicin levels (ug/ml) were 127 +/- 117 at 4 hours, 18 +/- 22 at 12 hours, 6 +/- 4 at 24 hours and 6 +/- 8 at 120 hours.

High concentrations of aminoglycosides were attained in perilymph from the avian vestibular system after pledget application. These levels were higher than typically seen with systemic injections. These ototoxic drugs appear to be rapidly cleared from vestibular perilymph without the systemic side effects seen with systemic application.

524 Short Term Metabolic Changes in the Avian Superior Vestibular Nucleus After Streptomycin Treatment or Labyrinthectomy

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The avian vestibular system demonstrates anatomical and functional recovery after damage to the inner ear, providing a means for study of CNS changes after damage to the peripheral end-organ. Rapid changes in mitochondrial activity, demonstrated by cytochrome oxidase (CO) histochemistry, have been previously described in the Superior Vestibular Nucleus (SVN). In this study, we investigated short-term, metabolic changes in SVN using 2-deoxyglucose (2DG) uptake after transient or permanent damage to the peripheral end organs. Transient damage was produced by a unilateral topical application of a streptomycin soaked pledget in the inner ear. The contralateral ear was treated similarly with a water soaked pledget. Inter-animal controls were treated bilaterally with water. A unilateral labyrinthectomy created permanent damage to the inner ear. The contralateral ear was not treated. Inter-animal controls for permanent damage received only anesthesia. Animals survived for 1 or 4 days after treatment and then received an IP injection of 14C-2DG. After 45 minutes of vestibular stimulation animals were sacrificed and brains were frozen. Alternate coronal sections were either stained for CO or exposed to X-Ray film. Film optical density measurements were taken bilaterally and the ipsilateral/contralateral ratio of SVN labeling was compared among groups. In control animals, the SVN ratio was 1 for both survival times. In both labyrinthectomy and streptomycin-treated birds the ipsilateral to contralateral ratio was not different from control at 1 day. At 4 days, the ratio for labyrinthectomy birds suggests an increase in 2DG labeling ipsilateral to the surgery. These results suggest that damage to the peripheral end-organs produces a surprising short-term increase in metabolic activity in this brainstem nucleus.

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525 Early carboplatin-induced damage to IHC and type I neurons in chinchillas: an excitotoxic effect?

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Carboplatin, a second-generation platinum antineoplastic drug, selectively destroys inner hair cells (IHCs) and type I spiral ganglion neurons in chinchillas. The mechanisms responsible for this unusual lesion are poorly understood, but preliminary anatomical evidence suggests that the afferent terminals of auditory and vestibular neurons are damaged before the hair cells. This suggests that auditory nerve activity might be altered soon after carboplatin treatment. To test this hypothesis, we measured the spontaneous discharge rate (SR) and driven discharge rate (DR) of single auditory nerve fibers shortly after administering a dose of carboplatin that would normally kill roughly 50% of IHCs. The SR and DR were measured at different time points 1 to 8 hours after carboplatin treatment. Surprisingly, a slight, but significant increase in SR was found between 1 and 2 hours following carboplatin treatment. In addition, a significant increase in DR, particularly at high intensities, was also evident at 1-2 hours post-treatment. The increase in DR gradually declined over time and reached normal values 8 hours post-treatment. The increase in SR and DR during the early stages of carboplatin toxicity may involve damage to the type I neurons, possibly excitotoxicity. This hypothesis was supported by morphological findings demonstrating that the earliest damage occurred at the synapses of type I spiral ganglion neurons. Significant swelling was observed on the afferent terminals beneath IHCs as early as 6 hours post-carboplatin. In addition, the myelin sheath surrounding the neuron was distorted and numerous vacuoles were present along the surrounding sheath. Remarkably, no signification IHC lesion was evident until 72 hours post-carboplatin, although histochemical changes were observed prior to IHC degeneration.

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526 The Role of Nitric Oxide and Free Radicals in Cisplatin-Induced Ototoxicity

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Cisplatin is known to cause high-frequency neurosensory hearing loss. Reactive oxygen species have been shown to play a role in cisplatin ototoxicity. Reactive nitrogen species have been implicated, but not proven to be involved, in cisplatin-induced ototoxicity. The purpose of the present study was to investigate the role of NO production in cisplatin ototoxicity by administering aminoguanidine (a specific inhibitor of inducible nitric oxide synthase (iNOS)) in conjunction with cisplatin.

Rats were injected with cisplatin (13 mg/kg, IP), aminoguanidine (50 mg/kg, IP, twice daily), or both. Auditory brainstem responses (ABRs) were measured, in response to clicks and tone bursts (8kHz and 16kHz), before and three days after cisplatin administration. The cochlear tissue was then assayed for NO (total nitrite) and malondyaldehyde (MDA), a byproduct of lipid peroxidation. Cisplatin alone caused significant ABR threshold shifts at all stimuli tested. There was a statistically significant reduction in threshold shift for clicks and 8kHz tone bursts

when aminoguanidine (AG) was given with cisplatin. There was no threshold shift from baseline when AG was given alone. While a reduction in cochlear NO concentration in the AG/cisplatin group was seen, it was not statistically significant when compared to the cisplatin group. However, the MDA concentration in the AG/cisplatin group was significantly lower than that of the cisplatin group. This is the first study in which a specific iNOS inhibitor has been shown to produce a statistically significant reduction in hearing loss and lipid peroxidation in the cochlea of cisplatin-treated rats. Our results demonstrate that the iNOS pathway plays an important role in the generation of free radicals and hearing loss resulting from cisplatin administration.

527 HMG1 Expression in Spiral Ganglion Neurons and the Influence of Cisplatin on its Expression

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Cisplatin (CDDP) is a commonly used chemotherapeutic agent that has severe ototoxic and nephropathic side effects. Although much attention has been directed at developing methods of protection against cisplatin ototoxicity, the mechanism by which cisplatin produces its ototoxic effects is not well understood. The high mobility group (HMG) domain is a DNA binding motif found in non-histone chromosomal proteins, HMG1, HMG2 and transcription factors. This domain plays an important role in regulation of DNA transcription, repair of damaged DNA, and the mediation of cisplatin antitumor activity. In this study, we investigated the relationship between HMG1 and cisplatin ototoxicity. We defined the pattern of expression of HMG1 in spiral ganglion neurons of the rat inner ear, and determined the effect of cisplatin treatment on HMG1 expression. Ten seven-weeks-old female Fisher344 rats were divided into two groups: Group I, untreated control and Group II, CDDP treated (5mg/kg every 72 hrs, 2x dosage). Immunohistochemical analysis of control specimens demonstrated a graded expression of HMG1 in the spiral ganglion at different turns of the rat cochlea. Neurons in the basal turn demonstrated the highest level of expression of HMG1, while neurons in the middle and apical turns demonstrated a moderate and low level of expression respectively. In cisplatin treated animals, there was an increased expression of HMG1 in each cochlear turn in comparison to the expression of HMG1 in corresponding turns of control group animals. However, expression of HMG1 remained highest in the basal cochear turn, i.e. the turn not susceptible to ototoxic damage by cisplatin. Our findings suggest the possibility that levels of expression of HMG1 in the basal, middle and apical turns of the cochlea may correlate with the degree of sensitivity of these cochlear turns to cisplatin ototoxicity.

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528 Cisplatin activates caspase-3 and the p53 inhibitor, pifithrin-alpha, attenuates caspase-3 labeling

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Cisplatin, a common antineoplastic agent with significant neurotoxic and ototoxic side effects, is believed to cause DNA damage leading to activation of P53, a tumor suppressor gene involved in cell death signalling. We had reported that pifithrin-alpha, a P53 inhibitor, could protect against cisplatin induced hair cell loss in P3 rat organotypic cochlear and vestibular cultures. In order to explore the mechanisms underlying cisplatin induced hair cell damage and the protective effects of pifithrin-alpha, we used a carboxyfluorescein-labeled, cell permeable caspase label that fluoresces in the presence of activated caspase-3. Fluorescence labeling was used to monitor caspase-3 activity following treatment with cisplatin or cisplatin plus pifithrin-alpha. Caspase-3 activation was examined in cochlear cultures treated for either 12 h or 24 h with (1) 10mg/ml cisplatin, (2) 10mg/ml cisplatin plus 100uM pfifthrin-alpha, and (3) normal controls. Tissues were examined with a confocal microscope. Little caspase-3 labeling was observed in control tissues cultured 12 or 24 h. Addition of cisplatin resulted in a significant increase in caspase-3 labeled hair cells and the number of labeled cells increased between 12 and 24 h. Addition of pifithrin-alpha to the culture medium greatly reduced the number of caspase-3 labeled cells. These results suggest that cisplatin, at the concentration used here, activates caspase-3 and that the protective effect of pifithrin-alpha is associated with signalling pathways involving caspase-3. Results of others members of the caspase family will be discussed.

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529 Influence of pH on the Ototoxicity of Cisplatin: a Round Window Application Study.

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Cisplatin is a commonly used antineoplastic agent that produces a number of dose-limiting side effects, including ototoxicity. Prior animal studies have shown that cisplatin toxicity may be modulated by pH. For example, it has been reported that cisplatin resistant cancer cells have a higher intracellar pH than cisplatin sensitive cells. In the present study, we investigated the effect of pH on cisplatin ototoxicity using a round window membrane (RWM) application model.

Healthy adult chinchillas were anesthetized with ketamine and pentobarbital and immobilized in a small animal stereotaxic apparatus with hollow chinchilla ear bars. Auditory brainstem responses (ABRs) were recorded in response to 1, 2, 4, 8 and 16kHz tone bursts. The middle ear cavity was surgically exposed, and 10 microliters of phosphate buffered saline (PBS) or alkaline PBS was applied to the RWM. After 30 minutes, any remaining solution was removed from the round window and 2 microliters of cisplatin solution (.66 mg/ml) was applied to the RWM. The skin was sutured closed and the animals were housed for 72 hours. After 72 hours, the animals were anesthetized and follow up ABRs were performed.

Pre-administration of normal PBS (pH7.4) resulted in profound cisplatin-induced threshold changes at all frequencies. However, alkaline PBS significantly reduced cisplatin-induced threshold changes at all frequencies except 16kHz. These results demonstrate that pH can modulate the ototoxic effects of cisplatin, probably by altering the ability of cisplatin to react with cellular components.

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530 Ultrastructural Changes in Gerbil Inner Ear Following Ouabain Application to the Round Window

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Ouabain applied to the round window in gerbils induced nerve deafness which physiological and histological studies attributed to ablation of type I spiral ganglion neurons (Schmiedt et al, JARO, in press). The present report concerns ultrastructural responses. Ouabain (0.001M) was administered by osmotic pump that delivered 0.25 µl/hr through a cannula to the intact round window. After treatment, anaesthetized animals were perfused with fixative prior to processing the temporal bones routinely into epon. Immediately after a 24 hr ouabain exposure, epoxy thin sections disclosed apoptosis of spiral ganglion neurons, confirming light microscopy. In addition, electron microscopy showed empty appearing spaces that surrounded the bases of inner hair cells and vestibular type I hair cells and represented explosive ballooning of the afferent terminals to these cells. The stria vascularis exhibited extensive edema separating the marginal cell bodies and primary

processes which latter lacked secondary processes. The edema coincided with a decreased endocochlear potential (EP) at this acute stage. After a 2 week recovery from a 24 hour treatment, afferent but not efferent nerves under inner hair cells and type I spiral ganglion cells were missing. Inner hair cells in the mid to upper cochlea retained the population of presumed synaptic vesicles that normally filled the lower half of the cells. In the basal cochlea, large pleomorphic islands of compacted vesicles occupied much of the cell's lower half in place of the synaptic vesicles. These islands apparently reflected an unique form of crinophagy. Two weeks post ouabain, resorption of strial edema corresponded with recovery of the EP. The data demonstrate a transient effect on stria versus permanent injury to neurons, presumably reflecting variable affinities of different ATPase isoforms for ouabain.

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531 Pharmacokinetics of Caroverine and its effect on cochlear function in the Inner Ear after Systemic and Local Administrations in Guinea Pig

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Caroverine, an NMDA and AMPA receptor antagonist, has been shown to protect the inner ear from excitotoxicity systemically and be effective in treating cochlear-origin tinnitus. Local administration of caroverine on the round window membrane (RWM) could be more effective and avoid the potential side-effects related to systemic administration. The present study shows the pharmacokinetics of caroverine in perilymph, cerebrospinal fluid (CSF) and plasma following intravenous and local applications at different dosages. High performance liquid chromatography (HPLC) was used to determine the drug concentration. Our results show much higher caroverine concentration in perilymph while with lower concentration in csf and plasma following local application compared with systemic administration. Auditory brainstem response (ABR) was performed in order to evaluate the alteration of auditory function of the cochlea. The effect on hearing was transient and reversible; the ABR threshold recovered to the value of pre-application at 24 hours after RWM application of caroverine. The findings suggest that local application of caroverine on the RWM for treating inner ear diseases, such as tinnitus, is both safe and more efficacious in the guinea pig model while avoiding the high blood and CSF levels of caroverine seen by systemic administration.

532 The Effects of Heptanol, a Gap-Junction Uncoupler, on Cochlear Function in the Gerbil

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Heptanol has been studied in many cell systems and is known to uncouple gap junctions. Here we determined the effects of heptanol on compound action potential (CAP) thresholds, distortion product otoacoustic emissions (DPOAEs) and the endocochlear potential (EP). Heptanol was applied to the gerbil round window by means of gelfoam placed in the round-window niche. The contralateral ear served as a control. Acute changes were studied over a two-hr period, and more chronic changes over 24- and 48-hr periods. After the appropriate time period, CAP thresholds, DPOAEs, and EPs were evaluated in each ear. Heptanol produced both acute and chronic changes in cochlear physiology with some variability among animals. With acute applications, the basal turn of the cochlea was most affected as judged by CAP threshold elevations. EP values were reduced by 10-40 mV throughout the cochlea with some loss of DPOAEs as well. With chronic applications, CAP threshold elevation was flat across the lower frequencies and increased at the higher frequencies. There was also a loss of DPOAEs at mid and high frequencies. Moreover, EP's under chronic applications seemed to recover somewhat as compared to the acute applications and were only 10-20 mV below that of the control ears. These results provide evidence that gap junctions are of fundamental importance to cochlear ion homeostasis. The potassium-recycling pathways, which involve several distinct networks of cells coupled via gap junctions, are likely targets of the heptanol. Histological studies are ongoing to determine which tissues and enzyme systems are being affected by exposure to heptanol.

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533 Alterations of basilar membrane velocity responses by scala tympani perfusion of quinine in guinea pig cochlea

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Quinine, an ototoxic drug, has been reported to be able to cause the outer hair cells (OHCs) to change the length. Such alteration of OHC motility would affect the performance of the cochlear amplifier. However, the effects of quinine on basilar membrane (BM) vibration remain somewhat controversial (Karlsson et al., 1991; Ruggero et al., 1996). The discrepancies were most likely due to differences in experimental conditions such as cochlear preparation, site of measurement, dosage of quinine, etc. In this study, we investigated the effects of quinine at both low and high concentrations on BM velocity responses in the basal turn of the living guinea pig cochlea. The BM velocity at the site corresponding to the frequency of around 17 kHz was measured from a reflective bead on the BM using a laser interferometer. The BM velocity responses were evoked by pure tones (2 to 24 kHz) delivered to the ear canal. Quinine in artificial perilymph (50 μM-5.0 mM) was infused locally into the scala tympani of the basal turn. Quinine resulted in decrease in both the magnitude and gain of BM velocity response around the characteristic frequency (CF) in a concentration dependent manner. The sharp peak of the gain-frequency curve near the CF completely disappeared and the velocity magnitude input/output function at CF became linear for concentrations of 1.0 mM and above. Isoresponse tuning curve of BM velocity shows sensitivity loss around the CF and broadening of tuning with downward CF shift when quinine was applied. The downward shift of CF could be as much as 1/2 octave with 5.0 mM quinine perfusion. The effects of quinine on BM response were reversible for concentration of 0.1 mM. These results suggest that the cochlear amplifier and BM mechanics are affected by quinine that may underlie the cochlear sensitivity loss.

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534 KCNQ channel blockade causes OHC degeneration and deafness

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The KCNQ genes encode a family of potassium channel comprising five members involved in various diseases (see for review Robbins, 2001 Pharmacol. Ther. 90:1-19). KCNQ1-4 mRNAs have been found in the cochlea using RT-PCR (Kubisch et al., 1999 Cell 96:437-46). In situ hybridization and immunohistochemistry studies showed that KCNQ1/IsK and KCNQ4 are expressed in the stria vascularis and in the basal membrane of the outer hair cells (OHCs) respectively (Sakagami et al., 1991 Hear. Res. 56:168-172; Neyroud et al., 1997 Nat. Genet. 15:186-9; Kharkovets et al., 2000 Proc Natl Acad Sci U S A. 97:4333-8). Therefore, we used a pharmacological approach to study the role of the KCNQ channels in the guinea pig cochlea in vivo.

Cumulative perfusions of increasing doses of linopirdine (0.01 to 1 mM), a specific blocker of the KCNQ channels, resulted in an elevation of compound action potential threshold and in a reduction of cochlear microphonic amplitude. Endocochlear potential (EP) was then recorded to investigate if linopirdine primarily acts on KCNQ1 channels expressed in the stria vascularis. Rather than decreasing the EP, linopirdine slightly increased it, suggesting that linopirdine does not affect the stria vascularis. In contrast, linopirdine caused a drastic reduction of DPOAE amplitude, reflecting an action on the function of OHCs. After the physiological recordings, cochleas were fixed and processed for electron microscopy. Ultrastructural examination of the cochleas revealed that the drastic effect on DPOAEs resulted from damage restricted to the OHC.

Consistent with Marcotti and Kros 1999 J. Physiol. 520:653-60, our results show that linopirdine acts on KCNQ channels located on OHCs (probably the KCNQ4 type). Our study further demonstrate that KCNQ channel activity is essential for OHC functioning and survival.

535 Behavioral model of salicylate-induced tinnitus in rats

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High doses of salicylate induce tinnitus and hearing loss. We designed a behavioral paradigm to measure the occurence of tinnitus in rats. Tinnitus was induced by daily injection of salicylate (300 mg/kg i.p. during 4 days). The behavioral protocol was an active avoidance conditioning task. Animals were trained to respond to a conditioned stimulus consisting of a 10 kHz tone burst of 3s duration. Two measurements were performed: the number of correct responses to sound (score) and the number of responses without sound (false positives). Hearing loss was assessed by recording distortion product otoacoustic emissions (DPOAEs) and compound action potential (CAP) of the auditory nerve from an electrode chronically implanted on the round window.

In behavioral experiments, salicylate treatment provoked a reversible reduction of the score and a drastic increase of the number of false positives. Concomitant changes in DPOAEs and CAP thresholds attested that salicylate treatment induced hearing loss. To test the hypothesis that hearing loss could be responsible for changes in score and false positives, the intensity of sound eliciting behavioral responses was adjusted as a function of salicylate-induced threshold elevation. In these animals, no significant decrease in the score was observed, suggesting that score is related to auditory threshold. In contrast, the increase of false positives still remained. We proposed that the increase of the number of false postives during salicylate treatment is due to the occurrence of tinnitus. The present behavioral paradigm may be thus a good model to study tinnitus in various pathologies (noise trauma, quinine…).

536 Input/Output Functions from Chinchilla Inferior Colliculus and Auditory Cortex to Ultrasonic (22 kHz) Tonebursts Delivered Via Air and Bone Conduction

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Many normal hearing and deaf humans can hear ultrasonic (>20 kHz) stimuli delivered via bone conduction (BC). The present investigation compares responses to 22 kHz tonebursts delivered via BC and air conduction (AC) in the chinchilla. Chinchillas were anesthetized and tungsten electrodes were chronically implanted in the inferior colliculus (IC) and auditory cortex (cortex). Following a recovery period, unanesthetized animals were passively restrained, and evoked response input/output functions were obtained. In one experiment, responses from the right IC and cortex were obtained by delivering AC stimuli to the left ear, and while delivering BC stimuli to the left superior surface of the skull, overlying the bulla. For AC, stimulus level decreased from 90 dB pSPL in 10 dB steps. For BC, tonebursts were delivered to an ultrasonic bone vibrator with a band-pass transfer function centered near 22 kHz. In a second experiment, stimuli were similar, but were delivered to both the right and left ears (or skull) while recording from the right IC and cortex. For AC and BC, in both IC and cortex, onset response latencies generally increased and response amplitudes decreased with decreasing stimulus level. Although AC stimuli produced responses over a more limited dynamic range, at the highest stimulus levels the AC responses were larger via BC, for both the IC and cortex. AC responses were larger when stimulating the ear contralateral to the IC and cortex electrodes. For BC stimuli, responses from the IC were similar for ipsilateral and contralateral stimulation, while cortex responses appeared larger for contralateral skull stimulation.

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537 Evoked Responses to 22 kHz Air and Bone Conducted Tonebursts: Masking and Gap Effects

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This study investigated responses to ultrasonic tonebursts presented via bone conduction (BC) and air conduction (AC) to adult chinchillas.

In a masking protocol, constant level 22 kHz tonebursts (1 ms cosine-squared rise/fall times) were presented via AC or BC, while presenting a continuous bandpass masker delivered via AC. Masker level was varied in 10 dB steps to 90 dB SPL. Six 4-kHz wide contiguous bandpass maskers were used, ranging from 8-12 to 28-32 kHz. We measured onset response amplitude and latency via electrodes implanted in the contralateral inferior colliculus (IC). Latency shift was greatest for masker frequencies close to the 22 kHz toneburst frequency, regardless of masker level and mode of toneburst delivery. Latency effects were similar for BC and AC. At masker levels that produced a 25% reduction in response amplitude, the most effective masker noiseband was centered at 0.67 octaves below the toneburst frequency for AC and BC. For low and moderate levels of masking noise, lower-frequency masking noise was less effective in masking BC stimuli, while higher frequency noise bands had similar effects. At higher masker levels, latency and amplitude changes were typically less for BC than AC stimuli.

In a gap protocol, two 50-ms duration (0.5 ms linear rise/fall times), 22 kHz tonebursts were presented via either AC or BC, separated by a gap time that varied from 0 ms to 32 ms. IC latency and amplitude results are similar. To toneburst 2 (TB2), onset response latencies were increased and amplitudes were decreased; these effects decreased with increasing gap time. For BC stimuli, the offset response to toneburst 1 (TB1) was not present at brief gaps, while the onset response to TB2 was present at substantially shorter gap times. In contrast, for AC tonebursts, the offset response to TB1 was seen at shorter gaps than the onset response to TB2.

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538 The Frequency Following Response from the Chinchilla Inferior Colliculus is Not Place Specific to 80 dB pSPL, 250 Hz Tonebursts

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In a previous study, we found that the inferior colliculus (IC) frequency following response (FFR) to a 250 Hz toneburst was greatly reduced in amplitude by masking noise high-passed at 500 Hz, and minimally affected by a masking noise low-passed at 500 Hz. This suggested that

the FFR to low-frequency stimuli was generated in the basal turn of the cochlea. The present investigation further evaluates the place-specificity of the FFR from the chinchilla IC.

Four chinchillas served as control animals (no gentamycin/ethacrynic acid), while six served as experimental animals (IM gentamicin and IV ethacrynic acid). An 80 dB pSPL, 250 Hz toneburst and masking noise (broadband, low-passed at 500 Hz, or high-passed at frequencies ranging from 500 to 8 kHz at levels ranging from 0 to 90 dB SPL) were presented to one ear. IHC and OHC loss was determined in each animal.

For control animals, the broadband noise produced a dramatic decrease in FFR amplitude, while the low-passed noise produced substantially smaller amplitude decrements. The high-passed masking frequencies from 500 Hz to 8 kHz produced progressively smaller effects on FFR amplitude. In four of six experimental animals, there was substantial OHC loss in the basal portion of the cochlea , with minimal, patchy IHC loss. In the four homogeneous experimental animals, FFR amplitude was much smaller than in the control group. The threshold was approximately the same for the control and experimental groups. Noise low-passed at 500 Hz had a greater effect on FFR amplitude in the experimental than the control group, with the highest high-pass masker cutoffs having less of an effect in the experimental group than in the controls.

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539 Digital Noise Reduction for Evoked Responses Performed in the Spectral Domain

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Noise with an identifiable periodicity is commonly seen in subcortically-recorded evoked responses due in part to the high impedance electrodes required for this type of recording. In extreme cases, a response can be so buried in noise that it is nearly invisible to the naked eye.

Often, filtering out specific frequencies (e.g. 60 Hz) does not get rid of the problem. High frequency noise in particular is difficult to remove without affecting the response.

We have collected auditory evoked response waveforms consisting of two parts from guinea pig medial geniculate nucleus. The first part (response) consists of a prestimulus period followed by the response of interest. The second part (noise) immediately follows the response. The noise is the same duration as the response, but because no stimulation had occurred, no response is present. The offending periodic noise is present in both the response and noise sections.

In the noise-reduction technique described here, the frequency content of both response and noise are determined by FFT. The frequencies present in the noise are subtracted from those present in the response. The remaining frequencies are reconstructed into a noise-free time-domain response.

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540 Immediate Changes in Response Properties of Cat Inferior Colliculus Neurons Following Acute Spiral Ganglion Lesions.

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In previous studies, we demonstrated that acute spiral ganglion (SG) lesions change the frequency organization of neurons in both the inferior colliculus (ICC) and primary auditory cortex. In those studies, we recorded the tuning of neurons along electrode penetrations before and after restricted (~1mm) mechanical SG lesions. In the present study, response areas (RA's) of single ICC neurons and multi-neuronal clusters were recorded before, immediately after (within 30-60 min) and long after (several hours) SG lesions produced by an ND:YAG laser. Lesion induced peripheral sensitivity changes were documented by recording pre- & post-lesion tone evoked CAP thresholds. Contralateral-tone evoked RAs were recorded using 16 channel silicon-recording probes, which were fixed in place prior to the lesion. Thus, pre- and post-lesion responses were recorded from the same neurons. Post-lesion IC neuronal responses to tones affected by the lesion underwent an elevation in threshold. Responses in the same neurons to frequencies not affected by the lesion were either unchanged or dramatically decreased in threshold. These changes in sensitivity produced shifts in characteristic frequency (CF) or ICC neurons that could be more than an octave. Thresholds at these new CF's matched the pre-lesion responses of ICC neurons tuned to the unaffected lesion-edge frequencies. These results suggest a model in which the frequency sensitivity of IC neurons is produced by discrete convergence of excitatory and inhibitory activity derived from auditory nerve (AN) fibers tuned to a wide range of frequencies. Activity derived from AN fibers tuned to each frequency act across a discrete frequency range within an IC RA. Removal of AN fibers tuned to a narrow range of frequencies produces a notch in the IC RA and immediate 'plastic' changes in its characteristic frequency without a change in its overall threshold within minutes.

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541 Temporal Response Properties of Inferior Colliculus Neurons For Acoustical and Electrical Cochlear Stimulation

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The temporal and spatial distribution of neural activation in the central nucleus of the inferior colliculus (ICC) varies depending on the cochlear stimulus. This study focuses on the temporal response properties of multi-neuronal responses to both acoustical and electrical stimuli. We measured ICC responses along the tonotopic axis using a standardized trajectory. Activity was recorded simultaneously at 16 depths using a single-shank multi-channel recording probe inserted into the ICC. In each animal, we tested responses to acoustical tones, clicks, and broadband noise. Then, we deafened the animal with an intrascalar injection of neomycin and studied responses to biphasic electrical pulses varying in phase duration and electrode configuration. Electrical stimuli were presented to the cochlea through either a Nucleus banded array (Cochlear Corp.) or pairs of platinum/iridium wires. For pure tone stimuli, neural responses consisted of sustained responses for neurons with characteristic frequencies (CFs) near the stimulus frequency, while neurons with adjacent CFs displayed onset-only responses. For broadband noise stimuli, neural responses were often pauser-like. Occasionally, they had weak periodic structure. For acoustical clicks, an onset burst of activation was observed and the duration of the response persisted for 5 to 10 ms. For all acoustical stimuli, the first-spike latency of responses decreased by a maximum of 10 ms with increasing stimulus level. For electrical stimuli, regardless of the electrode configuration or the phase duration, a sustained periodic response was observed. The response lasted from 5 to 20 ms and varied from channel to channel. The first-spike latency decreased bu a maximum of 5 ms as a function of increasing current level.

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542 Effects of Cochlear Nucleus Stimulation or Blockade on Neuronal Firing in the Inferior Colliculus

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Recent studies have raised the relative importance of the direct input from the cochlear nucleus into the inferior colliculus (IC) in coding of the acoustic message. Defining the precise nature of the effect of the input to the IC from the dorsal cochlear nucleus (DCN) will improve the understanding of input output relationships in the central nucleus of IC (ICc). To evaluate the relationship between DCN and ICc the present study examined the effect of electrical stimulation or focal blockade of the contralateral DCN on ICc neuronal firing in ketamine-anesthetized rats. Glass microelectrodes were advanced stereotaxically into ICc and a bipolar concentric stimulation electrode or a cannula (26-gauge) was placed in the contralateral DCN. The responses of ICc neurons to acoustic stimuli at CF were evaluated. Electrical stimuli in DCN consisted of single constant current pulses (10 μA), and the focal microinjection involved the local anesthetic, lidocaine (2%, 0.5 μl, infused over 2 min). In the experiments conducted thus far, 88% of ICc neurons responded to electrical stimuli with an excitatory response, consisting primarily of an onset response. Focal blockade in DCN by lidocaine produced a reduction of ICc neuronal firing in 100% of neurons examined. The mean degree of blockade was 56% reduction of acoustic responses in neurons examined, thus far. The mean onset of the effect was 6 min, and complete recovery of the response to control levels was observed by a mean of 25 min after the microinjection. These data provide direct evidence for the important actions that the DCN exerts over the acoustic responses of ICc neurons. Further experiments will attempt to define the nature of this input to the IC from the DCN more precisely.

543 Rate Dependent Sharpening of Frequency Selectivity of Inferior Colliculus Neurons in the Little Brown Bat, *Myotis lucifugus*

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Understanding how sound frequency and amplitude are represented in the auditory system is fundamental to our understanding of auditory perception. Responses to pure tones have been used to define a neuron's basic response characteristics such as frequency tuning, rate-level function and temporal firing patterns. These stimuli are usually presented at a low stimulation rate (SR) to reduce inter-stimulus interaction. Basic response properties derived in this way have been assumed to accurately represent the unit's overall response properties under various conditions including behavioral situations. However, natural sounds featuring complex amplitude envelopes and frequency spectra often occur in rapid succession. In light of this, an essential question is whether such conclusions are valid, especially in real life situations. Galazyuk et al. (2000) recently showed that nearly half of the inferior colliculus (IC) neurons studied showed a systematic increase in their amplitude selectivity with an increase in SR. To determine whether SR also influences frequency selectivity, we studied the frequency tuning characteristics of 40 IC neurons over multiple SRs in unanesthetized bats. SR altered the frequency response ranges of 95% of IC neurons studied. Most notably, when measured at 40 dB above threshold 77% of the IC neurons studied showed a reduction in frequency response range as the SR was increased. We suggest that this rate dependent sharpening of frequency selectivity of auditory neurons may confer a greater perceptual frequency resolution. The role of inhibition as the mechanism responsible for this phenomenon is discussed.

544 Responses to Communication Calls are More Complex in the Inferior Colliculus than in the Dorsal Nucleus of the Lateral Lemniscus

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Here we report on how neurons in the DNLL and IC of Mexican free-tailed bats respond to species-specific calls, and what changes in response properties occur when inhibition is blocked. Single units were recorded with multibarrel pipettes, where some barrels were filled with bicuculline or bicuculline and strychnine. A response area, defined as the range of frequencies that evoke discharges at a given intensity, was first obtained for each neuron. Responses evoked by 10 natural calls were then obtained. Of the 10 calls, eight were social communication calls and 2 were echolocation calls.

The main findings from the DNLL are that responses evoked by species-specific signals are both homogeneous and non-selective. They are homogeneous in that DNLL neurons with similar best frequencies (BFs) respond to a given signal with similar response patterns. They are also non-selective, in that they respond to most signals that encroach upon their excitatory response area.

In marked contrast, IC neurons with similar BFs respond to the same calls with a diversity of response patterns. The response diversity is expressed in 2 principal ways. The first is that any one of the species-specific signals we presented evoked a wide variety of response patterns among isofrequency IC cells, whereas the same call evoked similar response profiles among isofrequency DNLL cells. The second is that whereas DNLL cells are non-selective, IC cells are highly selective. Finally, blocking inhibition at the IC changes the response profiles and greatly reduces response selectivity. Based on these findings, we propose that one of the principal transformations that occurs in the IC is a change from processing that emphasizes similarity in lower nuclei to one that emphasizes diversity and selectivity in the IC.

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545 Excitatory-Inhibitory Interactions In The Inferior Colliculus Of The Big Brown Bat (*Eptesicus fuscus*) As Revealed By Two-Tone Testing Of Duration Tuned Neurons.

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The inferior colliculus is the first nucleus in the mammalian central auditory pathway that contains neurons tuned to specific signal durations. Existing data indicate that duration tuning is created in the inferior colliculus through the convergence and temporal interplay of excitatory and inhibitory synaptic inputs. To examine the strength and time course of the inputs, we recorded extracellularly from duration tuned cells while presenting pairs of pure tone pulses (same frequency) differing in their duration and temporal relationship. One tone in the pulse pair was set to the cell's Best Duration (BD tone); the other tone was set to a duration that elicited little or No Response (NR tone). The onset time of the BD tone varied relative to the NR tone. Because the BD tone always elicited spikes when presented alone, differences in spike count and latency when both tones were presented reflect the interaction of inputs activated by the two signals. Spike counts evoked by the BD tone were reduced or eliminated as it approached the NR tone, as it moved through it, and for some time following the NR tone. Spike counts were reduced even when stimulus energy in the BD tone was equated with that of the NR tone and regardless of whether the starting phases of the two signals matched. The interval of reduced spiking (i.e. inhibition) persisted for at least as long as the duration of the NR tone, a result consistent with conceptual models of the neural mechanisms of duration tuning, but could be shortened by increasing the amplitude of the BD tone relative to the NR tone. That inhibition

evoked by the onset of the NR tone can inhibit spiking to the BD tone—even before the BD tone temporally overlaps with the NR tone—demonstrates that the latency to inhibition is as short, or shorter than, the latency to excitation.

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546 Sensorimotor Integration in the Awake, Behaving FM-bat, *Eptesicus fuscus*

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The bat's sonar receiver determines the direction and distance of a target from the features and timing of returning echoes. It uses the sonar echoes to build a 3-D representation of the world that is used to adjust the features of its vocalizations. Echolocation thus requires the dynamic interplay between auditory information processing and adaptive motor responses. Neural mechanisms supporting audiomotor integration in *Eptesicus fuscus* were investigated in the midbrain superior colliculus (SC).

Our earlier studies have shown that the functional organization of the bat SC exhibits specializations that are potentially important to acoustic orientation by sonar. We have shown that a population of auditory neurons in the bat SC shows echo-delay tuning, a response property hypothesized to encode target range. In addition, microstimulation of the SC elicits sonar vocalizations, and anatomical pathways have been identified connecting the SC with pre-motor vocal control nuclei. These findings suggest that the SC may play an important role in the audiomotor integration required for echolocation.

Here, we describe results of neurophysiological recording experiments with awake, behaving bats. Bats were trained to remain on a platform in a dark room, while tracking a food reward using echolocation. The reward was moved in a space that extended 15-45 cm in front of the bat and up to 30° laterally from center, at zero elevation. Multi- and single unit neural activity was recorded from the intermediate and deep layers of the SC using high impedance electrodes, while simultaneously recording the bat's head position and vocalizations. We are studying pre-motor and auditory responses in the SC of bats performing echolocation behavior to deepen our understanding of the mechanisms supporting sensorimotor intergration in bat sonar.

547 Possible neuronal mechanisms underlying paradoxical latency shift in the little brown bats, Myotis lucifugus that is important for target ranging

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Many central auditory neurons in echolocating bats are tuned to the time delay between a pair of sound pulses of unequal amplitudes. Sullivan [1982] proposed that paradoxical latency shift (PLS), characterized by an increase in response latency to loud sounds, is critical for delay tuning. We showed that neural oscillation in combination with ordinary inhibition may be responsible for PLS [Galazyuk and Feng 2001]. To gain further insight into the origins of PLS we studied responses of inferior colliculus (IC) neurons to tone pulses at CF having different sound durations (from 1 to 16 ms). Most neurons showing PLS demonstrated one of two major response patterns. For one group of IC neurons an increase in stimulus duration had no effect on PLS. This result supports the main conclusion of our previous study as described above. Conversely, the latency shift of a second group of IC neurons was dependent on stimulus duration; i.e. the amount of latency shift increased with duration. For these neurons, the PLS appeared to be due to a change in firing pattern from an on-set transient or sustained response at low sound levels into a stimulus offset response ; created by an initial inhibition over the duration of the stimulus followed by an inhibitory rebound. Additionally a small group of neurons showed PLS only when the duration of tone pulses was 1 – 2 ms, suggesting an interaction between duration selectivity and PLS.

Duration-independent and duration-dependent PLS may serve two different functions. Duration-independence would allow discrimination of absolute target range while duration-dependence would allow the tracking of a moving target. Neurons with constant or stimulus dependent pulse-echo delays ("tracking neurons") have been shown in auditory cortex of the same bat [Tanaka and Wong, 1993].

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548 Auditory Cueing Modulates the Sensitivity of Neurons in the Superior Colliculus

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Attention can be directed to a location in the absence of overt signs of orienting, a phenomenon termed *covert orienting.* Posner devised a task to define operationally covert orienting of attention: with a subject fixating centrally, a visual peripheral cue is presented followed by a peripheral target (Posner, M.I., 1980, *Q.J.Exp.Psychol.* **32:** 3). In 80% of the trials, the (valid) cue indicates the side of the subsequent target, in the remaining 20%, the (invalid) cue indicates the side opposite to the target. The difference in reaction time between validly and invalidly cued trials is referred to as the *validity effect.*

We recorded neural activity in three awake rats performing an auditory version of the cueing paradigm. For cue and target two white noise pulses separated by 3 randomized delay periods (100, 300, 500 ms) were used. The duration of the cue was 100 ms. The target was presented until the animal reacted, but not longer than 300 ms. In each animal six electrodes were implanted in auditory responsive areas of the superior colliculus (SC).

Preliminary data showed that during the delay period the activity in the SC contralateral to the cued side was increased compared to spontaneous activity. In contrast, activity in the ipsilateral SC was not increased. Activity during target presentation was decreased in the valid stimulus configuration compared to the invalid stimulus configuration. Thus, a selective modulation of SC neurons based on stimulus validity was observed, indicating an influence of spatial attention.

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549 Functional Connections between Heschl's Gyrus and Auditory Field PLST on the Lateral Superior Temporal Gyrus in Humans

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The functional organization of human cerebral cortex engaged in hearing and speech is poorly understood. We used direct electrical stimulation and recording methods to study functional connections between auditory fields on Heschl's gyrus and the acoustically responsive posterior lateral superior temporal gyrus (field PLST). Averaged auditory evoked responses were recorded from multicontact (64) subdural recording arrays chronically implanted over the STG and from modified depth electrodes inserted into HG in patients undergoing diagnosis and treatment of intractable epilepsy. Experimental protocols were approved by the Univ. Iowa IRB. Single, imperceptible, bipolar, constant current, 0.2 ms pulses were delivered to HG sites while recording from the electrode array over acoustically responsive STG

cortex. Stimulation of sites along the medial-lateral extent of HG resulted in evoked complex waveforms distributed over restricted areas of STG. These areas overlapped each other and field PLST. For any given HG stimulus site, the morphology of the electrically evoked waveform varied across the STG map. Waveforms recorded at any STG site could change with shifts in the HG stimulus site. Data indicate widespread convergence and divergence of input to posterior STG from HG arriving over multiple pathways. Evidence was also found for a functional projection from STG to HG.

550 Multi-Modal Source Localization of the Auditory Steady State Response (ASSR)

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The ASSR is an oscillating potential elicited by amplitude modulated signals. Its generation site remains controversial. The purpose of this study is to determine the neuroanatomical sources of the ASSR. We mapped the 40 Hz ASSR in five subjects using PET (H_2O^{15}) imaging and EEG source localization. During separate PET and EEG data collection sessions, 1 kHz tones amplitude modulated at 40 Hz were presented to the right ear. PET scans collected were compared with resting scans. Statistical parametric maps were computed both for individual and group PET data. Four trials (~30,000 25 ms epochs) of 64-channel EEG data were collected from each subject, filtered (10-120 Hz) and averaged. Source localization with an MRI derived head model for each subject was performed. Analysis of individual subjects showed PET activation patterns involving one or both auditory cortices, thalamus, brainstem, inferior colliculus, medial frontal lobe and lateral cerebellum. Individual EEG current density maps show peaks in temporal, frontal and occipital lobes, as well as cerebellum, thalamus and brainstem. Group PET data show activations in right cerebellum, both auditory cortices, and left frontal lobe. Preliminary analysis of average EEG data show activation in subcortical and infratentorial sites leading cortical loci. These results provide new insights on the dynamic network of neural circuitry underlying the ASSR, an evoked potential with growing clinical relevance.

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551 Effects of continuous masking noise on 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. In the last meeting, we reported auditory evoked magnetic fields (AEFs) were enhanced at high stimulus intensities and prolonged at low stimulus intensities in patients who had inner-ear hearing loss with loudness recruitment. To elucidate whether there is difference or not between the cortical representations of these psychoacoustically similar phenomena, we measured AEFs in normal hearing subjects under two different conditions, with and without continuous noise masking.

Methods The sound stimulus 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. AEFs were recorded with a 122-channel whole-head magnetometer (Neuromag Ltd.).

Results The equivalent current dipole (ECD) moment of N100m increased as a function of sound intensity in both conditions. The moment was smaller and the latency was longer under noise than under without-noise conditions at all stimulus intensities, particularly at low stimulus intensities.

Discussion The results of under noise conditions differed from our previous findings 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.

552 Is there a Time and a Place for Distortion Products in Amplitude Modulation Processing?

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When two sinusoidal amplitude modulations (SAM) with different frequencies of modulation applied to the same carrier are played to a listener, a distortion product at the difference frequency between the modulation frequencies is recorded in steady state evoked responses. This distortion product is not present in the modulation spectrum of the stimulus; and must be generated by a non-linear process. To determine whether evoked responses to components in the stimuli, and responses to evoked distortion products, have the same source and phase in the brain, we have recorded responses to combined AM stimuli at 21 sites on the scalp.

One of the modulation rates was fixed at 40 Hz the other was set between 5 Hz and 75 Hz. Each modulation lasted nine seconds, but only the last eight seconds were analysed to avoid onset effects. Electrode positioning followed the International 10-20 system.

Steady state evoked responses to modulation rates above 15 Hz were mostly localized to the vertex, which may indicate a deep origin for these responses. Rates below 15 Hz were often lateralised. The evoked response to components in the stimuli tended to localise away from evoked responses of similar rates that were generated by distortion products.

The phase of evoked responses to rates of SAM that localised to the vertex, were in phase all across the scalp. In contrast, evoked responses at 5Hz to components in the stimuli showed that the two sides of the scalp were out of phase with each other. When the evoked response to distortion products at 5 Hz was measured, this phase disparity was shifted by approximately 90 degrees.

These results indicate that rates of SAM above 15 Hz may have a deep origin, whereas, rates below 15 Hz have a more cortical origin. The results also indicate that responses to components in the modulation spectrum of the stimuli have a different origin to responses from the distortion product.

553 Temporal Resolution of the Human Auditory Cortex in Gap Detection Tasks as Revealed By Magnetoencephalography (MEG)

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We examined the temporal resolution of primary auditory cortex in response to noise-bursts with late and early gaps using a 122-channel MEG system. In the late gap experiment, stimuli were 600ms white noise bursts (65 dB SPL) with gaps of 3, 6, and 9ms inserted after 300ms. Bursts without gap served as control stimuli. Middle latency auditory evoked fields (MAEF) were recorded from 18 listeners. 420 responses were averaged in each condition. Filtered source waveforms

(15-150Hz) of the spatio-temporal source model with one dipole in each hemisphere were used for analysis of the gap specific MAEF responses. Bootstrap based t-intervals revealed significant MAEF responses in each condition and both hemispheres except for the 3ms condition in the right hemisphere. Individual MRI's showed that the equivalent dipoles projected to the medial portion of the auditory cortex. The observed thresholds of 3ms correspond well with psychoacoustic gap detection thresholds of about 2ms. In the related early gap experiment we investigated the effect of (i) the length of the leading noise burst (5, 20, and 50ms) and (ii) the gap duration (3, 6, 10, and 30ms) in 100ms white noise bursts on the MAEF gap response. Eleven listeners participated in this experiment. 700 sweeps were averaged in each condition. Source analysis of the difference waveforms revealed significantly larger gap responses for (I) increased duration of the leading noise burst, and (II) for longer gap durations. Thus both experiments provide evidence for a close correspondence of behavioral thresholds and neurophysiological responses at the level of the primary auditory cortex.

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554 A New Technique For Analysis Of Temporally-Overlapped Auditory-Evoked Responses

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Analysis of human evoked responses to auditory stimuli are usually confined to slow stimulus rates to allow the responses to return to baseline prior to the next stimulus, thus limiting stimulus rates to below those encountered in natural situations. We present a new method enabling the recovery of transient neural responses to auditory stimulation rates even above perceptual fusion (>20 Hz, up to 100 Hz so far). Our pseudo-periodic stimulus sequences and the averaged responses to them are circular (100% duty cycle A-D) permitting deconvolution in the frequency domain. Using this method, WAAD (Wrap-Around Average Deconvolution) allows us to recover the mean brain response to individual stimuli despite a high stimulus rate, using naturally-binaural stimuli from wall-mounted loudspeakers.

Our data indicate that the classic "40-Hz response" is not "steady-state" but instead consists of overlapped transient responses to each single stimulus. Similar transient responses are found at other suprafusion repetition rates, as well, but such responses are not usually detected because the positive and negative waves cancel at the stimulation rate, whereas they sum at 40 Hz. These suprafusion responses may be the brain's response to a sustained "background" of sound.

The recorded waveforms show considerable similarity to Local Field Potentials recorded from the auditory system in animals. Thus, the method may provide access to such potentials from the human scalp.

The WAAD technique has demonstrated a clear, reliable attentional-state amplitude modulation of the responses as early as 25 ms post-stimulus (which is possibly sub-cortical in origin) as well as changes with latencies of 100 ms. There are indications of gender differences.

NS26209, MH54922, NS36880, RR14002 (DJ

555 Quantifying signal-to-noise ratio of the mismatch negativity

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Mismatch negativity (MMN) is thought to represent an index of auditory information processing independent of attention. It is elicited within an oddball paradigm where the difference between a frequently occurring standard and an infrequent target stimulus is indexed by an enhanced negativity in the averaged waveforms (latency and temporal distribution encompassing ~100-200 ms). Given this latency range, MMN overlaps with several well-known long-latency auditory evoked potentials, and is delineated as a difference wave between the standard and target responses. However, there are several outstanding issues that need to be resolved in order for MMN to evolve as a viable tool in the clinical arena. These include consistent identification of MMN within individuals (vs groups) and its reliability/stability over time.

Herein, we assess related statistical approaches to evaluate MMN in individual subjects in terms of signal-to-noise ratio. Pearson's r, the coefficient-of-variation (r2) or the t statistic, all provide similar indices of this metric. These statistics were applied to the N1 component versus a prestimulus baseline interval and the P300 and MMN components by comparing the intervals that contain the oddball and standard responses. Whereas signal-to-noise ratios for N1 and P300 were similar, signal-to-noise ratio for MMN was considerably lower. These results suggest that MMN is not as robust as these other long-latency components. In combination with strategies necessary to enhance MMN based on stimulus and recording parameters, development of methods that quantify MMN detection in individual subjects will ultimately enable decisions to be made regarding its clinical viability.

556 Mismatch Negativity Cortical ERP Measures of "Central" Auditory Gap Detection

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Recently, Phillips and colleagues [1997, JASA 101, 3694-3705] introduced the concept of "central" gap detection, where the frequency content of the leading and trailing markers differ. In this "between-channel" case, the underlying processes must be performed centrally by some process monitoring the output across many cochlear frequency channels. Gap thresholds increase as the frequency difference between the markers increases. The present study sought to determine whether the auditory cortical processing reflected by the mismatch negativity (MMN) reflects this between-channel processing.

Ten adults participated. Stimuli were narrowband (0.25 oct) noisebursts, consisting of a 2000-Hz noiseburst leading marker, a gap, and a trailing noiseburst of either 500, 1000, 2000, 4000 or 8000 Hz (the 2000-Hz trailing noiseburst is the "within-channel" condition; all others are "between-channel" conditions). Total duration of the stimuli (including both markers and the gap) was 100 ms. "Standard" stimuli contained a 1-ms gap; "deviant" stimuli contained a 20-ms gap.

Subjects' behavioural %correct results indicate significantly (p=.01) better performance in the within-channel (mean=93%) compared to between-channel (mean across conditions=80%) conditions. MMN results show essentially the same pattern: MMN amplitude in the within-channel condition (mean=-2.0uV) is significantly (p=.009) larger than the between-channel conditions (mean across conditions=-0.91uV). Latency results show a trend towards longer latencies for the between-channel conditions (221.0 ms compared to 207.4 ms for the within-channel condition).

These results indicate the MMN reflects the output of the central process monitoring multiple frequency channels. Because the MMN reflects auditory cortex processes, these results suggest this monitor is likely below this level.

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557 Nicotine and the Auditory Systems of Non-Smokers

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Nicotine exerts its action by binding to, and activating, nicotinic acetylcholine receptors found throughout the brain, including in the auditory system. Yet, the role of nicotinic mechanisms in auditory function is unclear. In general, results from previous behavioral and electrophysiological studies of smokers and non-smokers wearing a

nicotine patch are consistent with a nicotine-enhanced gating of task-irrelevant, disruptive stimuli so that irrelevant auditory stimuli are prevented from ascending to higher levels of cortical processing. To test this, complex auditory tasks that utilize central inhibitory mechanisms (detection of tones and discrimination of consonant-vowel (CV) stimuli in the presence of a noisy background) were conducted. Long-latency responses and mismatch negativity elicited by tones in noise and CV stimuli in quiet and in noise were measured in the presence and absence of transdermally-delivered nicotine and were correlated with results of psychophysical auditory tasks that incorporated the same non-smoking subjects and stimuli. Preliminary results indicate (1) poorer detection of threshold-level tones in noise and (2) improved discrimination of suprathreshold CVs in noise and in quiet. Implications of these findings regarding (1) the role of nicotinic mechanisms in the central auditory nervous system and (2) the role of nicotine in auditory communication will be discussed.

558 Patterns of Cortical Hemispheric Asymmetry to Speech Sounds in Normal and Learning Impaired Children

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Distinctive patterns of central dominance have been documented in children with learning problems. We investigated the hypothesis that normal children (NL) and children with learning problems (LP) can exhibit different patterns of hemispheric activation in response to complex speech stimuli. Auditory cortical evoked potentials (AEP) to the speech sound /da/ (right-ear stimulation) were measured in 23 NL and 55 LP age-matched children. A measure of relative RMS response amplitude was utilized to determine levels of hemispheric asymmetry across temporal electrodes for 9 latency epochs between 40-220 msec. Both NL and LP subjects demonstrated predominance for left-hemisphere activation throughout the response. However, NL subjects exhibited significantly increased, and later occurring, asymmetry compared to LP subjects. Additionally, LP subjects who scored within one standard deviation of the NL subjects' average on a standardized measure of reading (n=10) exhibited significantly more lateralized responses compared to LP subjects who scored below one standard deviation (n=45) of the NL subjects.

To examine the plasticity of this measure of hemispheric asymmetry, we recorded AEPs from 18 LP children before and after commercial, computer-based auditory training. A representative subset from the aforementioned study was included in the training group. As expected, the training group's pre-test responses were consistent with the LP responses discussed above. At post-test, 7 subjects exhibited significantly more left-hemisphere dominant responses following training. This increase in response lateralization was accompanied by greater improvement in a number of behavioral and learning measures compared to subjects that did not show increased asymmetry. Taken together, these findings suggest that distinctive patterns of hemispheric dominance may underlie normal learning and that auditory training can modify the asymmetry of response.

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559 The Effects of Aphasia on the N1-P2 Complex in Response to Speech Stimuli

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We have previously reported that the mismatch negativity response (MMN) is present less often in subjects with aphasia than in normal control subjects. However, no significant differences have been found between the two groups for MMN amplitude, latency, and duration measures, when present. This study reports on the N1-P2 complex recorded from aphasic and age-matched normal subjects.

Stimuli were synthesized /ga/ and /da/ syllables presented at 85 dB SPL in an oddball paradigm. Stimuli were presented to the right ear via an insert earphone at a rate of 1.43 per second. Responses were recorded from Fz, C3 and C4 electrode sites. N1 and P2 were analyzed in the responses to standard /ga/ stimuli as well as deviant /da/ stimuli.

Results indicated that the response, measured at the Fz electrode site, was identified in 100% of normal control subjects, but only 82% of subjects with aphasia. No significant group differences were seen for N1-P2 amplitude. Group differences were noted for N1 and P2 latency in the deviant waveform, with the normal group demonstrating shorter wave latencies. Additionally, P2 latency showed a group by electrode interaction. Shorter latencies were seen for C3 compared to C4 in the normal group. This hemispheric difference was not seen in the aphasic group. The relationship between the results and subjects' auditory comprehension and site of lesion will be discussed.

560 Middle Latency Response Differences in Children with Dichotic Left-Ear Deficits

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A left-ear deficit in the processing of competing auditory information is used to diagnose an auditory processing disorder (APD) in children and adults. Whether this deficit is the result of a speech or language deficit or due to a failure of the auditory system to normally process input directed toward the left ear has been the subject of debate. The presence of a difference in left-ear processing of non-linguistic stimuli would suggest a more purely auditory perceptual deficit.

This study compared the processing of non-linguistic stimuli in two groups of 11-year-old children: those with left-ear deficits during behavioral dichotic listening tests and those with normal dichotic listening results. The middle latency response (MLR) was used to explore scalp-evoked responses following the presentation of tone complexes (1000 Hz and 4500 Hz) and clicks to both the left and right ears at presentation rates of 6 and 12 Hz. Responses were recorded from a total of 32 electrode sites (Neuroscan QuickCap) and analyzed at T7 and T8.

In general, the children with left-ear deficits produced responses with shorter latencies and larger amplitudes in the earlier components of the MLR. Significant differences occurred in the amplitude of Na at both of the temporal electrode sites in response to stimuli presented to the left ear. Significantly larger amplitudes also occurred for Nb at both temporal electrode sites in response to stimuli presented to the right ear. Grand average waveforms for each group revealed larger peak-to-peak amplitudes from Na to Pa for left ear presentations that occurred for both rates of stimuli at both temporal electrode sites.

This preliminary data suggests evidence of interaural differences in the thalamo-cortical processing of non-linguistic stimuli in children with left-ear deficits observed during standard behavioral testing.

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561 Learning-Impaired Children Exhibit Timing Deficits in Cortical Responses to Speech Stimuli Presented in Background Noise

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Many researchers have suggested that auditory perception deficits can contribute to learning problems in some children. These deficits are enhanced when background noise is introduced. This study compares auditory evoked cortical potentials (P1/N1/P2/N2) elicited by a synthesized /da/ in quiet and in continuous background noise (0dB S/N) in normal children (N=25) and children with learning problems (N=73). The timing of these potentials was assessed to determine how the wave morphology changes between quiet and noise in each group. This was done by cross-correlating the quiet and noise waveforms for each child and comparing these values across groups. The correlation value was considered to represent the amount of degradation in neural synchrony from quiet to noise. Normal children exhibited relatively high correlation values between quiet and noise waveforms. A subset of the children with learning problems (22%) had poor quiet-to-noise correlations due to an increase in latency between the P1 and N1 peaks in the noise condition. This result indicates that some children with learning problems demonstrate a distinctive disruption of neural timing at the cortical level to auditory signals presented in background noise.

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562 Preferential Loss of Low-Frequency Hearing in Mice Deficient for the Barhl1 Homeobox Gene

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The Drosophila BarH1 and BarH2 genes, termed BarH-like (Barhl1) in mice, encode homeodomain proteins that are required for determination of external sensory organs. To study the role of Barhl1 during inner ear development, we generated a targeted Barhl1 deletion in mice by replacing the Barhl1 coding region with the LacZ reporter gene. In embryonic and postnatal mice heterozygous for the Barhl1 deletion, Barhl1 was highly expressed in cochlear outer hair cells (OHCs) but weakly expressed in cochlear inner hair cells and vestibular hair cells. All null mutants exhibited reduced startle reflexes by two weeks of age but lacked any sign of vestibular dysfunction. Auditory brainstem responses (ABRs) showed no difference in click thresholds for 3 month old Barhl1 and Barhl1 mice, whereas Barhl1-/- mice had greatly elevated thresholds. Tone pip ABRs revealed preferential low frequency hearing loss, suggesting that apical and middle regions of the organ of Corti were more severely affected than basal regions. X-gal and phalloidin staining showed disorganization of OHCs at P6 in the mutant, spreading into the middle turn by P16-P19. At two months of age, only a few residual OHCs remained in the apical and middle turns in the mutant, whereas the basal turn of the organ of Corti remained largely intact. This progression of OHC degeneration correlates with the severe to profound deafness at low and medium frequencies and mild hearing loss at high frequencies. SEM and LM analyses of 2-4 month old mice confirmed these anatomic observations. In summary, targeted Barhl1 disruption causes severe hearing loss to low and middle frequencies as a result of a gradual degeneration of OHCs that progresses along the organ of Corti. Barhl1 seems required for the maintenance and perhaps terminal differentiation of cochlear OHCs, but is not involved in their fate commitment.

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563 Role Of The *Brn4/Pou3f4* Gene During Mouse Inner Ear Development

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Mutations in the *Brn4/Pou3f4* gene cause congenital hearing loss in man and mouse. These mutations result in congenital malformations of the both auditory and vestibular regions of the inner ear. To gain a greater understanding of the role of *Brn4* during otic development, we have generated a targeted mutation in the gene using ES cell technology (Phippard *et al*., J. Neurosci., 19: 5980-5989, 1999). Analyses of this mutant demonstrate that a number of structures derived from the otic capsule, which normally expresses the *Brn4* gene, are malformed.

One approach to analyzing the role of *Brn4* during development is to assess the outcome of *Brn4* null cells in an embryonic inner ear that also contains wild type cells, otherwise referred to as chimera analyses. These chimera analyses are facilitated by the fact that the *Brn4* gene is located on the X chromosome, and is subject to X chromosome inactivation. Therefore, in heterozygous females we would expect half of the cells to be null for *Brn4* and half to be wild type. However, preliminary quantitation of cells in heterozygous females indicates that more cells than expected are immunopositive for Brn-4. To determine whether the *Brn4* gene on the inactive X chromosome becomes activated in some mutant cells, we have undertaken double label immunohistochemical analyses in which cells expressing the knockout allele are identified by expression of the lacZ reporter, which has been incorporated into the knockout allele. In our preliminary analyses, we have not detected cells that double label for the expression of lacZ and the endogenous Brn-4 gene. These data indicate that Brn-4 expression from the wild type allele is not activated in mutant cells, and suggests that the overabundance of Brn4 positive cells in the heterozygous mice is due to a selective advantage of Brn-4 positive cells during otic capsule development.

Our preliminary data suggest that there are no significant differences in cell growth during early otic development at the level of the vertical plate, the horizontal plate or the tip of the cochlear duct, as assessed by BrdU incorporation. We are currently examining the rates of apoptosis in mutant animals to determine whether differential cell death could play a role in the selective advantage of Brn-4 positive cells in the heterozygous animals.

564 POU-Domain Genes in the Inner Ear of the Chicken and Canary

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In the mammalian inner ear (IE), the class 4 POU-domain gene Brn-3.1 is specifically expressed in hair cells and is required for their differentiation and survival. Three class 4 POU-domain genes have recently been identified in the chicken (Artinger et al. J. Neurobiol. 36: 572, 1998). We evaluated the expression of these homologues in the IE of the chicken and the canary. cDNAs were prepared from the apical portion of the basilar papilla in both avian species, so that samples contained sensory epithelium but not cochlear ganglion cell bodies. A primer set was designed to amplify specifically the chicken gene with greatest homology to mammalian Brn-3.1. Primers were also designed to amplify the chicken gene with greatest homology to mammalian Brn-3.2. Two consensus primer sets were also designed to amplify Brn-3.1 sequence from mouse, human or chicken. Finally, consensus primers

designed by Artinger et al. to amplify any class 4 family gene based on mouse, Drosophila and C. elegans sequences, were used.

In chicken, strong PCR products of the expected length were obtained from the chicken sensory epithelium with chicken Brn-3.1-specific primers, but no product from canary. Weak PCR products were obtained from the sensory epithelium in both chicken and canary with the chicken Brn-3.2-specific primers. Consensus primers designed to amplify all three class 4 genes produced products in both chicken and canary. Products from chicken sensory epithelium, when cloned and sequenced, proved to be Brn-3.1. Additional cloning and sequencing is in progress.

In conclusion, Brn-3.1 is strongly expressed in the sensory epithelium of the avian IE. Brn-3.2 is also present, although perhaps at lower levels. Similar expression also appears to occur in the canary IE.

However, significant sequence differences exist between chicken and canary Brn-3.1.

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565 Analysis of COUP-TFI Signaling in the Inner Ear

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Nuclear receptors are ligand-activated modulators of transcription that bind to hormone responsive elements where they recruit coactivator proteins that stimulate high level transcription at a target promoter. In the absense of a ligand they may be repressors of transcription. Loss-of-function COUP-TFI mouse mutants have defects in the glossopharyngeal nerve, which impair both sensory and motor functions of the pharynx and the tongue, compromise feeding behavior and usually result in perinatal death. Surviving COUP-TFI mutants have subplate neuron differentiation defects and altered thalamocortical axon guidance that result in apoptosis of cerebral cortical layer IV, which is required for perception of sensory stimuli. In addition, the spatial and temporal COUP-TFI expression pattern during inner ear organogenesis suggests that it may play a critical role in regulating processes that lead to formation of a functional inner ear. COUP-TFI mutants are profoundly deaf with a complete absence of auditory brainstem responses and have balance difficulties. There are multiple mechanisms underlying these sensory defects. Paint-filling COUP-TFI mutant ears revealed a foreshortened cochlear duct and severely malformed vestibular chambers. By P20, the basal coils of the organ of Corti are degenerated and the saccular macula is significantly smaller with few otoconia and is poorly innervated. Early stages of differentiation occur in these structures but there are proliferation deficits in both the saccular and cochlear primordia. This suggests that COUP-TFI is an essential gene for development and differentiation of both the hearing and balance systems. We are currently analyzing possible COUP-TFI downstream signaling molecules, which may be responsible for these phenotypes, using gene chip and biochemical analyses.

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566 FGFR4 Homozygous Mutant Mice are Defective in Hearing

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Fibroblast growth factors and their receptors are important in the biogenesis of many organ systems, including the inner ear. For example FGF3, FGF10, FGFR2 and FGFR3 have all been shown to be critical in the development of the inner ear. Mutations in FGFR3, specifically, appear to cause cochlear development to stall at an early developmental stage such that no pillar cells develop and the tunnel of Corti fails to open resulting in a completely deaf animal. We examined physiological indices of hearing in the FGFR4 knockout mouse. The homozygous null FGFR4 animal appears normal and healthy. We examined the hearing at 8-10 week of age in FGFR4 -/- mice using auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE). Input-output functions were collected for both measures over the frequency range 4 kHz to 32 kHz. We have found that these animals (n=13) have a 10-40 dB threshold shift in both the ABRs and the OAEs across all frequencies, compared to normal strain control animals. We also examined the cochleas of these animals by scanning electron and light microscopy and find that histologically they look qualitatively normal. Examination of the expression of FGFR4 mRNA in mouse cochlea reveals that at P3-5 expression is specifically localized to the spiral limbus. These finding suggest that while FGFR4 does not appear to be necessary for normal histological development of the cochlea it is necessary for the ontogeny of optimal hearing function. Loss of expression of FGFR4 in the spiral limbus somehow blocks normal functional maturation.

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567 Combining Human and Mouse Genetics Helps Unravel the Function of Proteins

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There has been a spectacular increase in our knowledge regarding the genetic basis of hearing loss in the last six years. The first autosomal nonsyndromic deafness genes were first identified in the mouse (myosin VIIA and myosin VI in 1995), and since then, 24 'deafness' genes have been cloned in humans. Our laboratory combines human and mouse genetics to map, clone and characterize genes contributing to hearing loss. We have identified several genes, and one example is our study of POU4F3 mutations associated with human and mouse deafness. We have found protein misexpression and aberrant cellular morphology in cells transfected with the human POU4F3 deafness mutation. Dramatically, most of the truncated POU4F3 protein is localized in the cytoplasm, due to the dysfunction of a putative nuclear localization signal (NLS). Our study of the dreidel mouse mutant with a deletion in Pou4f3 allows further insights into DFNA15 inner ear pathophysiology and molecular mechanisms of deafness. Our latest progress in studying conductive hearing loss, vestibular dysfunction, and sensorineural hearing loss will be presented.

568 Myosin VI - Expression, Deafness and Abnormal Cellular Morphology

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Mutations in the gene encoding myosin VI cause both human and mouse deafness (Melchionda et al. AJHG 2001), emphasizing that although the biological activity of myosin VI is not completely understood, this protein has a vital role in the sensory hair cell cytoskeleton. Myosin VI is a unique member of the myosin superfamily, having the ability to move towards the pointed (-) end of actin. Myosin VI plays a major role in cell motility and shape change events, including invagination of the plasma membrane, observed during pseudocleavage in Drosophila embryo blastoderm and sperm individualization in Drosophila and C. elegans. Reduction or loss in the levels of myosin VI in the hair cells of mice leads to disorganization and progressive deterioration of the hair cell stereocillia. In mice embryos myosin VI can be observed as early as embryonic day 10.5. In the developing mouse cochlea, myosin VI is expressed in the sensory epithelium prior to the differentiation of hair cells, and is restricted to the hair cells following differentiation. We have identified regulatory sequences approximately 70 kb upstream of the first coding exon that drive myosin VI expression. Transgenic mice with the human myosin VI missense mutation have been generated and crossed onto a myosin VI-null background. Most interestingly, fibroblasts derived from human deaf individuals are larger in size and have pronounced actin stress fibers, reinforcing the function of myosin VI in forming the cellular architecture.

569 Characterization Of Protocadherin 15, The Gene Associated With The Mouse Mutation Ames Waltzer (av)

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The av mutation causes deafness and vestibular dysfunction associated with the hair cell pathology 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. Scanning electron microscopy of hair cells from Pcdh15av-Tg and Pcdh15av-3J alleles show severe disorganization of stereocilia bundles as early as P0. Hair cell stereocilia from Pcdh15av-J and Pcdh15av-2J alleles, appear fairly normal at P0. However, some cuticular plates of hair cells from Pcdh15av-J and Pcdh15av-2J alleles appear rotated on the apical cell surface by P2, compared to age matched controls. By P5, this rotation is more conspicuous. Expression studies (RNA and protein) show that Pcdh15 is expressed in the inner ear. Analysis of the predicted amino acid sequence of Pcdh15 shows 11 cadherin repeats, a transmembrane domain and an intracellular domain that is unique and contains 2 proline-rich regions, which 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 efforts are under way to identify interacting proteins. Based on current observations it appears that Pcdh15 plays a role in the cellular process that controls bundle organization during hair cell development, and that Pcdh15 may mediate its function through interacting proteins.

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570 Cochlear Expression and Localization of WFS1, the Wolfram Syndrome 1 Gene, Which is Responsible for Nonsyndromic Low Frequency Sensorineural Hearing Loss

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Nonsyndromic sensorineural hearing loss is a disorder with marked genetic heterogeneity. Low frequency sensorineural hearing loss (LFSNHL) is the exception, as DFNA6/14/38 are all caused by mutations in WFS1, a gene that also causes the recessive Wolfram syndrome. WFS1 encodes a protein of unknown function, wolframin, localized to the endoplasmic reticulum. To investigate WFS expression in the cochlea, in situ hybridization was performed with mid-modiolar cochlear sections from 6 week old C57Bl/6 mice. IMAGE clone #4719145 (Research Genetics) from a 3' murine WFS1 cDNA was sequenced to confirm its identity and used to prepare an RNA probe. Specific expression was seen in the apical turn of the cochlea in Deiters cells and in the spiral ganglion. Wolfram syndrome mutations are usually null alleles, but rare homozygous missense mutations appear to cause a less severe Wolfram phenotype. All LFSNHL mutations are heterozygous missense mutations in the part of exon 8 that encodes the C-terminal domain, which might act in a dominant-negative fashion by interfering with or enhancing the specific function of the C-terminal domain, such as protein phosphorylation or protein-protein interactions. Since WFS1 mutations lead to progressive neural degeneration in many systems, localization in the spiral ganglion suggests that wolframin is necessary for maintenance of cochlear function and may help explain why WFS1 mutations cause progressive, delayed onset LFSNHL. Correlating genotype with phenotype is the first step toward understanding the function of this gene in the development and maintenance of normal hearing.

571 Age-Related Changes In CD-1 and CBA/CaJ Mouse Strains Of Endocochlear Potential And Potassium Concentration

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The CD-1 mouse strain has been used to express targeted genetic mutations that result in inner ear dysfunction, including disturbances of endolymph homeostasis. However, this mouse strain has been found to have early sensorineural hearing loss that worsens with age, as assessed by auditory brainstem response (ABR). This loss of hearing with age potentially complicates analyses of the effects of genetic mutation. In this study, we determined the effect of aging on the upstream driving forces for transduction, endocochlear potential (EP) and potassium concentration ([K]) in both CD-1 and CBA/CaJ mice, a strain in which the ABR threshold does not change with age. Mice were divided into YOUNG (1-2 months) and OLD (5-9 months) groups. Double-barrel electrodes were used to measure EP and [K] in the basal and apical turns and the –EP during anoxia in the apical turn. There was a significant decrease of [K] with age in both the apical and basal turns of CD-1 mice but no changes in the EP. This disturbance was restricted to the cochlea; there was no change in [K] in the utricle with age. By contrast in CBA/CaJ mice, there was no significant difference of [K] with age but a significant increase in the EP. Histologic observation of the lateral wall of OLD CD-1 mice showed an obvious loss of type IV fibrocytes (proposed to participate in K recycling) in the spiral ligament beneath the outer sulcus epithelia. The reduction of endocochlear [K] with age in the CD-1 strain suggested that the disturbance in K homeostasis may be due to either reduced K secretion or increased K

efflux. Caution must be exercised in using the CBA/CaJ mouse as an age-invariant strain since there is an age-related change in the EP.

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572 The Importance of the Class D L-Type Ca $^{2+}$ Channel in the Mouse Inner Ear

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Calcium channel activity in hair cells of the cochlea has been characterized as predominantly voltage-gated L-type Ca $^{2+}$ channels (LTCCs). The LTCCs evidenced in the inner ear contain a pore forming a1D subunit (D-LTCC) found widely expressed in mammals. D-LTCCs recently evidenced in hair cells of the chick basilar papilla suggest this isoform may play a participatory role in the afferent synaptic transmission of the cochlea. Depolarization of the sensory cell opens voltage-activated Ca $^{2+}$ channels which couples membrane depolarisation to neurotransmitter release.

Ca $^{2+}$ currents which couple sound-evoked depolarization with neurotransmission in mouse cochlear inner hair cells, are primarily mediated by D-LTCCs. The aim of the present study was to investigate structural changes during postnatal development in the a1D KO mouse cochlea. 40 a1D KO mice cochleae ranging from age's 1 day to 1 year were processed by the block surface method. 11 wild type mice were included as a control. Measurements of click-evoked auditory brainstem responses confirmed a profound deafness in all neonatal KO mice. Histological findings using light and electron microscopy revealed a beginning degeneration at the age of 7 days in both inner and outer hair cells, at synaptic contacts, and along afferent nerve fibers while efferent nerve fibers remained intact. At 15 days of age, a notable degeneration of spiral ganglion cells could be seen and by 1 year, nearly all spiral ganglion cells and sensory cells of the organ of Corti were absent. Serial ultrathin sectioning of 15 day old á1D KO mice point out more frequent direct synaptic contacts of efferent fibers with IHCs. Synaptic bodies were lacking in KO mice in the afferent nerve endings. This is true for all agegroups investigated. The functional role of LTCCs in the cochlea is discussed.

573 Central pathways in alpha 1 D L-type calcium channel knock-out mouse

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Voltage-gated Ca $^{2+}$ channels mediate depolarization-induced Ca $^{2+}$ influx across the plasma membrane of electrically excitable cells. A variety of studies in bullfrog, chick and turtle have shown that voltage-gated Ca $^{2+}$ channels are largely found at discrete sites in hair cell membranes that colocalize with presynaptic dense bodies (the presumed sites of neurotransmitter release) and that these are important in regulating the excitation of the auditory nerve fibers. L-type calcium channels are widely expressed in mammalian organisms. In skeletal smooth, and cardiac muscle, they couple membrane depolarization to muscle contraction. In (neuro)endocrine cells, including pancreatic ß-cells, Ca $^{2+}$ influx LTCCs trigger hormone secretion. In neurons, LTCCs are preferentially located at cell bodies and dendrites.

In a recent study, we evaluated the inner ear of different age groups of L-type calcium channels knock out (KO) mice. The findings show that the neonatal KO mice were in fact deaf with evidence of sensory structure degeneration.

The aim of the present study was to investigate the cochlear and vestibular nuclei in the brainstem by the use of immunohistochemistry. Antibodies against Synaptophysin, Synapsin, Catestatin were applied on frozen and paraffin sections of the brainstem in KO and Wild-type mice. To find out any structural and functional changes in the neurons of the central auditory system, these markers for presynaptic vesicles were used. Results from the central system were discussed in regard to the peripheral findings in the inner ear.

574 Age-Related Effects of GATA-3 Haplo-Insufficiency on the Auditory Brainstem Response of Alert Mice.

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GATA-3 haplo-insufficiency causes deafness in patients suffering from HDR syndrome (Hypoparathyroidism Deafness and Renal abnormalities; Van Esch *et. al.*, Nature, 406, 2000). As GATA-3 is distributed in both peripheral and central parts of the auditory system, it is unknown how the deafness in HDR syndrome comes about. To investigate the potential underlying peripheral and central mechanisms we have created GATA-3 mutant mice and subjected them to longitudinal Auditory Brainstem Response (ABR) recordings and histological analyses. GATA-3 heterozygous mice (ht) and wild type littermates (wt) varying from 3 to 19 months of age received an acrylic head fixation pedestal with recording electrodes, and their ABR thresholds were measured following stimulation with clicks and tone pips (4,8,16,32 kHz, pip duration 1 ms; repetition rate 80/s). As compared to wt littermates the thresholds of GATA-3 ht mice were elevated in all age groups. The latency of peak 1 in the ht mice was significantly longer than that of controls, while the interpeak latencies did not differ between ht and wt mice. The inner ear of ht mice showed abundant degeneration of hair cells, supporting cells and spiral nerve fibers, while no morphological abnormalities were observed in the brainstem or cerebral cortex. Moreover, outer or middle ear problems were not observed. These data strongly suggest that the deafness following GATA-3 haplo-insufficiency is caused by inner ear aberrations that lead to delayed responses of the cochlea and/or VIII nerve and that this deafness is not prominently enhanced by central effects.

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575 Cochlear damage due to germanium-induced mitochondrial abnormality

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Long-term administration of high-dose germanium may cause renal failure, anemia, emaciation, and muscle weakness. It has been reported that, when chronically given to rats, germanium induces ragged-red fibers and cytochrome-c oxidase-deficient fibers in the skeletal muscles and reduces biochemical activity of enzymes in the mitochondrial respiratory chain. Hitsopathological findings in these muscles are similar to those seen in mitochondrial encephalomyopathy.

In the current study, guinea pigs were orally given germanium at different concentrations (0.05%, 0.15%, 0.50%, and 1%). Animals treated with 1% germanium died within a few weeks. Animals treated with 0.05% or 0.15% germanium did not die until 6 months but they did not develop any abnormalities in any organ examined. Animals treated with 0.5% germanium survived for two months, but did not gain body weight. Their skeletal muscles were apparently atrophic. TEM

observation revealed degeneration and germanium inclusion in a lot of mitochondria in the skeletal muscle, heart, and kidney. ABR measurements revealed moderate threshold shifts. A lot of germanium inclusion and degenerative changes were found in the stria vascularis and its adjacent areas. Germanium was also scattered in the supporting cells, the sensory epithelium in the vestibule, and areas around the cochlear and vestibular nerve fibers, but these tissues showed virtually normal appearance. These findings indicate that 0.5% germanium administration induces mitochondrial damages in multiple organs including the cochlea in the guinea pigs, suggesting that this experimental model is useful to investigate cochlear damage in mitochondrial encephalomyopathy. The threshold shifts may be due chiefly to damage to the stria vascularis.

576 Neomycin-induced hair cell death in the lateral line of zebrafish: a preparation for studying the genetics of hair cell survival

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In order to systematically study endogenous genes that influence hair cell death or survival, we have developed an in vivo preparation for phenotype screening using wild-type zebrafish embryos. First, the survival of hair cells in lateral line neuromasts after neomycin exposure was assessed over a range of drug concentrations (0-500 μM). Four hours after initial exposure to neomycin, fish were scored for the presence or absence of hair cells in each of the lateral line neuromasts. Neuromasts were labeled with the fluorescent vital dye, DASPEI, which is preferentially taken up by hair cells. We have established a dose-response relationship between neomycin concentration and hair cell survival, with approximately 50% of neuromasts retaining hair cells after treatment with 125 μM neomycin. Total lateral line hair cell loss occurred following exposure to 500 μM neomycin. Second, the time frame of lateral line hair cell regeneration was analyzed. The number of proliferating cells per neuromast was identified using bromodeoxyuridine (BrdU). BrdU labeling of neomycin damaged neuromasts did not differ from control animals at 4 hours post-drug exposure. At 12 hours BrdU labeling increased approximately 4-fold and regenerated hair cells were first observed between 12 and 24 hours following neomycin treatment. We believe this in vivo neomycin damage paradigm in the lateral line system is an efficient preparation for studying hair cell death and survival. Current work focuses on screening genetically modified animals for endogenous genes that modulate hair cell susceptibility to environmental insults such as aminoglycoside antibiotics.

577 Ultrastructure of Normal and Neomycin-exposed Embryonic Zebrafish Lateral Line Hair Cells

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We have undertaken a transmission electron microscopic study (TEM) of hair cells in embryonic zebrafish neuromasts. Representative head and trunk lateral line neuromasts are studied in 4-5 day old embryos. Neomycin exposures are designed to match our studies of hair cell death and regeneration (Harris et al., this meeting). Our goals are to thoroughly describe mature and immature hair cells in the embryonic zebrafish and to carefully document the ultrastructural changes that take place between aminoglycoside exposure and hair cell death. Of interest is to evaluate the similarities and differences between the cascades of cellular events leading to hair death in these cells and those of the mammalian inner ear exposed to similar ototoxic agents. Our initial observations have been on the patterns of hair cell synaptic innervation in normal and neomycin-exposed hair cells.

In normal embryonic neuromasts the classical pattern of afferent and efferent synapses is seen. Notably, even at an early developmental stage (E4-5) both afferent and efferent endings are seen making recognizable synaptic contacts with newly differentiated hair cells. Afferent synapses are characterized by the typical large presynaptic body, while efferent synapses show the characteristic postsynaptic specialization (cistern). Meanwhile, some immature features are noticed, such as the multiple contacts that single afferent neurites make with one or several neighbouring hair cells. Reorganisation of neurites and synapses is followed immediately after neomycin exposure and a few days later, when neuromasts grow new hair cells.

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578 Compensatory Upregulation of Glutathione Peroxidase May Protect Middle-Aged SOD1 KO Mice from Noise-Induced Hearing Loss

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Exposure to noise is one of the major causes of hearing loss in the adult population. However, susceptibility to noise-induced hearing loss (NIHL) varies greatly among individuals. The factors that influence individual susceptibility are poorly understood but may include the relationship between reactive oxygen species (ROS) produced during cellular metabolism and endogenous antioxidants such as copper/zinc superoxide dismutase (SOD1) and glutathione peroxidase (GPx). In this experiment, we examined the effects of altering the balance between antioxidants and ROS on susceptibility to impulse noise. Subjects included 7-month-old wild type (WT) mice with normal levels of SOD1, heterozygous knockout (HET) mice with 50% of normal levels of SOD1, and homozygous knockout (KO) mice with no SOD1. Auditory brain stem responses were measured at 3, 6, 8, and 12 kHz before and 2 weeks after the mice were exposed to 150 dB peak SPL impulse noise. Based on previous evidence regarding the relationship among ROS, antioxidants, and NIHL, we expected that the KO mice would be more susceptible to noise than the WT and HET mice. However, results indicated that the WT and HET mice had significant NIHL at 3 kHz, whereas the KO mice did not have significant NIHL at any frequency. Enzyme assays performed on a separate group of middle-aged (8-month old) and aged (16-month old) SOD1 mice showed enhanced levels of GPx in KO mice. Thus, middle-aged SOD1 KO mice may actually be protected from NIHL by compensatory upregulation of GPx. The results provide insights into the biological pathways for NIHL and emphasize the need for caution when interpreting data from genetically altered mice.

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579 Glucocorticoid Suppression of Cochlear Lesions due to Repeated Adenovirus Gene Transfer

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Gene transfer using recombinant adenovirus is a powerful tool for neurobiological research and a potential treatment for a variety of human diseases. One limitation in the use of a recombinant adenovirus as a gene transfer vehicle is immune response. The immune response is directed against the adenovirus antigen and the transgene product. The immune response becomes severe upon a second application of the gene transfer vector, following the primary exposure to the virus. Glucocorticoids such as dexamethasone (DEX) have been used effectively as potent immunosuppressive agents against inflammatory disease. The goal of this experiment was to determine the effect of DEX on the outcome of repeated adenovirus gene transfer in the guinea pig inner ear. Pigmented guinea pigs were divided into 3 groups. The experimental group received the DEX (1 mg/kg SC) for 29 days. The animals were inoculated with 5 µl of the adenovirus expressing bacterial *lacZ* gene driven by a cytomegalovirus promoter (10^{11} viral particles per ml) in the left ear on day 5, followed by a second inoculation on day 26. Animals were sacrificed on day 29. One control group consisted of animals that were inoculated with the viral vector but did not receive DEX. The other control group consisted of animals that received no DEX, and the vector was replaced with artificial perilymph. ABRs (at 4, 12 and 20kHz) were measured on days 1, 8 and 29. Inner ear tissues collected on day 29 were processed for hair cell counts.

The results show a significant difference in hearing thresholds between the experimental group and each of the control groups. Preliminary cytocochleogram data show that DEX treatment protects hair cells in the experimental group. Our data demonstrate that treatment with DEX can reduce the loss of hair cells and hearing loss caused by repeated administration of recombinant adenovirus to the inner ear.

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580 Expression of c-RET and the RET/PTC Fusion Oncoprotein in Hashimoto's Thyroiditis and Papillary Thyroid Carcinoma

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Objectives: To evaluate the expression of c-RET proto-oncogene and the RET/PTC fusion protein in Hashimoto's thyroiditis and papillary thyroid carcinoma.

Introduction: The RET proto-oncogene encodes a receptor-type tyrosine kinase which has been shown to be expressed in cells and tissue of neural crest origin. This expression is thought to play a role in cell differentiation and survival. Until recently, demonstration of RET expression in thyroid follicular cells has been limited to RET/PTC translocation products and this expression was shown to have a positive correlation with presentation of Hashimoto's thyroiditis and papillary thyroid carcinoma.

Methods: Immunohistochemical staining of human thyroid tissue was performed. Normal thyroid, Hashimoto's thyroiditis, papillary thyroid carcinoma and anaplastic carcinoma were evaluated using antibodies specific against the extracellular domain and tyrosine kinase domain of c-RET.

Results: Expression of the extracellular and tyrosine kinase domains of RET proto-oncogene was demonstrated in the majority of patients presenting with autoimmune thyroiditis and papillary thyroid carcinoma but was not observed in normal human thyroid or in anaplastic carcinoma.

Conclusions: Expression of the tyrosine kinase and extracellular domains of the c-RET proto-oncogene was demonstrated in Hashimoto's thyroiditis and papillary thyroid carcinoma. Variations in staining localization indicate expression of the RET proto-oncogene in three possible configurations: 1) as the wild-type c-RET receptor; 2) as a RET/PTC fusion oncoprotein; and/or, 3) as the reciprocal translocation product of the above fusion protein. Implications of these data may hold promise in diagnostic, surveillance and treatment options in Hashimoto's thyroiditis and papillary thyroid carcinoma.

581 Identification of Differentially Expressed Genes in a Healing Fetal Wound

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Adult mammalian tissues heal injury with scar formation. While scarring seals a wound, it can also compromise essential body functions, such as respiration, speech, hearing, smell, and movement. In contrast, mammalian fetal tissue can heal *without* scar. We have undertaken to identify genes regulated in fetal tissues that are important in scarless wound healing.

Tissues were obtained from wounded and control New Zealand white fetal rabbits. Total messenger RNA (mRNA) from each tissue type was reverse transcribed into complementary DNA (cDNA) and amplified via the polymerase chain reaction (PCR). This cDNA was then used to carry out PCR suppression subtraction hybridization; a dual subtraction experiment was carried out to identify genes that were both upregulated and downregulated in fetal wound compared to fetal control. The subtracted DNA was shotgun cloned into a TA vector; cloned inserts were then sequenced and analyzed by BLAST searching on the GenBank database.

A total of 37 genes have been identified that are putatively differentially expressed in healing fetal tissue. These include a transcription factor, multiple splice factors, and elements of the cellular translational machinery. Each of these genes may be involved in the further regulation of other downstream gene products. The majority of genes recovered, however, are either previously undescribed or correspond to expressed sequence tags of unknown function.

We are currently obtaining more sequence information of novel genes through RACE (rapid amplification of cloned ends), and confirming the differential expression of these gene products by real-time PCR using "molecular beacon" technology. We hope thereby to arrive at a broader understanding of the molecular mechanisms that allow scarless healing in fetal tissues.

582 Histopathologic Evaluation of Electroporation-Assisted Luciferase DNA Delivery to Porcine Skin

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Objectives/Hypothesis: The purpose of this study is to evaluate the histopathological consequences and effectiveness of delivering various volumes of luciferase plasmid DNA to the skin of a large animal using in vivo electroporation. Electroporation is a means of enhancing DNA transfection into cells by delivering a pulsed electrical field. The electrical field makes the cell membranes porous allowing the luciferase marker gene to enter the cell. The production of luciferase in the cell can be measured in light units. The potential clinical benefits for this type of gene therapy are broad and include the treatment of genetic and acquired skin diseases.

Study Design/Methods: A prospective, matched design experiment was performed to evaluate gene transfection and histopathologic changes of electroporation-assisted luciferase plasmid DNA delivery to porcine skin. Ten micrograms luciferase plasmid DNA was intradermally injected into porcine skin to compare transfection utilizing seven variations of volume and two treatment parameters: no electroporation and electroporation with the meander surface electrode. A similar volume of phosphate buffered solution was intradermally injected and electroporated to serve as a matching control. All sites were biopsied on study day two. Each specimen was sent for histopathologic evaluation and determination of luciferase relative light unit activity.

Results: Electroporation significantly increased the transfection of luciferase plasmid DNA into porcine skin ($p=0.004$). The volume injected did not significantly improve transfection. There was no statistical difference in tissue damage by histological grading between electroporated and non-electroporated specimens.

Conclusions: Electroporation is an effective and safe manner to transfect plasmid DNA into porcine skin.

Key Words: Electroporation, gene therapy, luciferase, swine, porcine.

583 Smooth Muscle of the Annulus Fibrosus in the Gerbil: Structure and Physiological Properties

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In a wide variety of mammals, the rim of the tympanic membrane (annulus fibrosus) has an array of contractile elements, either smooth muscle (Henson and Henson, JARO 1:25, 2000) or myofibroblasts (Kuijpers et al., Hear.Res. 128:80, 1999). In this study, TEM micrographs of the gerbil annulus were used to study the ultrastructure and distribution of the cells. Cells with the ultrastructural characteristics of smooth muscle were congregated along the circumference of the sulcus tympanicus; they were attached to the central part of the tympanic sulcus and radiated toward the tympanic membrane. They had numerous processes that extended into the dense collagenous matrix of the annulus. Their arrangement suggests that they are involved in the control of tympanic membrane tension.

Cochlear microphonic (CM) threshold changes were recorded in gerbils to study the physiological effects of these contractile elements. It was demonstrated that: the application of substances known to make smooth muscle contract (vanadate and norepinephrine) caused concentration dependent elevations in CM thresholds. Maximum changes of 8-9 dB occurred with the lowest frequency tested (2.16 kHz). The application of muscle relaxing drugs reversed these effects. Controls showed that the threshold changes were not induced by effects on middle or inner ear structures. These results add to growing evidence that the tympanic membrane may have intrinsic control of tension and is potentially able to have some control over energy levels reaching the cochlea.

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584 Morphometric Study of the Carotid Canal Dehiscence in the Middle Ear

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A bony wall dehiscence of the carotid canal in the middle ear is occasionally present. The internal carotid artery to the middle ear (ME) through a dehiscence creates a hazard during surgery. For example, fatal aural bleeding from the carotid on myringotomy has been reported. This study examines the location and prevalence of ME carotid canal dehiscence in 109 human temporal bone specimens; obtained from 68 individuals, whose ages were 6 days to 90 years at death, with no known congenital anomalies or either a clinical history or histological evidence of ear disease.

The specimens were processed histologically and prepared for light microscopic study. The bony dehiscence of the carotid canal and its surrounding structures were identified and both its size and the distance from anterior-superior margin of the tympanic annulus were noted. In one case, the images of those structures were entered into the personal computer through the digital camera (960 x 1280 pixels) and reconstructed by an image analysis program (NIH Image 1.62).

A ME dehiscence of the carotid canal was observed in 5 (4.6 %) of 109 temporal bones. Four of those bones were obtained from four individuals whose ages were over 40 years. They were located anterior-superior to the promontory (medial wall of bony eustachian tube) and varied in sizes from 0.6 x 0.3 mm to 3.7 x 4.4 mm and their distance from the tympanic annulus were from 4.6 to 6.1 mm.

Although dehiscence is relatively uncommon, it is usually located near the ear drum. Hence, routine clinical care should be paid to avoid rupture during clinical procedures such as myringotomy.

585 Simultaneous Measurement of DPOAEs, Middle-Ear Input Impedance, and Forward/Reverse Middle-Ear Transmission in Cat

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We developed a novel paradigm for measuring forward and reverse middle-ear transmission that exploits distortion-product otoacoustic emissions (DPOAEs) to drive the middle ear "in reverse" without opening the inner ear. The technique allows measurement of DPOAEs, middle-ear input impedance, and forward/reverse middle-ear stapes-velocity transfer functions simultaneously in the same animal. We applied the technique to measure middle-ear characteristics in anesthetized cat. Stapes velocity was measured with a laser-Doppler velocimeter through a small hole drilled in McEwen's triangle lateral to the superior-posterior quadrant of the tympanic membrane (Tonndorf and Taber, 1962, *Ann. Otol. Rhinol. Laryngol.*, **71**:5.) Sound stimuli were generated and recorded using a calibrated acoustic assembly placed within a few millimeters of the tympanic membrane. The sound stimulus consisted of two primary tones, at frequencies f_1 and f_2 with $f_2/f_1=1.2$, presented simultaneously. Intermodulation distortion in the cochlea generated a DPOAE, measurable in both the ear-canal pressure and the stapes velocity, at the frequency $2f_1-f_2$. Middle-ear input impedance and forward transfer functions were computed across frequency from stapes velocities and corresponding ear-canal pressures measured at the two primary frequencies. The reverse transfer function was computed from velocity and pressure measurements at the DPOAE

frequency. We present and discuss results from consistent measurements on five animals.

586 A Re-Examination of Middle-Ear Transmission in Chinchilla

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Two contrasting views of mammalian middle-ear transmission are commonly held. One is that the middle ear acts as a resonant tuned filter (with low- and high-frequency velocity magnitude slopes of +6 and –6 dB/octave) that narrowly focuses acoustic energy into the cochlea. Another is that the middle ear is a wide-band pressure transformer with a linear phase-vs.-frequency characteristic. Both views can claim experimental support from measurements of stapes vibrations (favoring the resonance hypothesis: Aibara et al., *Hear.Res.* 152: 100-109, 2001, in humans; Rosowski et al., *Audiol.Neuro-Otol.* 4: 129-136, 1999, in gerbils; favoring the wide-band transformer hypothesis: Olson, *J.A.S.A.*, 103: 3445-3463, 1998, and Overstreet and Ruggero, *Assoc. Res. Otolaryngol Mid-Wint. Meet. Abst.*, 23: 115, 2000, and *J.A.S.A.*, in press, both in gerbils). Although, among mammals, the chinchilla is an especially appropriate species to investigate the question of middle-ear bandwidth because of its unusual low-frequency hearing, the one previous study of middle-ear vibration in this species (Ruggero et al., *J.A.S.A.* 87: 1612-1629, 1990) could not address this question because it used an inefficient vibration recording technique (the Mössbauer method) and because the acoustic-stimulus delivery system had limited high-frequency response. We are re-investigating stapes vibrations in chinchilla, now using a laser velocimeter and a wide-band stimulus system. Initial results support the wide-band transformer hypothesis in that the magnitudes of stapes vibrations exhibit a relatively flat frequency spectrum (~ 0.1 mm/s/Pa) up to at least 26-31 kHz (i.e., near the 60-dB high-frequency cut-off of hearing, 33 kHz) and the phase-vs.-frequency curve is approximately linear.

However, these results do not rule out that the steep high-frequency slope of the audiogram is determined by the middle ear.

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587 The Mobility of the Incudo-Malleolar Joint and the Middle Ear Transfer Function

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The mechanical behavior of the incudo-malleolar joint was investigated and described by Gundersen and HØgmoen in 1976. The motion of Malleus and Incus was described as a single body motion mainly consisting of a rotatory and a translatory component at the position of the joint (effect of tensor tympani excluded). These experiments were performed in temporal bones which lack the cochlear impedance load. Removing the cochlear load might have changed the system dramatically but was inevitable for the applied technique.

In this study we describe the dynamic behavior of the incudo-malleolar joint by means of laser Doppler scanning vibrometry in fresh temporal bones which have an intact cochlea. Our measurements show that draining the cochlea indeed changes the behavior of the joint in a crucial way. The rotation around the axis between the anterior process of Malleus and the posterior ligament of Incus constitutes the major motion component of the ossicles. Our results furthermore demonstrate a frequency dependent loss of this rotational motion from Malleus to Incus. This loss of rotation coincides closely with the transfer function between the Umbo displacement and the displacement of the lenticular process of Incus.

Based on our results we conclude that the transfer function of the middle ear is mainly defined by the characteristics of the incudo-malleolar joint.

Gundersen T and HØgmoen K, 1976. Holographic vibration analysis of the ossicular chain. Acta Otolaryngol 82: 16-25.

588 Measurements of Stapes Vibration Modes Following Surgical Middle Ear Reconstruction In The Cadaveric Human Ear.

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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. These reconstructions change the force vectors and energy flow path in the middle ear significantly when compared to the normal anatomic state. Changing the force vectors with surgical reconstruction may change the 3D motion of the stapes.

Fresh human cadaveric temporal bones were harvested within 48 hours after death. Measurements on the stapes footplate were carried out at several sites using a Laser Doppler Vibrometer to determine the 3D movements. Measurements were carried out first for intact temporal bones. Following this, for 2 types of reconstruction in which the incus had been removed and the middle ear reconstructed with either a prosthesis from the stapes head to the malleus or a prosthesis from the stapes head to the eardrum (partial ossicular replacement prosthesis (PORP)).

The tympanic membrane was stimulated with a sound input of 80 – 120 dB SPL over a frequency range of 0.1 to 10kHz.

We present the change of motion of the stapes before and after surgical reconstruction. How the rocking motion of the stapes changes is emphasised

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589 Inter-Subject vs. Intra-Subject Variability in Ear-Canal Impedance and Reflectance of Living Human Ears

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Measurements of acoustic impedance and reflectance made in the ear canals of living humans exhibit substantial inter-subject variability. These impedances are often calculated from a pressure measurement made in the ear canal along with the sound-delivery system's Thévenin equivalent, which can be determined through pressure measurements in well defined cavities [e.g., Allen, J.B. (1985). "Measurement of eardrum acoustic impedance," Peripheral Auditory Mechanisms, edited by J.B. Allen, J.L.Hall, A. Hubbard, S.T.Neely, and A. Tubis, Springer-Verlag, New York]. This work begins to quantify inter-subject variability relative to intra-subject variability in ear-canal impedance and reflectance measurements that might occur as a result of either methodological issues or changes in an individual ear over time.

The ear-canal impedance and reflectance from repeated measurements of ear-canal pressure on five subjects (10 ears) were calculated to examine the intra-subject variability that occurs over a time period of four weeks. The Thévenin equivalent of the sound delivery system was measured at each measurement session, and care was taken to obtain approximately equal ear-canal insertion depths of the eartip across all sessions. At 500 Hz, in six of the ears, the impedance magnitude's standard deviation for a given ear is less than half of the population's

standard deviation of 1.11 (units of impedance normalized by the ear-canal's characteristic impedance). However, in four ears, the individual standard deviations approach the population standard deviation. The variability in the reflectance domain is similar to that in the impedance domain. We conclude that measurements used to calculate the ear-canal impedance and reflectance that employ a method similar to Allen (1985) can exhibit intra-subject variability that approaches the variability measured in a population of ears. Further work is needed to quantify the source of this variability.

590 Scanning Laser Vibrometry of the Reconstructed Middle Ear:

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Ossicular chain reconstruction gives unpredictable hearing results: Despite new materials, a better understanding of middle ear disease, a plethora of prosthesis designs and apparently simple reconstruction situations, clinical results have not materially improved in the last 25 years. In an acoustically favorable reconstruction situation with an intact stapes superstructure, 60% of patients achieve disappointing results with an air bone gap of greater than 15 dB. This presentation attempts to investigate why.

Fresh temporal bones were harvested within 12 hours of death and the middle ear exposed using conventional otosurgical techniques. The ear was stimulated acoustically at 94 dB SPL. The tympanic membrane and ossicular chain motion was investigated at varying frequencies using, a scanning and single point laser doppler vibrometer. The ossicular chain was then reconstructed using one of two methods. Some specimens had the incus removed and the ossicular chain reconstructed using a conventional partial ossicular replacement prosthesis. To emulate incus erosion, other specimens underwent a laser excision of the distal 2mm of incus and the chain was reconstructed with ionomeric cement. The tympanic membrane and ossicular chain were again scanned.

A comparison of the intact and reconstructed chain motion was then made. The limitations of current PORP design are demonstrated. Certain surgical interventions are investigated to determine techniques to obtain more effective ossicular reconstruction results.

This presentation makes extensive use of video animation to demonstrate the motion of the tympanic membrane and ossicular chain.

591 Simple Motion of the Stapes Revealed using Scanning Laser Doppler Vibrometry

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The human ossicular chain performs impedance matching between air (low impedance) and cochlear fluid (high). Synovial joints in the ossicular chain allow slip to protect the oval window from excessive displacement due to static pressure variations, yet remain functionally rigid to conduct sound. This presentation addresses another important ossicular function, that of ossicular averaging.

To remain acoustically sensitive, the tympanic membrane is highly flexible. A flexible membrane introduces vibration modes resulting in complex tympanic membrane motion that varies across frequency. A tympanic membrane free of vibration modes would be too stiff for acoustic pickup. The ossicular chain serves to progressively reduce the degrees of freedom and convert complex motion occurring at the malleus to piston-like motion at the stapes.

Using scanning laser vibrometry, we demonstrate this in a cadaver temporal bone model. Video animations of the ossicular function are shown. The malleal head moves about an elliptical orbit with additional roll. The incus exhibits similar motion, but significantly less roll and yaw. Slip at the incudo-stapedial joint ensures that stapes motion is nearly piston-like with minimal rocking at high frequencies.

592 Detailed Analysis of the 3-D Vibration of the Malleus Described in an Intrinsic Malleus-Annulus Reference System.

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Our earlier studies of the malleus vibration in the cat middle ear have shown that the rotation axis of the malleus is not fixed and the motion is three-dimensional (Decraemer and Khanna, 1996. Proc. Diversity in Auditory Mechanics, Berkeley 115-121). The observation was done through the narrow ear canal limiting the viewing angle. All measured points were inherently located on a narrow strip which is not optimal for calculating the 3-D motion of the malleus. In order to characterize the 3-D motion more precisely it is necessary to make vibration measurements from a larger portion of the malleus at widespread viewing angles. To obtain good optical access below the malleus, it is necessary to open the wall and the floor of the middle ear cavity in fresh temporal bones. Measurements from different viewing angles were taken on the anterior surface of the malleus from the tip to the neck of the malleus, using a heterodyne laser interferometer. 3-D components of vibration were extracted from these measurements assuming rigid body behavior. The precise anatomical dimensions of the malleus and the annulus in the experimental middle ear were obtained using high resolution CT scan. To compare these results with our earlier findings, they were transformed to the intrinsic reference system with the x-y plane parallel to the annulus plane and the origin at the umbo. The results confirm our earlier findings and extend them.

The motions of the malleus are best seen in the animation of a precise 3-D model of the malleus that is based on the present experimental results.

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593 Directionality of the Lizard Tympanum

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Many lizards (Lacertilia) have highly developed, sensitive ears with an upper frequency of hearing extending to about 8 kHz. However, most lizard heads are small compared to the wavelengths of sound at their most sensitive frequencies. Therefore,the time and intensity differences at the two ears due to sound diffraction around the lizard will be very small, and for their directional hearing lizards might therefore exploit the inherent directionality produced by the coupling of the two tympana through the mouth cavity (probably a primitive characteristic of the ear of lizards and other tetrapods).

We have investigated tympanic membrane motion in four lizard species: *Gecko gekko* (a gekkonid), *Mabuya macularia* (a skink), *Leiolepis belliana* (an agamid) and *Ctenosaura similis* (an iguanid) using laser doppler vibrometry and free-field sound stimulation. Eardrum vibrations of all four species showed band-pass characteristics within peak amplitudes at 1-3 kHz. Within this range a frequency band showed pronounced directionality with ipsi-contralateral differences of up to 20 dB. The directionality was abolished by enclosing the contralateral eardrum in Vaseline.

In conclusion, we have demonstrated that the auditory periphery of these lizard species is highly directional and the directionality is generated by acoustic coupling of the two eardrums. Furthermore, the

measured directionality are similar to the directionality produced by a simple model of the acoustic periphery based on electrical analogues and assuming coupling of the two tympana through a central cavity. Therefore, the lizard ear is probably the clearest vertebrate example of pressure-difference directionality generated by coupling of the tympana.

594 Middle Ear Biomechanics - Using Double Laser Interferometry on Human Temporal Bones

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The human middle ear, including the tympanic membrane (TM) and 3 ossicles: malleus, incus and stapes, is a complex mechanical system for sound transmission from the air in the ear canal to the cochlear fluid in the inner ear. Different experimental methods were used in the past to investigate middle ear mechanics with animals and human temporal bones. Recently, a new method using double laser interferometry to measure vibrations of the eardrum and stapes footplate in a human temporal bone model was developed in our lab. A hearing laser vibrometer (Polytec PI) was used to measure the vibrations of the TM and simultaneously, a single point laser vibrometer was used to measure the movement of the stapes footplate. We sought to derive the relationship between the displacements of the tympanic membrane and the stapes footplate in both magnitude and phase with intact and drained cochlea, respectively.

Experiments were performed on 10 human cadaver temporal bones. Results show that the sound transmission ratio (STR) between the displacement magnitudes at the stapes footplate and TM reached a stable, highest level at low frequencies (0.25-0.75k Hz), remained unchanged at frequencies of 1-1.5k Hz, and decreased at high frequencies (2-8k Hz). The phase angles of the displacements at the TM and stapes footplate were negative (delayed) at the lower frequencies and positive (advanced) at the higher frequencies in comparison with the input sound. The cross-over frequency of the displacement phase was around 2k Hz for the TM and 3k Hz for the stapes footplate. Experimental data were then simulated using MATLAB for future analysis.

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595 Lumped-Parameter Model of Human Middle Ear for Dynamic Simulation

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Transfer function of the middle ear for sound transmission from the air in ear canal to the cochlear fluid has been measured with different methods. A new direct method, simultaneously using double laser Doppler interferometers, to measure vibrations of the tympanic membrane (TM) and the stapes footplate in a human temporal bone model was developed in our lab and first reported in ARO 2000 Midwinter Meeting. In this paper, we report a currently completed 6-mass lumped-parameter model for simulating dynamic behavior of the middle ear. The parameters of the model were determined based on double laser interferometry data obtained in ten human temporal bones.

The six-mass lumped model consists of 6 masses including the air column in ear canal, the tympanic membrane, malleus, incus, stapes and the cochlear fluid, and 9 pairs of springs and dashpots. All elements together represent the ear canal-TM coupling, malleus-incus body, incudostapedial joint, and stapes-oval window coupling. To determine 18 parameters of the model, fundamental equations of the system were first established. The experimental data of the TM and stapes footplate displacements across auditory frequencies were then incorporated into the derived equations. All 18 parameters for springs and dashpots in the model were calculated through numerical process and parameter-perturbation using MATLAB. Finally, statistic analysis on 10 groups of parameters derived from 10 temporal bones was carried out to provide the parameters with statistic significance.

Using this lumped model we computed displacements of the TM and stapes footplate and compared the results with the temporal bone experimental data. Our results show that the 6-mass lumped model gives a good simulation of middle ear dynamics for sound transmission. The model will be further employed for studying middle ear biomechanics with implants.

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596 EarLab: Species Specific Modeling of the Mammalian Auditory System

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Numerous features of the auditory system are shared across the mammalian taxonomic order. Similarities in morphology and psychophysical abilities are the basis for comparative studies in audition, which in turn form the foundation for much of our current understanding of auditory system physiology in humans and other animals. Physiological and phenomenological models of auditory systems traditionally follow experimental data; as a result, their predictions are usually limited not only to a class of stimuli but also the species from which the data were taken. Few attempts have been made to develop "scaling factors" between species, so generalizations of model predictions across species are usually made qualitatively.

EarLab offers a modular approach to modeling that incorporates computational models of the auditory system controlled by species-specific parameter sets. The auditory system is divided into functional modules, which are modeled independently and connected to other modules. For instance, the model of cochlear mechanics is parameterized by a frequency-place map developed for the species of interest. Likewise, when possible, the middle ear module uses species specific transfer function coefficients. Each module accepts and outputs defined and measurable quantities so that different models for a given structure can be interchanged and predictions can be verified experimentally. Early success in predicting audiograms suggests that it is possible to develop scaling factors to quantitatively transform models of one species into another.

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597 Three-Dimensional Model of the Cat Middle-Ear Cavities

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A simplified three-dimensional (3D) model has been developed for the coupled middle-ear cavities (MEC) of the domestic cat, using a finite element program to solve the Helmholtz equation for interior acoustics (Oberai, et al., 1998, Applied Numerical Mathematics). Simpler models of the MEC have been previously studied, using lumped elements and 1D models. We use the 3D model to test the validity of these previous models. Model impedances for normal and surgically modified MECs are compared both with Lynch's measurements (PhD Thesis, MIT 1981) and with a lumped element model. From these comparisons, we see good correlations below 10 kHz, but find that only the 3D model captures the higher order resonances, such as a resonance at 40 kHz not seen in the lumped-element model. The effect of the removal of the septum has also been studied using the 3D model. In the absence of the septum, the first resonance and anti-resonance frequencies increase,

while the magnitude of the smaller resonances between 10 and 40 kHz increase. This suggests that the role of the septum is to minimize the magnitude of the impedance peaks in the 10-40 kHz region, which may otherwise interfere with cat localization cues (Puria 1991, PhD Thesis). Finally, the 3D pressure distribution allows us to understand both qualitatively and quantitatively the effects of anatomical variations on pressure differences between oval and round windows. For example, in the normal MEC, the oval and round window pressures are the same for frequencies under 1 kHz, but as expected show significant differences above this range.

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598 Dynamic Analysis of the Middle Ear

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Investigations of the middle ear function can be divided into static and dynamic investigations. For sound stimuli which vary dynamically in the frequency range between 100 and 20000 Hz dynamic analysis methods should provide the most useful information to understand the vibrational behaviour of the middle ear.

Therefore, dynamic analysis of two components of the middle ear was performed.

Firstly, the tympanic membrane vibration patterns were investigated via a modal parameter analysis using a set of measurements performed on fresh human temporal bones. From a theoretical point of view a vibrating structure is completely characterized by its modal parameters (natural frequencies, modal damping factor and vibration mode shapes). Secondly, the dynamic behavior of the isolated middle ear ossicles (from the same fresh human temporal bones) was investigated in order to determine their contribution to the vibration of the whole ossicular chain.

The results show that the TM vibration pattern is complex and composed of more resonance frequencies heavily damped. Because of this high damping factor satisfactory modal analysis would be performed only up to 5 kHz. The modal analysis performed on the isolated middle ear ossicles showed that they act as a rigid body in the audible frequency range.

These results will be used to fit a Finite Element Model of the middle ear. Comparison of the resonance frequencies and modeshape (measured and simulated) will help in estimating correct material parameter for the tympanic membrane. Knowing that the isolated ossicles behaves as a rigid body also help in estimating their material parameters.

The Finite Element Model could then be used to analyse different kind of middle prosthesis

599 Characterizing Cochlear Mechano-Electric Transduction (MET) with a Nonlinear System Identification Technique: the Influence of the Middle Ear

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Previously, with a nonlinear system identification technique (NLSI) applied to the cochlear microphonic (CM), MET was characterized by providing a third-order polynomial equation relating ear canal pressure to the CM (Chertoff et al., 1996, JASA 100(6)). The equation explained 80 to 90% of MET. One possible reason for the limited coherence is that the NLSI procedure assumed a nonlinear system followed by a linear system. Anatomically, however, the middle ear intervenes between the ear canal and the CM and the system should be considered a linear system followed by a nonlinear system. The purpose of this study was to determine the influence of the middle ear on the fit of the polynomial model of MET.

Gaussian noise (0.1 kHz to 10 kHz) was presented at 78, 88, and 98 dB SPL to ten gerbils. The acoustic signal (AC) was measured near the tympanic membrane with a probe microphone (ER7C). The CM was recorded from an electrode placed on the round window. The stapes velocity (ST) was measured with a laser (Polytec HLV 1000) focused on the stapes footplate. Twenty different noise signals were delivered and their responses recorded. Transfer and coherence functions relating ST to AC (ST/AC) and CM to ST (CM/ST) were computed.

The ST/AC transfer function was independent of signal level and the coherence was close to 1.0 indicating linearity of the middle ear. The CM/ST transfer function was dependent on signal level and the coherence did not improve from our previous study. This suggests that the middle ear was not the reason for the limited description of MET by the third-order polynomial equation. Other nonlinear functions may be necessary to improve the functional description of MET.

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600 Localization of Myosin XVA in Hair Cells

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Mutations of myosin XVA gene cause recessive, non-syndromic congenital sensorineural deafness (*DFNB3*) in humans. In mice, myosin XVA mutations cause deafness and vestibular disorders producing the shaker 2 phenotype which is characterized by abnormal development of stereocilia. Full-length myosin XVA transcripts contain 66 exons, and encodes ~ 390 kDa protein that is unique among myosins in possessing a very long ~1200 amino acid N-terminal extension preceding the motor domain. The tail region of myosin XVA contains two MyTH4, an SH3, and two regions with similarity to the membrane attachment FERM domain. There are several splice variants of myosin XVA, which may have different functions and different cellular or subcellular localizations. In order to extend our previous results on the localization of myosin XVA in the sensory hair cells of the inner ear (Anderson at al., 2000), we have now generated new affinity purified antibodies raised against fusion proteins or peptides corresponding to the different domains of this protein. Using confocal immunofluorescence microscopy we studied the localization of myosin XVA in whole mount preparations of mouse organ of Corti. Six antibodies raised against the N-terminus region and two raised against the FERM-like domain labeled a plaque-like structure at the synaptic region of the outer hair cells (OHCs). Three antibodies raised against peptides corresponding to the region between the MyTH4 domain and the first FERM-like domain labeled the stereocilia of the inner and OHCs. Another antibody targeting this same region labeled both the stereocilia and the plaque-like structure at the synaptic region of the OHCs. Our results indicate that there are at least two distinct localizations and possibly two different functions for myosin XVA in hair cells. Myosin XVA reactivity in the stereocilia confirms its previously proposed essential role in development and elongation of stereocilia during the maturation of the organ of Corti. Another function of myosin XVA may be related to the synaptic structure and function in the OHCs. It remains unclear whether the targeting to opposite ends of the hair cell is based on splice variants of myosin XVA.

601 Twisted Hair Bundles Depend on ATPase Activity

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Hair bundle mechanoreceptors similar in structure and function to those in mammalian ears occur on the surface of sea anemone tentacles. Anemone hair bundles are dynamically tuned to detect swimming

movements of prey through an elegant regulation by chemoreceptors sensitive to prey derived compounds. N-acetylated sugars, which diffuse into the seawater from prey surfaces, activate chemoreceptors that induce actin polymerization and elongation of hair bundles. We previously found that during tuning, elongating hair bundles change from a twisted state to an untwisted state. We now find that treatment with vanadate, a phosphate analog that inhibits ATPases, causes control hair bundles to untwist and elongate. The vanadate-treated hair bundles are similar in morphology to sugar-treated hair bundles except that the base widths decrease with vanadate. However, in contrast to sugar-mediated tuning, vanadate renders hair bundles unresponsive based on electrophysiology and whole animal assays. These effects are observed at 0.5 mM vanadate, a concentration typical for inhibition of myosin ATPase activity. Based on immunological evidence, myosin VIIA is located in anemone hair bundles. With sugar-mediated tuning, myosin VIIA shifts from a base to tip location within the hair bundle. We propose that myosin VIIA may actively regulate morphodynamics associated with sugar-mediated tuning. Somewhat surprisingly, these results also suggest that maintaining control hair bundles in a twisted state requires energy expenditure.

602 Dihydrostreptomycin is a Permeant Blocker of the Outer Hair Cell Transducer Channel.

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Hair cells are sensitive to damage by aminoglycoside antibiotics such as dihydrostreptomycin (DHS). Protracted exposure leads to intracellular accumulation of the drug and subsequent cell deterioration. Aminoglycosides are large polycationic molecules able to block the hair cells' transducer channels (Kroese et al., 1989). The possibility that DHS enters into the cells via the transducer channel was investigated.

Transducer currents were recorded from outer hair cells (OHCs) in acutely isolated organs of Corti of CD-1 mice (ages P5-P7). When applied extracellularly, DHS rapidly and reversibly blocked the currents in a dose- and voltage-dependent manner with positive membrane potentials releasing the block. The exponential relaxation of the currents in the presence of the drug indicates open-channel block. The half-blocking concentration at -84 mV was 8 μM and the Hill coefficient was close to 1, suggesting that one drug molecule blocks the transducer channel. The block was strongly dependent on extracellular Ca, being most effective when Ca was reduced. These effects are consistent with a drug binding site within the channel pore. In 1.3 mM Ca the block was partially removed when potentials negative to -100 mV were applied, suggesting the drug molecules dissociate from their binding site and move inside the cells.

Our findings are consistent with DHS entering OHCs through the transducer channels by acting as a permeant blocker. Sufficient electrical driving force must be generated across the membrane in order to "punch through" the drug molecules toward the cytoplasm. The minimal diameter of DHS (about 1 nm) suggests the transducer channel pore is larger than the 0.7 nm previously suggested (Howard et al, 1988).

Howard et al (1988) Ann Rev Biophys Biophys Chem 17:99-124.

Kroese et al (1989) Hear Res 37:203-218.

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603 Unconventional Myosins in Vertebrate Hair Cells

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Mechanosensory hair cells are the primary auditory and vestibular receptors in all vertebrate inner ears. While these cells exhibit some divergence in form such as cochlear inner and outer hair cells, the many similarities among all hair cells suggest recent common ancestry. These similarities include ciliary bundle structure and adaptation responses. Recent mutant screens and molecular studies have determined that some unconventional myosins are critical hair cell proteins. Myosins VI, VIIa, and XV appear to be involved in ciliary bundle development and/or maintenance, while myosin Iβ may play a role in adaptation. This leads to the hypothesis that all of these myosins are ubiquitous in all vertebrate inner ears and were inherited from an ancestral mechanosensory or epithelial cell. This study examines the distribution of myosins VI and VIIa within the inner ears of fishes as a first step in tracing the evolution of vertebrate inner ear hair cells. Species from three of the four major vertebrate radiations (Agnatha, Chondrichthyes, and Osteichthyes) were chosen to provide a diverse representation of vertebrate taxa. Indirect immunofluorescense using polyclonal antibodies to these two proteins reveals that both myosins are present in all three of these taxa, as they have been found in all vertebrate hair cells studied to date. This finding strengthens the hypothesis of vertebrate hair cell homology. However, the distribution of myosin VI within hair cells differs between vertebrate taxa. These differences may be used to study hair cell divergence from the common sensory ancestor, and to indicate the direction of hair cell evolution. In addition to providing these evolutionary insights, these findings should be useful to researchers studying unconventional myosins in hair cells, as they open up a wider selection of species for study.

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604 Calcium channels in turtle auditory hair cells

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Turtle auditory hair cell calcium channels regulate both membrane excitability and synaptic transmission. Coupled with BK potassium channels, calcium channels establish electrical resonance and coupled to synaptic release proteins dictate vesicle fusion. The purpose of the present investigation was to characterize the biophysical and pharmacological properties of hair cell calcium channels to determine if multiple channel types exist and if channels linked to either function behave differently. Whole-cell recordings were obtained from hair cells of the intact auditory papilla. Calcium currents were isolated with a cesium based intracellular solution and 100nm Apamin in the external solution to block the SK calcium-activated potassium channels. Current amplitudes showed a marked run-up upon obtaining the whole-cell configuration, increasing several fold over the first ten minutes. No noticeable shift in the current-voltage relationship was found during run-up. Most cells showed an inactivating component that was typically about 20% of the current during a 20ms depolarization. Several kinetic components of inactivation were found. Maximal inactivation occurred near 20mV, the potential where current is maximal suggesting calcium induced inactivation. Nimodipine was the most effective blocker of this current, 5μM blocking about 80% from a holding potential of -80mV. Activation curves for currents both sensitive and insensitive to blocker were identical. ω-conotoxin GVIA was also effective at blocking this current, 1μM blocking about 30% of the total current. Here too no difference in activation properties were observed for sensitive and resistance components of the current. Data suggests the predominant channel is of the L-type, perhaps α1D as suggested for chick auditory papilla (Kollmar et al.,1997).

605 Tonotopic Variations in the Kinetics and Calcium Sensitivity of Large-Conductance, Calcium-Activated Potassium Channels in Chick Cochlear Hair Cells

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Models of electrical tuning in hair cells of turtles, frogs, and chicks require tonotopic variation in the biophysical properties of large-conductance, calcium-activated potassium (BK) channels. Expression of cloned BK channels suggests that this diversity can arise from

alternative splicing of the gene encoding those channels and by co-expression with an accessory beta subunit. To support these ideas, we surveyed hair cell BK channel properties along the tonotopic axis of the chick's cochlea using inside-out patch-clamp recordings from patches with few channels (1 to 8). Multiple patches from restricted regions along the cochlea (0.1 mm) revealed wide ranges in calcium sensitivity, up to 20-fold. This range is attributed partially to the presence or absence of accessory subunits.

The resonant frequency of the hair cell from which the patch was taken was estimated on the basis of the deactivation time constant for channels in that patch. The estimated resonant frequency approximated the cell's characteristic frequency predicted by its tonotopic location, particularly for patches with a higher number of channels. Patches with few channels deviated further from the predicted tonotopic frequency, presumably reflecting the wide single channel heterogeneity in hair cells. Finally, the kinetics and calcium sensitivities in more than 20 patches with limited numbers of channels were compared to see if distinct populations of channel configurations emerged. There were no obvious groupings from our data set, suggesting a wide diversity in the variants, possibly employing multiple molecular mechanisms.

606 Immunological Characterization of a Potassium Channel Beta Subunit

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Voltage-gated potassium channels can show various degrees of inactivation upon opening. Among these channels is the A-type, which inactivates within a few milliseconds of activation and is found in the short hair cells of the chick cochlea. The rapid inactivation observed in A-channels can be regulated by either the N or C-terminal domains of the alpha subunit, which contains the voltage-gated pore. Additionally, these channels can be inactivated by an accessory beta subunit. Previously, using a cDNA library of the chick cochlea, we cloned a member of the beta subfamily known as cKvβ1.1 [Rajeevan et al., (1999) Mol. Br. Res., 66, 83-93]. We raised a rabbit polyclonal antibody to cKvβ1.1, using a synthetic peptide, corresponding to amino acids 12-29. This peptide sequence is unique to cKvβ1.1 and shares a 67% identity with human and rat Kvβ1.1. With this antibody, we have localized beta as well as determined potential interacting alpha subunits. Immunostaining of the adult chick cochlea shows that cKvβ1.1 is found in the cuticular plate of hair cells, nerve terminals synapsing at the base of hair cells, VIIIth nerve fibers, and the plasmalemma and nuclear membrane of ganglion cell bodies. Additionally, using tissue from the adult chick brain, we found that cKvβ1.1 is expressed in cytosol, microsome, and membrane fractions. The result that cKvβ1.1 is found in the cytosol suggests cKvβ1.1 may interact with non-membrane-bound proteins. Also, we have begun immunoprecipitation experiments using chick brain tissue to determine which alpha subunits may interact with beta. These studies show that the anti-cKvβ1.1 antibody brings down members of the Shaker subfamily, including Kvα1.2, and 1.4. We will discuss these results as well as immunoprecipitation studies using cochlear tissues.

607 Expression of OTRPC4 in the Rat Inner Ear

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OTRPC4 is a new mammalian nonselective ion channel that is responsive to changes in extracellular osmolarity in a physiologically-relevant range, suggesting that it is involved in cellular osmoregulation. OTRPC4 has been identified in kidney, heart and liver (Strotmann et al, Nature Cell Biol, 2000, 2, 695-702). In the mammalian inner ear, a related receptor for osmoregulatuon, the vanilloid receptor-related osmotically activated channel (VR-OAC) has also been found (Liedtke et al. Cell, 2000, 103, 525-535). We investigated the immunocytochemical distribution of OTRPC4 in adult rat inner ear to determine if this protein is potentially involved in cochlear osmoregulation.

Formaldehyde-fixed rat cochleae and kidneys were immunolabeled with a rabbit anti-OTRPC4 antibody, and localized using Alexa-488-conjugated secondary antibodies. Specimens were observed using confocal microscopy techniques.

Positive OTRPC4-labeling was identified in the rat kidney and cochlea. In the kidney label was found at the lining of the tubules. In the cochlea labeling was observed in the endothelial cells of blood vessels within the stria vascularis and in the cell bodies of the outer and inner hair cells, and in supporting cells (Hensen's cells, interdental cells) of the organ of Corti.

This distribution pattern in the rat cochlea suggests that OTRPC4 might act as an osmotic sensor of the fluid homeostasis of the stria vascularis, the hair cells and certain supporting cells of the organ of Corti.

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608 Calcium homeostasis in cochlear inner hair cells

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The inner hair cell (IHC) is the primary sensory cell of the cochlea exciting the auditory nerve in response to incoming sound. Mechanoelectrical transduction causes Ca^{2+} influx into the stereocilia and results in activation of voltage-gated Ca^{2+} influx by depolarizing IHCs. Sound-stimulation can reach high intensities and last for long periods of time. The resulting Ca^{2+}-influx causes a substantial intracellular Ca^{2+}-load, which has to be cleared by the IHCs. Various mechanisms could be implicated in the Ca^{2+}-homeostasis and extrusion: Ca^{2+} pumps including plasma-membrane Ca^{2+} -ATPase and/or sarco-endoplasmatic pumps, Na^{+}/Ca^{2+} exchanger as well as the mitochondrial uniporter. Here we performed fura-2 recordings of spatially averaged $[Ca^{2+}]_i$ on patch-clamped IHCs from the mouse to study their Ca^{2+} homeostasis and the relevant extrusion mechanisms. The IHC displays a very efficient Ca^{2+} clearance setting the resting $[Ca^{2+}]_i$ to 29 ± 9.6 nM (n=13 cells) and removing Ca^{2+} even after strong stimulation. The decay of $[Ca^{2+}]_i$ following Ca^{2+} influx showed a single exponential time course for $[Ca^{2+}]_i$ transients with peak amplitudes lower than 400-500 nM but double exponential kinetic for bigger $[Ca^{2+}]_i$ signals.Plasmamembrane Ca^{2+}-ATPases and Na^{+}/Ca^{2+} exchanger play an important role in the extrusion of Ca^{2+} from IHCs into the extracellular space. Mitochondria shape the Ca^{2+} signal by rapidly sequestering Ca^{2+} from the cytosol and probably releasing Ca^{2+} later on. Sarco-endoplasmatic pumps did not significantly contribute to the Ca^{2+}-clearance in our experiments*Supported by grants of the DFG and the MPG to T.M.*

609 Dynamic Expression of Voltage-Dependent Ca^{2+} Channel γ Subunits in Rat Cochlear Hair Cells

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Voltage-dependent Ca^{2+} channels (VDCCs) are expressed in the neuronal and neurosensory epithelium of the inner ear. The VDCCs exist as a heteromultimeric complex consisting of α1, α2δ, β, and γ subunits. Multiple α1 and β genes are expressed in the cochlea with α1D being the predominant hair cell isoform. At least eight γ subunit genes, Cacng1-8, have been identified and have a wide range of tissue

expression. Our goal was to characterize the γ subunit expression pattern in cochlear hair cells. RT-PCR experiments were done using gene-specific oligo primers to amplify isoform-specific cDNA fragments from rat cochlea, inner hair cell (IHC), outer hair cell (OHC), and vestibular type-I HC λZAP unidirectional cDNA libraries and to characterize these products by Southern blot analyses and direct sequencing. Of the eight Cacng isoforms γ2, γ4, γ5, and γ6 were present in the cochlear library, while γ2 and γ4 were detected in only the OHC library. Whole mount *in situ* hybridization using transcript-specific riboprobes were done on P0, P8, and P21 cochleae. Both γ2 and γ4 had similar patterns of expression with overlapping reciprocal longitudinal gradients being present in IHC and OHCs. The highest levels of γ2 and γ4 were in IHCs in the base. γ6 had a partially overlapping reciprocal pattern, where OHC expression was in the middle of the cochlea, while IHC γ6 had the lowest levels in middle turn. OHCs weakly expressed γ5 in the upper half of the cochlea except for the apical tip, while IHCs were positive in the lower half except in the hook region. *In vitro* co-expression analyses of Cacng with α1, α2δ, and β subunits showed γ can cause channel inactivation to increase in rate and to shift to more negative potentials. These data suggest that variation in HC γ expression may be reflected by changes in VDCC electrophysiological properties along the length of the cochlea.

610 Different kinds of outward rectifying K^+ currents in Deiters' cells

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Deiters' cells are the supporting cells in organ of Corti and are suggested as playing an important role in mechanical modulation of outer hair cells. Recently, very large voltage activated, outward rectifying potassium currents were found in these cells and the presence of more than one type of K+ selective channel was suggested. In this study, we tried to isolate the types of K+ currents using K+ channel blockers such as class III anti-arrhythmic K+ channel blocker clofilium and tetraethyl ammonium (TEA) and showed their electrical characteristics. We isolated Deiters' cells from the organ of Corti of guinea pig. To record K^+-current, whole cell patch clamp technique was applied. With high K+ pipette solution, the outward currents were activated by depolarizing step pulses. Clofilium blocked this outward rectifying K+ currents in a dose-dependent manner. At a concentration of 30 μM of clofilium, the residual current showed clear transient outward current (ITO) and was completely different from clofilium-sensitive outward current. Clofilium-sensitive outward current looked like fast activating delayed outward rectifying current. The addition of 5 mM TEA further blocked the transient outward K+ current remaining a very fast inactivating transient outward current. Therefore we could isolate at least three different kinds of K+ current in Deiters' cells, such as fast activating delayed outward current, transient outward current and very fast inactivating K+ current. The inactivation time constants of each current are 505, 27.6 and 5.6 msec, respectively. The range of the reported inactivation time constant of ITO is between 25-50 msec and the very fast inactivating transient outward current has not been reported. In conclusion, Deiters' cells contain at least three different kinds of outward rectifying K+ current. The physiological roles of these outward rectifying K+ currents are to be established.

611 The α9α10 nAChR is permeable to and modulated by Ca^{2+}, Ba^{2+} and Mg^{2+}

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The α9 nAChR subunit is a main component of the cholinergic receptor present at the base of outer hair cells (OHC). Some biophysical features of the α9 nAChRs do not match those seen in isolated OHC. Co-expression of the α9 with the α10 subunit yields a heteromeric receptor with pharmacological and biophysical characteristics closely resembling those of the native OHC receptor. Divalent cation permeability and modulation of the α9α10 receptor were studied by the two-electrode voltage-clamp technique in X. laevis oocytes injected with α9 and α10 cRNA. The relative divalent to monovalent cation permeability was ~10 for Ca^{2+}, Ba^{2+} and Mg^{2+}. Acetylcholine (ACh)-evoked currents were potentiated by either Ca^{2+} or Ba^{2+} up to 500 μM but were blocked by higher concentrations of these cations. Voltage-ramps (-120 to +50 mV) performed at different Ca^{2+} concentrations showed that potentiation by Ca^{2+} is voltage-independent whereas blockage is stronger at hyperpolarized potentials. In the absence of Ca^{2+}, the EC_{50} for ACh was higher (48 μM) than with 1.8 mM Ca^{2+} (14.3 μM), suggesting that potentiation by Ca^{2+}involves changes in the affinity of ACh for this receptor. Currents were almost undetectable when Ca^{2+} was substituted for Mg^{2+}. However, in the presence of 0.5 Ca^{2+}, Mg^{2+} blocked the ACh-evoked currents (IC_{50}= 0.38 mM). Three major functional characteristics of the α9α10 nAChR can be inferred: it is highly permeable to Ca^{2+}, Ba^{2+} and Mg^{2+}. It is potentiated and blocked, through different mechanisms, by external Ca^{2+} in the physiological range. Both Ba^{2+} and Mg^{2+} block this receptor, whereas only Ba^{2+} substitutes for Ca^{2+} in its potentiating effect.

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612 Inward-rectifier K+ Channel (Kir 7.1) Expression in OHC-IHC Subtracted Library

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The outer hair cell (OHC) is one of two sensory cells in the mammalian inner ear. This special neuroepithelial cell has unique function and electrical characteristics. Potassium current has an important role in controlling the OHC transmembrane potential. At least two K+ conductances have been reported in OHC: (1). Voltage-gated K+ outward current named IK,n; its channel possibly encoded by the KCNQ4 gene. The mutation of KCNQ4 causes nonsyndromic dominant deafness DFNA2 (Kubisch et al., 1999). (2). Outwardly rectifying K+ current appears similar to the calcium-activated K+ current (Housley and Ashmore, 1992). No inward rectifier K current has been reported in OHCs. It has been shown that an inward rectifier K current does exist in chicken hair cells (Pantelias et al 2001). It can help set the resting membrane potential in some hair cells, and has been shown to play a role in frequency tuning in the turtle ear (Goodman & Art, 1996). In order to identify genes that are associated with OHC function, we created an OHC-IHC subtracted plasmid library using a combination of suppression subtractive hybridization and differential screening strategies. We have identified an inward-rectifier K+ channel (Kir 7.1) clone from the OHC-IHC subtracted library. The inward-rectifier K+ channel is a relatively new family of ion channels. Kir 7.1 was first identified from brain (Krapivinsky et al., 1998). It is also expressed in multiple tissues including thyroid intestinal epithelial cells, intestinal epithelial cells, kidney and retinal pigment epithelium. In many of these cases, Kir 7.1 is located in the basolateral membrane of the cells. It has been suggested that it provides a pathway for recycling of K+ and contributes to ion homeostasis. The function of Kir 7.1 in the

mammalian inner ear remains unknown. We are now identifying Kir 7.1 expression pattern and function in the cochlea.

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613 Effect of intra- and extracellular ATP on gap junctional coupling of Hensen-cells in guinea pigs organ of corti

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It is widely accepted that supporting cells in the mammalian organ of Corti are responsible for recovery and recycling of endolymphatic potassium ions „used and released“ by the sensory cells for mechanosensory transduction. Thus Hensen-cells, which show strong gap junctional coupling, play an essential role for the maintenance of the endolymphatic potential and of cochlear ionic homeostasis.

In order to evaluate the physiological mechanisms and the corresponding signal transduction pathways which influence the gap junctional coupling of Hensen-cells we applied the double-whole patch-clamp technique. The work was focused to the effect of ATP containing bath and pipette solutions on gap junctional coupling. We present data which show that in the absence of ATP in the pipette solution spontaneous gap junctional uncoupling occurs. Addition of millimolar concentration of ATP was found to be sufficient for stable gap junctional coupling for recording times up to 60 min. It is proposed that gap junctional coupling depends on intracellular phosphorylation mechanisms. Whereas, as reported earlier, extracellular ATP inhibits gap junctional coupling in a dose dependent manner. In the latter case it appears to be likely that intracellular chloride is not involved, but an increase of cytoplasmic free calcium concentration.

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614 Glutamate Transporters in the Guinea-Pig Cochlea: Partial mRNA Sequences, Cellular Expression and Functional Implications

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In the cochlea, the glutamate (Glu) is the neurotransmitter between the inner hair cell (IHC) and the primary auditory neurons. In order to prevent excitotoxic mechanisms, extracellular Glu concentration must be maintained below a certain level. The external Glu concentration is regulated by excitatory amino acid transporters (EAAT), plasma membrane proteins which remove Glu from the synaptic cleft and neighboring synapses.

The present study was design to study, the expression and the activity of three EAATs in the Guinea-pig cochlea: GLAST, GLT-1 and EAAC1. A partial mRNA sequence was determined for each of these transporters by a degenerated primed PCR performed on guinea-pig brain cDNA. A PCR screening was then done on a guinea-pig dissected organ Corti cDNA library. The cellular expression and distribution of these transporters was examined by confocal microscopy. Consequences of the inhibition of glutamate uptake were evaluated by recording cochlear potentials during intracochlear perfusion of L-trans-pyrrolidine-2,4-dicarboxylic acid (PDC) and subsequent examination of the cochleas by electron microscopy.

Our results show that only GLAST mRNA is detected in the organ of Corti. Confocal microscopy reveals that GLAST is localized in the IHCs' supporting cells and in the ganglion satellite cells. Nevertheless, GLT-1 and EAAC1 are localized in the neurons of the ganglion of Corti and in the spiral lamina. Treatment with PDC (0.01-10 mM) results in a dose dependent reduction of the amplitude of the cochlear compound action potential, leaving the cochear microphonic and the summating potentials unaffected. Ultrastructual examination of these cochleas showed a swelling of afferent endings typically seen after excitotoxicity.

615 Immunohistochemical localization of voltage-gated potassium channel Kv3.4 subunit in the mammalian inner ear

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It is generally accepted that the gap junction networks in the cochlea form the route by which potassium ions can be recycled back to the endolymph (Kikuchi et al., Brain Res. Rev. 32: 163-166, 2000; Kikuchi et al., Medical Electron Microscopy 33: 51-56, 2000). Detailed information about the expression of various types of potassium channels in the inner ear can provide an important information for the better understanding of the potasium ion recycling mechanism in the cochlea. In our preceding study, we showed the expression of a voltage-gated potassium channel Kv3.1b subunit in the type I, III, IV and suprastrial fibrocytes in the cochlear lateral wall (So et al., NeuroReport 12: 2761-2765, 2001)

In the present study, we have shown the immunohistochemical localization of a voltage-gated potassium channel Kv3.4 subunit in the guinea pig cochlea. Intense Kv3.4-like immunoreactivity was observed in the type I and suprastrial fibrocytes in the spiral ligament and the basal cells in the stria vascularis. In the spiral limbus, Kv3.4-like immunoreactivity was also present in interdental cells and the underlying connective tissue cells. In the organ of Corti, immunostaining was found in the sensory cells and the neighboring supporting cells. The results obtained in the present study suggest that the voltage-gated potassium channel, containing Kv3.4 subunit, may play some important roles in regulating the flow of potassium ions in the inner ear.

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616 The BK channel in spiral ligament fibrocytes is regulated by phosphorylation

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BK channel has been identified in the spiral ligament fibrocytes and the physiological role of this channel has been linked to maintain the membrane potential in these cells (Shen et al., 2002), which is important to form the electrochemical gradient for recycling K+ from perilymph to endolymph. In this study, regulatory effects of phosphorylation on BK channel were investigated.

The activity of the BK channel was continuously monitored from inside-out patches. With Na-rich pipette solution and K–rich ATP-free bath, a significant increase of the open probability (NPo) of BK channel was observed. NPo was gradually increased from 0.021 ± 0.005 to 0.26 ± 0.08 (n=10) within 10 minutes (holding at 0 mV) and held steady thereafter. Adding 1 mM ATP to the bath solution significantly reduced the NPo from 0.276 ± 0.15 to 0.031 ± 0.02 (n=5) and it was reversible. The up-regulation of BK channel was also seen at whole cell level. An average increase of 3.9 ±1.2 times of its original whole cell current value was observed within 14 minutes (n=7). The effect was completely prevented by adding 1 mM ATP in the pipette solution (n=3).

To investigate if the effect of cytosolic ATP is mediated by phosphorylation of the channel protein, cells were pre-incubated with 20 nM calyculin A (a non-specific inhibitor for protein phosphortase)

for 20 minutes. The BK channel failed to show up-regulation in single channel recordings when the inside-out patches faced ATP-free bath. NPo was extremely low even at strong depolarization of 100 mV, from 0.001 to 0.005 (n=3). Raising cytosolic [Ca^{2+}] from 1 μM to 1 mM only partially restore the activity of the channel.

The data demonstrated that the BK channel of spiral ligament fibrocytes is reversibly modulated by protein phosphorylation. This process greatly affects the calcium and voltage dependence property of the channel, thus affecting membrane property of these cells.

617 Intracellular Sodium Increases During Recovery of Isolated Goldfish Hair Cells from an Acid Load

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In goldfish hair cells, pH regulation via Na^+/H^+ exchange accounts for about half of the total resting sodium influx normally balanced by Na^+/K^+-ATPase. We examined the influence of these processes on intracellular Na^+ concentration during the stress of recovery from an acid load.

We measured intracellular Na^+ concentration in isolated goldfish hair cells with the ratiometric fluorescent sodium ion indicator SBFI. In 5 cells, the control Na^+ concentration was 13±5 mM. During reversible inhibition of the Na^+/K^+ pump, the initial rate of increase of intracellular Na^+ was 11±5 mM/min, providing an estimate of the steady-state Na^+ influx normally balanced by the Na^+/K^+ pump. Following recovery from the pump inhibition, acid loads of about 0.5 pH units were imposed on these cells by washout of 10 mM NH_4Cl pre-pulsed for 5-10 minutes. During recovery from the acid load, Na^+ concentration increased at an initial rate of 15±12mM/min and eventually returned to control values (18±8 mM).

Based on estimates of pH buffering capacity and pH responses to acid loads (measured using the dye BCECF), the recovery of pH is associated with an initial 50% increase of Na^+ influx over its steady-state value. The rate of Na increase measured during recovery from an acid load is close to the rate of change expected if the Na^+/K^+ pump were completely inhibited while Na^+/H^+ exchange was stimulated.

Intracellular acidification thus might inhibit the hair-cell Na^+/K^+ pump, as occurs in other cell types, in addition to increasing the rate of Na^+/H^+ exchange. The functional result is a temporary tradeoff of Na^+ regulation in favor of pH regulation.

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618 Expression of H1 and H2 receptors mRNAs in the Cochlea

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It is reported that histamine has a function as a neuromodulator within the vestibule. However, the function of histamine within the cochlea has not been investigated in detail and it remains unclear if histamine has a cochlear function. Previously, we reported that low concentrations of histamine increase the CAP (Compound Action Potential) amplitude without a change of CM (Cochlea Microphonic Potentional) amplitude by perilymphatic cochlear perfusion. In this study, we will investigate the presence and the distribution of H1 and H2 receptor mRNAs in the cochlea using RT-PCR and in situ hybridization.

Materials & Methods; Male Wistar strain rats at 4 weeks of age were used for all experiments. The cochleas were removed, and immersed and dissected in phosphate buffered saline (PBS). The organ of Corti, stria vascularis, and spiral ligament were immediately isolated from the cochlea. Total RNA was extracted by guanidine isothiocyanate method and total RNAs were treated with DNAase. After phenol extraction and ethanol precipitation, the cDNAs were synthesized in reverse transcriptase. Primers to H1 and H2 receptors were designed from full sequence data, and PCR was performed.

Results; In the spiral ligament, H1 and H2 receptor mRNAs were strongly expressed. Additionally, in the stria vascularis H1 histamine receptor mRNAs were weakly expressed. In the organ of Corti, H2 histamine receptor mRNAs were also weakly expressed.

Discussion; Our results suggest that histamine receptor mRNAs exist within the cochlea. Furthermore, considering our previous electrophysiological data, it seems that histamine has a function as a neuromodulator for afferent nerves in the auditory system. We will also present results of in situ hybridization at the meeting.

619 Permeation of FM1-43 and Styryl Dyes Through Multiple Sensory Channels

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Hair cells quickly internalize a number of styryl pyridinium dyes such as FM1-43. The rapid entry and the progression of dye loading from the stereocilia to the cell soma suggested that FM1-43 entered via the transduction channels. Consistent with this hypothesis, focal application of FM1-43 at the distal tips of stereocilia resulted in dye entry, while basolateral application did not. Mechanical gating of transduction channels with a fluid stream resulted in FM1-43 internalization in positively deflected cells, but not in cells deflected negatively. FM1-43 internalization was inhibited by transduction channel blockers, by depolarization, and by agents that disrupt the mechanical gating, but not by micromolar extracellular calcium. This evidence supports the hypothesis that FM1-43 permeates through the transduction channel.

FM1-43 also labeled cells transfected with the vanilloid (VR-1) or purinergic (P2X2) receptors when these channels were opened with their agonists and loading was blocked with specific antagonists. To determine whether cell types other than hair cells would load under physiological conditions, mice were injected with FM1-43 and examined after 1-15 days. FM1-43 labeled mechanosensory structures such as hair cells, Merkel cells, and muscle spindles, as well as other sensory structures including taste buds, DRG neurons, and cranial sensory ganglia. Labeling of neuronal cell bodies was blocked by nerve ligation at the distal end, indicating that FM1-43 entered the neurons through distal sensory channels.

The ability of a large organic molecule to pass through non-selective cation channels, including the hair cell transduction channel, unidentified channels in other sensory cells, P2X2, and VR-1 suggests similarity in the pore domains. FM1-43 permeation also enables rapid assay of sensory channel function, screening for mutations in transduction, and visualization of various sensory cell types in vivo.

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620 Contribution of BK-like Ca^{2+}-activated K+ channels to mammalian cochlear neurotransmission

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Large conductance, calcium-activated potassium (BK) channels are known to play a prominent role in hair cell function of lower vertebrate where these channels determine electrical tuning and regulation of neurotransmitter release. Very little is known, by contrast, about the role of BK channels in the mammalian cochlea. In the present study, we

perfused various specific toxins in the guinea pig cochlea to characterize the role of BK channels in cochlear neurotransmission. Intracochlear perfusion of charybdotoxin (ChTX) at 2 or 5 μM (n = 3) reversibly suppressed within 3-5 min the compound action potential (CAP) of the auditory nerve, evoked by a 8 kHz tone burst. The perfusion of iberiotoxin (IbTX), a more specific blocker of BK channels, at 0.5 to 5 μM (n = 5) also largely reduced within minutes in a dose-dependent manner the amplitude of CAP. The block was reversible within 45 min of rinse with saline. The cochlear microphonic (CM) during the toxin perfusion did not decrease but, on the contrary, slightly increased by about 2-4.5 dB. The perfusion of apamin (5 μM; n = 5), a toxin specific to SK channels, did not significantly affect CAP or CM. We also tested the effects of these toxins on the whole-cell voltage-dependent membrane current of isolated guinea pig inner hair cells (IHCs). Apamin (up to 5 μM) did not show any effect. ChTX and IbTX (0.5 μM; n = 5) reversibly blocked a fast outward current (activating above -35 mV, peaking at 0 mV with a mean activation time constant t < 5 ms). A similar block of a fast outward current was also observed with the extracellular application of 1 to 4 mM barium ions (n = 5) which we believe permeate through Ca^{2+} channels and block BK channels. Overall, our results clearly revealed the importance of BK channels in mammalian cochlear neurotransmssion and that at the presynaptic level, fast BK-like channels are a significant component of the repolarizing current of IHCs.

621 The dependence of exocytosis at the afferent hair cell synapse on Ca^{2+}-influx during modulation by L-type Ca^{2+}-channel block

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Ca^{2+}-dependent exocytosis of glutamate mediates synaptic transmission at the inner haircell (IHC) afferent synapse. Using patch-clamp membrane capacitance measurements we previously demonstrated a steep Ca^{2+}-dependence of the underlying fusion of readily releasable vesicles and showed that L-type Ca^{2+}-channels are involved in stimulus-secretion coupling (Beutner et al., 2001, Neuron 29:681-690; Moser and Beutner, 2000, PNAS, 97, 883-888). However, nifedipine (10μM), a dihydropyridine (DHP) inhibitor of L-type Ca^{2+}-channels, blocked both Ca^{2+}-current and exocytosis to the same degree (~50%), which was not expected given the high power dependence of exocytosis on Ca^{2+}. The incomplete, linear block by the DHP prompted us to further investigate the dependence of the fusion of the readily releasable vesicle pool (RRP) on L-type Ca^{2+}-influx. IHCs were stimulated by short (20ms) depolarizations, which selectively recruit the RRP, in the increasing presence (up to 10 μM) of nifedipine or isradipine. Thereby, we modified the Ca^{2+}-influx over a range of ~80% of control. In support of our previous result inhibition of exocytosis related linearly to the reduction of available L-type Ca^{2+}-channels. This finding demonstrates that at least 80% of the IHC Ca^{2+}-channels are of L-type, which is in agreement with the conclusions from experiments on IHCs lacking the alpha1D-subunit of Ca^{2+}-channels (Platzer et al., 2000 Cell 102, 89-97). Our results show that influx through non-L-type channels maximally contributes linearly to release of the RRP, if it contributes at all. Moreover, the linear relationship of Ca^{2+}- channel block and exocytosis suggests that the Ca^{2+}- concentration at each release site is controlled by very few L-type channels, maybe a single Ca^{2+}- channel.

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622 Prestin Expression in The Reeler Mouse

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The electromotility of outer hair cell (OHC) has been hypothesized to be the foundation for mammalian cochlear sensitivity and frequency selectivity. A generally accepted theory is that motor proteins in the OHC's basolateral membrane change their conformation in the presence of electrical stimulation, thereby leading to a change in OHC length. We recently cloned a novel gene, known as prestin, from gerbil OHCs. It was demonstrated in vitro that this prestin protein is the major, if not the only, component of the OHC motor and that it provides the molecular basis for electromotility. However, in order to learn if electromotility is responsible for the sharp tuning and signal amplification in the mammalian cochlea, an in vivo system is required. Unfortunately, there is no animal model currently available for studying prestin function in the intact ear.

Our data indicate that the prestin gene is located on chromosome 5,close to the reelin gene (symbol Reln) locus. Reelin is an extracellular protein whose function in brain development has been studied using the reeler mouse, such as Relnrl, in which the reelin gene is mutated. In fact, Relnrl mice have 150kb deletion in the Reln locus. This 150 kilobase (kb) deletion could very well include portions of the nearby prestin gene, i.e., its coding regions and/or regulatory elements.

In order to determine if prestin expression is influenced by deletion of the reelin gene, homozygous Relnrl mice from The Jackson Laboratory were compared with their ungenotyped controls. In addition, in vivo experiments were performed under Pentobarbital anesthesia to learn if compound action potential (CAP) thresholds were affected by the deletion. Results indicate that prestin mRNA and protein are expressed in both control and homozygous mutant mice. CAP thresholds are also similar in the two groups. Consequently, the reeler mouse does not provide a naturally occurring prestin mutation.

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623 The Effects of Prestin's N-glycosylation Sites on Prestin Protein Distribution and Function.

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Outer hair cells (OHCs) possess 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 the following: There are voltage-dependent "motor proteins" in the membrane of OHCs. When transmembrane potential changes, these motor proteins alter their conformation, resulting in a surface area change. Prestin is a gene we recently cloned from mammalian OHC. The localization and gene expression profile of prestin, as well as its physiological function suggest that it is the motor protein of OHCs.

Prestin has 744 amino acids; it belongs to a newly discovered anion transporter family called SLC26. This family includes Pendrin, DRA, and DTDST. Prestin shares similar structure with this family, such as a conserved sulfate transport motif, and conserved glycosylation sites. Western blot analysis of prestin protein suggested possible post-translational modifications: glycosylation or phosphorylation. Whether the conserved N- glycosylation sites play an important role in prestin's distribution and its normal physiological function remains unknown.

To address this question, we have generated three mutants with altered N-glycosylation sites: N163Q, N166Q and a double mutant NN163/166QQ. These mutants were transiently transfected into a TSA 201 cell line. The cellular distribution of these prestin mutants was investigated using immunofluorescence. Our preliminary results suggest that all prestin mutants localize to the plasma membrane. Electromechanical properties of these prestin mutants were studied by examining the voltage-dependent charge movement, which is evidenced as non-linear capacitance. All three mutants had nonlinear capacitance

functions similar to wild-type. These results suggest that glycosylation does not interfere with prestin's localization or function.

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624 Expression of Prestin, a Membrane Motor Protein, in the Mammalian Auditory and Vestibular Periphery

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Hair cells are specialized mechanoreceptors common to auditory and vestibular sensory organs. Hair cells from mammalian and non-mammalian species are believed to share common features related to their mechanosensory function. In addition, it has been shown that hair cells possess various forms of motile properties that enhance their receptor function. Electromotility is a form of hair cell motility observed in isolated outer hair cells (OHCs) of the cochlea. In this study we explore whether prestin, a recently cloned OHC plasma membrane protein postulated to be responsible for electromotility, is also present in hair cells of the vestibular system. We initially cloned and sequenced the mouse prestin gene and compared the sequence to previously reported rat and gerbil prestin sequences. We determined regions of maximal sequence similarity and made riboprobes and peptide-specific antibodies. Using RT-PCR and in situ hybridization we show that prestin is not only expressed in OHCs but it is also expressed in vestibular hair cells (VHCs) in the three rodent species studied. Using various combinations of primers we could not detect prestin in chicks or frogs. Immunolocalization studies using three different prestin-specific antibodies confirmed the presence of prestin in VHCs in the mouse, rat and gerbil saccule, utricle, and cristae ampullaris. However, in the VHCs, prestin could not be detected in the lateral plasma membrane or in the stereociliary membrane, but was present in vesicular organelles in the cytoplasm. This suggests that unlike OHCs, the VHCs do not have an efficient mechanism to target prestin to the plasma membrane. Whole-cell patch-clamp recordings showed that VHCs do not possess the voltage-dependent capacitance associated with electromotility. We conclude that although prestin is expressed in VHCs, it is unlikely that it supports a form of somatic motility like the one in OHCs.

625 Effects of Streptomycin 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, the effects of streptomycin on OHC motility were investigated by using isolated guinea-pig OHCs with the microchamber and whole-cell voltage-clamp techniques and a photodiode-based motility measurement system. Motility was first measured using the microchamber method. The cell was 25% inserted into the microchamber and 50 mM streptomycin was applied to the extruded segment (ciliated pole) for 2 minutes through a puff pipette. Motility was reduced by approximately 25 to 50%. The effect was partially reversible in 3-4 minutes after washing out. When 20 mM streptomycin was applied intracellularly through a patch pipette, motility was eliminated. In addition, streptomycin also causes severe deflation of the cell. The elimination of motility was not due to loss of cell turgo, since re-inflation of the cell did not bring back motility. Possible mechanisms of the effects of streptomycin on motility are discussed.

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626 The Influence of a Goitrogen on Prestin Expression and Cochlear Function in CBA/CaJ Mice

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The incidence of hearing loss in humans with acquired hypothroidism is ~25%. Although this statistic is motivation enough to study the influence of thyroid hormone on auditory function, our inducement was based on the observation that a thyroid binding site is located near the beginning of prestin's coding region (Knipper et al., ARO 2001). This information suggests that expression of the outer hair cell (OHC) motor protein might be influenced by thyroid hormone. This study was, therefore, designed to study the influence of a goitrogen (methimazole, MMI) on cochlear function and on prestin expression.

Male CBA/CaJ mice received MMI (0.02%) in their drinking water beginning at 3 weeks of age to avoid interferring with normal cochlear development. During the ~5 months of MMI administration, the water consumption of both controls and MMI mice was determined. These measures indicated that MMI mice drank ~1/3 less than controls, most likely due to the unpleasant taste of the treated water. Eliza tests, administered after ~5 months on MMI, indicated that T3 and T4 levels were well below those in controls. After the thyroid blood tests were administered, the mice were anesthetized with Sodium Pentobarbital and a round window electrode positioned to allow compound action potential (CAP) thresholds and CAP tuning curves to be collected. The animals were then euthanized and their prestin mRNA and protein determined. For each animal, one cochlea was used for immunohistochemistry to assay protein using an anti-prestin antibody; the other cochlea, for RNA isolation and comparative RT-PCR. Results indicate that cochlear sensitivity and frequency selectivity, as well as prestin expression, were not affected by the reduction in thyroid hormone. Hence, the adminstration of a goitrogen will not result in reduced levels of native prestin in animal models designed to reduce expression of the OHC motor protein.

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627 Cellular force and molecular electromechanical transduction of outer hair cells in thyroxin deficient rats

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Outer hair cells (OHC) produce mechanical force in response to change of their membrane potential. The axial component of this electromechanical force provides a basis for the exquisite sensitivity, frequency selectivity and dynamic range of the cochlea. The protein responsible for the motor action of the OHC is located in the lateral plasma membrane; it has been identified and named prestin. It is believed that the aggregate conformational area change of the motor proteins, reflected by a voltage-dependent charge transfer across the plasma membrane, generates the somatic mechanical force. Using hypothyroid rats, we show here that it is possible to differentially influence the number of motor elements and the amplitude of the force generated by the cell. At postnatal day 17 (P17), an age when in control animals prestin is known to exhibit a mature distribution along the OHC lateral membrane, the cells from hypothyroid animals exhibited an immature distribution of prestin immunoreactivity around the entire cell

membrane, together with a seven-fold reduction of voltage-dependent charge transfer and an eleven-fold reduction of electromechanical force. By P28, the charge transfer had developed to its normal value, but both the distribution of prestin molecules and the electromechanical force remained abnormal. These results provide a new experimental system for investigating molecular mechanisms of motor function and the generation of force by the whole cell.

628 No correlates for somatic motility in freeze-fractured hair-cell membranes of lizards and birds

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In mammals, it is commonly accepted that a cochlear amplifier enhances vibration amplitudes in response to near-threshold stimuli. The most favoured mechanism is a fast electromotility shown by outer hair cells (OHC) in vitro. The motile element is thought to be the protein Prestin that is present at high density in the lateral plasma membrane of OHC. In freeze-fracture preparations, densities of up to 6000 particles per μm² of membrane have been reported.

The inner ears of nonmammals can also actively generate mechanical energy. The widespread existence of spontaneous otoacoustic emissions is undisputed evidence for this. However, the mechanism underlying it is believed to reside in the mechanosensitive hair bundle at the top of the cell, not in the cell body.

To further explore the candidate mechanisms of hair-cell motility in nonmammals, we have freeze-fractured the plasma membranes of basilar-papilla hair cells from a lizard, the Tokay gecko and from a bird, the barn owl. Both species are known to generate spontaneous otoacoustic emissions.

Freeze-fracture replicas of basilar-papilla hair cells were obtained by standard procedures. The density and size of particles was determined from patches of membrane P-faces of standard size. Our preliminary data show that in both gecko and owl, the means of the particle size distributions fall between 8 and 10nm, and that there are average densities of around 1000 membrane particles per μm² in gecko hair cells and up to 3000 particles per μm² in barn owl hair cells. The particle size distributions and the densities differ substantially from the values typically seen in mammalian OHC and are more similar to data from mammalian IHC. Thus we suggest that in our species, these particles do not reflect motor proteins.

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629 RC time constant paradox of outer hair cells

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Outer hair cells (OHC) are a critical element in the sensitivity and sharpness of frequency selectivity of the ear. It has been believed that the fast motility of these cells is essential for this function. Indeed, force produced by outer hair cells (OHC) follows the membrane potential very closely, responding up to 60 kHz. However, the receptor potential is attenuated by a low pass RC circuit, inherent to these cells. The RC roll-off frequencies of these cells are significantly lower than their operating frequencies, rendering the motility quite inefficient in producing force. Why, then, should these cells possess the exceptionally fast motility?

To address this puzzle, we formulated a theory by assuming that multiple degrees of freedom and vibrational modes due to the complex structure of organ of Corti provide optimal phases for OHC input and output to cancel viscous drag. The derived frequency limit depended on the membrane capacitance but not on the membrane resistance. If we assume that the drag coefficient per an OHC is equivalent to a sphere of 30 μm diameter, the limiting frequency was 5 kHz. Although our choice of viscous drag is rather arbitrary, the uncertainty of the limiting frequency is not too large because the square root of viscous drag affects the limiting frequency. Our analysis also showed that fast activating potassium current can further extend the frequency limit by counteracting the capacitive current.

To test our predictions, we performed patch clamp experiments on isolated OHCs. We found fast activating potassium currents in OHCs whose operating frequencies exceeded about 5 kHz. Such currents were absent in OHCs whose operating frequencies were less than 5 kHz. In addition, slow activating potassium currents observed in apical cells were absent in high frequency OHCs. We propose that these fast activating currents are the key to the paradox.

630 Electromotile Hearing: Evidence that Tone-like Percepts are Produced by Electrical Stimulation of Cochlear Outer Hair Cells

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Sinusoidal electric stimulation of the intact (basal) cochlea in guinea pigs produces a tonal perception corresponding to the stimulation frequency (Le Prell et al., 2000). This conclusion was based on frequency-specific masking of electric stimulation by pure-tone acoustic stimuli. Here, we extend our psychophysical testing to additional masking frequencies and evaluate the cochlear tissues after long-term electrode implantation (i.e., 2-3 years) in two subjects. Both subjects showed evidence of frequency-specific masking. For the first subject, masking was sharply tuned at 8 kHz and 11.2 kHz electric stimulation frequencies, and hair cells were intact at the end of the experimental procedures. For the second subject, masking was broader, extending beyond the critical bandwidth described by Greenwood (1991). However, the masking function shape indicated masking was generally frequency specific. Near the end of the experimental testing, this subject developed a profound hearing loss. The loss became evident when acoustic stimuli stopped masking the electric stimulus; then we saw a general decline in sensitivity to electric stimulation. A significant loss of outer hair cells was evident in the basal cochlea, a finding consistent with the idea that electromotile perception is produced by electrically stimulating outer hair cells in the intact organ of Corti. Pure tones did not mask detection of electric stimulation of the cochlea at 5.6 kHz for either subject. Because electric stimulation below 6 kHz does not produce strong EEOAEs (e.g., Ren & Nuttall, 1996; Le Prell et al., 2000), this result was not surprising. Taken together, the psychophysical and morphological findings support the hypothesis that outer hair cell motile action, resulting from sinusoidal electric stimulation at frequencies above 6 kHz, results in a sharply tuned tonal perception.

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631 An Unusual Cl- Conductance In Isolated Guinea Pig Outer Hair Cells

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Outer hair cells (OHCs) mediate sharp tuning in the basilar membrane. The energy for enhanced basilar membrane motion might be fueled by fast OHC electromotility originating from the membrane protein prestin. It changes its conformational state following membrane potential resulting in instantaneous changes in membrane surface and cell length. Recently it was shown that binding of small anions to the cytoplasmic residue of prestin is necessary for its voltage sensitivity (Oliver et al., 2001). The Cl- anion is thus a main candidate to produce chemical modulation of OHC motility. The contribution of different Cl channels and transporters to [Cl-]i regulation in OHCs is not thoroughly

investigated. Recent RT-PCR experiments (Kawasaki et al., 1999; 2000) revealed the presence of mRNA for ClC-1/2/3 and ClC-K1 voltage-dependent chloride channels. However, their physiological role in OHCs is not clear. We carried out our study of transmembrane chloride currents to investigate the possible match with properties of currents mediated by electrophysiologically well-characterized types of Cl channels. Chloride currents (Icl) in isolated OHCs revealed linear I-V curves around 0 mV in the range of membrane potentials of ~ -40 / +40 mV. They demonstrated moderate outward rectification at more positive membrane potentials and pronounced inward rectification at more negative potentials outside the linear I-V zone. Icl were flat without on/off time-dependent activation/relaxation phases within the range of -60/+60 mV. This observation indicates that the massive influx of Cl- at depolarizing potentials does not derive from any known type of ClC-family channels. The membrane structure responsible for these currents is not presently known. A slow component resembling ClC-2-mediated currents was activated at membrane potentials more negative to -80 mV and might be responsible for the efflux of Cl- from the cell at hyperpolarizing potentials.

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632 Three-Dimensional Reconstruction of Electrically-Evoked Motions in the Gerbil Cochlea

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In order to understand the role of electromotility in cochlear micromechanics we have developed a stroboscopic video microscopy system to visualize the mechanical responses within the organ of Corti (OC) due to outer hair cell (OHC) contractions. Using two dimensional cross correlation techniques we have been able to calculate the amplitude and phase of motion for each structure or interest within the organ. We have previously reported data from only one or two focal levels per experiment (Karavitaki and Mountain, ARO Abstracts 2000, 710:204). In particular most of the data were collected at the level of the OHC nuclei. At that level, OHC motions were large compared to inner hair cell and pillar cell motion. In addition, at low frequencies below the characteristic frequency of the region, OHC nuclei moved out of phase with respect to each other suggesting that multiple vibration modes contribute to the frequency response of individual structures in the OC.

In this study we have collected images at multiple focal levels per experiment and used them to reconstruct the motion of the organ in three dimensions. Data were collected from the middle turn of excised gerbil cochleas. The tissue was stimulated electrically using sinusoidal currents ranging from 30-9000 Hz. The experimental set-up and motion estimation techniques have been described previously (Karavitaki and Mountain, ARO Abstracts 1998, 719:180).

Preliminary data at low frequencies suggest that, with electrical stimulation, motion of the three rows of OHCs and the Hensen cells is large near the level of OHC nuclei compared to motion at levels close to the cuticular plate and the basilar membrane (BM). Similar to previous observations (Karavitaki and Mountain, ARO Abstracts 2001, 819:229), MOC fibers showed large longitudinal displacements suggesting fluid flow in the tunnel of Corti during OHC contractions. There was little or no motion at the tectorial membrane and the BM levels.

633 Hints for pillar movements by VASP-expression

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Background: Vasodilator-stimulated phosphoprotein (VASP) is , a member of the ENA/VASP-family and found to be a crucial factor in the regulation of actin dynamics which involves processes such as motility and cell adhesion. Zyxin acts as an important binding partner of VASP and is concentrated at sites where VASP-dependent actin movements occur. Since cochlear mechanical properties are often determined by actin filament dynamics and cross-linked microtubule bundles, we tested whether any actin/tubulin-expressing cochlear cells might show signs of active movement linked to expression of VASP and/or zyxin.

Materials and Methods: We investigated expression of VASP and zyxin in the postnatal and adult rat cochlea using polymerase chain reaction, western blot, immunohistochemistry, and confocal microscopy.

Results: In addition to the expression in vessels and fibroblasts in the stria vascularis, limbus, and spiral ganglion, the most prominent staining for VASP and zyxin was observed, somewhat surprisingly, at the time of hearing onset in the head of the outer pillar and foot plates of both pillar cells. In these pillar regions, VASP and zyxin expression was co-localised with pan-actin, thus indicating actin dynamics.

Conclusions: Our observations led to a significant alteration in our present understanding of the mechanical properties of the cochlea. Considering VASP/zyxin-mediated active movements within the pillars, we assume a hinge-like connection in the centre of the organ of Corti. Pillar movements would best explain the rise of the inner hair cells, thus enabling their stereocilia to come into contact with the tectorial membrane. Dynamic pillar actions might also explain the observed radial movements within the organ of Corti.

634 Intracellular calcium and stiffness of pillar cells after the application of adenosine triphosphate (ATP)

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Inner and outer pillar cells are long slender rods packed with a continuous bundle of parallel microtubules and actin filaments that determine the stability and architecture of these cells. The rigidity of the microtubular bundles depends on the degree of their cross-linking, and damage of connections between parallel microtubules decreases the stiffness of pillar cells (Tolomeo and Holley, Biophys. J. 73(4): 2241-7, 1997). Physiologically, these cross-links are controlled by microtubule-associated proteins (MAPs) which, in turn, are regulated by calcium-dependent phosphorylation. Since intracellular calcium is modulated by adenosine triphosphate (ATP) in pillar cells (Chung and Schacht, JARO, 2001) we hypothesized that cell stiffness may be controlled by ATP. This hypothesis was tested in gerbil pillar cells.

Inner and outer pillar cells were isolated from different sections along the basilar membrane. After the pillar cells were transferred into an experimental chamber, they were maintained in L-15 solution and bending stiffness for cells of different lengths was measured with a calibrated glass fiber. Stiffness of gerbil pillar cells was in the same range as the stiffness of guinea pig pillars reported by Tolomeo and Holley. Furthermore, bending stiffness decreased with increasing cell length. The response of intracellular calcium to the application of extracellular ATP was determined using the fluorescent indictor fluo-3.

The effect of ATP on bending stiffness of the cells and potential consequences for cochlear physiology will be discussed.

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635 Pathology of a New Mutant Mouse showing Circling Behavior and Deafness

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Mutant mice with inner ear abnormality provide a model for studying the inner ear biology and related pathophysiology. Recently, the authors reported newly found mutant mice which showed circling behavior and deafness. They are inherited in autosomal recessive pattern. Preliminary genetic analysis revealed that affected gene locus was different from the other known mutant mice. The detailed pathological findings in this mutant mice are essential for differentiating themselves from other mutants and getting the clue of unidentified etiologic protein. The inner ear pathology in this mutant mice (we call it '*cir*' mouse) was investigated using light and electron microscopy.

In the cochlea, the spiral ganglion neurons were sparse in Rosenthal's canal, and the hair cells were absent in basal and middle turn. In the vestibular end organs, the ratio of type I and type II hair cells was changed. Number of the stereocilia in the crista had decreased in the central region. In TEM, the nerve endings in type I hair cells showed irregular pattern. Calyxeal structure of type I hair cells and cytoplasmic organelles in type II hair cells were changed.

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636 Analysis Of Spinner Mutants With Sensory Gene Microarrays

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Gene expression profiling with DNA microarrays is a valuable approach for delineating the underlying cellular pathways involved in development, in response to traumatic injury, and in disease states. We are generating profiles of gene expression in the auditory system, including the mature (Cho et al., JARO, in press) and the acoustically damaged rat cochlea, and the cochleae of mouse deafness mutants. One limitation of most commercially available gene arrays is that they often do not include genes that are cochlear-specific or expressed preferentially in sensory epithelia. Many genes exhibit dual expression in tissues of the eye and the inner ear. With this in mind, we have produced mouse cDNA arrays in the sensory gene microarray facility at the University of Michigan, which has developed a comprehensive resource of genes expressed in the developing and mature mouse eye (Farjo et al. in press, http://www.umich.edu/~retina/farjo-2001-visres.html). We are using these cDNA arrays, along with membrane arrays from Research Genetics to evaluate gene expression profiles in the inner ears of the spinner mutant, a mouse genetic model of deafness and vestibular dysfunction. RNA from cochleae of spinner homozygotes and control heterozygotes at 3 weeks and 3 months of age have been analyzed. With the Research Genetics arrays, we detected no differences in gene expression between spinner homozygotes and control heterozygotes at 3 weeks of age; however, there appear to be differences at 3 months of age. We are currently repeating these microarray experiments and confirming any consistent changes by RT-PCR. Understanding the genes that change should provide insight into the molecular pathways impacted by the spinner mutation.

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637 Development of Auditory Brainstem Responses (ABRs) in Normal and Hypothyroid (Tshr Mutant) Mice

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Tshr mutant mice express a point mutation in the gene encoding the thyrotropin receptor and affected animals are congenitally hypothyroid and profoundly deaf under some conditions. Developmental changes in ABRs to click and tone bursts were studied in mice that were homozygous for the hypothyroid trait (hyt/hyt), as well as in euthyroid heterozygous (+/hyt) individuals and their wild type counterparts (BALB/c) between P13 and P90. All homozygous (hyt/hyt) mice were born to hypothyroid (hyt/hyt) dams and were consequently deprived of thyroxin throughout development. Normally, ABR thresholds develop rapidly between P13 and P15, decreasing by as much as 80 dB, and gradually acquire maturity by P24. Although responses to low frequencies were observed before those to high frequencies, responses to high frequencies matured faster than responses to low frequency stimuli. Slopes of both amplitude and latency vs. intensity curves were relatively steep early in development, progressively becoming adultlike by the beginning of the third postnatal week. In contrast, hypothyroid mice were profoundly deaf at P24. Thereafter, thresholds improved as much as 30 dB between P24 and P60. Unlike control animals, sensitivity developed along a linear time line in hypothyroid animals, with maturation occurring at a faster rate in the mid-frequency range than for either lower or higher frequencies. Slopes of both amplitude and latency vs. intensity curves were steeper than age matched controls. Although hypothyroid mice develop partial auditory function over a protracted time period ending around P60, normal function is never achieved and affected animals exhibit hearing losses in the range of 50 dB relative to control animals. These findings support the conclusion of previous studies suggesting that the enduring deficit of hypothyroidism lies in the cochlear amplifier.

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638 Neurochemical abnormalities in the auditory pathway of the congenital hypothyroid (hyt/hyt) mouse

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The hyt/hyt mouse has a homozygous recessive mutation that results in a severe congenital primary hypothyroidism, due to abnormalities in the thyrotropin receptor in the thyroid gland. Hereditary hypothyroidism is associated with sensorineural hearing loss in animal models and human patients. We looked for changes in the glycoconjugate composition of the auditory receptor by means of lectin cytochemistry, as well as neurochemical alterations of the central auditory pathway, using immunocytochemistry for parvalbumin, calbindin, and calretinin, in adult hyt/hyt mice.

Homozygotes showed: (1) a gross distortion and enlargement of the tectorial membrane, together with abnormal extracellular deposits of carbohydrates below the outer hair cells, (2) a reduction of parvalbumin-immunopositive cells in the dorsal cochlear nucleus, (3)

the disappearance of calbindin-positive terminals in the lateral superior olivary nucleus and superior paraolivary nucleus, and (4) a decrease of parvalbumin-immunostained terminals in layers II and III of auditory cortex. The auditory system of heterozygous mice was comparable to that of normal animals.

Thyroid hormone is essential for the normal development of neurochemically-specific circuits in the brain, because its absence results in long-term alterations in the expression of calcium-binding proteins in selected auditory nuclei. These data provide a ground for evaluating the effects of inherited hypothyroidism on the auditory pathway.

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639 Pathologic Basis Of Hearing Loss In Anderson Fabry Disease And In Fabry Mice

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Anderson Fabry disease is an X linked glycosphingolipid storage disorder in which a deficiency of lysosomal hydrolase, -galactosidase, leads to the accumulation of uncleaved glycosphingolipids, predominantly globotriosylceramide (Gb3). Auditory and vestibular abnormalities are frequently found in this condition (K. D. MacDermot, personal observations). In this study we describe histologic changes in the temporal bone of a patient with Anderson Fabry syndrome and ultrastructural changes in the cochleas of Fabry mice(1). The patient was a 41 year-old sufferer from Anderson Fabry disease who died of a heart attack. He had had bilateral asymmetrical sensorineural hearing loss. The modiolus was empty of any spiral ganglion cells; its cavities were filled with a fibrillar material in which many birefringent, Luxol fast blue positive crystalloid structures were present. The vestibular ganglion cells showed reduced numbers of ganglion cells. In about 40% the cytoplasm was distended by a pale-staining Luxol fast blue positive faintly birefringent material, which displaced the nucleus to one side. Sections of the cochleas of 7 Fabry mice were examined by transmission electron microscopy and changes compared with appearances in 6 C57BL/6 control mice. The most striking observation was the presence of large osmiophilic inclusion bodies within the cytoplasm of the Fabry mice. Deposits of lipid material, presumably Gb3, are thus present in the spiral ganglion cells of temporal bones in both the human disease and in Fabry mice and in the vestibular ganglion cells in the human disease. This would account for the prominent audiovestibular symptoms in the human disease. (1) Ohshima T, Murray GJ, Swaim WD, et al. alpha-Galactosidase A deficient mice: a model of Fabry disease. Proc Natl Acad Sci U S A. 1997;94:2540-4

640 Having Hair Cells is not Sufficient for Healthy Hearing

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Disruption of the p27kip1 gene in mice results in a multitude of phenotypic abnormalities in a variety of tissues (e.g. Fero et al, Cell 85, 1996). In the inner ear ongoing proliferation of cells in the organ of Corti, in postnatal and adult animals is observed (Loewenheim et al., PNAS 96, 1999; Chen and Sigel, Dev. 126, 1999). This leads to a disarrangement of the regular cochlear cytoarchitecture. Later in life loss of outer hair cells (OHC) in the cochlear base occurs. To elucidate the role of the cytoarchitecture for hearing and its development, we evaluated the cochlear thresholds and morphology of these mice during 3rd to 6th week of life.

Mice of a 129Sv genetic background with a disruption of p27kip1 were used. All three genotypes were examined, +/+, +/-, and -/- (wildtype, hetero- and homozygous p27-deficient). Acoustically evoked auditory brainstem responses were measured using standard equipment. Hearing was evaluated at postnatal day (Pnd) 15, 18, 22 and 30, respectively, at different frequencies in the hearing range.

In +/+ mice, a clear development of hearing was observed. At Pnd 15 most sensitive thresholds were around 60 dB SPL at 11 to 16 kHz. At Pnd 18 there was an overall improvement of about 20 dB. At Pnd 22 an additional improvement of approx. 20 dB only at the high frequencies (22-32 kHz) was observed. The +/- mice had audiograms similar to +/+ mice. In contrast, -/- animals at Pnd 15 showed thresholds of 80 to 90 dB SPL at the most sensitive frequencies (8-32 kHz). There was no improvement of these thresholds at Pnd 18 or 22.

From these data it can be concluded that the previously described impaired hearing of adult -/- mice is not exclusively caused by a secondary loss of OHCs, but that hearing thresholds are extremely elevated from the onset of hearing. This could be due to the disarranged cellular architecture, but remains to be compared to more detailed histological data.

641 Persistent Neural Deficits in Acetylcholinesterase Knockout Mice

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In the complete absence of acetylcholinesterase (AChE), as in the case of the AChE knockout mouse that is the object of this investigation, the development of auditory function is delayed and the phenotype of affected adults is complex: Some individuals exhibit normal acoustic sensitivity, whereas auditory brainstem response (ABR) thresholds are elevated in other cases. The pattern of otopathology associated with abnormal mice is also complex in that some individuals express what appear to be purely conductive deficits, while sensorineural deficits have also been observed in other cases. Histological studies suggest that middle ear abnormalities may account for the conductive deficits observed in affected animals. Otherwise, cochlear anatomy appears to be normal even in mice where sensorineural abnormality was observed. Results from the earlier study also suggested that the percentage of normal appearing individuals from the experimental group increased as a function of age, such that the mean threshold difference between control and experimental animals was smaller at P40 (~40 dB) when compared with differences observed at P24 and P30 (~50 dB). In an effort to test the hypothesis that AChE deficient mice eventually acquire normal auditory thresholds, affected animals were studied between 3 and 4 postnatal months and ABRs and distortion product otoacoustic emissions (DPOAEs) were examined. Whereas DPOAE thresholds overlapped those observed in normal animals considerably, ABR derived tuning curve tip and tail thresholds were elevated by roughly 24 dB and 13 dB respectively. These findings suggest that nearly normal sensory function is acquired in AChE deficient animals, and that an enduring neural abnormality persists, at least until the end of 4 months.

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642 Temporary and permanent threshold shift from loud sound in the PARP-1 -/- mouse mutant

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Poly adenosine diphosphate ribose polymerase (PARP) is a nuclear enzyme that is activated by single DNA strand breaks. It catalyzes ADP-ribose units from the substrate nicotinamide adenine dinucleotide (NAD) to various nuclear proteins. When strongly activated, PARP depletes NAD resulting in inhibition of mitochondrial function which may lead to cell death. To determine if PARP has a role in noise induced hearing loss, we exposed the PARP-1 -/- mice to broad-band noise and compared auditory brainstem evoked responses (ABR) to the identically treated wild type mouse. Mice, in groups of 5, were sound exposed for 3-hour periods/day. ABRs were recorded 1) before, 2) immediately following an exposure series, 3) one week following exposure and 4) two weeks following sound exposure. The exposure series were 1, 2 and 5 days. Sound level was 122 dBA SPL for each series and 110 dBA SPL for the 1 and 2 day series. After the last ABR for the 5-day series, mice were killed, the cochleas harvested, fixed with 10% formalin; the organ of Corti stained with succinate dehydrogenase and hair cells counted for cytocochleargrams. The 1-day/110 dBA SPL did not produce significant permanent and temporary threshold shift (PTS and TTS) in either group. For all other series the amount of the TTS and PTS were markedly and statistically significantly less in the knockout mouse. For example, 5-day/122 dBA produced in PARP +/+ an average (across frequency) TTS of 45 dB and a PTS of 31 dB while PARP -/- had an average TTS of 31 dB and PTS of 10 dB (TTS, $p < .05$; PTS, $p < .001$). The PTS following trauma is consistent with protection against cell death by inhibition PARP and indicates a noise damage mechanism that involves reactive oxygen species damage to nuclear DNA.

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643 The Wild-Derived Inbred Strain MOLF/Ei has Normal DPOAEs but shows Exceptional Resistance to NIHL

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Several investigators (Yoshida et al, 2000; Candreia et al, 2001) have shown that the 129/SvEvTac mouse is resistant to noise-induced hearing loss (NIHL). Unfortunately, this strain has abnormal hearing (Yoshida et al, 2001; Candreia et al, 2001) in that at high frequencies it exhibits elevated ABR thresholds and poor distortion-product otoacoustic emissions (DPOAEs). As part of a screening study, we discovered that the wild-derived strain MOLF/Ei (MOLF) had normal DPOAEs, but exhibited exceptional resistance to NIHL. The present study was designed to confirm these findings. DPOAEs were recorded in the form of DP-grams (DPOAE level as a function of primary-tone frequency), with geometric–mean (GM) frequencies ranging from 5.6-48.5 kHz (f_2=6.3-54.2 kHz), in 0.1-oct steps. DP-grams were collected at three primary-tone levels (L_1=L_2=55, 65, 75 dB SPL). At 2 mo of age, MOLF mice were exposed to an octave-band noise (OBN) centered at 10 kHz for 8 h. DPOAEs were assessed prior to noise exposure, and at 2 d and 1 and 2 wk after noise. DPOAE loss was minimal at 2 d post-exposure and DPOAEs returned to normal levels by 1 wk after acoustic overexposure. In an older group of mice given a similar exposure, DPOAEs were tested immediately after removal from the noise-exposure chamber. These measurements revealed the expected NIHL pattern with maximal DPOAE loss around the OBN. When these data were compared to those obtained from CBA/CaJ (CBA) mice with similar noise exposures and comparable ages, CBA mice showed much slower recovery from NIHL. Together, these findings suggest that an unusual ability to recover from noise exposure, rather than the lack of initial damage, accounts for the exceptional resistance to NIHL exhibited by the MOLF strain. The MOLF strain may be an ideal model for studying susceptibility to NIHL in that unlike the 129/SvEvTac strain, the MOLF strain has normal auditory function as measured by DPOAEs and ABRs (Zheng et al, 1999).

644 Steroid Treatments Restore Stria Vascularis Capillary Size in MRL/MpJ-Fas^{lpr} Autoimmune Mice

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The MRL/MpJ-Faslpr(MRL/lpr)mouse lacks the fas gene necessary for eliminating self-recognizing t-cells and develops autoimmune disease and hearing loss as it ages. Studies have shown that the stria vascularis endothelial cell tight junctions are disrupted and the capillaries become dysmorphic and enlarged. Treatment of autoimmune mice with the glucocorticoid prednisolone or the mineralocorticoid aldosterone preserves cochlear function, and the stria morphology improves. One hypothesis is that steroids improve stria Na+-K+ transport to restore endolymph ion balances. This study was designed to measure stria vascularis capillaries in MRL/lpr mice treated with prednisilone and aldosterone to determine if vessel pathology was affected. Age-matched BALB/c mice were used as a normal albino control for vessel size to compare treated and untreated autoimmune mice. After 2 months of oral steroid treatments, all ears were embedded in GMA and mid-modiolar sections of the stria were drawn and measured. The median vessel size in the three turns of BALB/c mice was 35 mm, 29 mm, and 32 mm, respectively. Vessel sizes in untreated MRL/lpr mice were significantly larger than normal ($p=0.005$), reflecting the dysmorphic vessels seen with advanced of disease. Prednisolone treated MRL/lpr mice had vessels that were close to normal size, although still significantly larger than the normal mice ($p=0.043$). There was no difference in vessel size between the aldosterone treated animals and the normal mice. These results quantify prior observations that stria vascularis capillaries become dysmorphic and enlarged as autoimmune disease progresses in MRL/lpr mice. The positive treatment results also demonstrate that the stria vascularis may be a site of steroid influence in treatments for autoimmune hearing loss. It is possible that the sodium transport function of both of these corticosteroid groups represents one of the steroid responsive mechanisms within the inner ear.

645 The hemicochlea: A feasible model of an intact electrically stimulated cochlea.

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Cochlear implants take advantage of the tonotopic arrangement of afferent neurons by delivering information through multiple stimulation contacts placed longitudinally within scala tympani. The aim is to provide spatially discrete spectral information that can be perceived distinctly and used to understand speech. Recent electrode development efforts have been aimed at enhancing the spatial selectivity of stimulation by placing the electrode array closer to the modiolar wall. However, the design of these electrodes is based primarily on indirect measurements, theoretical assumptions, and model predictions. Few real measurements of the field potentials resulting from electrical stimulation within the cochlea have been made. The paucity of empirical data primarily stems from difficulty in accessing an intact cochlea. In this poster, we show that the hemicochlea is a feasible model for approximating potential fields generated by electrical stimulation in an intact cochlea. The potential distributions generated by a pair of electrodes placed in a saline bath were compared to results from similar electrodes placed in the gerbil hemicochlea in various monopolar and bipolar configurations. Comparisons of length

constants, potential gradients, and field shapes indicate that the hemicochlea is a reasonable model of an intact electrically stimulated cochlea if stimulating electrodes are about 500 μm below the cut edge of the preparation. The comparisons also show that the length constant for the hemicochlea is longer than that measured in saline (250 vs. 150 μm), and that cochlear electroanatomy significantly affects the location of maximum potential difference across the organ of Corti and spiral ganglion cells. Field potential measurements, along with estimates of resistance of cochlear tissues, will be used to approximate current density patterns resulting from electrical stimulation.

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. 646 A Comparison of Inferior Colliculus (IC) Spatial Selectivity and Electrically Evoked Auditory Brainstem Responses (EABR) to Spiral Ganglion Cell (SCG) Survival in an Animal Model

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Theoretical considerations and past empirical studies have suggested that EABR amplitude relates directly to the number of excited auditory nerve neurons. Previous intracochlear electrical stimulation studies have shown that stimulation near threshold excites a restricted area within the tonotopic organization of the IC. Moreover, increasing intensity increases this area. Thus, growth of magnitude with increasing intensity may be related directly to increasing neural recruitment in both the cochlea and auditory CNS. This study compares EABR response properties with IC spatial selectivity in animals with varying SGC survival.

Animals received unilateral scala tympani implants and chronic stimulation (6 months). EABR intensity series were recorded in response to stimulation of apical and basal bipolar electrode pairs, and the amplitudes of wave III and V were measured. In terminal experiments, IC neuronal thresholds were plotted as a function of depth to create spatial tuning curves (STC). STC widths were measured to determine the relative spread of excitation (spatial selectivity) within the IC to the stimulated electrode pairs. Cochleas were examined, the electrode contact positions determined, and the spiral ganglion cell density calculated. Comparisons were made between apical and basal electrode pairs and the slopes of the EABR functions, STC widths and related to the spiral ganglion cell.

The slope of growth of the EABR evoked by the most apical pair was significantly steeper than that for the basal electrode pair. This finding likely reflects better positioning of the apical electrode pair in relation to the SGC population. The growth of the EABR amplitude showed a modest correlation with STC width. In addition, greater SGC survival corresponds to greater IC selectivity for the apical pair.

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647 Temporal Processing of Unmodulated and Amplitude-Modulated Intracochlear Electrical Pulses in the Inferior Colliculus of the Deaf Cat

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The use of high frequency, amplitude modulated pulse trains in modern cochlear implant speech processors has led to significant improvements in speech perception. To understand the mechanisms underlying successful 'electrical hearing', we investigated the temporal resolution of single neurons in the central nucleus of the inferior colliculus (ICC), as defined by their maximum following frequency [Fmax] to intracochlear electrical pulse trains. Cats were neonatally deafened, unilaterally implanted and chronically stimulated with carrier frequencies of 500-900 pps that were sinusoidally amplitude modulated (SAM=20-60 Hz, 100% modulation depth). In acute electrophysiological experiments, we recorded the responses of single ICC neurons to unmodulated and SAM pulse trains (biphasic pulses, 0.2 ms/phase) of increasing frequencies. Adult, acutely deafened, unstimulated cats served as controls.

Previous studies have shown that the normal range of Fmax to unmodulated pulse trains is ≈10-330 pps, and that chronic stimulation with SAM carrier frequencies of 300 pps (SAM 30 Hz) resulted in a significant increase in average Fmax (133 pps) as compared to control animals (102 pps). Thus, chronic stimulation with carrier frequencies near the high end of the normal range of Fmax improved temporal resolution of ICC neurons.

In contrast, the present study suggests that chronic stimulation with higher SAM carrier frequencies (≥500 pps) does not significantly alter Fmax to unmodulated pulse trains (98 pps) from that of controls. Moreover, acute recordings of responses to SAM carrier frequencies ≥500 pps show that ICC neurons generally phase lock exclusively to the lower modulation frequency (8-60 Hz) rather than the carrier. These results suggest that at SAM carrier frequencies exceeding the neurons' capacity to follow unmodulated pulse trains, the (lower) modulation frequency provides the dominant temporal information.

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648 Temporal Integration in Electrical Hearing: Psychophysical Thresholds and Responses of Inferior Colliculus (IC) Neurons in the Deaf Cat

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Temporal integration refers to the lower (more sensitive) detection thresholds observed when the duration of a brief stimulus is increased. This report presents behavioral and electrophysiological results obtained in a study of temporal integration in a deaf animal model.

Cats (n=8) were deafened neonatally and were trained subsequently to avoid a mild electrocutaneous shock when electrical stimuli (biphasic rectangular or sinusoidal pulses; 5.0 ms/phase) were delivered to the cochlea. Psychophysical detection thresholds (50% avoidance) and reaction times were estimated as functions of stimulus duration. At the conclusion of behavioral testing, neuronal responses were recorded in the IC during acute physiological experiments in the trained animals.

The results include: 1) increasing the duration of a train of sinusoidal pulses from 30 ms (3 cycles) to 1000 ms (100 cycles) reduced the mean psychophysical threshold by 11.4 dB; 2) mean psychophysical threshold decreased by 1.2 dB/doubling of stimulus duration for trains of rectangular pulses that varied in duration from 10 ms (1 pulse) to 1000 ms (80 pulses, interpulse interval=2.5 ms); 3) at psychophysical threshold, the fastest average reaction times were obtained for short duration, high intensity stimuli, whereas slower average reaction times were associated with longer duration, low intensity pulse trains; and 4) a sample of sustained-response IC neurons exhibited time-intensity trading, i.e., a neuron's cumulative response magnitude was similar for high intensity, short duration stimuli and for low intensity, long duration stimuli.

The results are consistent with the predictions of a model for neural spatial-temporal integration in electrical hearing (Beitel et al. J. Neurophysiology, 2000). The discussion will emphasize this conclusion.

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649 Effects of electrode-to-fiber distance on temporal variation of neural spikes

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We have been interested in better understanding mechanisms of electrical excitation of auditory nerve fibers and their stochastic response properties. It is still unclear how the distance between the nerve fiber and the stimulus electrode influences the temporal property of the fiber. In this presentation, the effects of the electrode-to-fiber distance on these properties is investigated by computer simulations.

The mammalian auditory nerve fiber was modeled by a multi-compartment cable model with 50 nodal sections. Each stochastic node accounted for current fluctuations arising from sodium and potassium channel kinetics. Electrode location was fixed near the midpoint of the fiber (above the 26th node of the fiber), while the electrode-to-fiber distance Z was systematically varied. Monophasic cathodic current pulses with a duration of 40 us were presented 500 times as stimuli. The transmembrane potentials at each node (1st-50th) were recorded for analyzing statistics of spike times.

For a fiber responding at about 50% FE, jitter was found to increase more than two-fold as Z was increased from 1 to 7 mm. Further investigation showed the origin of the jitter. The nodes ``initiated'' by stimuli ranged the 23-28th nodes for Z=1mm, the 22-29th nodes for Z=4mm, and the 21-30th for Z=7mm. This may imply ``spatial influence''. Likewise, the distribution of post-stimulus time histograms at each node initiated was wider with increasing the distance Z. This may imply ``temporal influence''.

We conclude that the temporal variation of spikes tends to increase as the electrode-to-fiber distance increases due to ``spatio-temporal influences''.

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650 Can stimulus-dependent refractoriness due to unstable subthreshold oscillations account for rate decay and variability in electrically stimulated neurons?

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Firing patterns of spiral ganglion cells in response to rapid electrical stimulation exhibit post-onset rate reductions and rate variability across trials (Moxon 1967; Litvak et al. 2001). Since rapidly fluctuating stimuli are used in cochlear implants, these effects could be perceptually important for users of these devices. It has been proposed that post-onset rate reduction and firing variability are caused by adaptation mechanisms such as ionic concentration changes (Litvak et al. 2001) and random influences such as membrane noise (Rubinstein et al. 1998; Litvak et al. 2001). We explore whether a different biophysical mechanism—stimulus-dependent refractoriness due to unstable subthreshold oscillations—might account for the transient rate reductions and the firing variability. This mechanism has recently been shown to operate in the squid giant axon and the Fitzhugh-Nagumo (FN) neuron model (Kaplan et al. 1996).

We numerically solve the FN model for stimulus conditions at and near those that have been previously shown to evoke subthreshold responses similar to those measured in squid axon and construct ensembles of such responses that differ slightly in initial conditions. The results demonstrate that for stimulus conditions that give rise to unstable subthreshold responses, the action potentials are initially highly synchronized across trails, but become desynchronized over the course of the stimulus. Firing rates decrease during the time that the responses desynchronize. Similar firing behavior has recently been observed in the electrically stimulated auditory nerve (Litvak et al. 2001). Also, the variance in the firing rate is within the range measured in the auditory nerve, which shows that a deterministic mechanism sensitive to initial conditions can in principle account for firing variability.

651 Threshold Prediction for a Noise-Modulated Electrical Stimulus using a Stochastic Auditory Nerve Model: Implications for Cochlear Implants

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An important factor that may play a role in speech recognition performance by cochlear implant subjects is that electrically stimulated nerves respond with a much higher level of synchrony than what is normally observed in acoustically stimulated nerves. Recent work has indicated that the addition of noise to the electrical stimulus may result in neural responses whose statistical characteristics are more similar to the characteristics observed in acoustically driven neurons. Initial psychophysical data has indicated that psychophysical performance on some tasks might also be enhanced by the judicious addition of noise [Zeng FG. Fu QJ. Morse R. "Human hearing enhanced by noise." Brain Research. 869(1-2):251-5, 2000]. It remains to be determined, however, how adding noise to a stimulus will impact performance on other basic psychophysical tasks and whether speech recognition can be enhanced. To address the first issue, we utilized a neural-behavioral model of the responsiveness of electrically stimulated auditory nerves to investigate the effect of additive noise on psychophysical performance for stimuli containing a single pulse as well as those containing multiple pulses. Although stochastic resonance per se does not occur for a single pulse, it is important to understand the impact of additive noise in both the single and multiple pulse cases. In this work, we consider the effect of additive noise on threshold, dynamic range, and intensity discrimination limens. Because simulations that are performed with this model are computationally intensive, we compare results obtained via simulation to those obtained via a theoretical analysis. We model the input amplitude stochastically, and model the two-down one-up or one-down one-up experimental paradigm using a Markov chain. We show that theoretical predictions match simulated performance, and that psychophysical performance is a function of the noise parameters and the experimental paradigm.

652 Electrode Configuration Affects the Ensemble Response Properties of the Auditory Nerve

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It is well known that intracochlear electrode configuration strongly influences auditory nerve fiber threshold and rate of recruitment with stimulus level. Single-fiber data of van den Honert & Stypulkowski (1987) indicate that, compared with monopolar stimulation, bipolar stimulation recruits fibers over a wider range of stimulus levels and a narrower spatial extent. Pfingst et al. (1997; 2001) and others have shown that electrode configurations believed to elicit wider excitation regions (i.e., monopolar or widely-spaced bipolar) can produce speech scores equal to or better than that produced by narrower configurations and also favorable reported sound quality.

If perceptual level is related to total (ensemble) spike activity within the nerve and broader excitation is assumed for monopolar stimulation, then one would expect monopolar stimuli to excite a relatively large proportion of fibers at relatively low firing efficiencies. The modeling work of White (1984) has demonstrated that this condition may be quite relevant to the neural excitation occurring in implant patients.

What remains unclear is how stochastic single-fiber response properties vary with both electrode configuration and stimulus level, particularly for the short-duration pulsatile stimuli used in most cochlear implant devices. To better understand these issues, we have obtained single-fiber and gross-potential (ECAP) responses to stimuli delivered through monopolar, bipolar, and tripolar electrodes. Measures were obtained using acute, deafened cats implanted with a Nucleus banded-electrode array. The results indicate that, at low stimulus levels (near the visual detection threshold for an ECAP response), a greater degree of ensemble jitter occurs with monopolar stimulation than with more focused stimulation. We suggest that this temporal aspect of the response may underlie the favorable results observed for monopolar stimulation in psychophysical studies.

653 Effects of Bipolar Electrode Contact Spacing on Psychophysical Strength-duration Contours in the Cat

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An important consideration in the design of intracochlear electrode arrays is the effect of electrode separation on the resulting electrical fields. The present study investigated the effects of small (~300 μm step) increments in the longitudinal spacing between bipolar contacts on psychophysical thresholds. Psychophysical thresholds were collected in a group of unilaterally deafened cats, each implanted with a multi-contact, scala tympani electrode array. Thresholds were measured for single presentations of charge-balanced biphasic pulses. The electrode array contained a series of six (numbered consecutively 1-6), 175 μm-diameter hemispheres, arranged in a linear array, with each separated by 305 μm center-to-center (125 μm edge-to-edge). The carrier was designed so that the six contacts would be in contact with the modiolar wall. Thresholds were measured for the longitudinal bipolar pairs 1-2, 1-3, 1-4, 1-5 and 1-6, using each contact for both source and sink. Stimuli had phase durations ranging from 25 to 6400 μs/phase. In general, for a given electrode pairing, absolute threshold levels varied considerably across subjects, likely reflecting a varying degree, and/or pattern of neural survival across animals. The effects of bipolar electrode contact separation on the strength-duration contours, however, were relatively consistent across subjects: Thresholds decreased, and strength-duration contour slope increased, in an orderly manner with increases in contact separation, from pair 1-2 (305 μm separation) to the pairing separation of 1-5 (1220 μm). With the longest separations tested (1220-1525 μm), the absolute threshold levels and contour slopes approached those for monopolar configurations using the same contacts with an indifferent extracochlear electrode. These data suggest that bipolar contacts separated by approximately 1200 μm or greater act similarly to two adjacent monopolar configurations.

654 Consequences of Phase Interactions for Asymmetric Biphasic Electrical Stimuli on Psychophysical Thresholds in the Cat

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The efficacy of electrical stimulation delivered through a cochlear implant depends critically on the characteristics of the stimulus waveform. Recent physiological and psychophysical studies have shown that the second phase of a *symmetric* charge-balanced biphasic pulse can significantly alter the response elicited by the first phase. This report describes psychophysical data collected in a group of unilaterally deafened cats, each implanted with a multi-contact, scala tympani electrode array. Thresholds were measured for presentations of single *asymmetric*, charge-balanced biphasic pulses under several monopolar conditions. The excitatory phase (the high-amplitude, short duration phase) of the pulse was varied from 25 to 100 μs. The charge-balancing phase varied in duration from 1 to 24 times the duration of the excitatory phase. The order of presentation and the polarity of the phases were varied. In general, thresholds varied systematically from the condition where both phases were of equal duration (and thus amplitude), and increased or decreased in an orderly manner with increases in the duration of the non-excitatory, or charge-balancing phase. The magnitude of the threshold shifts observed was greater for short excitatory phase durations. The changes in threshold with the duration of the non-excitatory phase were apparent to durations of 16 to 24 times the excitatory phase duration. Thresholds could change in either direction by as much as 8 dB. Whether thresholds increased or decreased from the equal phase duration condition varied within and across animals and appeared to be unrelated to absolute threshold or electrode location. These findings suggest that phase interactions for *asymmetric* biphasic pulses can have significant consequences for the psychophysical percept.

655 Auditory Nerve Fiber Responses To Sinusoidal vs. Biphasic Electrical Pulses

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Cochlear implants typically employ biphasic electrical pulses, modulated in amplitude and interleaved across electrodes (e.g. CIS processing). In practice, this stimulation paradigm serves both to ensure an injection of zero net charge and to minimize interactions of the electrical field presented at each site of the cochlea. Theoretically, simultaneous presentation of analog currents at each electrode site would provide greater bandwidth for information transfer. Practical implementation of this type of stimulation strategy (e.g. SAS processing) is complicated by the high likelihood of electrical field interactions and the need to provide a high probability of excitation for neurons near each electrode. One possible "hybrid" design would retain the continuously interleaved presentation order, but use a variety of excitation waveforms in order to convey additional information about the sound environment. A thorough understanding of the neural response driven by each candidate current waveform is a prerequisite for the development of such a signal encoding strategy. As a starting point, the goal of this study was to characterize differences in the neural response driven by two elementary, charge-balanced waveforms: sinusoidal and biphasic.

Single auditory nerve responses were studied in acutely deafened, anaesthetized cats with a single electrode placed within the scala tympani. Fibers were tested with both sinusoidal and biphasic current pulses of various periods, presented at intervals of 20 msec to avoid influences related to the refractory period. Input/output curves were taken to characterize the dynamic range of the driven response and peristimulus time histograms were computed to characterize the time course of the response at each level. This later response property will be used to judge the capability of each current waveform to drive a neural response that might be discriminable from the standard stimulus, a 100μsec/phase biphasic current pulse.

656 Potential Distribution and Efficiency of Electrical Stimulation with Multi-Channel-Electrodes in Brain Slices

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This study was to investigate the spatial selectivity of a multi-channel electrode by determination of the current density distribution around the electrode and to record the activity evoked in electrically stimulated neurons.

Brain slices from the auditory cortex of young rats were used as neuronal substrate. For stimulation a 28-channel electrode array (channel diameter 100 um) with a concentric design was used. The stimulus electrode was placed on the white matter, neuronal activity was recorded intra- and extracellularly from layers III to V. The potential distribution of the electrode within the slice chamber was measured 3-dimensionally with a resolution of up to 1 um with a microelectrode used as a probe. Current density distributions were calculated from these data. Different stimulus configurations (number of channels, current amplitude, polarity) were tested.

The electrical field produced by the multi-channel electrode could be shaped by simultaneous activation of different channels. Stimulus configurations producing `lateral inhibition´ resulted in a decrease of current spread (e.g. peaks of potential distribution become narrower). Within the brain slice neurons could be stimulated with different direction of the current vector, e.g. parallel or perpendicular to cortical layers. This is important with respect to stimulation of the brainstem (e.g. the selective activation of subnuclei). The neuronal responses evoked by electrical stimulation most frequently consisted of excitatory potentials (EPSPs). Inhibitory responses (IPSPs) were found only in a few cases.

Conclusions drawn from these in-vitro experiments may be useful for designing new cochlear- and brainstem implants and more generally for the development of all neuroprotheses (e.g. visual system), where efficiency of artificial electrical stimulation on excitable tissue is of importance.

657 Auditory Response to Electric Pulse Trains Before and After Furosemide Treatment

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The issue of how the presence of residual hair cells affects the electrical stimulation of the auditory nerve is of particular concern given that more individuals with significant residual hearing are receiving cochlear implants. Previous results from our laboratory have demonstrated differences in the electrically evoked auditory nerve response to electrical stimulation of the cochlea, before and after destruction of hair cell function by injection of kanamycin and ethacrynic acid. This paper reports similar responses in animals injected with furosemide to affect hair cell function. Since the effects of furosemide can be reversible, this protocol can be used as an experimental model to evaluate the time course of changes with loss of hair-cell function as well as recovery. In this study, the acoustically evoked compound action potential was used to monitor state of hair cell function and the electrically evoked compound action potential to pulse trains were used to assess the effects on the response. Electric stimuli were presented through a monopolar intracochlear electrode and the auditory responses were recorded through electrode on the auditory nerve. Responses were sampled over an interval of several hours after the first injection of furosemide. Responses to pulse trains demonstrated a typical pattern of adaptation over time and alternation of response amplitude to successive pulses. Three measures were used to assess changes with hearing loss: (1) amplitude of the first pulse in the train, (2) amplitude of response alternation between adjacent pulses, and (3) adaptation as measured by the asymptotic average amplitude relative to the first pulse. It was found that there was a tendency for both of the first pulse amplitude and alternation to increase following with hair cell function disappeared, otherwise adaptation significantly revealed to decrease slightly.

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658 Effect of systemic treatment with antioxidant on auditory function in deafened guinea pigs

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Cochlear prosthesis benefits depend in part on survival and responsiveness of auditory neurons in the deaf patient. Studies in animals have shown these factors can be influenced by chronic electrical stimulation and neurotrophins. The "neurotrophin hypothesis" suggests that formation of oxygen free radicals, with neurotrophin deprivation (Estus,1998), may contribute to nerve degeneration, and hence intervention with free radical scavengers may prevent nerve degeneration. We examined the effect of systemic trolox (vitamin E) and ascorbic acid (vitamin C) to maintain nerve responsiveness following deafness. Twenty-four guinea pigs were divided into 3 groups of 8 each. All subjects were implanted with an intracochlear Pt-Ir stimulating electrode, epidural recording electrodes and intracochlear cannula. Two groups were deafened by intracochlear infusion of 10% neomycin for 2 days. The third group only received intracochlear artificial perilymph. Following deafening 8 animals received daily IP injections of trolox (10mg/kg) and ascorbic acid (200mg/kg) for 4 weeks. Deafened-untreated and hearing-control groups were injected with the same volume of saline for 4 weeks. EABRs were recorded on days 5 to 44. In both deafened treated and untreated animals, EABR thresholds increased during the first 16 days, compared to undeafened controls. EABR thresholds of antioxidant treated subjects decreased 3 weeks after start of treatment, while those of untreated group remained elevated. There were statistical significant differences between these two groups on day 23, 30, 37 and 44. Systemic treatment with antioxidants clearly increased electrical sensitivity of the deafferented auditory nerve.

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659 Auditory neuron growth on cochlear and brainstem implant settings.

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In implant surgery, the interaction between the host cells and the alloplastic material is essential for the adaptation and function of the new system. In cochlear and brainstem implants, a close contact between auditory neurons and the platinum electrodes embedded into a silicone array is desirable to optimize the function the hearing devices.

To study the growth of auditory neurons on electrode settings, cochlear neurons of postnatal day 4 rats were cultured on platinum for 3 days and the resulting neurite growth compared to control explants. On platinum, the average number of neurites (n=15.2) extending from the spiral ganglion (SG) explants was significantly lower ($p<0.013$) than in the control group (n=26.4). However, the length of the neurites was significantly higher ($p<0.0001$) in the explant group cultured on platinum (509µm) than in the control group (359µm).

Additionally, SG explants were cultured on brainstem electrodes (provided by MedEl, Austria) and the neurite behaviour at the borders of silicone and platinum was observed. In a preliminary experiment, the

neurites tended to grow along the silicone/platinum border. However in some regions, neurites grew from silicone onto the platinum surface. These growth patterns suggested a mechanical border to be responsible for the observed effects. To eliminate border effects, flat electrodes with no mechanical border were designed in cooperation with MedEl. On these electrodes, the majority of outgrowing neurites extended from the silicone surface to the platinum electrode and not along the material border.

The results of this investigation indicate that SG neurite extension is stimulated by contact with platinum. They further suggest that the accurate embedding of the platinum electrode into the silicone array could increase neurite/electrode contact and might help to optimize neuronal responses of an auditory prosthesis.

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660 Neurons of the Central Nucleus of the Inferior Colliculus exhibit Different Firing Patterns to Repetitive Synaptic Waveform Injection

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Neurons in the auditory brainstem are able to follow a wide range of repetition rates of acoustic stimuli. In contrast, neurons in the inferior colliculus (IC) respond well only to relatively low repetition rates. Since inhibitory processing seems to be only partially responsible for this modification of response properties, we were interested in exploiting whether intrinsic properties of the cells (e.g. voltage gated channels) might be also involved.

We recorded the responses of neurons in the central nucleus of the IC to 400ms long depolarizing current pulses in the whole cell current clamp configuration from P16-P19 rats. Consistent with previous reports (Peruzzi et al. 2000), we found both onset and sustained type cells. Onset type neurons respond with only one spike to the beginning of a current pulse, whereas sustained type cells respond with spikes through the depolarizing step. In the present study, we found that in onset cells the current step was followed by a large (up to 10mV) hyperpolarization that lasted up to 50ms. This after-hyperpolarization was much smaller or absent in sustained firing neurons. Next, we recorded responses to simulated repetitive synaptic stimulation. For this we injected current waveforms modeled after excitatory synaptic currents obtained previously from voltage clamp recordings of IC neurons (risetime: 0.3ms, decay τ=3ms). Onset neurons were unable to fire to repetition rates higher than 20Hz, while sustained type neurons responded to frequencies of 200Hz.

This suggests that in onset neurons a voltage or Calcium activated K-current influences the firing to repetitive current stimulation.

661 Physiological Properties of NMDA Receptors in the Rat's Inferior Colliculus

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Our previous studies have shown that AMPA and NMDA receptors in the central nucleus of the inferior colliculus (ICC) contribute respectively to the early and late components of EPSPs evoked by electrical stimulation of the lateral lemniscus (LL). Removal of Mg^{2+} from the extracellular fluid enhances the NMDA receptor mediated EPSP amplitude, indicating that NMDA receptors in ICC neurons are susceptible to Mg2+ blockade. To study further the properties of NMDA receptors in ICC neurons, we made whole-cell patch clamp recordings in voltage clamp mode. Wistar rats, 10-14 days old, were used. Brain slices were cut in the frontal plane and submerged in oxygenated artificial cerebrospinal fluid (ACSF). EPSCs were evoked by stimulation of the LL at holding potential of -60 mV. Strychnine and bicuculline were added to the bath to block glycinergic and GABAergic inhibition. NMDA receptor mediated EPSCs were further isolated pharmacologically by adding CNQX to the bath. The NMDA receptor mediated EPSCs showed longer rise time and decay time constants compared to AMPA receptor mediated ones, and had non-linear current-voltage relationships. The I-V curves for NMDA receptor mediated EPSCs showed a negative conductance at membrane potentials more negative than -25 mV. The sensitivity of NMDA receptor mediated EPSCs to Mg^{2+} was tested with the slice in Mg2+ -free ACSF. In most neurons (12/16) the EPSCs increased up to 9 fold relative to control EPSCs. In 6 out of these 12 cells, the increase was less than 3 fold. These results suggest that NMDA receptors in ICC neurons vary widely in their sensitivity to Mg^{2+} block, but generally are less sensitive to Mg^{2+} compared to those in hippocampal pyramidal cells. NMDA receptors in ICC neurons are activated at voltages near the resting potential, which may be attributed to lower sensitivity of Mg2+ blockade.

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662 The Inferior Colliculus (IC) uses Two Cellular Mechanisms for Spike Frequency Adaptation During a Synaptic Stimulus.

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In the IC, spike frequency adaptation is thought to contribute to auditory motion cues. The most common ionic currents underlying adaptation are Ca^{++}-dependent K^{+}; slow, voltage-dependent K^{+}; and Na^{+}-dependent currents. We have examined the cellular mechanisms used by IC neurons to adapt during a synaptic stimulus.

Whole-cell patch clamp recordings were made from IC neurons in rat brain slices. Stimulation of the lateral lemniscus (LL) with a bipolar electrode resulted in excitatory postsynaptic potentials (EPSPs) in all IC neurons examined. Evoked EPSPs had long decay times of 0.6-1.2s, similar to the decay times of spontaneous EPSPs. Comparisons of synaptic currents with synaptic potentials indicated that IC neurons use two different mechanisms to prolong EPSPs. In neurons that show regular firing during current injection (sustained-regular, rebound-regular, pause/build neurons), EPSP decay was determined mainly by slow membrane time constants, whereas in neurons that showed adaptation (rebound-adapting cells), EPSP decay was determined by the decay of the NMDA-mediated synaptic current.

Prolonged EPSPs evoked by LL stimulation generated sustained firing in all IC neurons. Moreover, all IC neurons showed spike frequency adaptation during EPSP-evoked firing, even those that did not normally adapt during current injection. Two distinct mechanisms accounted for adaptation during synaptic stimulation. In rebound-adapting cells adaptation during an EPSP was due to an apamin-sensitive current. The majority of IC neurons, however, adapt during firing because of the decreasing membrane potential during the prolonged decay phase of the excitatory synaptic potential. Thus, all IC neurons exhibit sustained firing and spike frequency adaptation during prolonged synaptic potentials.

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663 Inferior Colliculus Excitatory Neurotransmitters in Sleep and Wakefulness

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The auditory is the main sensory system remaining active while asleep. Thus, it may exist a special relation between audition and sleep(1). Inferior colliculus unit firing was reported increasing, decreasing and without changes during sleep in comparison with wakefulness. No neuron stopped its firing on passing from wakefulness to sleep (2).

Firstly, single-unit activity was recorded before and after iontophoretic ejection of a receptor specific NMDA antagonist, 2-amino-5-phosphonovaleric acid (AP5), during sleep and waking (n=21). Secondly, inferior colliculus auditory neurons were studied during both kynuretic acid (Kyn) iontophoresis and auditory cortical electrical stimulation in anesthetized guinea pigs and during wakefulness (n=37). Bipolar cortical stimulating electrode was placed in AI region. Double barreled micropipettes were used in both preparations filled with, 3M Sodium acetate and AP5 or Kyn acid.

The AP5 determined a decrease in most of the units recorded, mainly in the later part of the response, while a low proportion of them exhibited a firing increase, during both wakefulness and sleep phases, i,e., slow wave and paradoxical sleep.

Both Kyn acid iontophoresis and cortical (AI) electrical stimulation evoked inferior colliculus units decreased firing rate, while a low number of them increased their discharge, in anesthetized and also in awake guinea pigs.

1. Actions of excitatory amino acid on the ICc cells are present during wakefulness, slow wave and paradoxical sleep. 2. AP5 may block actions of cortical descending system and/or ascending incoming auditory signals. 3. The cortico-ICc efferent effects are at least partially mediated by ionotropic excitatory amino acid receptors. 4. Both, Kyn acid and AP5 provoke ICc firing decreases and increments that may include inhibitory neurons through direct and indirect circuital actions.

1. Biol. Signals Recept. 9: 297-308, 2000.

2. J. Sleep Res. 4: 242-251, 1995.

664 Effects of TEA on Neural Responses in the Inferior Colliculus to Tone Bursts and Amplitude Modulated Sounds

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We studied the effect of a potassium channel blocker, tetraethylammonium (TEA), on responses in the rat inferior colliculus to tone bursts and sinusoidal amplitude modulated (SAM) sounds. Single unit activity was recorded and iontophoretic drug delivery was made using a multi-barrel electrode assembly. We found that TEA increased the duration of spikes in response to acoustic stimulation. For the majority of IC neurons, TEA increased firing rate in response to both tone bursts and SAM sounds. For some neurons, although there was no drug effect with tonal stimulation, there was an increase in firing rate with SAM stimulation. For other neurons, there was a reduction of firing rate in response to tone bursts, and either an increase, a decrease, or no change in response to SAM sounds. In about half of the neurons, TEA affected the synchrony of responses to SAM sounds. The vector strength was reduced at off-peak modulation rates during drug application, especially at the low frequency flank of the modulation transfer function. A reduction in vector strength was usually accompanied by an increase in firing rate. However, there were cases in which a reduction in vector strength was found without any change in firing rate. Also, in some neurons, there was an increase in firing that was not accompanied by a reduction in vector strength. The results suggest that potassium channels play an important role in the regulation of sound-evoked responses in the inferior colliculus and contribute to the synchrony of responses locked to the envelope of amplitude modulated sounds.

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665 Influence of the NMDA-Antagonist Memantine on Salicylate-Induced Tinnitus in Mongolian Gerbils: A 14C-2-Deoxyglucose-Study

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Subjective tinnitus, an auditory sensation without corresponding acoustic stimulus may be the perceptual correlate of altered spontaneous neural activity. Experimentally tinnitus can be evoked by salicylate intoxication that also causes an elevation of hearing threshold. The latter effect originates in the inner ear. Therefore and because of easy accessibility in humans most pharmacological studies focus on the cochlea. PET-studies, however, revealed altered activation related to tinnitus at higher levels of the auditory pathway (e.g. Arnold, Bartenstein, Oestreicher et al., 1996). In the present study the effect of memantine on tinnitus-related activity was investigated with the 14C-2-deoxyglucose (2DG)-method. Gerbils received i.p. injections of salicylate (350mg/kg body weight) which was followed by an i.p. injection of memantine (1mg/kg body weight) or saline two hours later. A third group received memantine only. Immediately thereafter gerbils received 18 µCi of 2DG i.p. and were left in a sound proof chamber in silence for 90 min. In all groups 2DG uptake was weak in ventral cochlear nucleus (CN), moderate in dorsal CN, and high in auditory cortex (AC). Differences between treatments were found in the inferior colliculus (IC): salicylate with saline produced weak 2DG uptake, whereas salicylate with memantine led to enhanced uptake, and highest uptake was seen after memantine alone. As described earlier salicylate reduces activity in VCN and IC but enhances activity in AC compared to saline controls (Wallhäusser-Franke, Braun, Langner, 1996). In contrast, memantine increases activation of IC and AC but not of VCN, the recipient of cochlear input. We conclude that memantine injected together with salicylate ameliorates the reduction of acticity found in IC after salicylate. With the doses used so far memantine does not, however, lead to a reduction of the enhanced cortical activation present after salicylate intoxication.

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666 Descending Neurons in the Medial Geniculate Body in the Gerbil

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Descending projections are found in most of the main auditory pathways from cortex to cochlea. These projections are thought to contribute to forming feedback circuits involved in various functions of auditory signal processing, including auditory and multimodal feedback systems. While a prevailing notion is that the hierarchical chain of descending projections bypasses geniculo-collicular pathway, a previous study (Kuwabara and Zook, Brain Res. 878:79-87.2000) demonstrated clear descending projections primarily from the medial and dorsal divisions of the medical geniculate body (MGB) to the external nucleus and part of dorsal cortex of the inferior colliculus (IC) in the gerbil. The above study used a combination of intra/extra cellular labeling and tract-tracing techniques applied to parasagittal brain slice preparations. The extent of the origin of descending axons within and around the auditory thalamus, however, has not been thoroughly investigated.

In this study, stereotaxic injection of either Fluoro-Gold or Fast blue was placed in vivo. Some of these animals were subject to subsequent quasi-parasagittal slice preparation for anterograde or intracellular labeling of identified area or single cells.

Neurons with descending projections to the IC were labeled retrogradely in the ventral part of the ventral division of the ipsilateral

MGB (MGBv) and even further ventral to the MGBv border, dubbed by Clerici and Coleman (JCN. 297:14-31., 1990) the auditory forebrain bundle area. Neurons in this region had a multipolar cell body with less extensively tufting dendrites. The injection site that yielded the most extensive retrograde labeling in this region was the caudal part of the dorsolateral division of the ICc and adjacent external nucleus. The results suggest the descsending projections are more extensive than previously thought.

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667 A novel projection from the dorsal cochlear nucleus to the medial division of the medial geniculate body in rat

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The inferior colliculus (IC) is thought to be an obligatory relay for ascending auditory pathways emanating from multiple cell types in the brainstem. However, several exceptions have been observed to this rule, but mainly from minor or peripheral cell groups in the auditory brainstem (e.g., Henkel, 1983, Brain res. 259:21-30). In the present study, we report a novel projection from the cochlear nuclear complex that bypasses the IC and ends directly in the medial geniculate body (MGB).

Two groups of experiments were performed. First, we made injections with anterograde tracers (biotinylated-, FICT-, and TRITC-labeled dextran) into physiologically defined regions of the DCN and VCN in rat. Second, injections of HRP were made into the MGB to study the afferent projections to it in the rat. A previously unreported projection was found that bypasses the IC and ends directly in the medial division of the MGB (mMGB) and possibly in the suprageniculate nucleus. This projection originates in the pyramidal cells in the DCN. The injections in the VCN fail to label terminals in the MGB. Collaterals of the labelled DCN axons also may terminate in the IC. Since the mMGB and suprageniculate nucleus is connected with the amygdala, we suggest that this projection may be implicated in the processing of the emotional significance of acoustic stimuli (e.g., LeDoux et al., 1984, J. Neurosci., 4:683-698). Thus, auditory information may reach the amygdala more quickly than previously thought.

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668 Projections from the Inferior Colliculus to the Tectal Commissural Column

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It has been previously noted that some of the inferior colliculus (IC) fibers that traverse the commissure of the inferior colliculus (CoIC) form two small terminal fields within the CoIC itself, one on each side of the midline (Fig. 4A-D of Saldaña and Merchán, 1992, J. Comp. Neurol., 319:417-437). With the recent discovery of the tectal commissural column (TCC), it is now clear that these previously reported terminal fields are located in the caudal third of the TCC.

To further characterize the projections from the IC to the TCC, we have injected the tracers *Phaseolus vulgaris* leucoagglutinin (PHA-L) or biotinylated dextran (BD) into either the medial IC (IC-m, which includes the central nucleus and the dorsal cortex of previous studies) or the lateral IC (IC-l, equivalent to the classical external cortex) of albino rats, and have studied the trajectory and distribution of the fibers labeled in the TCC.

Although the IC-m and IC-l differ markedly in their intrinsic and commissural projections, their projections to the TCC are remarkably similar. All subdivisions of the IC send bilateral projections to the TCC. While virtually the entire rostrocaudal extent of the ipsilateral TCC is reached by IC fibers, only the caudal third or half of the contralateral TCC is innervated by the IC. On both sides the density of the terminal fields decreases progressively from caudal to rostral. We have found no obvious topography/tonotopy in the IC-to-TCC projections.

IC fibers enter the TCC by two routes. Most terminal fibers are collaterals of axons that travel in the CoIC. Others are collaterals of colliculo-geniculate axons; from the brachium of the IC, these latter collaterals travel in the deep layers of the superior colliculus to reach the ipsilateral TCC, where they end.

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669 Reciprocal Connections between the Superior Olivary Complex and the Tectal Commissural Column

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The tectal commissural column (TCC), a novel nucleus of the midbrain tectum, was discovered when many of its neurons were labeled after injections of FluoroGold into the ipsilateral superior olivary complex (SOC) of the rat. Such large injections did not reveal which SOC nuclei are innervated by the TCC. On the other hand, the injection of the bidirectional tracer, biotinylated dextran (BD) into the TCC labels cell bodies in numerous neural centers, including the superior paraolivary nucleus (SPN). These data indicate that the TCC and the SOC of the same side are reciprocally connected, but additional information is needed about the SOC nuclei or cell types involved in this loop.

To identify the targets of tecto-olivary projections, we made small injections of BD into different nuclei of the rat SOC. The number of neurons labeled in the TCC was highest following injections into the SPN. Fewer neurons were labeled after injections into the ventral nucleus of the trapezoid body (VNTB) or the dorsal ribbon of the SOC (dSOC). No TCC neurons were labeled after injection in other SOC nuclei. Only in cases with SPN injection were fibers labeled in the TCC. To innervate the TCC, some SPN axons by-pass the IC and branch in the deep layers of the superior colliculus before reaching the ipsilateral TCC at different rostrocaudal levels.

The injection of BD into the TCC revealed the morphology of tecto-olivary projections, which are largely ipsilateral. As expected, labeled terminal fields were found along the entire SPN, and also in the VNTB and dSOC, but not in the SOC principal nuclei. Other cases with BD injection into areas surrounding TCC suggest that tectal projections to the SPN originate only in TCC, whereas those to the VNTB and dSOC may arise partly from the periaqueductal gray matter.

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670 Cytochrome oxidase histochemistry and soybean agglutinin staining label complementary regions of the IC in the gerbil

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We have shown previously that different regions in the inferior colliculus (IC) of the gerbil can be distinguished using cytochrome oxidase (CO) histochemistry. A central core of dense CO staining is surrounded on all sides by areas of less intense staining. Projections to the IC from the lateral and medial superior olivary nuclei terminate

almost exclusively in the CO-dense core of the nucleus. We show here that soybean agglutinin (SBA), a plant lectin, also exhibits a differential staining pattern in the gerbil IC. (The pattern of SBA staining in the gerbil is very similar to that obtained with Wisteria floribunda agglutinin as reported recently in rat IC [Horta, Cruz-Rizzolo, Merchan, Saldana and Lopez, International Symposium on the Central Auditory System, Salamanca, Spain, 2001]). A central core in the IC is stained lightly, if at all, with SBA; this core corresponds almost exactly to the part of the nucleus that is most heavily labeled with CO. Surrounding this SBA-free core on all sides are regions of dense, but not uniform, SBA staining. In the dorsolateral and dorsocaudal IC (in the deep layers of the classically defined external cortex) SBA staining appears as a series of dense patches separated by SBA-free areas. In the ventrocaudal IC and in the rostral IC (rostral to the location of the CO-dense core), the SBA staining is more uniform. Ventromedially, the SBA staining is quite dense and may overlap the most ventral region of dense CO-staining. Otherwise, the staining seen with the two markers appears complementary. Markers such as CO and plant lectins appear to distinguish regions within the IC which probably receive different patterns of inputs. These labeling methods, which are simple to perform, may provide a means to visualize different functional domains within the IC.

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671 Development of Auditory Projections to the Ferret Superior Colliculus

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The deeper layers of the superior colliculus (SC) contain a topographic representation of auditory space. Retrograde tracing studies have shown that the adult ferret SC receives a topographically-organized projection from the ipsilateral nucleus of the brachium of the inferior colliculus (nBIC; King et al., 1998, *J. Comp. Neurol.* 390:342-365). We have examined the postnatal development of this pathway, by making anterograde tracer injections (BDA-3000) in the nBIC, on postnatal days (P) 30, 60 and 90. After surgery and 1-3 days survival, the animals were perfused and the brains processed using the ABC method.

Our results show that the nBIC projects to the SC at all ages studied. The labelled fibers from the injection site in the nBIC run dorsomedially into the SC, where most of the terminals and *en passant* boutons that we observed were restricted to the deepest layers. Terminals were located along the trajectory of the incoming axons over the entire mediolateral extent of the SC. Estimation of the number of boutons using the 'Optical Fractionator' revealed that these were distributed in a Gaussian fashion along the rostrocaudal axis of the SC. The location of the peak of the distribution varied with the location of the injection site in the nBIC at all ages, but at P30 this distribution appeared to be more focussed than in older animals. In addition to the anterograde labelling, some retrogadely-labelled cells were seen in the external cortex and central nucleus of the IC, and may have contributed to the terminals observed in the SC.

672 Differentiation of the auditory pathway of adult tree shrews revealed by calcium-binding proteins

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The auditory pathway of the tree shrew, an archaic mammalian species with a vocalization repertoire within the human hearing range, was studied with the calcium-binding proteins (CBPs) calbindin-D28k (CB), calretinin (CR) and parvalbumin (PA) to characterize the subdivisions of auditory nuclei from the brainstem to the auditory cortex (AC) using immunohistochemical methods.

Within the CN the superficial layers of the DCN showed few or no IR-somata for CB and CR, but many PA-IR neurons. In the deep layers cells labeled for all CBPs were present. Both parts of the VCN were devoid of CB-IR cells, while many somata and fibers were stained for CR and PA. In the SOC somata of the MNTB were labeled with all CBPs, MSO and LSO were devoid of CB-IR cells, while there were labeled cells for CR and PA. In the central band of the MSO fibers were labeled for CB and neurons plus fibers for CR and PA. In the LL dorsoventrally-running fibers were stained for CBPs. In the DNLL somata with mediolaterally-oriented dendrites were labeled for all CBPs. While the ICc showed only little labeling for CB and CR, there were many cells and fibers labeled for PA. In contrast, the ICp was devoid of PA-IR cells, while there were many cells labeled for CB and CR. Compared to the ICc the ICx was densely labeled for CR-IR fibers. Compared to the MGBd and MGBm the highest number of labeled cells was present in the MGBv for all CBPs. In the AC cells were labeled in layers II-VI (CB, PA) or IV-VI (CR). While CB-IR cells were equally distributed within the AC, there were more CR-IR cells within the inner belt and core and more PA-IR cells within the outer belt and core. In conclusion, the CBPs used in our study are powerful markers for characterizing neuronal subpopulations in the different nuclei of the auditory system and are especially promising tools for the investigating of the different divisions of the auditory cortex of the tree shrew.

673 Immunolocalization of Homer 1a and Homer 1b/c in the rat central auditory system.

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Group I metabotropic glutamate receptors (mGluRs) are G-protein coupled receptors that are coupled to phospholipase C and are important in excitatory neurotransmission. Homer was isolated as an immediate-early gene that is induced during increased levels of activity in the central nervous system, but is now known to be a member of a multigene family of scaffolding proteins that target and regulate the function of class I mGluRs and regulate the activity of N-type Ca++ and M-type K+ voltage-sensitive channels. Although much is known about Homer's in vitro binding properties and of Homer's importance in mGluR1a/5 targeting, little anatomical information about Homer distribution in the intact CNS is known. We examined the distribution of Homer 1a (Santa Cruz Biotechnology, Inc; sc- 8922) and Homer 1b/c (Paul Worley, Johns Hopkins Medical School) in the central auditory pathways of the adult rat using peroxidase immunocytochemistry. Homer 1a was found to be expressed in several structures of the central auditory pathways, including the dorsal cochlear nucleus [DCN], the central nucleus of the inferior colliculus [CIC] and in primary auditory cortex [Au1]. The distribution pattern of Homer 1a was punctate, and localized to dendritic processes and neuropil. Homer 1b/c was found to be expressed in several central auditory structures as well, including DCN, CIC, and in layer 5 pyramidal neurons in Au1. Puncta of Homer 1b/c immunoreactivity localized to somatodendritic compartments. Surprisingly, the overlap in the distribution of Homer proteins with mGluR1a is limited in Au1. These data suggest that Homer may function in targeting and regulation of group I mGluRs and/or other important signaling proteins, but that the overlap in the distributions of Homer proteins and mGluR1a varies by region in the central auditory system.

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674 Cross-Modality Matches for Long and Short Tones: Testing the Equal-Loudness-Ratio Hypothesis

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This study tests the Equal-Loudness-Ratio hypothesis [Florentine et al., J. Acoust. Soc. Am. 99, 1633-1644 (1996)], which states that the loudness ratio between equal-SPL long and short tones is independent of SPL. The amount of temporal integration (i.e., the level difference between equally loud short and long sounds) is maximal at moderate levels. Therefore, the Equal-Loudness-Ratio hypothesis predicts that the loudness function is shallower at moderate levels than at low and high levels. Equal-loudness matches and cross-modality string-length matches were used to assess the form of the loudness function for 5- and 200-ms tones at 1 kHz and the loudness ratio between them. Results from nine normal listeners show that (1) the amount of temporal integration is largest at moderate levels, in agreement with previous studies, and (2) the loudness functions are shallowest at moderate levels. For eight of the nine listeners, the loudness ratio between the 200- and 5-ms tones is approximately constant, except at low levels where it tends to increase. The average data show good agreement between the two methods, but discrepancies are apparent for some individuals. These findings support the Equal-Loudness-Ratio hypothesis except at low levels.

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675 A Comparison of Psychophysical Procedures for Level-Discrimination Thresholds

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Five different psychophysical procedures were used to measure level-discrimination thresholds for 1-kHz tones at two levels (30 and 90 dB SPL) and two durations (5 and 500 ms). The set of procedures included: The classic adaptive staircase method with a 2AFC paradigm, two variations of the Method of Maximum Likelihood (MML) with a cued yes-no paradigm (15- versus 50-trial blocks), and two variations of ZEST (a cued yes-no versus a 2AFC paradigm, both with 18-trial blocks). Adjusting for different d' convergence points among the different procedures, results obtained from nine normal listeners show that all the procedures produced the similar estimates for all four conditions. The within-subject variances of threshold estimates within a stimulus condition were statistically indistinguishable among the procedures, except that the MML with 15 trials had significantly greater variance. The up-down staircase took the most number of trials (around 85) to complete a threshold estimate and took the longest time; the ZEST and short MML procedures required the least times. One measure of the performance of psychophysical procedures is the sweat factor (SF)--defined as the product of the variance and the number of trials. The SFs for the tested procedures show clear trends among them. In order of increasing performance, the sweat factors were: MML (15 trials)=5.54, MML (50 trials)=3.29, ZEST (yes-no)=1.71, up-down=1.43, and ZEST (2AFC)=1.15. The yes-no paradigms were the worst, whereas the 2AFC paradigms were the best. The ZEST method compared favorably to the established up-down method; ZEST required the relatively fewer trials per block, but more assumptions. The difference in the performance between the two paradigms used with the ZEST method may reflect the effects of response-criterion shifts across sessions, as suggested by Florentine, Marvit, and Buus (2001, J. Am. Acad. Audiol. 12(3):113-120).

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676 Differential Influence of Temporal Parameters on Level Discrimination Within a Sequence of Sound Pulses

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Recent studies determined that a listener's ability to identify differences in sound pulse level within a sequence depends on the time course of pulses. Listeners attempted to detect differences in sound level between standard pulses and pseudorandomly presented oddball pulses when pulses were presented at different rates. Overall, increasing the pulse rate enhanced the ability to discriminate small increments in the level of oddball pulses. However, it was unclear how temporal parameters that changed with pulse rate contributed to differences in level discrimination. The focus of the present study was to determine to what degree interonset interval of pulses or interpulse interval contributes to a listener's ability to discriminate level differences.

Adult participants with normal hearing thresholds listened to pulse sequences monaurally. Listeners were trained in the oddball paradigm to respond to sound pulses when they were perceived to be louder than the standard pulses. Standard (30 dB above threshold in response to a fixed amplitude pulse train) and oddball pulses (1 dB, 2 dB, and 3 dB re: standard pulse level) were spectrally identical broad band noise bursts. At each oddball sound level discrimination performance was measured for different pulse interonset intervals (40 ms to 250 ms) and interpulse intervals (10ms to 160 ms). Pulse duration (10 ms to 90 ms, 0.5 ms rise/fall time) was shifted accordingly to achieve the different pulse intervals. The accuracy of responses and reaction time (re: oddball pulse onset) were evaluated.

Interpulse interval showed a stronger influence on level discrimination ability than interonset interval. Poorer performance in response to pulse sequences with longer interpulse intervals could be improved by increasing pulse duration.

677 Influence of Component Phase on the Loudness of Complex Tones

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Sounds with strongly modulated envelopes undergo differential compression in the peripheral auditory system; medium to high level portions are amplified less than low level portions. One might expect this to affect the loudness of sounds with the same magnitude spectrum but differing phase spectra; phases giving waveforms with a high peak factor would be expected to lead to lower loudness than phases giving a lower peak factor. This was investigated by asking four listeners to match the loudness of complex tones and noise. The complex tones had a fundamental frequency of 62.5 Hz and were filtered into a frequency range from 625 Hz (10th harmonic) to 5000 Hz. The Gaussian noise was filtered in the same way. The components of the complex tones were added either in cosine phase (CP), giving a high peak factor, or in random phase (RP), giving a lower peak factor. Three tasks were used: (1) matching the loudness of the CP tones with that of the RP tones, and vice versa; (2) matching the loudness of the CP tones with that of the

noise, and vice versa; (3) matching the loudness of the RP tones with that of the noise, and vice versa. The task were performed using six different levels of the fixed stimulus, ranging from 30 dB SPL to 80 dB SPL in 10 dB steps. Results were not consistent with the above hypothesis. CP tones were adjusted to a lower rms level than RP tones and than noise at the point of equal loudness, indicating that at equal levels the CP tones would sound louder. RP tones were adjusted to a lower level than noise at the point of equal loudness. The differences in level were greatest for mid-range levels of the fixed stimulus. In a second experiment, a continuous bandpass noise was present, in order to mask distortion products which might have caused the loudness differences. The bandpass masker reduced the observed differences in loudness somewhat, but the basic pattern of results was unchanged.

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678 The Mid-Level Hump in the Intensity-Difference Limen at 2 kHz Enlarges Significantly Under Forward Masking

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Detection and discrimination thresholds were determined for a 2-kHz tone-pip shaped by a Gaussian envelope 8 msec long (equivalent to a rectangular duration of 2.51 msec). Nine subjects participated. First, intensity-difference limens (DLs) were obtained as a function of level. These DLs peaked at 50 dB SPL, replicating earlier observations (Nizami et al., JASA, in press 2001). Pip-detection thresholds were then obtained as a function of the level of a 200-msec 2-kHz forward masker, at delays of 0, 10, 30, or 100 msec. Subsequently, intensity-difference limens were obtained for the tone-pip as a function of tone-pip level at 10 msec and 100 msec after the offset of a 50 dB SPL forward masker, and at 100 msec after the offset of a 70 dB SPL forward masker. For all 3 masker/delay conditions, the level-dependence of the tone-pip DL shows a mid-level hump that is significantly larger than the hump found without the forward masker. Within each forward-masking condition, the DLs are a constant multiple of the DLs obtained without the forward masker. Sensation-level (SL) scales were constructed for each masker/delay condition by subtracting the forward-masked detection thresholds from the pedestal levels. In these scales, the levels at which the largest DLs occur are comparable for all 3 forward-masking conditions. The level-dependence curves of the DLs for non-forward-masked 2-kHz Gaussian-shaped tone-pips of D=1.25 msec and D=2.51 msec, from Nizami et al., also have peaks that line up in SL scales, but 6.6 dB higher, a difference that is significant at $p<.05$. An SL transformation was also performed for the DLs for forward-masked mid-frequency tones presented in notched noise (data of Zeng, JASA 103, 2021-2030, 1998). The curves for lower notched-noise levels also tend toward alignment in SL scales. Sensation level, rather than SPL, may be the appropriate scale with which to examine the position of mid-level humps.

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679 Psychometric Functions for Variable Masker Levels in the Context of Peripheral Nonlinearity

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A recent model of forward masking that incorporates a peripheral nonlinearity assumes that response growth is nonlinear at the signal place for on-frequency forward maskers, but linear for maskers at frequencies well below the signal frequency. Psychometric functions (PFs) can be reconstructed from procedures in which signal level is adapted, but also can be obtained when masker level (ML) is adapted. When ML is held constant and signal level is adapted, the signal passes through the same nonlinearity regardless of masker frequency. In this case, the model predicts that slopes of PFs should be similar for on- and off-frequency maskers. When ML is adapted and signal level is constant, only on-frequency maskers pass through the nonlinearity at the signal place. In this case, slopes of PFs should differ for on- and off-frequency maskers. PF's as a function of ML have not been reported in the literature to our knowledge. In the current study, signals were fixed at 30 and 50 dB SPL, and ML at threshold was determined for 4 normal-hearing adult subjects in a two-interval forced choice adaptive procedure. The signal was a 4-kHz, 10-ms tone. The on- and off-frequency maskers were 200-ms tones presented at 4 and 2.4 kHz, respectively. All signal delays were 0 ms. MLs at threshold were similar for the 30-dB signal for on- and off-frequency maskers. A higher ML was required in the off-frequency than on-frequency masker case to mask the 50-dB signal. Slopes of PFs as a function of ML for on-frequency maskers were shallower for higher MLs, whereas slopes of PFs for off-frequency maskers either showed no effect or increased at higher MLs. For the same subjects, slopes of PFs as a function of signal level were similar for on- and off-frequency maskers. The results are consistent with the model.

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680 Examining reaction time (RT)-intensity functions in normal-hearing infants and adults

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Reaction time (RT) to sound is related to loudness in adults. Recently, we trained infants to make head turns in response to suprathreshold tones and measured the RT of the head turns recorded by an observer's button press. A systematic decrease in RT with increasing intensity was found, suggesting that RT holds potential as a measure of loudness in infants. An examination of the RT-intensity functions suggested that infant slopes were steeper than adult slopes. However, considerable variability existed, making the interpretation difficult. One potential source of variability is the observer's contribution to the overall RT. The purpose of this study was to replicate the initial findings, using methods to reduce variability. Specifically, RT was measured offline using videotape to eliminate the observer's contribution to the overall RT. Pure tones at 4000 Hz were presented monaurally through an ER1 insert earphone for 500 msec. 5 tones ranging in 10 dB steps from 50-90 dB SPL for infants and 40-80 dB SPL for adults were presented 7 times each. A conditioned head-turn procedure was used to test infants. Adults were instructed to turn their head to the right whenever they heard a tone. An observer sat outside the booth and started presentations when the subject was quiet and facing ahead. Reinforcement was provided after each correct head turn. RT was scored offline from the videotape by measuring the time required for a 45-degree head turn following the signal onset. The median RTs measured offline using videotape were compared to the mean RTs obtained in the initial study. Consistent with the original investigation, RT significantly decreased with increasing intensity for both age groups. The effects of intensity on RT were similar in the current results to that observed in the initial investigation, suggesting that the apparent difference between infants and adults in RT-intensity function slope is not an artifact of the observer's contribution to the overall RT.

681 Measuring Informational Masking Using the Multiple-Bursts Procedure

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In the "multitone masking" paradigm introduced by Neff and Green (Percept. Psyphys, v41, 1987), the masker typically is a small number of tones having frequencies and levels that are randomly drawn on

every presentation. Large amounts of masking for a pure-tone signal often occur that are thought to reflect central, rather than peripheral, limitations. Previous work from our laboratory has indicated that playing a rapid succession of randomly-drawn multitone maskers in each observation interval dramatically reduces the amount of masking that is observed relative to a single multitone masker burst (SB). In this "multiple-bursts different" (MBD) procedure, the signal tone is the only constant frequency component during the sequence of bursts and tends to segregate perceptually from the masker. In this study, we varied the number of multitone masker bursts and the interburst interval (IBI). The goal was to determine how many bursts were needed to reduce informational masking relative to SB and to examine whether lengthening the IBI would cause each burst to be processed independently, which might disrupt the perception of signal coherence. The number of 50-ms masker bursts was varied from 1 to 8 and the IBI ranged from 0 to 500 ms. For subjects showing large amounts of masking for SB, substantial reduction in masking occurred as the number of masker bursts increased. Further, the amount of masking increased as the IBI increased. Subjects demonstrating little masking in SB were much less affected by the number of masker bursts or the IBI. The results indicated that even two masker bursts are sufficient to produce a significant reduction in masking relative to SB, and that the MBD advantage persists for IBIs as long as 100 ms. Discussion will focus on the expected improvement in performance due to multiple "looks" compared to that obtained in the various burst number/IBI conditions.

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682 Release from energetic and informational masking using spatial separation of speech.

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The effect of spatial separation of sources on the masking of a speech signal was investigated for 3 types of maskers, ranging from energetic to informational. Normal-hearing listeners performed a 4- by 8-alternative identification task embedded in CRM (Coordinate Response Measure) sentences of the form: "Ready baron go to [color] [number] now." Listeners identified color and number in the presence of a masker at various signal-to-noise ratios. The stimuli were presented in a quiet soundfield. The signal was played from the front speaker (0 deg) and a masker was played either from the same speaker or from 90 deg to the right. All sentences were pre-processed through a modified version of a cochlear-implant simulation program. Each sentence was filtered into 15 frequency bands. The envelope was extracted from each band and used to modulate a pure-tone at the center frequency of the band. On every presentation, 8 randomly-chosen bands from the 15 bands available were selected, summed and played as the signal. The 3 maskers were 1) 6-band CRM sentences: processed as described above, but only 6 bands (different from the 8 chosen for the signal) were selected and summed to form the masker (primarily informational); 2) 6-band speech-shaped noise: the long-term spectrum of the 6-band sentence (in #1) was multiplied by broad-band Gaussian noise; and 3) 8-band speech-shaped noise: the sentence used in #1 was processed into the same 8 bands as the signal sentence and its spectrum was multiplied by broad-band Gaussian noise (primarily energetic). Results revealed that in the 6-band sentence masker, the effect of spatial separation on intelligibility ranged from 15 to 25 dB, while in the 8-band and 6-band noise maskers the effect was less than 10 dB. These results suggest that, in these conditions, the advantage due to spatial separation of sources is much greater for informational masking than for energetic masking.

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683 Sensation Level and Decision Weights as Factors Affecting Informational Masking in Hearing-Impaired Listeners

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Thresholds for a 0.8, 2.0, and 5.0-kHz signal were measured in 16 normal-hearing and 9 hearing-impaired listeners in the presence of a simultaneous multitone masker. The masker consisted of fixed-frequency tones that occurred independently and at random with a probability $p = 0.1$-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. Listener decision weights were estimated from correlations of the listener's response with the presence or absence of the signal and individual masker components on each trial. Sensation levels (SLs) for signal and masker components were also determined from quiet thresholds for each component. The results showed a wide range of amounts of informational masking in normal-hearing listeners (approximately 40 dB), but little or no informational masking in the hearing-impaired listeners when the signal was in a region of hearing loss. Decision weights were similar for both groups. The decision weights and SLs were incorporated as factors in a model (no free parameters) that accurately predicted performance based on the binomial variance in the SL of masker components [Lutfi, J. Acoust. Soc. Am. 94, 748-758 (1993)]. Model results suggested that reduced sensitivity accompanying a hearing loss may effectively reduce masker uncertainty, and so the amount of informational masking, by limiting the potential range of audible masker levels having the greatest decision weights.

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684 Testing the Theoretical Viability of Spectral Enhancement

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Extensive research has documented the spectral resolution of the normal and impaired mammalian auditory system. This research consistently shows that an impaired auditory system has reduced spectral resolution. More than simply increasing threshold, such peripheral impairments reduce speech intelligibility, especially when competing sounds are present. The goal of hearing aid design is to restore a human's partially impaired ability to hear. Traditionally, the main aspects of design include amplification and some sort of amplitude compression. These, however, do not account for the loss in frequency resolution. More recently, spectral enhancement processing has been offered that putatively restores aspects of frequency resolution. The main feature of this system is to increase the peak to trough ratio of the speech spectrum. Cochlear models have suggested that since the auditory filters are already wider in an impaired ear, the enhancement introduced to a signal will be lost through the broadened filter (Giguere & Smoorenburg, 1998). However, some evidence exists suggesting significant benefits to particular implementations of spectral enhancement (Miller et al., 1999, Simpson et al., 1990). The goal of this study was to test the idea of spectral enhancement without using a specific spectral enhancement algorithm. In that regard, subjects listened in two psychophysical experiments: detecting a spectral increment in broadband noise and discriminating center frequency change of a narrowband noise in the presence of broadband noise. Preliminary results show that normally hearing subjects have an improved ability to detect and discriminate frequencies when there is a

spectral decrement at frequencies adjacent to the increment. These results suggest that the idea of spectral enhancement is theoretically viable.

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685 Carboplatin-induced inner hair cell loss: effects on threshold, threshold in noise, gap detection and tuning.

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Previous studies have shown that selective outer hair cell (OHC) lesions can lead to threshold increases of 40-60 dB and impaired frequency selectivity. However, the effects of inner hair cell (IHC) loss are poorly understood because it is difficult to selectively destroy IHCs without also damaging OHCs. The obstacle has been overcome in the chinchilla where it is possible to use carboplatin, an antineoplastic drug, to selectively destroy IHCs and type I auditory nerve fibers without affecting the OHCs. Thus, carboplatin-treated chinchillas provide a powerful model for determining how IHC lesions influences different aspects of hearing function. The present study extends our previous behavioral measures of hearing performance measures in chinchillas with selective IHC loss by obtaining gap detection thresholds and tone thresholds in notch noise. Chinchillas were trained to respond to the appropriate stimulus conditions using a shock-avoidance conditioning procedure. Behavioral measures obtained before and after carboplatin-treatment included threshold in quiet (0.25-11.2 kHz), tone thresholds in wideband noise (50 dB SPL), gap detection thresholds (silent intervals) in continuous wideband noise (40-75 dB SPL), and tone thresholds in the presence of notched-noise maskers. Preliminary results show that significant IHC loss (20-90%) had almost no effect on pure tone thresholds in quiet, but tone thresholds in noise increased up to 10-20 dB after carboplatin treatment; the increase of threshold in noise was closely related to the amount of IHC loss suggesting that this measure may be a good indicator of IHC function. The new measures on gap detection and tone threshold in notch noise will be discussed in relationship to the amount of IHC loss.

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686 Dichotic Presentation of Spectro-Temporally Correlated Noise Create Illusory Moving Ripples

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Experimentation on binaural beat and binaural pitch phenomena have long been conducted by psychoacoustic researchers. In these experiments interaural time difference (ITD) cues are used to create illusory percepts that are not embodied by the physically presented sounds to each ear. Here we use interaural level difference (ILD) cues to create illusionary moving ripple sounds. To do this, we create uncorrelated spectrotemporal m-sequence stimuli for the contralateral and ipsilateral ears. Next a spectrotemporal correlation map is created between the contra- and ipsi-sounds. The spectrotemporal correlations of the ipsi-sound are then adjusted so that the correlation map resembles a moving ripple pattern. Two alternative forced choice tests confirm that correlated dicotic stimulation produces a strong percept of a moving ripple whereas its uncorrelated counterpart produces no effect. These effects were observed over a restricted range of modulation rates and ripple densities.

687 Binaural gap detection and interaural correlation discrimination under various interaural configurations

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We examined detection of interaural correlation (IC), using binaural gap detection (Akeroyd &Summerfield,1999) as a measure of temporal acuity for IC change, and the IC just noticeable difference (jnd) as a measure of acuity for static IC. In binaural gap detection, perhaps better construed as binaural change detection, a sequence of 3 contiguous wideband dichotic noises are presented in which the first and last (markers) have an IC of X, and the center ("gap") noise has an IC of Y, i.e. X/Y/X. The minimum duration of Y required for detection is determined, and this threshold has been shown to be related to the IC jnd from a reference of X. Thresholds were obtained for all X and Y configurations of homophasic (+1), antiphasic (-1), or uncorrelated (0) noise. These were compared with the IC jnd from corresponding reference points of +1, -1, and from 0 in a positive(0+) or negative direction (0-). For binaural gap conditions in which there was a reduction in IC from +1 or -1, the sequence and mean (n=8) thresholds(ms) were 1/0/1(2.39), -1/0/-1 (7.66). The corresponding reference points and mean (n=5) jnd's obtained were +1 (0.025) and -1 (0.055), mirroring the asymmetry observed in the binaural gap task. For binaural gap conditions with uncorrelated markers, thresholds (ms) were elevated and asymmetric for homo- and anti-phase "gaps": 0/1/0 (20.12), 0/-1/0 (41.43). This elevation and asymmetry is consistent with the elevated jnd's observed for 0+ (0.24) and 0- (0.385) conditions. For each listener, the ERD calculated for the binaural temporal window was similar across tasks, but varied between listeners. In general, the data indicate that discrimination of IC is easier within the positive range. This asymmetry in thresholds will be discussed in terms of different response properties of peaker and trougher neurons, which fire preferentially to homophasic and antiphasic sounds respectively.

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688 Distance Judgements in a Reverberant Room

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In an earlier study, listeners were asked to make relative depth judgments by indicating which of six loudspeakers in front of them had emitted a brief noise burst while seated in a reverberant room. Listeners indicated an ability to discriminate depth when the loudspeakers were atop a table, but not when suspended from the ceiling. It was concluded however, that an absolute judgment of distance was preferable to the relative judgment of depth which was used. In addition, since listeners were seated in a lighted room, visual cues may have influenced the results. The present study seeks to refine the procedure by attempting to remove any visual cues available to listeners, and to ask listeners to make absolute judgments of distance by entering a number of feet and/or inches on a computer keyboard. In one condition, listeners were seated in a darkened room, in which they could see neither the dimensions of the room nor the configuration of loudspeakers. In another condition, listeners were seated in a sound attenuating chamber, and listened over headphones to stimuli recorded by an acoustic manikin (KEMAR) seated in the room. Preliminary results indicate that while performance in either condition is not as good as in the lighted room condition, listeners perform as well in the KEMAR condition as they do when seated in the darkened room.

689 Binaural decoloration as a function of spatial separation

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A delayed copy of a sound (an echo) interacts with the original sound to produce a coloration of the sound. However, it has been demonstrated that when the origin of the echo differs significantly from that of the original sound in space, this coloration is greatly reduced. The current experiments were designed to investigate how this decoloration changes with increasing spatial separation of the original sound (lead) and the echo (lag). Using 500-ms noise bursts, listeners were asked to indicate the interval with the higher pitch corresponding to the shorter lead-lag delay. In an adaptive 2-AFC procedure, uncorrelated noise was added to the lead and lag with increasing relative level until a 6% pitch difference between the lead-lag combinations in the two intervals became inaudible. The relative level of uncorrelated noise at threshold is thus a measure of the pitch strength produced by the lead-lag combination. Results show that with increasing spatial separation between lead and lag, the pitch strength decreases rapidly within the first 20 to 30 degree azimuthal separation. In the median-sagittal plane, however, pitch strength remains strong independent of lead-lag spatial separation. Control experiments with a unilaterally hearing impaired subject confirm that binaural interaction is essential over and above the influence of head-shadowing effects. A unilateral pitch-strength model can predict the data of the unilaterally impaired listener quantitatively.

690 Generalization of Object Size in Echolocation

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From architectural acoustics, it is known that reflections of an object, defined by its acoustic impulse response, scale with object size. This means that the acoustic size of a virtual object can be manipulated by compressing or expanding its impulse response in time. Compression in the time domain is accompanied by expansion in the frequency domain, resulting in an upward shift of the center of gravity, and vice versa. The current study is designed to investigate to which extent echolocating bats can generalize these manipulations of virtual-object size due to object constancy. Four Megaderma lyra were tested in a 2-AFC playback experiment. The stimuli were generated by real-time FIR filtering of the bats' sonar call, using filter coefficients spectrally centered around either the calls' second (41 kHz) or fourth (82 kHz) harmonic. Random transmission characteristics within the pass band resulted in individually different impulse responses with the same spectral center of gravity. The bats were trained to discriminate impulse responses with a high center of gravity from those with low center of gravity. Then it was investigated to which extent the bats are able to generalize known, but spectrally compressed or expanded impulse responses. The performance was compared to the generalization of impulse responses with the same center of gravity but unknown characteristics within the pass band. Preliminary results show that spectral compression of known impulse responses was tolerated significantly more than compression of unknown impulse responses. This suggests an object constancy for known objects increasing in size. Unknown virtual objects, whether manipulated or not, seem to be categorized in terms of absolute spectral similarity. This is in good agreement with the results obtained from an auditory computer model, simulating the categorization of complex virtual objects in M. lyra in terms of similarity of the resulting excitation patterns.

691 Effects of Aspirin on Extended High-Frequency Audiometry, Otoacoustic Emissions (OAEs) and Binaural Performance

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Binaural changes in auditory function induced by ingestion of aspirin were monitored by pure-tone audiometry up to 16 kHz and by OAEs in normally hearing subjects. Narrow-band noise (NBN) stimuli were used to measure monaural sensitivity thresholds near spontaneous OAEs (SOAEs) and for binaural centered-image tests. Aspirin-induced hearing loss (AIHL) that developed over the period of drug treatment was bilateral with a predominance above 3 kHz and especially above 8 kHz. Most SOAEs progressively decreased in amplitude and disappeared during aspirin treatment, whereas some remained present. Aspirin created asymmetrical shifts of sensitivity thresholds between the two ears (up to 12 dB) when the NBN spectrum corresponded to the SOAE frequency. For the centered-image task performed with stimuli less than 30 dB SL, subjects kept the same difference in dB SL between the two ears during aspirin treatment as in the pre-aspirin condition. For stimuli presented at higher levels, subjects required signals to be roughly equal in dB SPL in the two ears to achieve a binaural balance prior to and during aspirin intake. Thus, subjects could not adapt to induced auditory imbalance for low-level stimuli but adapted quickly for more intense signals. Distortion product OAEs (DPOAEs) evoked by moderate-level primaries ($L_2 \geq 55$ dB SPL) persisted with little change during the course of the drug. For low-level primaries ($L_2 \leq 40$ dB), aspirin treatment resulted in a progressive reduction of DPOAEs across the tested frequency range. This result, linked to the finding that AIHL predominantly affects high frequencies, supports the view that the basal region of the cochlea contributes to the generation of lower-frequency DPOAEs. Because aspirin exerts differential ear effects when SOAEs are present, AIHL provides a model to study short-term changes in binaural performance for low-level signals.

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692 Lack of Tonotopy in the Midbrain of *Wnt-1* Overexpressing Mice

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The mammalian central auditory system is characterized by multiple tonotopic maps, but the mechanisms underlying their development are unknown. One way to study them is to use malformations in a known genetic context. We have started to investigate the tonotopy in the inferior colliculus (IC) in the Wnt-1 knock-in mutant mouse.

Wnt-1 is a secreted protein expressed around E9 at the rostral portion of the mid-hindbrain boundary. Wnt-1 overexpression under the Engrailed-1 promoter increases cell proliferation causing a several-fold enlargement of the IC (Panhuysen et al. 2000, ISDN-Abstract). Normally, neurons in the IC are arranged in iso-frequency contours with low best frequencies (BF) in the dorsal and high BFs in the ventral portions. Typically, frequency tuning curves are V-shaped. Many of the IC neurons are bipolar with their dendrites residing within an iso-frequency contour.

Comparing orientation of the dendrites or dendritic branching patterns of ballistically DiI-labeled neurons using confocal microscopy, we found no significant difference between the IC of mutant and outbred mice. However, our electrophysiological recordings revealed the lack of tonotopy in the IC of Wnt-1 mice. Wildtype littermates and outbred mice showed the typical tonotopic arrangement. Moreover, IC neurons

in both control groups showed mainly V-shaped frequency tuning with BFs ranging from 3-40 kHz. In contrast, all IC neurons in Wnt-1 mice had BFs below 10 kHz. Aditionally, tuning functions of Wnt-1 IC neurons were unusual in that they showed a strong nonmonotonicity at BF and above, but monotonicity in the low frequency tail, resulting in "banana-shaped" tuning functions.

Assuming that the auditory periphery and brainstem is not affected by the Wnt-1 overexpression, the lack of tonotopy in the IC is likely to be due to a misguidance of the incoming axons during development. Therefore, the Wnt-1 mouse might help us to reveal the molecular basis for tonotopic map formation.

693 Tonotopic Expression of Eph Receptors and Ephrins in the Developing Chick Nucleus Laminaris

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In the chick, auditory information from the cochlea arrives at nucleus magnocellularis (NM) via the VIIIth nerve. NM projects tonotopically to nucleus laminaris (NL) on both sides of the brainstem. We have previously shown that proteins in the Eph family of receptor tyrosine kinases (RTKs) and their associated ligands, the ephrins, are expressed in the developing chick auditory brainstem nuclei. Here, we sectioned tissue parallel to the tonotopic axis of NL and used immunohistochemistry to examine expression patterns of these proteins along this axis. We then performed densitometry to determine if levels of protein expression correlated with tonotopy at several ages. At embryonic day (E)10, gradients of Eph RTKs and ephrins were observed along the tonotopic axis of NL in the neuropil and somata, as well as in NM axons innervating NL. In the neuropil, EphA4 and ephrin-B1 are expressed at a higher concentration at the high frequency (rostromedial) end of NL, decreasing in concentration monotonically toward the low frequency (caudolateral) end. EphB2 is also expressed in the neuropil, but it does not appear in a gradient. In the somata, EphA4 and ephrin-B2 are more concentrated rostromedially. Finally, in the axons innervating NL from NM, ephrin-B2 is expressed more heavily in the rostromedial region. These gradients disappear by E14, after establishment of tonotopy.

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694 Short Term Maturation of the Neonate Auditory Brainstem Response

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Maturational changes that occur in various portions of the peripheral neonate auditory system can interfere with interpretation of maturation effects for other components of the system. The purpose of this study was to investigate maturational changes in the human neonatal peripheral auditory system up to the level of the brainstem using near simultaneous measures of related structures to help separate maturation effects for individual components of the auditory system.

Distortion product otoacoustic emissions and auditory brainstem responses were measured in full term neonates with identical stimulus transducers for both measures and all stimuli adjusted for real ear effects. This approach allowed the two types of measures to be made nearly simultaneously and eliminated several sources of variability. Distortion product otoacoustic emission levels did not vary significantly between genders nor did they vary significantly with age over a period of 13 through 40 hours after birth providing evidence that the conductive mechanisms were functioning normally and not influencing the auditory brainstem measures. Auditory brainstem responses were measured with linear averaging and a fixed number of frames and quantified using a variance ratio which is a signal to noise ratio. The variance ratios of the auditory brainstem responses in neonates with normal otoacoustic emissions did not differ with respect to gender but were directly proportional to neonate age in hours. If an assumption is made that the noise was constant, the increased signal to noise ratios can be attributed to increases in brainstem responses. The results suggest that auditory brainstem function can undergo maturation in full term neonates over the first hours of life independent of middle ear or cochlear effects.

695 Cortical Auditory Evoked Potential Maturation

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The purpose of this study was to examine changes in the topography of the obligatory cortical auditory evoked potentials and the mismatch negativity. Five age groups were examined: adults (n=12), and 105-138 month (n=14), 84-103 month (n=14), 67-82 month (n=15), and 48-65 (n=10)month old children. Stimuli were 85 dB peSPL 1000 and 1200 Hz tones presented bilaterally using an oddball paradigm (deviant probability = 0.15). Auditory evoked potentials were collected using electrodes placed at 31 locations on the scalp and referenced to an electrode on the nose. Subjects ignored the stimuli during the recording. The topography of the evoked potential was evaluated using scalp current density analysis. Systematic changes in latency and topography across the age groupse were observed for both the obligatory potentials as well as the mismatch negativity. P1 showed changes in the strength of activation and anterior shifting of the major focus of activation with maturation. The two major foci of activation seen for N1 showed large changes in strength and small changes in location with maturation. The mismatch negativity showed maturational changes that included a shift to more medial locations in the older age groups and greater hemispheric asymmetry in the strength of activation in children. These topographic changes indicate that the generators of the cortical auditory evoked potentials, or their orientation, are not mature by 11 years of age.

696 The Timecourse of Development and Plasticity of the Human Central Auditory System in the Presence of Auditory Deprivation.

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We are investigating the time course of development, deterioration, and plasticity of the human central auditory system. We have recorded the P1 cortical evoked potential and EABRs in normal-hearing children, children with cochlear implants and children with auditory neuropathy who are fit with cochlear implants. Our results show that congenitally deaf children implanted after age 4 had delayed P1 responses even after years of stimulation. Children implanted by approximately age 3 years evidenced normal latency cortical responses within months of electrical stimulation. These data suggest that in the absence of normal stimulation, the central auditory system remains relatively non-degenerate for about 3 years. P1 latencies in implanted children were

retested at different times after the onset of electrical stimulation. We found decreases in latencies within the first year following the onset of electrical stimulation in children implanted at ages 2- 6 years. However, children implanted after age 11 did not demonstrate decreases in P1 latency, suggesting that too long a period of deprivation may result in a loss of plasticity in central auditory pathways. Furthermore, it appears that the degenerative effects of auditory deprivation are more readily apparent at the thalamo-cortical levels of the auditory pathway compared to at the auditory brainstem. Preliminary data show that late-implanted children and adults show EABR wave V latencies that fall within the range of EABR wave V latencies seen in early-implanted children. Preliminary data from auditory neuropathy patients shows that electrical stimulation via a cochlear implant restores normal ABR responses almost immediately, however cortical responses take longer to develop. In sum, our data suggest that there are different timelines for the development, degeneration and plasticity of different levels of the human central auditory system. Studies are ongoing to better define these time periods.

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697 Long-term Potentiation of Inhibitory Transmission in the Developing LSO.

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We have previously examined developmental plasticity of inhibitory synapses from the medial nucleus of the trapezoid body (MNTB) to the lateral superior olive (LSO) in gerbils. Activation of the MNTB inhibitory afferents at 1 Hz produces long-term inhibitory depression (LTD, Kotak and Sanes, 2000) during a time when these synapses undergo rearrangement (Sanes and Takács, 1993). Therefore, we are also interested in exploring whether inhibitory strength can be enhanced. Whole-cell voltage-clamp recordings were obtained from LSO neurons in brain slices from P8 15 gerbils while MNTB afferents were stimulated at a very low rate (0.03 Hz) while inhibitory postsynaptic currents (IPSCs) were recorded in LSO neurons for an hour. In the post-hearing onset group (P12-15), this led to a robust enhancement in the IPSC amplitudes by 30 mins, from 135 ± 15 pA to 564 ± 110 pA (means ± SEM; p=0.003; n=6). This persisted for one hour (Initial IPSCs, 135 ± 15 pA vs. IPSCs at 60 mins, 588 ± 115 pA; p = 0.003, N=6). In two cases, 60 mins following LTP, stimulation of MNTB at 1 Hz that induces inhibitory LTD did not alter the potentiated IPSCs. There was also an associated increase in the number of spontaneous IPSCs with frequencies of up to 20 Hz not seen in control neurons. MNTB stimulation at 0.03 Hz prior to hearing onset (P 8-11) did not produce any change in evoked IPSC amplitudes (Initial IPSCs: 183 ± 55 pA vs. IPSCs at 60 mins: 95 pA ± 35 pA; p=0.2, n=7). Together, while our previous findings predict that a weakening in inhibitory synaptic strength induced by one activity pattern (1 Hz) may underlie synapse elimination (Kotak and Sanes, 2000), the present evidence implies that in older animals, a much lower pattern (0.03 Hz) may strengthen these inhibitory synapses.

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698 Glutamate receptors contributing to synaptic Ca^{2+} responses in developing LSO neurons.

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Glutamatergic synaptic transmission and intracellular calcium are important for maturation and survival of neurons in the lateral superior olive (LSO). Immature LSO neurons express all major classes of glutamate receptors (GluRs) but it is not known under what synaptic stimulus conditions specific GluRs are recruited. In this study we addressed this question using calcium imaging in Fura-2 labeled brainstem slices prepared from C57/BlJ6 mice aged between P0-P7. Synaptic Ca^{2+} responses were elicited by electrical stimulation of the ipsilateral ventral acoustic stria. In order to mimic spontaneous activity patterns described in vivo before hearing onset, electrical stimuli consisted of single stimuli or stimulus trains (10 pulses @ 5-100 Hz). Responses evoked by single stimuli were primarily mediated by AMPA/Kainate receptors as they were reduced to 8 ± 2 % (n=40 cells) by CNQX (20 μM). However, responses that exceeded $\Delta[Ca^{2+}]_i$ of 50 ± 5 nM also involved NMDA receptors which then contributed 55 ± 3 % (n=8 cells). Low frequency stimulus trains (5-10 Hz) activated NMDA and AMPA/Kainate receptors. The contribution of NMDA receptors to total Ca^{2+} response was 59 ± 4 % (n=32 cells). Finally, high frequency stimulus trains (20-100 Hz) additionally recruited metabotropic GluRs (mGluRs) which accounted for 6 ± 1 % (n=39 cells) of the total Ca^{2+} response.

Taken together, our results demonstrate that distinct synaptic activity patterns before hearing onset activate distinct classes of GluRs, which in turn elevate $[Ca^{2+}]_i$ via distinct sets of pathways. This finding is consistent with the idea that spontaneous activity in the immature auditory system has an instructive role in the early development of the LSO.

699 Dendritic Ca^{2+} responses elicited by glycinergic/GABAergic synapses in immature LSO neurons

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In the neonatal LSO the glycinergic/GABAergic input from the MNTB is depolarizing and increases $[Ca^{2+}]_i$. In this study we combined whole-cell patch clamp recordings and confocal Ca^{2+} imaging to characterize in detail the spatial organization of synaptic glycinergic/GABAergic Ca^{2+} responses in individual neurons.

Electrical stimulation of the MNTB elicited depolarizing PSPs that were accompanied by Ca^{2+} responses in proximal and distal dendrites. These responses were locally restricted, extending over 2-15 μm of dendritic length (mean 4.9 μm, n=39). Amplitudes of local Ca^{2+} responses were comparable to spontaneous Ca^{2+} changes and did not correlated with the amplitudes of simultaneously recorded PSPs. This suggests that local dendritic Ca^{2+} responses reflect transmitter release from one or a few closely spaced synapses.

Stronger or burst-like synaptic stimulation elicited action potentials (APs), which were accompanied by Ca^{2+} responses that spread throughout the entire dendritic tree. Similar global Ca^{2+} changes could also be elicited by suprathreshold somatic current injections suggesting that global Ca^{2+} responses are generated by back-propagating APs. In support of this suprathreshold current injections in the presence of TTX did not produce global Ca^{2+} responses.

Our results indicate that glycinergic/GABAergic synaptic activity in immature LSO neurons can produce two distinct types of postsynaptic Ca^{2+} responses. Subthreshold PSPs give rise to local responses, which can preserve the dendritic location of active synapses whereas suprathreshold inputs elicit global responses by virtue of dendritic sodium APs. This functional distinction might play a role in the activity-dependent sharpening of the developing MNTB-LSO pathway.

700 Early Ultrastructural Changes in Mouse Anteroventral Cochlear Nucleus After Unilateral Cochlea Removal

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Development of brainstem auditory nuclei is influenced by the presence or absence of auditory afferents. For example, in the chick, neuronal

degeneration occurs in the auditory nuclei after experimental removal of auditory input. Cell death following afferent deprivation is a calcium-dependent, apoptotic-like process with well-characterized early changes in ribosomes and mitochondria. Afferent innervation also influences the survival of neurons in the mouse anteroventral cochlear nucleus (AVCN). In the mouse, experimental removal of afferent inputs results in the degeneration of up to 70% of neurons in the AVCN. Neuron loss only takes place if sensory input is removed during a critical period between postnatal days (P)5 and 14. In this study, we are examining the ultrastructural changes in AVCN following deafferentation during this critical period. Unilateral cochlea removal is performed in P5 mice. After various survival periods from 6 to 24 hours, mice are perfusion-fixed and processed for conventional transmission electron microscopy. Control tissue shows at least two populations of cells and synaptic contacts in varying stages of maturation. As early as 6 hours after deafferentation, we observe degeneration of axons, synapses, and neuronal cell bodies within AVCN, and find evidence for both necrosis and apoptosis. These changes are more pronounced by 18 hours after cochlea removal. The data presented herein suggest that AVCN neuron survival may depend on the state of maturation of afferent synapses. Ultrastructural studies, together with ongoing molecular studies, will help provide insight into the basis for the critical period.

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701 Deafferentation-Induced Bcl-2 mRNA Expression in the Avian Cochlear Nucleus: Effect of Chloramphenicol

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Cochlea ablation results in the death of ~30% of neurons in the avian cochlear nucleus, nucleus magnocellularis (NM). The factors that determine an individual NM neuron's fate remain to be clarified. One potential factor is bcl-2, a protein known to regulate cell death cascades in other systems. Previous results show a subpopulation (~30%) of NM neurons exhibit a transient increase in bcl-2 mRNA 6-12h following deafferentation. This percentage corresponds to the percentage of neurons that eventually die following afferent deprivation. If bcl-2 regulates cell death in this system, then one would expect manipulations that increase cell death to alter bcl-2 mRNA expression. One manipulation that dramatically increases cell death in NM is pharmacological inhibition of mitochondrial protein synthesis with chloramphenicol (CAP). Injections of CAP (1200mg/kg/d) enhance deafferentation-induced cell death, increasing the number of dying NM neurons from 22% to 65% (Hyde and Durham, J. Neurosci., '94). In this experiment we explored the effect of CAP on the expression of bcl-2 mRNA following deafferentation.

A unilateral cochlea removal was performed on posthatch chicks (P7-P9), and administration of CAP followed the procedure described by Hyde and Durham ('94). Tissue sections from subjects surviving 12h following surgery were processed for in situ hybridization using an oligonucleotide probe to chicken bcl-2 (Eguchi et al., Nucleic Acids Res '93) and examined by emulsion autoradiography. CAP administration did not lead to an increase in the number of neurons exhibiting a deafferentation-induced upregulation of bcl-2 mRNA. If anything, some cases suggested that CAP may reduce the number of cells showing upregulation of bcl-2 mRNA. These data suggest that bcl-2 expression does not mark dying neurons following deafferentation. Alternatively, CAP may activate cell death cascades that differ from those activated in untreated neurons.

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702 Effects Of Unilateral Cochlear Ablation On The Development of Callosal Connections Of Rat Auditory Cortex

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In the adult auditory cortex, EE and EI units form intercalated, rostrocaudal bands. The former are the origin and target of interhemispheric connections, while the latter are innervated by thalamocortical input. We studied the postnatal development of the auditory callosal connections after unilateral cochlear ablation.

The critical period for deafferentation was determined by looking at the expression of enzymes (cytochrome-oxidase, NADPH-diaphorase, and acetylcholinesterase) and calcium-binding proteins (parvalbumin, calbindin, and calretinin), in the cortex contralateral to the lesioned cochlea. We found profound alterations in cortical layers III and IV, when the cochleotomy was peformed between P0 and P6.

The auditory callosal connections were labeled by injection of axoplasmic tracers (BDA, WGA-HRP) in Te1 in adut animals. In rats that had been cochleotomized between P0 and P6, the anterograde and retrograde labeling in the cortex ipsilateral to the cochleotomy was similar to that of control animals. Yet, the labeling of the incoming axons and callosal neurons in the cortex contralateral to the lesioned cochlea showed an abnormal topographical and laminar distribution, reflected in a smaller lateral extent of callosal axons and presence of ectopic callosal neurons in layer IV.

Unilateral cochleotomy in the early postnatal period produces that the thalamocortical axons of the opposite side would be incapable to induce the normal development of specific features in the auditory cortex to which it projects, including the callosal system.

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703 NMDA R1 is expressed in the Auditory Brainstem of embryonic and post-hatch Chicks

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NMDA receptors may mediate a component of the excitatory response in chick NM (Zhang and Trussell, 1994; Nemeth, Jackson and Parks, 1983). We have examined the developmental expression of NMDA receptor 1 (NR1). NR1 is an essential component of the NMDA receptor channel, whereas four NMDA receptor 2 subunits (A-D) modulate functional properties. We used immunohistochemical techniques to examine the temporal and spatial changes of NR1 expression in the nucleus magnocellularis (NM), nucleus laminaris (NL), nucleus angularis (NA) and the superior olive (SO) of chickens from E10 to adult. NR1 was first observed in E10 NL cell bodies but not in NM. From E11 -12 expression of NR1 in the NL was greater than that in NM although levels in both were low. At E13, labels in both NM and NL had increased and were similar. After E13, NR1 expression in NM was greater than in NL. Between E15-20 NM displayed a gradient in NR1 expression with medial, future higher best frequency, neurons being more immunoreactive than lateral neurons. NR1 levels appeared to increase after hatching, and the gradient in expression in NM disappeared. After E14, NL staining extended into the dendrites. NR1 immunoreactivity also appeared in NA at E11. NR1 expression in NA was heterogeneous and increased over time. NR1 immunoreactivity was found in the SO from E11, and increased during development. After hatching, the number of immunoreactive neurons in SO decreased.

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704 Development of Calyx of Held-MNTB Cell Adherent Complexes

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Each globular bushy cell of the cochlear nucleus projects tonotopically to one or two MNTB cells via Calyces of Held, perhaps the largest nerve terminals in the CNS. Adult calyces in cats contain specialized organelle assemblies, which we named mitochondria associated adherens complexes (MACs), that include a punctum adherens tethered via filaments to a mitochondrion with interposed tubular or vesicular membranous structures. We have hypothesized that MACs maintain cell-cell contact during rapid turnover of synaptic vesicle membrane resulting from high rates of activity by these neurons. Furthermore, the punctum adherens structure may stabilize the calyceal contact from its early stages of formation. Therefore, we investigated calyx terminals in mice in order to describe the developmental sequence for MAC formation.

Puncta adherentia and tubular membranes are common features in the the mouse adult calyx (3 months old), but are not as frequently associated with mitochondria as in the cat. This adult-like organelle arrangement is also found just before the onset of hearing (postnatal day 11). Younger animals (PD 5 and 4) have a proliferation of puncta adherentia and a paucity of extended extracellular spaces between the calyx and MNTB cell, normally containing glial processes, that characterize the adult nerve terminal. MACs are found at PD5, but mitochondria are located too far from the presynaptic membrane on PD4 to be part of the organelle assembly. Therefore, the puncta adherentia and tubular membranes are the first MAC components to be put in place. We are investigating their presence at earlier postnatal and late embryonic ages when contact between calyceal growth cones and MNTB cells is initiated.

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705 Low Levels of Lead Induce Neuronal and Glial Changes During Development of the Auditory Brainstem.

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Lead continues to be an important environmental toxin even though it has been removed from paint and gasoline. Early exposure to lead is a risk factor for reading disability (RD) and attention deficit hyperactivity disorder, but it is not clear how lead exerts its effects on language and perception. In recent studies, children with language impairment have demonstrated deficits in backward masking and our preliminary studies indicate that children who have measurable lead levels also have deficits in backward masking. We have shown that chicks exposed to very low levels of lead during development (< 10 mg/dL) show a deficit in backward masking that is similar to the deficit in children with RD. The present study was undertaken to determine whether the deficits in backward masking observed in chicks exposed to low levels of lead had an anatomical correlate in the auditory brainstem. Fertilized white leghorn eggs received injections of lead acetate (88 mg/kg-low dose or 132 mg/kg-high dose) on day 14 of incubation. Brains were then processed for GFAP (Glial Fibrillary Acidic Protein) and neurofilament histochemistry on day 0 and day 4 after hatching. GFAP immunoreactivity is significantly increased over controls within the fiber tracts surrounding the cochlear nucleus, n. Magnocellulars (NM), at both doses of lead at both ages. Of particular importance, neurofilament immunostaining is significantly decreased both within and around NM at both doses and times. The loss of neurofilament labeling appears restricted to axons within and surrounding NM and to fibers that cross the midline. The rest of the brainstem of the leaded birds have similar levels of neurofilament staining to that of unleaded controls. These findings demonstrate that exposure to low levels of lead during development cause significant changes in both neurons and glia within the avian auditory brainstem that are consistent with our observed behavioral deficits.

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706 The effects of aging on frequency modulated sweep responses in rat inferior colliculus

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A common complaint among the elderly is a difficulty in understanding speech. One central factor that may contribute to this difficulty, is a deterioration in the ability to accurately encode the dynamic aspects of speech such as the formant transitions. It has been suggested that processing speed deteriorates with age. Thus, for the aging auditory system, this deterioration may be manifest as a deficit in encoding time-varying sounds that contain rapidly changing frequencies such as formant transitions. A useful stimulus for studying this decline is the FM sweep which shares features in common with formant transitions. Mendelson and Ricketts (2001) recently showed that auditory cortical cells recorded from young animals responded best to fast FM sweeps while those recorded from aged animals preferred slower sweeps. In order to determine if this age-related effect is exclusive to the auditory cortex we examined the responses of units to FM sweeps in a subcortical structure, the inferior colliculus (IC). In the present study extracellular single-unit recordings were obtained from the IC of young (2-4 months) and old (24-30 months) Long-Evans Hooded rats in response to linear FM sweeps. FM sweeps were varied in both speed and direction. Results showed that there was no significant difference in the preferred speed of FM sweep between the two age groups. In particular, the majority of neurons recorded from both age groups preferred faster sweeps. In addition, for preferred direction of FM sweep, most of the cells in both groups were non-direction selective. The results indicate that temporal processing speed does not appear to be affected by aging at the level of the inferior colliculus. These results support the hypothesis that aging appears to affect temporal processing speed at a level higher than the midbrain.

707 Mouse Inferior Colliculus Neurons Alter Their Coding of Amplitude Modulated Signals Under Conditions of Spatial Masking

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A large body of psychoacoustical research shows that the effect of masking noise is most pronounced when the signal and the noise are co-localized. Here we examine the effect of varying the spatial separation of signal and noise on the rate encoding of sinusiodally amplitude modulated (SAM) tones in single inferior colliculus units of young CBA mice. The response of 43 IC neurons to SAM tones (-60°, best frequency, 20 dB above threshold) was measured as continuous broadband maskers were varied in azimuth. The best modulation frequency (BMF) of each unit was defined as the MF that elicited the most spikes during the signal onset in the quiet. A unit's pass band was defined as extending from the MF eliciting 50% of the BMF response (lower cutoff frequency) to that eliciting twice the BMF response (upper cutoff frequency). By these criteria, 31% of the units were low-pass, 30% band-pass, 16 % high-pass, and 23% were all-pass when measured in the quiet. When 50 dB SPL background noise was presented at -90°, it resulted in a 3-fold increase in the number of all-pass units MTFs. This result was due to the masker capturing the cell's response and the suppression of the number of spikes at BMF, i.e., the masker was most effective in the same hemifield as the signal. Spatially separating the noise from the signal restored MF tuning in 40% of the affected units,

and 37% of the sample showed substantially increased SAM responses at certain azimuths of background noise, suggesting a degree of spatial tuning. These data will provide a baseline for comparison to the responses of units from old CBA mice, in order to elucidate the neural correlates of age-related declines in the use of spatial cues which contribute to presbycusic speech perception difficulties in background noise.

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708 Inferior Colliculus (IC) Near-field Evoked Responses Following Central Reorganization in the C57BL/6J Mouse

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The C57 mouse develops a progressive high to low frequency sensorineural hearing loss that begins at about 2-3 months of age. It is accompanied by significant neural plasticity in the IC that is secondary to the loss of afferent input: ventral regions once responsive to the high frequencies become sensitive to only the mid-frequencies (8-12 kHz) with thresholds approximating those seen in normal mid-frequency BF neurons in more dorsal regions. We examined the effects of tonotopic reorganization on neural gap detection, by recording near-field evoked potentials (NFEPs) from dorsal (300 um) and ventral (1300 um) IC locations in normal hearing (NH, < 8 week old, n = 5) and hearing-impaired (HI, > 6 month old, n =5) C57 mice, using bipolar electrodes. Silent gaps (.25 - 64 ms duration) were imbedded into two pairs of 1/3 octave band noise bursts (NB1 and NB2, 50 ms durations, 60 and 80 dB SPL, and centered at either 6 or at 12 kHz). Gap thresholds, the slope of the recovery functions for the post-gap response, and peak latencies of the post-gap response were compared and found to be similar for the NH and HI groups for each condition of level, depth, and band-pass frequency. Although it may be expected that more IC neurons would be excited by the 12 kHz narrow-band markers in the HI mice, these data suggest that the mechanisms underlying neural reorganization after the loss of afferent input act to maintain temporal acuity from ensembles of cells as measured by this gap detection paradigm.

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709 Calbindin Immunoreactivity in the Cochlear Nucleus of Aging Mice and Old Mice Deafened as Young Adults

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Intracellular calcium regulation deficits are associated with neuronal aging. Because intracellular calcium homeostasis is controlled in part by calcium binding proteins, alterations in their expression may play a role in neuron survival or death. In previous mouse model studies of presbycusis, we have demonstrated that calbindin (CB) decreases, parvalbumin increases, and calretinin (CR) may increase or decrease with age in the brainstem auditory system. The present study examines calbindin immunoreactivity in the mouse ventral cochlear nucleus (VCN). The CBA strain maintains good hearing until late in life, whereas in contrast, the C57 strain exhibits a severe high frequency hearing loss at an early age. Immunoreactivity patterns were characterized and cell counts performed for young adult (3-4 mon) and old (>24 mon) mice. CB immunoreactivity declined with age in the anterior PVCN in CBAs, with the greatest age-related declines occurring in CBA mice deafened as young adults who were allowed to survive until old age. However in C57s, CB remained unchanged in the anterior PVCN, but the number of CB+ neurons declined with age in the posterior AVCN. While a few studies in other brain systems report increases, most report an overall decrease in calcium-binding protein expression with age. The present results support the hypothesis that CB expression is down regulated in the PVCN with age, and that this age-related decline is enhanced due to deafening. Ongoing analyses involve counting CR and CB+ neurons in all cochlear nucleus divisions for both strains, and measuring size and shape-related structural properties of immunoreactive and total neurons that are present, with image-analysis software.

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710 Acetylcholinesterase Staining in the Auditory Brainstem of Young Adult and Old C57 Mice

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Previous anatomical and biochemical investigations have established that acetylcholine (ACh) is an inhibitory neurotransmitter for the descending auditory system. In particular, it is localized in pathways projecting from the superior olivary complex (SOC) to the cochlear nucleus (CN) and cochlea. Optimal processing of sound features by the central auditory system depends upon a delicate balance between excitation and inhibition. In aging, it is likely that uneven degenerative changes in this circuitry disrupt this delicate balance. For example, in CBA mice we have found that the activity level of acetylcholinesterase (AChE), an enzyme that degrades ACh, is upregulated with age. As part of our ongoing investigations of the neural bases of presbycusis (hearing loss associated with aging), we are beginning to examine these changes. In the present experiment, a method of enzyme histochemistry for detecting AChE involving treatment with diaminobenzidine (DAB) and H2O2 was utilized to visualize AChE-positive cells and neuropil in the central auditory system of C57 mice, both young adult (3 mon) and old (24 mon) and to compare the results with our earlier study of changes in AChE presence with age in CBA mice. Initial findings show very similar intense staining of fibers and/or cells in periolivary (PO) regions such as rostral PO, ventromedial PO, and ventrolateral PO, as well as the trapezoid body (TB), anteroventral CN and posteroventral CN. In addition, there were many lightly labeled cells in the medial nucleus of TB, and darkly stained cells in and near the lateral superior olive. The superior colliculus and sagulum also displayed pronounced neuropil labeling. Current efforts are aimed at comparing age-related changes in AChE presence with age in CBA and C57 mouse strains, to see if the CBA upregulation occurs in old C57s who are deaf.

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711 Choline acetyltransferase activity in young and aged CBA mouse brainstem auditory structures

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Evidence from behavioral studies suggests that cholinergic pathways to the cochlear nucleus may play a role in the recognition of signals in a noisy background. Studies of central nervous system disorders such as Alzheimer's disease have indicated age-related loss of some cholinergic neurons in the brain. This study explored age-related changes in brainstem auditory structures of the activity of choline acetyltransferase (ChAT), the enzyme responsible for acetylcholine synthesis. CBA mice 3 months, 12 months, and 24 months old were anesthetized and their brains frozen. Frozen sections were cut and freeze-dried, and regions in the brainstem were sampled by microdissection of the freeze-dried

sections. ChAT activity was measured for each sample by a radiometric assay. The preliminary results found a significant age-related decrease of ChAT activity in the facial nucleus. Mice 12 months old showed a 20% decrease in ChAT, and mice 24 months old showed a 44 % decrease. There was suggestive evidence of a decrease in ChAT activity in the anteroventral cochlear nucleus, but there were no clear changes in the posteroventral or dorsal cochlear nucleus, granular regions or superior olivary regions. We speculate that there may be significant age-related decreases in acetylcholine metabolism in facial motoneurons, but such decreases may be less in cholinergic pathways of the auditory system.

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712 Age related changes in ChAT and VAChT immunoreactivity of CBA mouse lateral superior olive and cochlear nucleus

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Acetylcholine plays modulatory roles in the auditory system. How cholinergic neurons in the central auditory system change with aging is not clear. Studies on central nervous system disorders such as Alzheimer's disease have suggested age-related changes in some cholinergic neurons in the brain. This study examined choline acetyltransferase (ChAT) and vesicular acetylcholine transportor (VAChT) immunoreactivity in the lateral superior olivary nucleus (LSO) and cochlear nucleus of young and old CBA mice. CBA mice 3 months, 12 months and 24 months old were anesthetized and perfused transcardially, then the brains were removed and frozen sections cut. Free floating sections were immunoreacted with antiserum to ChAT (from Chemicon) or VAChT (from DiaSorin). In the LSO, ChAT-positive neurons were analyzed with a computer program, Neurolucida, from MicroBrightField. There was a slight increase in cell body areas and perimeters from young to older age. In both the sections labeled for ChAT and VAChT, average LSO area in 24-month-old mice was significantly smaller than those in 3 and 12-month-old mice. However, the numbers of ChAT- and VAChT- positive neurons were comparable among the 3 age groups. In the cochlear nucleus, puncta presumably corresponding to VAChT-labeled terminals were present in all subregions, although there was a slight decrease in density of positively labeled terminals in the 24-month-old group. In conclusion, this study found that, unlike in some other central nervous system regions, there was little evidence of age related loss of cholinergic neurons in the centrifugal pathways to the cochlear nucleus.

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713 KCNA1 Knockout Mice Have Deficits In High Frequency SAM Evoked Potentials Recorded in the Inferior Colliculus

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Speech perception relies on encoding the rapid amplitude modulation (AM) of its envelope, and some auditory neurons possess properties well suited to this task. Octopus cells of the PVCN respond to coincident AM presented across their input array, and follow AM at high rates. Their selective response to synchrony depends on a fast low threshold K+ current that suppresses small EPSPs from asynchronous input. We predicted that KCNA1 knockout mice would show deficits in temporal acuity because the Kv1.1 channel that they lack has the lowest threshold. The stimuli were 200 ms long, 80 dB SPL SAM noise bursts, having modulation frequencies between 10 Hz and 1000 Hz, and presented 1/s to awake mice. Phase locking to SAM in -/- KO mice was compared with that of +/- and +/+ mice. Near-field EPs were recorded using bipolar electrodes from the IC of 15 young mice (4 +/+, 6 +/-, and 5 -/-). Modulation transfer functions (MTF) were calculated as the ratio of the power in the EP at the driven SAM rate to the power in the EP to non-modulated noise. Group differences were small in the low and mid SAM frequencies, showing a peak at 100-150 Hz, followed by a steep decline at 200 Hz. The +/+ and +/- mice then increased to a second lower peak at 400 Hz, followed by a gradual decline, while -/- mice EPs fell rapidly beyond 300 Hz. We have shown that old mice also exhibit MTF deficits at high SAM rates, and preliminary data show reduced mRNA expression by 25-30% for KCNA1 and KCNA2 genes in the old CN. Our *in vivo* EP data in KO mice reveal that the KCNA genes contribute to the ability of young listeners to follow high rates of AM, and we suggest their reduced expression with age may contribute to presbycusis in the elderly. [Supported by NIA and the Schmitt Program on Integrative Brain Research]

714 Normal Threshold and Suprathreshold ABR and ASR Responses to Acoustic Onsets in KCNA1 Knockout Mice, but a Reduced Response to Offsets.

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Data obtained in vitro from slice preparations suggest that the ability to follow rapid level changes in an acoustic envelope depends on fast voltage gated potassium channels heavily represented in the brainstem auditory system. Here we report on threshold and suprathreshold measures of spectral and temporal acuity to acoustic onsets and offsets in KCNA1 knockout mice (3-4 wk old) missing low threshold Kv1.1 ion channels. ABR thresholds from 6 kHz to 48 kHz were identical in -/-, +/-, and +/+ mice ($n = 9, 21,14$) though the -/- mice had a lower threshold at 3 kHz ($p < .05$). ABR latencies and amplitudes for 90 dB tone pips were identical over this range. Acoustic startle reflex (ASR) thresholds and amplitudes to tone bursts (4 kHz to 32 kHz, levels from 60 to 120 dB) were also identical, though -/- mice showed higher activity ($p < .05$) in the absence of any stimulation. Null-mutant mice showed less prepulse inhibition (PPI) to gaps in a noise carrier than the +/+ (with +/- intermediate). They also showed a slower increase in PPI for an abrupt noise offset versus a ramped offset ($p < .05$). The reaction to offsets in KO mice is similar to that of old mice, which have 25 - 30% less expression of KCNA genes in the cochlear nucleus, though old mice also show reduced reactions to acoustic onsets while KCNA KO mice do not.

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715 Age-related Changes in the Subunit Makeup of the GABAA Receptor in Rat Primary Auditory Cortex

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Primary auditory cortex (AC) is the final structure in the series of nuclei dedicated to the processing of acoustic information. The inhibitory neurotransmitter GABA has been shown to have a key role in the processing of complex acoustic signals at many levels of the auditory neuroaxis. Primary auditory cortex is endowed with a rich network of GABAergic neurons and extrinsic GABAergic inputs. Damage at the level of the cochlea, in adult animals, results in a dramatic increase in the amplitude of superthreshold cortical evoked potentials. Since aging can be conceptualized as a slow peripheral degradation of the auditory input into the brain, these findings imply a cumulative down-regulation of inhibition at subcortical structures and/or primary changes at the level of AC. The present study examined age-related changes in the

heteromorphic layers of the rat primary AC. Results of western blots from punches from AC found an age-related decrease in the level of the alpha 1 subunit protein throughout primary AC. Cellular level in situ hybridization techniques were used to determine mRNA levels for the alpha subunits of the GABAA receptor. Layers 3 and 6 showed significant age-related loss of alpha 1 mRNA, modest reductions in alpha 1 mRNA were seen in layers 2, 4 and 5. Reduction in alpha 2 mRNA levels were seen throughout the layers of AC with the greatest changes in layers 4 and 5. Significant elevations in mRNA levels for the alpha 3 subunit were found in layers 2 and 5 with modest increases in layers 3 and 4. These preliminary data are suggestive of age-related subunit changes within selective layers of primary auditory cortex. These layer specific age-related changes in the makeup of the GABAA receptor may have important implications for both pharmacologic and physiologic functioning in the AC of normal elderly.

716 Prestin and the electromechanical responses of outer hair cells

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Recent work on outer hair cell (OHC) electromechanical responses (fast electromotility and voltage-controlled stiffness) is considered in the context of the recently identified molecular motor protein, prestin (Zheng et al., Nature 405, 149-155, 2000). Specifically, we consider the modulatory effects of efferent neurotransmitters on OHC static stiffness and electromotility and the probable underlying mechanism. Further, we discuss the properties of dynamic stiffness changes and various schemes to explain these changes. Finally, we ask which properties of electromechanical responses are directly attributable to the nature of the prestin molecule itself.

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717 Hysteresis in the outer hair cell

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The outer hair cell lateral membrane harbors a dense population of motor proteins that enable the cell to mechanically influence basilar membrane mechanics. These motors, which are voltage driven, can be inspected by measuring the membrane-bound charge movement that accompanies displacement of the motors' voltage sensors. Through this means and by measuring mechanical activity of the cell, it has been found that the voltage dependence of the cell's mechanical activity depends on initial conditions of membrane voltage and tension. Here we will review these data and attempt to reconcile through models the interactions between these two influential parameters that contribute to hysteresis in OHC activity.

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718 Membrane Based Motor Mechanisms

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Membranes act as electro-mechanical force generators while functioning as permeability barriers. These phenomenologically described "membrane motors" have been studied in outer hair cells and in more generic tissue cultured cells. Both types of cells convert voltage to mechanical force at acoustic frequencies without utilizing cellular energy stores. A model based on interfacial physics has been developed for the membrane motor found in native human embryonic kidney (HEK) cells. The surface tension at both the inner and outer leaflet of the cell membrane is modulated by the transmembrane potential. The resulting change in membrane tension is a linear function of the potential difference in the physiological range of potentials. The HEK cell's native electromotility is increased when it is transfected by prestin and more closely matches that of the outer hair cell. A membrane-bending model based on membrane flexoelectricity has been developed for the outer hair cell. Flexoelectricity is a general property of polarizable interfaces and describes how bending a membrane moves charges that can change the transmembrane potential, or inversely, how changing the potential can cause movement. The membrane-bending model takes into account a broad range of morphological, physiological and biophysical observations. It identifies transmembrane potential gradients and membrane tension as key independent variables controlling the passive and active mechanics of the outer hair cell. The ability of the membrane-bending model to match the response properties of the outer hair cell membrane motor is compared with some of the other models presented in this session. The lack of specialized structures in the HEK cell model could provide insight into how prestin functions in the OHC.

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719 'Area motor' model of OHC motility

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The motile activity of outer hair cells (OHC) is accompanied by charge transfer across the plasma membrane, which is observed as a voltage-dependent component of the membrane capacitance. The simplest model that can describe these motile properties is an `area motor model,' which assumes two states in a membrane element. These states differ in both their fixed charge and their membrane areas. Here we examine this model using recent experiments.

One prediction of the model is that the peak of the nonlinear capacitance shifts to a more positive potential as increased pressure is applied to the membrane. Such shifts are experimentally observed, providing values for area changes.

Another prediction is that area constraints inhibit conformational transitions of the motor. Such a constraint can be imposed on hair cells that are made spherical by injecting a trypsin containing medium by applying short waveforms to impose a volume constraint. When the pressure applied to the cell through the pipette is above 0.3 kPa, the voltage-dependent component of the membrane potential recorded during a quick voltage scan is significantly reduced.

The area motor model is a special case of piezoelectricity, in which the system has a small number of polarization states. This feature leads to its prominent nonlinearity in contrast to the linear responses observed in most piezoelectric materials, which have continuum of polarization states. A recent experiment showed that OHCs satisfy the piezoelectric reciprocal relationship.

This model appears to be consistent with a motor protein recently identified as `prestin'. Both hair cells and other cells that express prestin have a membrane capacitance, for which the voltage dependence is bell-shaped. Application of tension to their membranes induces shifts of the voltage dependence that is proportional to the magnitude of tension.

720 Coupling of electromechanical force into the organ of Corti

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The mechanism by which the outer hair cell (OHC) force is coupled into the organ of Corti to produce frequency tuning of the travelling wave on the basilar membrane (BM) at low sound pressure levels remains unknown. In order to elucidate coupling mechanisms, vibration measurements are required from the apical surface of the hair cells and the tectorial membrane (TM). However, to date these structures still remain optically inaccessible in the high-frequency region of the cochlea, where tuning is most pronounced. As a first step in addressing

the coupling question, we made vibration measurements in three orthogonal directions from the apical region of the in-vitro cochlea, where these structures are optically accessible [Gummer et al., 1996; Hemmert et al., 2000]. Evidence was found that the TM is tuned in the radial direction and that the resonant frequency is about 0.5 oct below that for the BM. According to this experimental data, the 90° phase lead of OHC force relative to passive BM displacement, required for active "amplification", derives from the 180° phase lag of TM displacement relative to BM displacement near the BM resonant frequency, together with the 90° phase lag of OHC force relative to stereocilia displacement, which in turn results from the time constant of the basolateral cell membrane. This time constant also causes the somatic mechanical response of the OHC to be attenuated, estimated to be up to 40 dB. It is still not understood how the attenuation problem has been resolved. In order to address this question, the force produced by OHCs in response to extracellular electrical stimulation in a cochlear explant was measured with a high-impedance cantilever. The force was independent of frequency up to at least the characteristic frequency and was a factor of ten larger than the force produced by isolated OHCs. Further experiments are being conducted to measure the extra- and intracellular potentials.

721 What Does the OHC Membrane Voltage Control, BM Displacement, Force, or Stiffness and Does it Matter?

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He and Dallos found that the relative change in the OHC's axial stiffness is greater than 2:1. The relative change in the length however less than 1.06:1. When the membrane is under tension, due to the turgor pressure, the voltage dependent axial stiffness is sufficient to account for OHC cell motility. A key question is, which is the controlling variable, the voltage dependent stiffness, or length?

It is widely assumed that the function of the OHC is to increase the frequency selectivity of the cochlea, and increase its sensitivity, via the OHC voltage dependent length change. According to this view the length of the OHC is assumed to follow the stimulus (phasic) to the upper frequency limit of hearing. This theory does not explain why the nonlinear OHC gains exists at the best frequency but not basal by half an octave, where the basilar membrane is linear, and the gain is greatly reduced. Why does the gain change so rapidly over such a short distance?

An alternative point of view is that the OHC controls the dynamic range in a parametric manner, such as via the cells longitudinal stiffness. In this case, the change in gain seen by the IHC does not require a phasic response at high frequencies. Via impedance changes, the OHC could mediate a fast acting gain control that follows the OHC membrane voltage envelope. Given a nonlinear change in dynamic range (i.e., compression), the tuning and sensitivity would necessarily change.

Both tonic and phasic (i.e., DC and AC) changes in OHC stiffness occur. The tonic changes are larger and the phasic smaller as frequency increases. What are the signal detection consequences of the tonic stiffness change?

722 Fgf-3 and Fgf-10 in mouse inner ear development

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Otic development initiates at embryonic day (E) 8.0 and several intercellular signalling molecules, including the fibroblast growth factors (FGFs), are likely to be involved in this process. Of the FGFs that have been targeted, both *fgf-3* and *fgf-10* play important roles in inner ear development. In mice that lack *fgf-3*, induction of the placode and formation of the otocyst occur normally, but defects were observed in the induction of the endolymphatic duct and the subsequent morphogenesis of the otocyst as well as in the formation of the otic ganglion. However, the penetrance and expressivity of the mutant phenotype varied, suggesting that other Fgfs and/or their receptors might also play roles in otic development. Indeed, *fgf-10* is expressed in the developing ear (Pirvola *et al*, 2000) and *fgf-10* mutants have small inner ears (Ohuchi *et al*, 2000). This hypothesis was also strengthened by the finding that mice homozygous for a targeted disruption of *fgfr2IIIb*, which encodes the preferred receptor for Fgfs-3 and -10, have an ear phenotype similar to, but more severe and penetrant than those of *fgf-3* and *fgf-10* mutants (De Moerlooze *et al*, 2000).

To determine the combinatorial roles of Fgf-3 and Fgf-10, we have initiated a study of mice carrying null mutations in both these genes. Surprisingly, the double mutant embryos lack otic vesicles. These embryos do not show otic expression of the placode marker *pax-2* and expression of additional placode markers, *gbx-2* and *dlx-5*, is mislocalised. Hindbrain patterning as assessed by in situ hybridisation analysis of *hoxb1* and *kr* expression in rhombomeres 4-6 in the double mutants is normal. In addition, an intermediate otic phenotype is present in embryos with 3 mutant alleles. Further characterisation of otic development and ganglion formation in double mutant embryos and those with three mutant alleles is underway. These results suggest that fgf signalling has a direct role in placode specification and/or growth.

723 Molecular Events in Inner Ear Induction

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The inner ear arises adjacent to the hindbrain from ectoderm which is induced to form the otic placode. In the past year, a number of candidate tissues and growth factors have been proposed to play a role in otic placode induction. At present, however, the necessity and sufficiency of these different candidate signals for the induction is not clear. We have begun to examine the induction of the otic placode in the chicken embryo, by first examining the necessity and sufficiency of the hindbrain and cranial paraxial mesoderm for the induction. We have also addressed this problem in mutant quail embryos in which the otic placode is greatly enlarged.

The Wnt and FGF families of growth factors has been suggested to play a role in the induction of the otic placode by a number of studies. We have begun to test the necessity of Wnt and FGF signaling in otic placode induction using pharmacological inhibitors. Finally, we are investigating the roles of transcriptional regulators in the induction process, in particular members of the Dlx gene family. The preliminary results of these studies will be presented.

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724 Role of FGF8 in the developing mouse inner ear as revealed by expression patters and conditional gene inactivation

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Recent data suggest that Fibroblast Growth Factor (FGF)/FGF receptor signaling regulates inner ear development at multiple levels. Motivated by the evidence that FGF8, a member of this multiligand family, is a key molecule in induction, patterning and growth of tissues such as mesencephalon/metencephalon, limbs and craniofacial structures, we are studying its role in the developing mouse ear. We show that Fgf8 is expressed in the mouse inner ear from the earliest developmental stages onward. The epithelium of the otic vesicle shows a restricted patch of

Fgf8 expression that extends into the early cochleovestibular neurons. As previously shown, in the late-embryonic cochlea, Fgf8 starts to be expressed in the differentiating inner hair cells along with the base-to-apex differentiation gradient that proceeds through the cochlear duct (Pirvola et al., ARO abstract #665, 1998). Additionally, FGF receptors, specifically their alternatively spliced IIIc isoforms that bind FGF8, are expressed adjacent to Fgf8 expressing cells. Due to early lethality, standard knock out approach can not be used to study the functional significance of Fgf8 during organogenesis.To circumvent this problem, we have analyzed ears of mice in which Cre/loxP system has been used to restrict Fgf8 inactivation to distinct regions of the head, including the otic epithelium. In the presentation, we will describe the impact of conditional inactivation of Fgf8 on inner ear development.

725 Effects of Fibroblast Growth Factors on Canal Formation

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The semicircular canals and cristae ampullaris are integral structures for inner ear vestibular function. Regions of the otic epithelium grow out to form the canal pouches, portions of which later fuse and resorb to form the three doughnut-shaped canals. The molecular mechanisms governing the process of canal formation remain largely unknown. Infecting developing chicken otocysts at embryonic day 3 (E3) with avian retrovirus encoding Fibroblast Growth Factor 3 led to overgrowth and thickening of the canal epithelia, and inhibition of resorption in the canal pouch. Implantation of beads soaked with basic FGF, but not acidic FGF, to E5 chicken otocysts caused a delay in the resorption process. This delay in resorption was associated with a delay in the thinning of otic epithelia and downregulation of SOHo-1 expression in the presumably resorbing regions. When the resorption process resumed later in development, the common crus was often obliterated, indicative of a loss of normal regulation of the resorption process. Together, these results suggest that FGFs may play a role in spatial and temporal regulation of canal growth and resorption.

726 Identification of an Enhancer from the Mouse Fgf3 gene

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The fibroblast growth factors (FGFs) comprise an important family of intercellular signalling molecules required for normal development in many different embryonic contexts. A complex spatial and temporal pattern of expression of Fgf3 during mouse development suggests multiple potential roles for the ligand during embryogenesis. Of particular interest are restricted domains of expression in the developing hindbrain in rhombomeres(r) r5 and r6 at 9.5dpc but more generally throughout the hindbrain at 8.0dpc, in addition to other areas of expression within the developing inner ear. Ectopic expression experiments[1] and antibody/oligonucleotide blocking experiments[2] in chick suggest a role for FGF3 in otic vesicle induction. The mouse Fgf3 mutant develops otic vesicles but an inner ear phenotype is still presented[3] thus illustrating the important role(s) Fgf3 plays in inner ear development.

In order to begin to dissect the molecular mechanisms governing normal Fgf3 expression, we have scanned the Fgf3 locus for regulatory elements by using a lacZ reporter coupled with test enhancer fragments to generate transgenic mice. Enhancer activity is detected by Xgal staining, and we have used this approach to identify an enhancer responsible for the major aspects of the normal domain of Fgf3 expression. We have generated a detailed time course for enhancer activity, and have delimited the enhancer to a 5.7kb fragment. We have sequenced this 5.7kb fragment and sequence analysis identifies multiple potential binding sites, thus providing a list of candidate upstream regulators.

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727 The Divergent Homeobox Transcription Factor, cProx1, Defines Proneural and Prosensory Areas in the Developing Avian Otocyst

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The otic neuroepithelium gives rise to a diverse array of sensory and non-sensory tissues of the inner ear. Many transcription factors are implicated in regulating gross morphogenesis and cellular patterning of the otocyst. cProx1 is a divergent homeobox transcription factor whose homologues in fruit flies and mice promote cell cycle withdrawal and terminal differentiation in numerous cell types. Therefore, we examined expression of cProx1 in the ear primordium of chicken embryos. Nuclear cProx1 protein was not evident in the otic placode but emerged in the anterior otic cup by stage 13 (E2.1). By stage 16 (E2.2), nuclear cProx1 protein was identified in nearly every section of the otocyst; anteriorly, it was ventral and posteriorly, it was dorsomedial. By stage 21 (E3.5), nuclear cProx1 protein was present in a continuous band of cells that wrapped around the otocyst, excluding most of the lateral compartment. Protein expression of cProx1 overlapped with that of Serrate1, but not Pax2. At stage 27 (E5), cProx1 expression was present in discrete patches separated by large regions of unlabeled epithelium. Co-analysis with a hair cell marker (HCA) at stages 27 (E5) and 29 (E6) showed that all cProx1-positive patches corresponded to vestibular or auditory sensory epithelia. Expression of cProx1 was down-regulated in most auditory hair cells by stage 37 (E11) and in many auditory supporting cells by stage 44 (E18). A sub-population of supporting cells in the neural third of the basilar papilla remained cProx1-positive after hatching. As early as stage 13 (E2.1), cProx1 protein was also detected in the nuclei of neural cells as they delaminated from the otic epithelium and migrated to the cochleovestibular ganglion. Our findings demonstrate that nuclear cProx1 protein is a marker for prosensory and proneural regions of the developing avian otocyst.

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728 Effects of Retinoic Acid and Antisense Morpholinos on Seven-up Expression and Inner Ear Development in the Zebrafish

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The vertebrate inner ear, including the zebrafish, Danio rerio, develops from an ectodermal otic placode. Although most vertebrates form the otocyst by placode invagination and the zebrafish otocyst forms by cavitation, many of the same genes are involved in inner ear development. We examined the role of the seven-up (svp) gene family in zebrafish and chick inner ear development. Svps are orphan receptors, homologues of COUP-TF genes that play a role in mammalian inner ear development. In the zebrafish, svp gene expression is sensitive to retinoic acid (RA), one of the regulators of seven-up function (Fjose et al,1995). We followed inner ear development in the zebrafish between hrs. 11- 30 of embryonic

development by time-lapse confocal microscopy. Wild type and inner ear mutant zebrafish were labelled with bodipy-ceramide. Antisense oligo morpholinos to svp genes (Gene Tools) were injected at the 1- cell stage, blocking translation of svp. Whole mount in situ hybridization was used to follow the expression of svp and downstream genes, including BMP4 which were affected by morpholinos and RA. Inner ear development was also affected at the gross morphological and cellular levels. The zebrafish provides an ideal model system to study gene cascades involved in inner ear development and the morphological events in semicircular canal formation. The entire process can be followed by time-lapse confocal microscopy, loss- and gain-of-function experiments can be done beginning at the one cell stage and gene expression observed in the translucent embryo and inner ear.

This work was supported by grants from the NIH, NSF, and Michigan Diabetes Research and Training Grant to KFB, by an IIRPG from the NIH to Monte Westerfield, Kate Barald et al. Thanks to Monte Westerfield for hosting a sabbatical year (KFB) at the U of O, to Dong Liu and Lisa Maeves for probes and helpful discussions and to Anders Fjose for providing svp probes.

729 Zsix-1 is involved in the specification of hair cell fate in the inner ear of the zebrafish (Danio rerio).

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The inner ear of zebrafish arises, as in other vertebrates, from a thickening of head ectoderm, called the otic placode. After the otic vesicle (or otocyst) forms, cells delaminate from the ventral floor and migrate medially to differentiate into neurons of the auditory ganglion. The first inner ear sensory organs (hair and support cells) also arise from the ventral region of the otocyst, though later. We are interested in investigating the genetic basis underlying the choice that those ventral cells have to make in order to acquire either a neuronal fate or a sensory cell fate. One set of candidates is the members of the so-called 'pax-eya-six' pathway. This pathway has already been demonstrated as being involved in the development of the eye in mammals and insects and of the muscle in mammals. Several of these genes are expressed during inner ear development, hence, we decided to study the involvement of one of the zebrafish six genes: Zsix-1. We cloned Zsix-1 and studied its expression pattern. Its expression started at 20hpf (hours post-fertilization) and is restricted to the otic and lateral line placodes. During otocyst formation, the Zsix-1 expression is restricted to the ventral region, then to the otic ganglion. The Zsix-1 expression is no longer detectable after 2 days of development. We chose to address the function of Zsix-1 in inner ear development by knocking down its expression in embryos by injecting a morpholino-modified oligonucleotide directed against Zsix-1. The preliminary results we obtained showed that hair cells of the anterior macula are either reduced in number or disorganized in the experimental versus control embryos. Furthermore, the number of neurons in the otic ganglion seems to be dramatically increased. Those results lead us to think that Zsix-1 and by consequence, the 'pax-eya-six' pathway could be an important actor in the specification of cell fates in the developing inner ear of the zebrafish embryo.

730 Evidence For A Compartmentalized Proneural Domain Within The Murine Otic Epithelium

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We have mapped the spatial extent of the murine otic neuroepithelium by expression analyses of neurogenin1 (ngn1), an Atonal-related gene with proneural activity, and NeuroD, both of which encode basic helix-loop-helix containing transcription factors with essential roles in vestibulocochlear ganglion development. Beginning at the onset of ngn1 expression, changes in the distributions of Eph and ephrin protein expressing epithelial cells result in contiguous expression domain boundaries that bisect the neurogenic zone into lateral and ventromedial sectors. At the interface of Eph and ephrin expression domains, boundary sharpening occurs as assessed by changes in the distribution of an independent cytoplasmic marker of lateral sector cells. The Eph-ephrin interface is the initial site of ngn1-positive cell delamination, a focus of heightened mitotic activity, and the boundary of an epithelial apoptotic domain. Mitotic activity varies independently in the adjacent epithelia flanking the interface, and the timing of sequential ngn1 and NeuroD expression relative to epithelial cell mitosis differs on either side of the interface. An apoptotic focus within the lateral sector exhibits horizontally dividing NeuroD-positive cells and other unique neurogenic cell types that are absent from the ventromedial sector. These results provide evidence that, in the murine otic epithelium, Eph-ephrin signaling plays a role in establishing a regionalized distribution of distinct neurogenic cell assemblies.

731 Lineage Analysis of the Developing Mouse Cochlea by Transuterine Injection of Ecotropic Retrovirus

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Fate mapping and lineage analysis of the inner ear has so far been confined to lower vertebrates. Some lineage questions may be specific to mammals in view of the increased complexity of cell types in the ear. In the cochlea one can ask about clonal relatedness of the following: within vs. beyond the organ of Corti (sensory vs. non-sensory); across the tunnel of Corti (inner vs. outer); hair cells vs. supporting cells; organ of Corti vs. neurons; or type I vs. II spiral ganglion neurons. Furthermore, analysis of clonal distribution might reveal lineage compartments important in cell fate specification.

We have established a transuterine injection method to target the mouse otocyst with replication-defective retrovirus. This will allow us to mark progenitors at early stages and map their progeny after cell differentiation is complete. A murine retroviral library encoding alkaline phosphatase (AP), CAP, was used so that clonal relationships can be confirmed by PCR-based sequencing of a 24-bp variable region in each viral genome (McCarthy et al., 2001, J. Neurosci. 21:6772-81). Dams were anesthetized at 11.25-11.75 days post coitus (plug day is 0.5) by ip injection of Nembutal/magnesium sulfate/ethanol/propylene glycol. The uterus was exposed by ventral laparotomy. Approximately 0.1 ul of virus (titer = 1 x 10^7 I.U./ml) with polybrene and fast green was injected through the uterine wall into the otocyst with a beveled glass micropipette. Embryos were harvested 3-4 days postinjection, paraformaldehyde fixed and stained for AP activity. Inner ear clones were obtained with high efficiency (51% of 33 injected embryos/5 dams). In pilot experiments, manipulated embryos from 3 dams were delivered vaginally and developed normally. With a reliable method now in hand, we plan to analyze the cellular composition of clones in the mammalian inner ear, with a special focus on the cochlea.

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732 Cellular patterning in the organ of Corti is dependent on interactions between epithelial and mesenchymal cells.

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Previous results have demonstrated that cellular differentiation in the organ of Corti proceeds in a gradient that begins in the base of the cochlea and extends towards the apex, suggesting that an organizing center located in the basal region may play a role in cellular patterning.

To test this hypothesis, cochlear ducts were dissected on E12 or E13 and the apical-most quarter turns were separated from the rest of the cochlea. The remaining portion of the cochlea was separated into 3 basal pieces. After 5 or 6 days in vitro, development of hair cells and pillar cells was determined using specific antibodies. Results indicate that 100% of apical and basal pieces develop normal cellular patterning, including rows of inner and outer hair cells with intervening pillar cells. These results demonstrate that the cues required for cellular patterning are already present throughout the cochlear duct by E12 and suggest that an organizing center is not required for the development of cellular pattern.

To determine whether underlying mesenchymal cells could act as a source for patterning signals, cochlear epithelial sheets were isolated from associated mesenchymal tissue, and then separated as described. Results indicate that at E13, 100% of apical and basal pieces develop differentiated hair cells and 86% of apical pieces and 93% of basal pieces develop a line or rudimentary row of hair cells. In contrast, only 58% of apical pieces isolated on E12 develop hair cells, compared to 100% in basal pieces, and only 14% of apical pieces develop an alignment of hair cells compared to 73% for basal pieces.

The results suggest that some of the cues required for normal patterning of the organ of Corti are present within the basal region of the cochlear epithelium by E12. The results also identify associated mesenchymal cells as a key factor in the early development of cellular pattern.

733 UB/OC-1 is a GER-derived Cell Line with the Potential to Differentiate into Hair Cells Without the Need of Math1

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We have used the conditionally immortal cell line UB/OC-1 to identify functionally related groups of genes associated with the early differentiation of hair cells. UB/OC-1 upregulates a number of key hair cell genes when it is cultured under differentiating conditions (39°C). During the first 1-2 days it transiently expresses mRNA for the bHLH transcription factor Hes1 and for the extracellular matrix protein b-tectorin. It does not express mRNA for the bHLH transcription factors Math1 or Hes5. This profile suggests that UB/OC-1 is derived from non-sensory epithelial cells from the greater epithelial ridge (GER), which are known to be competent to form sensory cells. At 39°C, all cells in UB/OC-1 upregulate the hair cell-specific transcription factor Brn3c, as well as other hair cell markers, without expressing math1. On the other hand, they failed to express several supporting cell markers, suggesting that they adopt a single phenotype. As a clonal cell line UB/OC-1 is particularly suited to analysis with gene arrays because it allows us to explore 'synexpression' groups of genes during differentiation. Genes within synexpression groups have a relatively high probability of being functionally linked. We analyzed UB/OC-1 at daily intervals for a period of 14 days with Affymetrix, oligonucleotide gene arrays. We then used a clustering algorithm to produce self organising maps of genes that shared similar expression profiles. We have generated clustered expression profiles for genes known to be involved in the Notch signaling cascade, as well as for other regulatory molecules. This information can be used to establish a catalogue of molecules involved in hair cell differentiation and to construct hypotheses that can be tested both in vitro and in vivo.

734 Isolation and In Vitro Differentiation of Sensory Hair Cell Progenitors from the Embryonic Mouse Inner Ear

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The progenitor cells that generate sensory hair cells in mammals are largely uncharacterized, in part because culture conditions that support precursor proliferation and differentiation have not been established. We have developed an in vitro assay system in which epithelial progenitor cells from embryonic mice can differentiate into myosinVIIa-positive hair cells. BrdU labeling indicates that some of these hair cells arise from cycling precursors and can survive for over one week. By adapting prospective isolation techniques commonly used in other fields, we can deplete differentiated hair cells from dissociated embryonic vestibular epithelial cells, and we show that the remaining population has the capacity to generate new sensory hair cells in vitro. We have begun the process of antigenically characterizing vestibular sensory progenitors by testing FACS-purified populations in this assay system.

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735 Stem-like cell phenotypes in cell culture of cochlear sensory epithelia of mammals

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Mammalian organ of Corti is a small organ with a fine mosaic structure consisting of terminally differentiated hair cells and supporting cells. Cochlear hair cells and supporting cells develop from the otic placode during embryogenesis and complete their proliferation in early postnatal development. A stem cell is a cell that is capable of self-renewing without limit and giving rise to various cell types throughout its lifetime. Tissue specific multipotent stem cells, which differentiate into all of the specialized cell types during organogenesis, have been found in many epithelial tissues. We have established the natural culture of the cochlear sensory epithelial cells (Zhao, Neurosci. Lett., 2001). In this experiment, cell proliferation/differentiation was examined by labeling with BrdU (bromodeoxyuridine), which is a DNA synthetic tracer and incorporates into the DNA during cell dividing. The micro-dissociated cells from sensory epithelia (organ of Corti) of adult guinea pigs were cultivated using our established cochlear cell culture technique. Some cells, but not all, exhibited a positive reactivity for BrdU labeling. The new cells could park tightly to form a compact spherical cell colony (ball) floating in medium. Within the ball, there were different morphotypical cells, implicating that cell proliferation and differentiation ensued. The ball continued growing expansively with cell division and finally divided or broken. Immunocytochemical staining revealed that the cells expressed with supporting cell markers or hair cell markers besides general epithelial cell markers. These data suggest that the cochlear sensory epithelial cells of mammals can be induced to proliferate and differentiate in culture, and that cochlear sensory epithelia may contain stem-like cells, just like other epithelial tissues.

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736 Targeted Ablation Of Connexin26 In The Inner Ear Epithelial Gap Junction Network Causes Hearing Impairment and Massive/Cell Apoptosis

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DFNB1 is an autosomal recessive form of isolated deafness that alone accounts for about half of the congenital hereditary deafness cases in western countries. It is one of the most frequent Mendelian disorders, affecting about 1 in 2000 children. DFNB1 is caused by mutations in the CX26 (GJB2) gene that encodes the gap junction protein connexin26 (CX26). Due to the embryonic lethality of Cx26-/- mice, the role of Cx26 in the inner ear is unknown. To address this issue, we performed targeted ablation of Cx26 specifically in one of the two cellular networks that it underlies, the epithelial network of the vestibule, the balance organ, and the cochlea, the auditory organ. No balance dysfunction was observed in homozygous mutant mice. In contrast, these mice exhibited hearing impairment and a progressive disorganization of the cochlear neuroepithelium. Notably, cell apoptosis appeared at postnatal day 14, i.e. just at the onset of hearing, initially affecting the supporting cells flanking the inner hair cells (i.e. genuine sensory cells). Subsequently, it extended throughout the epithelium. Our results show that Cx26-containing epithelial gap junctions play an essential role in cochlear physiology. We propose that Cx26 serves to clear glutamate released by the hair cells upon sound stimulation.

737 How the Duration and Level of Intense Tones Affect the Loudness Reduction (Recalibration) of Subsequent Weaker Tones

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The loudness of a moderate-level tone drops when preceded by a more intense tone, an effect called loudness recalibration by Marks (J. Exp. Psych. Hum. Perc. and Perf. 20, 382-396, 1994). The amount of recalibration (i.e., the decrease in the weaker or test tone's loudness level) was measured by a 2AFC procedure as a function of the duration and level of the stronger recalibration tone. A 500-Hz test tone at 60 or 70 dB SPL was matched in loudness to a 2500-Hz variable-level comparison tone; both lasted 200 ms. Results for twelve listeners showed that the amount of recalibration depended on the duration of the recalibration tone, but not on its level; 5-ms recalibration tones, whether at 80, 95, or 110 dB SPL, yielded only 3 to 4 dB of recalibration. Long recalibration tones at 80 (200 and 500 ms) and 95 dB SPL (200 ms only) yielded 7 to 11 dB of recalibration, again with no apparent effect from the level of the recalibration tone, but with more recalibration for a 70-dB than for a 60-dB test tone. Although the 5-ms recalibration tones at 95 dB SPL were about as loud as the 200-ms recalibration tones at 80 dB SPL, they yielded much less recalibration. A follow-up experiment addressed the following question: Did 5-ms recalibration tones induce little recalibration because they were so brief in absolute terms or because they were so brief relative to the 200-ms test tones? The test and comparison tones were now shortened to 5 ms. Results for twelve normal listeners showed that recalibration tones at 80 dB SPL--whether 5 or 200 ms long--yielded the same average amounts of recalibration for the 5-ms test tones: 4 dB for 60-dB test tones and 9 dB for 70-dB test tones. Taken together, these experiments suggest that (1) recalibration is governed neither by the loudness nor by the sound pressure level of the recalibration tone and (2) the amount of recalibration is small when the recalibration tone is briefer than the test tone.

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738 Effect of masker variability on forward masking: A test of temporal window models.

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One account of forward masking attributes the threshold elevation following the offset of a masker to adaptation, perhaps at the level of the eighth nerve. Another account attributes the effect to a continuation or persistence of neural activity after stimulus offset, resulting in temporal overlap of signal and masker at some more central point in the auditory system. Models based on the second account describe the persistence in terms of a temporal window and assume that the limitations on performance are comparable to those observed in simultaneous masking. If so, then variability in masker level from interval to interval should have the same effect as in simultaneous masking, where the resulting limitation on performance is described in terms of the properties of the ideal observer. To test this hypothesis, four subjects were tested in a forward masking task with a 4-kHz, 10-ms signal and 2.4-kHz tone, 200 ms masker. Signal delays of 0 and 30 ms were used. The masker was well below the signal in frequency to reduce the effects of peripheral nonlinearity. In separate blocks of trials, masker level was either fixed at 70 dB SPL or randomly drawn for each interval from a Gaussian distribution (in dB), with a mean of 70 dB SPL and a standard deviation of 2, 4, or 6 dB. There was no significant effect of masker-level variability on forward-masked thresholds. Control conditions using forward maskers and signals of the same frequency also showed no effect. Control conditions using simultaneous maskers showed a marked effect, as predicted by the ideal observer model. These results provide strong evidence against the temporal window account of forward masking. Correlational analyses of masker levels and subjects' responses on a trial-by-trial basis support the alternative, adaptation account.

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739 Temporal Course of Suppression

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Studies of two-tone suppression measured in the auditory nerve (AN) have shown that suppression is instantaneous and does not depend on the duration of the suppressed tone and the suppressor (Sachs and Kiang, 1968). In contrast, data from psychophysical studies appear to suggest that it takes about 40 - 100 ms for suppression to build up (e.g., Shannon, 1976). The reason for this discrepancy may lie in different measures of suppression used in AN and psychophysical studies. The present study investigates suppression as a function of duration in an experiment designed to keep the inferred output of the BM constant for the masker presented alone and with a suppressor. In a forward-masking task, a 10-ms, 4-kHz probe followed a longer-duration masker in one condition and the masker and suppressor in another condition. The masker was a 1-ERB band of noise geometrically centered at 4 kHz. The suppressor consisted of two 1-ERB bands of noise, the lower centered at 2 kHz and the upper centered at 4.8 kHz. The levels of the probe and the suppressor were fixed and the level of the masker was varied until it masked the probe. The difference between the masker levels at threshold in the presence and the absence of the suppressor was used to estimate the magnitude of suppression. Masker and suppressor durations between 20 and 300 ms were examined. The results are

consistent with AN data, i.e., no change in the magnitude of suppression was apparent for these different durations.

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740 Cueing effects in backward masking in adults and children

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Backward masking is a test condition in which a brief signal is immediately followed by a masking noise. It is notorious for its large within and across subject variability, late maturation, and potentially dramatic improvements of the detection threshold with practice. It has been suggested that listeners find backward masking particularly difficult because of its inherent temporal uncertainty (Puleo JS & Pastore RE, JASA 1980; 67:947-51). Experiments presented at last year's ARO meeting supported this hypothesis. They showed that a temporally informative noise cue with a fixed delay time to the signal onset improved the detection threshold in naive listeners by up to 40 dB. The current experiments were designed to test the "temporal uncertainty" hypothesis by making the noise cue temporally uninformative, using a random delay time between cue and signal. The temporally informative cue reduced the amount of masking in adults on average by more than 20 dB, which is close to the values obtained in previously reported experiments (Rose MM et al, ARO abstracts Vol. 24, 2001 #886). On average, the cue was equally effective in both the temporally informative and temporally uninformative conditions. This implies that temporal uncertainty is not a major factor in backward masking. Alternatively, the noise cue might provide a template which aides detection of a deviant sound by effectively changing a 2I-2AFC recognition task into a 4I- 2AFC "odd one out" detection task. Implications for the use of backward masking as a "magnifying glass" on processing efficiency (Hartley DEH, PH.D. Thesis, Oxford University, 2000), and as a tool to study attention and memory processes in children and adults will be discussed.

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741 Monaural phase sensitivity: All in the ear?

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We propose a model which accounts for the facts that listeners are sensitive to phase differences between the envelopes of sounds occupying remote frequency regions, and between the fine structures of partials that interact within a single auditory filter, but are insensitive to such phase differences when the frequency separation between the partials is large enough to preclude such interactions. It is argued that the model is superior in this regard to approaches which explicitly discard all across-channel timing differences, and which therefore cannot account for listeners' sensitivity to small across-channel differences in envelope phase. Instead, we propose that insensitivity to phase differences in the fine structure of widely-separated tones is due to the rapid phase transitions that occur near the peak of the basilar membrane travelling wave, which means that auditory neurons responding to each tone do so at a wide variety of phases. No such transition occurs with broader-band stimuli, such as pairs of bandpass filtered pulse trains, and between which listeners can detect small ongoing timing differences. It is shown that sensitivity or otherwise to phase differences can be predicted qualitatively by visual inspection of "auditory spectrograms" of the stimuli. Sensitivity is predicted quantitatively by analyzing the auditory spectrograms using spectro-temporal response fields based on the responses of neurons in auditory cortex, and by computing a distance metric between the responses to two stimuli to be discriminated. Discriminations successfully modeled include phase differences between pairs of bandpass filtered pulse trains and between pairs of sinusoidally amplitude modulated tones, discrimination between amplitude and frequency modulation, and discrimination of transient signals differing only in their phase spectra ("Huffman sequences").

742 A Monaural Cross-Frequency Coincidence-Detection Model for Masked Detection of Low-Frequency Tones

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Last year at this meeting, we presented a model for detection of tones in reproducible noise waveforms that was based on a population of cross-frequency coincidence detectors. The model was able to predict the differences in detectability of a tone across an ensemble of reproducible noises. The model has also been shown to predict thresholds for detection in a roving-level paradigm, a case for which the power-spectrum (or "energy") model of masking fails. The strongest cue for detection in both of these cases is a reduction in the response of some cross-frequency coincidence detectors upon addition of the tone to a noise masker. The reduction in response is due to phase differences between auditory-nerve fibers tuned to different frequencies; we refer to this model as the phase-opponency (PO) model for masked detection.

In this study, we explore the ability of this model to explain several classical phenomena associated with detection of tones in noise. We will present estimates of the model's critical ratio based on simulated detection of tones in noise maskers at several spectrum levels. We will also present estimates of critical bands based on both simulated band-widening experiments and on the notched-noise method for estimating equivalent rectangular bandwidths. The general trends in the results of these simulations are appropriate for human listeners. The detailed values of the estimated thresholds and bandwidths can be used to adjust the parameters of the auditory-nerve model and the model coincidence-detecting cells for further studies of masking phenomena.

743 Upward Spread of Masking by Harmonic Complexes with Different Phase Spectra in Normal-Hearing and Hearing-Impaired Listeners

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In normal-hearing listeners, harmonic complexes with phases selected according to the Schroeder algorithm can produce large differences in masking, depending on whether phases increase (positive Schroeder) or decrease (negative Schroeder) with frequency. The masking difference, which has been attributed to an interaction between stimulus phase and the phase characteristics of auditory filters, is greatly reduced in hearing-impaired listeners. In this study, masking by harmonic complexes was investigated for signal frequencies above the frequencies in the masker in normal-hearing and hearing-impaired listeners. Maskers were Schroeder-phase complexes with frequencies from 200 to 2000 Hz and a fundamental frequency of 100 Hz, at masker levels of 70, 80, and 90 dB SPL. Signal frequencies ranged from 1000 to 4000 Hz. As has been reported many times, for signals within the frequency range of the masker (2 kHz or less), more masking was observed in normal-hearing listeners for negative Schroeder-phase maskers than for positive Schroeder-phase maskers, with smaller differences in masking shown by hearing-impaired subjects. For signal frequencies above the masker, more upward spread of masking was observed for hearing-impaired than normal-hearing listeners. Further, most subjects in both groups showed a reversal in the effectiveness of

the maskers, with the positive Schoeder-phase complex providing more masking. The reversal in masking effectiveness was more pronounced at higher stimulus levels. This shift in the Schroeder masking effect may be related to phase changes occurring in the low-frequency skirts of the auditory filters.

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744 Effect of Modulator Phase on Modulation Detection Interference

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Past studies of modulation detection interference (MDI) have shown either a small or inconsistent effect of the phase relationship between the probe and masker modulators. The present work examined the effect of modulator phase in two experiments which utilized stimulus manipulations intended to promote perceptual segregation of the probe and masker. In both, the common probe and masker AM rate was either 4 or 10 Hz with the task detection of probe modulation. In the first experiment, the probe carrier was either a pure tone or a three-component harmonic complex. The intent was to determine if use of a harmonic complex for the probe carrier would encourage segregation to reveal an effect of modulator phase. For both the single- and three-component probe carriers, the amount of MDI was not significantly affected by the modulator phase relationship. The second experiment examined the relationship between asynchronous gating of the probe and masker carriers, modulator waveshape, and modulator phase. In the asynchronous conditions, probe onset was delayed 500 ms. Modulator waveshape was varied in terms of envelope slope while maintaining either the 4- or 10-Hz envelope periodicity. Significant phase effects were obtained with the 4-Hz modulators. Increasing modulator slope in the synchronous-gating conditions resulted in less MDI with anti-phasic than phasic probe and masker modulation. In contrast, the phase effect in the asynchronous-gating conditions diminished with increasing envelope slope. This opposite trend arises from the fact that at a low AM rate, anti-phasic modulation with a steep modulator slope is in some ways similar to asynchronous carrier gating. There was no significant effect of the envelope phase relationship with 10-Hz modulation. Results indicate that a consistent phase effect in MDI can be obtained at a low AM rate in conditions which allow for perceptual segregation of the probe and masker by manipulating gating synchrony.

745 Modulation gap detection: No evidence for ringing in the modulation filters

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We searched for evidence of "ringing" at the output of the modulation filters that have been proposed to exist in the auditory system. In experiment 1, the task was to detect a gap in the sinusoidal amplitude modulation imposed on a 4-kHz carrier. The modulator preceding the gap ended with a positive-going zero-crossing. The modulator started at the end of the gap with one of three phases: zero-phase, at a positive-going zero-crossing; π-phase, at a negative-going zero-crossing; "preserved" phase, at the phase the modulator would have had if it had continued without interruption. Modulation frequencies were 5, 10, 20 and 40 Hz. For the zero-phase and preserved-phase conditions, the detectability index, d', measured in a 2AFC task, increased monotonically with increasing gap duration. For the π-phase condition, performance was good (d' > 1) for small gap durations, and initially worsened with increasing gap duration, before improving again for longer gap durations. This is the pattern of results predicted from "ringing" at the output of a modulation filter broadly tuned to the modulation frequency. However, it is also possible that a rhythm cue was used to improve performance in the π-phase condition for short gap durations; the introduction of the gap markedly disrupted the regular rhythm produced by the modulator peaks. In experiment 2, the rhythm cue was disrupted by varying the modulator period randomly around its nominal value, except for the modulator periods immediately before and after the gap. This markedly impaired performance, and resulted in psychometric functions that were very similar for the zero-phase and π-phase conditions. This pattern of results is not what would be predicted from "ringing" at the output of a modulation filter tuned to the modulation frequency. We conclude that either the modulation filters do not exist, or they do not ring in the same way as mechanical or electrical filters.

746 Persistence in Audition

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Persistence and adaptation have been proposed as mechanisms to account for forward masking, but there is no strong empirical evidence to support either explanation. Given that high-intensity sounds produce more forward masking for a longer period of time after masker termination than low-intensity sounds, the persistence explanation predicts that high-intensity sounds have a longer perceived duration than low-intensity sounds. The adaptation explanation predicts equal perceptual durations for low and high intensity sounds. To test this idea, we measured duration-matching functions for rectangular-gated broadband noise for two levels (33 dB SPL and 93 dB SPL) for standard durations of 50 ms and 500 ms. Ten listeners with normal hearing participated. The high-level noise was perceived to be statistically significantly longer than the low-level noise for both durations, but the effect was larger for the 50 ms standard (factor of 1.23) than for the 500 ms standard (factor of 1.08). Temporal masking patterns for these stimuli collected from three listeners are in quantitative agreement with the subjective-duration judgements. These findings support the notion that forward masking represents a persistence of perception.

747 Deficits In Processing Efficiency In Individuals With Specific Language Impairment And Dyslexia

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Individuals with specific language impairment (SLI; Wright, B.A., et al. Nature, 1997; 387:176-178) and dyslexia (Wright, B.A. et al., unpublished data) have been found to have severe deficits for the perception of a brief tone presented before a masking noise (backward masking). However, when the tone was presented during the noise (simultaneous masking) participants with language and reading impairments performed nearly as well as controls. It was concluded from these studies that language and reading impairments are associated with poor temporal resolution. Here we report an analysis of data in terms of a model of temporal resolution (Moore, B. C. J. et al. JASA, 1988; 83:1102-1116). The model consists of four stages: i) a filtering device, ii) a linear device, which simulates the compressive input-output function of the basilar membrane, iii) an asymmetric temporal window and, iv) a decision device. In terms of the model, poor temporal resolution suggests a widened temporal window. Our analysis suggests that children with SLI and dyslexia have a normal temporal window. Instead, the model suggests that poor processing efficiency can account for deficits in backward masking amongst individuals with SLI and dyslexia. In terms of the model, this suggests that, at threshold, the decision device in subjects with SLI and dyslexia requires a higher signal-to-noise ratio for detection to occur, compared with controls. It has been suggested that poor processing efficiency encompasses factors such as poor attention, cognition and motivation. We also analysed other properties of backward masking in terms of the model of temporal resolution. The model correctly predicted that backward masking i) is worse in younger children, ii) is more prone to training effects, iii) has

greater inter- and intra-subject variability and, iv) increases less with masker level, than other masking tasks.

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748 Spatial Frequency Channels in Audition?

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Several investigators have suggested the existence of "channels" in the auditory system tuned to spectral envelope frequency that function in a manner analogous to spatial frequency channels in the visual system. Complex luminance patterns across retinal space are effectively decomposed into a series of simple (sinusoidal) luminance gratings by cells in the visual cortex (VI) tuned to different spatial frequencies (cycles/degree) and angles of orientation. One hypothesis is that complex amplitude patterns across cochlear space are similarly decomposed into a series of simple (e.g., sinusoidal) spectral patterns by "channels" or cells in the auditory pathway tuned to different spatial frequencies (cycles/octave). In vision, studies of selective adaptation to spatial frequency have provided strong psychophysical support for the existence of spatial frequency channels. Analogous measures in the auditory system are reported here. The listening task involved determining a contrast threshold for sinusoidal spectral modulation (in cycles/octave) superimposed upon bandlimited noise carriers (200 to 12800, 800 to 1600, or 6400 to 12800 Hz). Spectral modulation transfer functions (SMTFs) were obtained without adaptation and following adaptation to either a flat or a sinusoidally modulated spectrum (1, 2, or 3 cycles/octave). The starting phase of the sinusoidal modulator was randomly varied (0 to 2π radians) to limit the extent of peripheral adaptation. For each of the three carriers, sensitivity to spectral modulation was reduced markedly near the adapting frequency and changed little at remote frequencies. The resulting adaptation tuning curves had a bandwidth of approximately one octave and a dynamic range of about 4 dB. The present results provide strong support for the hypothesis that spectral shape perception is mediated by auditory channels tuned to spatial frequency.

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749 A Genetic Screen in the Zebrafish for Mutations that Affect the Ear and Lateral-Line Organ

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To discover novel genes that are involved in the function of the vertebrate ear, we have taken a classical genetic approach. As our experimental animal, we have chosen the zebrafish, *Danio rerio.* Its ear structure resembles that of humans; its external development and transparency allow us to study the ear in the intact animal; its fecundity provides us with large numbers of mutant progeny; and its extensive genetic tools facilitate the molecular cloning of mutated genes.

We conducted an F_3 screen in zebrafish using assays focussed solely on the acoustico-lateralis system. To identify mutations that interfere with early development, from the induction of the otic placode to the differentiation of the first hair cells, we scored the morphology of the developing ear under a compound microscope on the second day of embryogenesis. To identify mutations that compromise the operation of the ear and lateral-line organ in the free-swimming larva, we observed balance, the startle reflex in response to sound, and dye labeling of neuromasts in the lateral-line organ between embryonic days five and seven.

We detected mutant phenotypes in 37 of the 240 F_2 families that have been analyzed completely; another 150 F_2 families remain to be screened. We have outcrossed the 19 most promising mutant lines and begun their histological and physiological characterization. At least four mutants exhibit no receptor potential in the internal ear, suggesting a defect in the sensory apparatus. We have also initiated the positional cloning of the mutated genes and so far have obtained linkage for four lines.

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750 Non-syndromic dominant and recessive deafness at the DFNA36 and DFNB7/B11 loci is caused by mutations of TDC1, a novel gene encoding a transmembrane protein of hair cells.

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Although nearly 20 human nonsyndromic deafness genes have been identified, the genetic dissection of the auditory system is incomplete. We have positionally cloned a novel human gene, TDC1, which underlies both dominant and recessive forms of nonsyndromic hereditary sensorineural hearing loss at the DFNA36 and DFNB7/B11 loci. Seven different recessive TDC1 mutations have been identified in 11 of 11 families segregating recessive nonsyndromic sensorineural deafness linked to this locus. These recessive mutations include nonsense mutations, missense substitutions, a single nucleotide deletion, and a genomic deletion of 2 exons, providing strong genetic evidence that the causative gene has indeed been identified. One heterozygous missense substitution in a conserved residue of TDC1 was observed to cosegregate with dominant, rapidly progressive sensorineural hearing loss in a single large family. The predicted amino acid sequences of this gene, the closely related gene TDC2, and their mouse orthologs indicate they represent a family of novel genes that are transcribed in the inner ear at very low levels. TDC1 and TDC2 are predicted to encode transmembrane proteins, and the orthologous mouse Tdc1 and Tdc2 mRNAs are expressed in the postnatal inner ear. This data indicates that TDC1, and possibly TDC2, are required for normal structure or function of the mammalian auditory system.

751 Identification of a Gene Involved in Stereocillia Elongation - Positional Cloning of the Mouse *Whirler* Deafness Locus

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Genetic deafness is highly prevalent in the human population, affecting 1 in 2000 births. Many of these show primary abnormalities of the sensory epithelia of the inner ear, as do several mouse mutants. In the *whirler* (*wi*) mutant the stereocilia of the inner hair cells of the cochlear duct are considerably shorter than wild-type while outer hair cell stereocilia take on a more rounded U shape compared to the normal V or W shape. Cloning of the defective gene underlying *wi* will provide insight into the molecular processes involved in normal development of stereocilia as well as providing valuable insights into the causes of neuroepithelial deafness.

High resolution genetic and physical maps have been constructed in the vicinity of the *wi* locus using large interspecific and intraspecific backcrosses segregating the *wi* mutation. A BAC/PAC/P1 clone contig has also been constructed across the *wi* region. The *wi* non-recombinant region is contained within a minimal tiling path of three overlapping clones consisting of 2 BACs and a PAC. One of the BACs has been used in transgenic rescue experiments and been shown to rescue the inner hair cell phenotype of *wi* mutant mice, while there is partial rescue of the outer hair cell phenotype. *Whirler* mice carrying the BAC also demonstrate a normal Preyer reflex up to P95 and an absence of the usual circling and head tossing behaviour normally associated with the *wi* mutant. One of the genes in the BAC carries a mutation in *wi* mice and is expressed from the BAC in the rescued mutant mice. The gene shows no homology to other genes in the mouse or human genomes and represents a provocative novel candidate molecule involved in stereocilia elongation.

752 Positional Cloning of Tilted (*tlt*), a Mouse Mutant Lacking Vestibular Otoconia

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Otoconia are biomineral particles that overly the macular sensory epithelium of the utricle and saccule and contribute to the perception of spatial orientation, linear motion and balance. Tilted *(tlt)* is a recessive mutation mapping to mouse Chr5 that causes vestibular dysfunction in mice. The *tlt/tlt* mutant exhibits head tilting and swimming difficulty. The defect in *tlt/tlt* mice is limited to the utricle and saccule, which completely lack otoconia. To identify the *tlt* gene we used a positional cloning approach that combined linkage, physical and comparative mapping strategies. Sequence analysis of a BAC-based contig that completely covers the *tlt* locus resulted in the identification of a number of known genes and expressed sequence tag clusters. Mutation screening revealed a point mutation in a novel gene encoding a putative transmembrane protein that we designated "Otopetrin". Otopetrin is expressed in the developing otocyst at E16.5 and P0, as determined by RT-PCR. The characterization of the physiological function and pattern of expression of Otopetrin is under investigation and should provide further clues to understanding of the development, biosynthesis and function of vestibular otoconia.

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753 Claudin 14 Knockout Mice Are Deaf: a Mouse Model for Autosomal Recessive Deafness DFNB29.

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Tight junctions are circumferential strands around cells that selectively modulate paracellular permeability between extracellular compartments. Tight junction fibrils can be composed of more than one of the 20 members of the claudin family. Recently it was found that autosomal recessive deafness DFNB29 is caused by mutations in human CLDN14 (Wilcox et al., Cell 104: 165-172, 2001). Mouse Cldn14 is expressed in the sensory epithelium of the organ of Corti, and in kidney and liver. To explore the role of claudin 14 in these tissues we created a mouse model for DFNB29 by a targeted deletion of Cldn14.

Cldn14-null homozygous and heterozygous mice and their wild type litter mates underwent auditory brainstem response (ABR) analyses at 4 weeks of age. Responses to 50 µsec duration clicks, and 8, 16, and 32 kHz tone bursts were recorded. Cldn14-null homozygous mice were found to be profoundly deaf, while the hearing of their heterozygous litter mates is normal.

In the Cldn14-null allele a lacZ cassette replaces the only Cldn14 coding exon, and is expressed under the Cldn14 promoter, thus serving as a reporter gene for Cldn14 expression. Specific b-galactosidase activity was detected in inner and outer hair cells of the cochlea, in the collecting ducts in the kidney, and around the lobules in the liver of a Cldn14-lacZ heterozygous mouse. We are currently using various techniques to analyze the physiological basis for deafness in homozygous Cldn14-knockout mice, which are a valuable model to study claudin 14 role in the hearing process.

754 Jerker Deafness Mutation: Is Hair Cell Espin A Mediator of Actin Polymerization in Stereocilia?

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Identified originally as an actin cross-linking protein concentrated in the parallel actin bundle of the hair cell stereocilium and as the target of the jerker deafness mutation, hair cell espin displays a number of the properties expected for a mediator of actin polymerization. First, hair cell espin exhibits a modular organization with intriguing similarity to other proteins involved in actin polymerization: hair cell espin contains a consensus WASp homology 2 (WH2) domain; a binding site for phosphatidylinositol-4,5-bisphosphate; and two proline-rich peptides, which include a consensus profilin-binding site and binding sites for SH3 and WW proteins. Second, when expressed in transfected cells, hair cell espin causes a dramatic increase in the level of F-actin. In fibroblasts, hair cell espin constructs elicit the formation of cross-linked stress fiber-like structures or surface projections resembling microvilli, whereas in neurons they elicit the formation of star-like F-actin displays in the centrosomal region. The introduction of the jerker frameshift mutation, which results in mislocalization of hair cell espin to the nucleus, causes actin polymerization and bundling ectopically within the nucleus. Third, hair cell espin can mediate the polymerization of actin in a cell-free actin polymerization assay. When coated onto beads and incubated with rhodamine-labeled actin monomer in the presence of soluble extract of rat brain, hair cell espin causes the formation of star-like F-actin displays similar to those elicited by coated with WASp, a known nucleation promoter of actin polymerization. Fourth, hair cell espin accumulates in developing hair cell stereocilia during a period of actin polymerization that results in an increased diameter of the core actin bundle. These results suggest that, in addition to providing cross-links to the parallel actin bundle at core of the stereocilium, hair cell espin may mediate actin polymerization.

755 Single channel analysis of inner ear gap junctions for deafness mechanisms of nonsyndromic hearing loss

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Most cases of nonsyndromic hearing loss are caused by the gap junctional connexin (Cx) gene mutations. There are at least 7 Cx genes co-expressed in the inner ear supporting cells. However, only single Cx gene mutation can result in serious hearing loss. Cochlear Deiter and Hensen supporting cells mainly contain Cx26&30. Recording of macroscopic current has demonstrated that gap junctional coupling in the supporting cells has variable transjunctional voltage (Vj) and membrane potential (Vm) dependences. Specially, the Vj and Vm dependences have predominant asymmetries of voltage gating and rectification in the cell directions. In this experiment, single channel activities of gap junctional coupling between the freshly isolated Hensen cell's pairs from the organ of Corti of guinea pigs were recorded using a double voltage clamp technique. The current traces exhibited three step-levels corresponding to the main open state, the residual state, and a substate in between. Single channel conductances at

the main open state, the residual state, and the substate were ~130 pS, ~35 pS, and ~95 pS, respectively, at -Vj, and were ~170 pS, ~60 pS, and ~110 pS, respectively, at +Vj. As the Vj increased, the channels descended to close, transiting from the main open state to the residual state, than to the close state. No direct transition from the main open state to the close state was observed. Asymmetrical Vj dependence was also found in the recording. The channels tended toward open instead of close at the +Vj. These single channel data were consistent with our previous macroscopic current recording, implicating that the configurations of gap junctional channels between the supporting cells are nonhomotypic and composed of at least two connexins. The functions of such hybrid channels can be impaired by one connexin mutation even other connexin components are normal. This may be responsible for single connexin mutation induced the serious hearing loss.

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756 Knockout Of KCNJ10 (Kir4.1) Potassium Channel Abolishes Endocochlear Potential

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Stria vascularis of the cochlea is known to generate the endocochlear potential (EP) and secrete potassium (K). K is the main charge carrier and the EP is the main driving force for the sensory transduction that leads to hearing. Stria vascularis consists of two barriers, marginal cells that secrete K and basal cells that are coupled via gap junctions to intermediate cells. The KCNJ10 (Kir4.1) K channel is expressed in strial intermediate cells [Ando & Takeuchi. Cell Tissue Res. 298:179-183, 1999; Sage & Marcus. Hear. Res. 160:1-9, 2001] and was proposed to be the site of EP generation. The EP and [K] of endolymph were measured with double barrel electrodes in the second cochlear turn and in the utricle. Homozygous gene knockout mice lacking the KCNJ10 (Kir4.1) K channel in strial intermediate cells did not generate an EP. Endolymph volume and [K] were reduced although thickness of the stria vascularis was not changed. The absence of EP was not due to systemic disturbance since vestibular endolymphatic [K] and electrical potential were normal. Further, the tunnel space in the organ of Corti of KCNJ10 knockout mice was widely open as in normal animals, indicating that a delay in cochlear development did not account for the absence of an EP. These studies establish that the KCNJ10 K channel provides the electromotive force for generation of the EP in concert with other transport pathways that establish the [K] difference across the channel. KCNJ10 was also found to be a limiting pathway for K secretion.

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757 *PDS* Gene (Pendrin) Knockout Abolishes Endocochlear Potential But Maintains Endolymphatic Potassium Concentration

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Pendrin is an anion transport protein with a distinct distribution in the inner ear, thyroid and kidney. In humans, mutations in its associated gene, *PDS*, cause Pendred syndrome. Within the ear, pendrin is expressed in the cation-absorptive outer sulcus and transitional cells and in the endolymphatic duct and sac; it likely plays an important role in ion homeostasis. As a step in understanding this role, we have used double-barrel electrodes to measure EP and potassium concentration ([K]) in the basal and apical cochlear turns and in the utricle, the –EP during anoxia in the apical cochlear turn and made histologic observations of the inner ear in *Pds*$^{-/-}$ mice. The cochlear and vestibular endolymphatic spaces were severely hydropic. Strikingly, the stria vascularis (the tissue responsible for generation of the EP and for K secretion) was only about two thirds the normal thickness and the spiral ligament was nearly absent. Consistent with the alterations in strial morphology, the EP was near zero in the apical turn and did not change significantly during anoxia, consistent with the reported loss of hair cells; the EP was negative in the basal turn. Surprisingly, the [K] was near normal in both the apical and basal turn of the cochlea and in the utricle and was therefore not the cause of hair cell degeneration in *Pds*$^{-/-}$ mice. The absence of the EP in the apical turn suggests pathology of the basal cells and/or the intermediate cells of the stria vascularis. We are currently attempting to define these defects and pendrin's role in the inner ear.

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758 Significance and diagnostic interest of high-intensity cubic difference tones

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Since their discovery, otoacoustic emissions (OAE) and particularly cubic difference tones (CDT) have been viewed as reliable tools for assessing the function of cochlear outer hair cells (OHC). This belief relies upon (1) circumstantial evidence that OAEs disappear when hearing loss due to OHC damage exceeds some limit and (2) physiological background, as OAEs are thought of as a by-product of the very mechanisms responsible for high sensitivity and tuning. However, in a number of OHC dysfunctions, CDTs elicited by primary stimuli > 60 dB SPL do persist: for the sake of consistency, they are often regarded as unsuitable and merely discarded. We consider instead that CDT level must depend at least on two cochlear characteristics, i.e. the input to its nonlinear elements (Inl) and the number of such elements (N), thus CDT level should be proportional to N.Inl^3. Two experiments were done, the first one with mutant CD1 mice with varying percentage of residual OHCs, hence varying N. Whenever cytocochleograms showed that N was near 0%, CDTs were absent whatever the primary intensity in the ear canal up to 80 dB SPL. The second experiment used sudden cochlear ischemia in gerbils (likely, N = 100%). Inl is involved because it depends on how the cochlear feedback loop works, however it is well-known that around 80 dB SPL, Inl tends to be unaffected by pathology. Indeed ischemic CDTs remained near-normal around 80 dB SPL, and generally varied as (input intensity)^3. We conclude that when primary intensities are low enough, Inl^3 gets so small when a pathology is present that low-intensity CDTs always disappear regardless of N. On the other hand, high-intensity CDTs no longer depend on how the cochlear loop influences Inl^3, thus their presence chiefly depends on N: It makes them highly valuable for diagnostic purposes (i.e. for gaining access to N). At lower levels, regrettably, the interplay of Inl and presumably other coefficients related to the exact shape of the nonlinearity at the origin of CDTs, make it illusory to expect a simple CDT_level-to-hearing_loss relationship.

759 DPOAE measurements reveal upward spread of suppression

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Decrements in DPOAE level due to a suppressor tone can be used as measures of response to the suppressor (e.g., Gorga et al., 2002). The present study used a similar paradigm to show how DPOAE decrements can be used to describe a pattern that is qualitatively similar to upward spread of masking. DPOAEs were measured in the presence of suppressor tones, f_3, fixed at either 2.1 or 4.2 kHz, and set to each of

seven suppressor levels (L_3) from 20 to 80 dB SPL in 10-dB steps. F_2 frequency was then varied from about 1 octave below to at least ½ octave above f_3, while L_2 was set to each of 6 values from 20 to 70 dB SPL in 10 dB steps. L_1 was set to maximize DPOAE level at each L_2 in ears with normal hearing (Whitehead et al., 1995; Janssen et al., 1998). At each L_2, L_1 combination, DPOAE level was measured in a control condition in which no suppressor was presented. Data were converted into decrements (in dB) by subtracting the DPOAE level in the presence of each suppressor from the DPOAE level in the control condition. Plots of DPOAE decrements as a function of f_2 showed maximum suppression when f_2 was close to f_3. As L_3 increased, the suppressive effect spread more towards higher f_2 frequencies, with little or no spread towards lower frequencies relative to f_3, especially when f_3 = 4.2 kHz. DPOAE decrement versus L_3 functions had steep slopes when $f_2 > f_3$, and shallower slopes when $f_2 < f_3$. These data are consistent with other findings that have shown that response growth for a characteristic place (CP) or frequency (CF) depends on the relation between CF and driver frequency, with steeper slopes when driver frequency is less than CF and shallower slopes when driver frequency is greater than CF.

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760 Cochlear Compression Estimates from Measurements of Distortion-Product Otoacoustic Emissions

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Evidence of the compressive growth of basilar membrane displacement can be seen in DPOAE levels measured as a function of stimulus level. When $L_1=0.4L_2+39$, the shape of the DPOAE level versus L_2 is similar (up to 70 dB SPL) to the classic Fletcher & Munson (1933) loudness function, plotted on a logarithmic scale. Below 70 dB SPL, these I/O functions appear to be fit well by a log-function, suggesting that DPOAE level is logarithmically related to stimulus level. If we define growth rate as the slope of the I/O function (dB/dB), then a cogent definition of compression is the reciprocal of growth rate. Normal cochlear compression varies from about 1 at threshold to about 4 at 70 dB SPL. When the I/O function is logarithmic, compression (α), becomes a linear function of stimulus level, $\alpha=(L+14)/22$. With cochlear hearing loss, compression is still about 1 at threshold, but grows more slowly above threshold. Median I/O data from ears with 25-35 and 40-50 dB HL still appear to be well fit by log-functions ($\alpha=(L+14)/32$ and $\alpha=(L+14)/52$, respectively). We also see evidence of cochlear compression in DPOAE suppression (threshold) tuning curves. In this case, both primary and suppressor tones exhibit compressive growth, but only their relative compression (RC) is observable in DPOAE suppression data. RC varies with frequency, but not with primary level at a fixed suppressor frequency (f_3). For normal ears, RC is about 1 when the f_3 is near f_2, indicating that cochlear responses to the two components grow at the same rate. RC rises to 4, when f_3 is an octave below f_2. The correspondence between DP I/O and loudness functions (group data) suggests that it might be possible to predict loudness growth from DPOAE measurements; but intra-subject variability makes such a comparison problematic.

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761 Adaptation of distortion product otoacoustic emission in awake rabbits

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Time-dependent changes in level of distortion product otoacoustic emission (DPOAE) were found to be partly effects of ipsilateral medial olivocochlear (MOC) reflex in anesthetized cats (Liberman et al., JASA, 1996). Here, we measured an analogous phenomenon in awake rabbits to gain information free from anesthesia. The two-tone stimulus had 5~10 ms rise/fall time, and 2.7~11 s burst duration. Time courses of level and phase of 2f1-f2 DPOAE were determined by using a heterodyne envelope analysis (Kim et al., JARO, 2001). At the onset/offset, DPOAE level often had a peak and/or trough. A rapid DPOAE phase change (0.1~0.6 cycle) accompanied these. A sufficiently wide bandwidth (320 Hz) of DPOAE analysis was needed to see the onset/offset events. The onset/offset events appear to be produced by a destructive interaction of two components of DPOAE. Subsequent to the onset event of about 10 ms, DPOAE exhibited a more gradual change not only in level but also in phase, to which we refer as DPOAE adaptation. We extended the method of fitting DPOAE adaptation with a complex-valued 2-exponential function so that both level and phase of DPOAE were fit. Here, each of the fast and slow components of adaptation has a time constant and amounts of level and phase change. The fast and slow time constants of DPOAE adaptation were on the order of 85 ms and 1.8 s, respectively. Time courses of DPOAE level and phase adaptation were typically biphasic. The initial part of DPOAE level was increasing or decreasing, and that of phase was lagging or leading. There was no fixed relationship between the polarities of level and phase changes. Large level changes of DPOAE adaptation were observed with stimulus conditions associated with a notch in input-output functions. We hypothesize that complexities in DPOAE time courses arise from interactions of two components of DPOAE and that the ipsilateral MOC reflex modulates the two components differentially.

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762 Chronic Intracochlear Strychnine Infusion Eliminates the Efferent Mediated Fast Adaptation of the Quadratic Distortion Product Oto-Acoustic Emission (DPOAE) in Guinea Pigs

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Efferent mediated fast adaptation of the quadratic DPOAE has been demonstrated in several species (Liberman et. al 1996, Sun & Kim 2000, Kim et. al. 2001). This measure of efferent nerve function has been shown to be a predictor of susceptibility to noise induced trauma (Maison & Liberman, 2000, Luebke et. al 2001) and to be eliminated by olivocochlear Bundle (OCB) sectioning (Liberman et. al 1996). This is consistent with studies showing that destroying efferent pathways through medial OCB sectioning or chronic infusion of strychnine (Yamasoba 1997) increases susceptibility to noise induced trauma. We have developed a system for measuring fast adaptation of the DPOAE, and show that the fast adaptation effect is eliminated after chronic infusion of Strychnine to cochlear perilymph. DPOAE adaptation was tested in anesthetized guinea pigs using a MATLAB ™ script developed in-house. Efferent-mediated fast adaptation was visible as a 1-3 dB drop in 2F1-F2 intensity over approximately the first 300-500 mSec of the DPOAE response. While animals were under anesthesia, auditory brainstem response (ABR) thresholds and DPOAE input-output functions were also obtained. A modification of the procedure described by Prieskorn & Miller (2000) was used to implant an osmotic pump (containing either 50 µM strychnine or artificial perilymph) and cannula terminating in the left scala tympani of each guinea pig. When tested 3-5 days post implant, subjects had no change in ABR thresholds or in DPOAE I/O functions. However, animals implanted with 50 µM strychnine showed elimination or a sharp reduction in the amplitude of the fast adaptation response.

These results demonstrate a technique for non-invasively testing for cochlear efferent function. In addition, the results confirm that Strychnine infusion is an effective method of eliminating cochlear efferent function, without affecting ABR or DPOAE sensitivity.

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763 Adaptation of the 2F1-F2 Distortion Product Otoacoustic Emission in Humans: The Effects of Binaural and Contralateral Stimulation

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Rapid adaptation of the otoacoustic emission cubic distortion tone (CDT) has been described in animal and human experiments. This phenomenon has been related to the normal activity of the medial olivocochlear (MOC) efferent system on the outer hair cells and, as such, provides a noninvasive measure of the activity the MOC system under varying stimulus conditions. In animals, binaural stimulation increases the adaptation response by approximately 30%, compared with monaural primary tones. The present data were collected in humans to characterize the effects of binaural stimulation and contralateral noise on the adaptation response. The CDT was measured under three different stimulus conditions. In the first, the primary tones were presented to only one ear. In the second condition, the two tones were presented simultaneously in both ears. In the third, contralateral broadband noise was presented at 80 dB SPL, beginning 4s after the primary tone onset in the test ear. F1 was presented at 70 dB SPL and varied from 1.5 to 8.0 kHz; the interstimulus interval was 2s. DPgrams were taken in both ears and the F1 producing the largest amplitude CDT at 70 dB SPL was used. The data were analyzed using the "heterodyne technique" as described by Kim et al. (JARO, 2001, 2, 31-40).

An important feature of the data is the substantial variability observed in measures from session-to-session within individual subjects and across subjects. Estimates of monaural rapid adaptation and time constants agreed well with the limited existent literature. The magnitude of the adaptation under binaural stimulation, as compared with monaural primaries, was 25% greater, and time constants were statistically shorter. With contralateral noise, the average suppression was approximately 1.2 dB SPL (0.25 – 2.7 dB SPL). The present data agree well with previous human and animal studies and indicate the value of the OAE rapid adaptation paradigm as a non-invasive measure of MOC function in humans.

764 Contralateral DPOAE Suppression In Humans At Very Low Sound Intensities

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It has been shown that reflex activation of the olivo-cochlear bundle system can excert a substantial effect on DPOAE. In animals, the suppression effect disappeared after sectioning the crossed olivo-cochlear bundle or middle ear muscles. In humans, sectioning is out of question. Thus, an involvement of the middle ear muscles in DPOAE suppression can not definitely be excluded. The purpose of the study was to find out whether contralateral DPOAE suppression (CSUP) occurs when applying contralateral sound intensities well below the reflex threshold of middle ear muscles.

CSUP was performed in 9 normally hearing subjects with outstandingly high emission levels at f2 = 2 kHz. Since DPOAE has been shown to reflect minute changes of outer hair cell function when elicited by close-to-threshold primary tone levels DPOAEs were measured at L2=20 dBSPL (L1=0.4L2+39). Broad-band noise (BB), narrow-band noise (NB) from 1830 to 2060 Hz, and a 2 kHz-tone were used for contralateral acoustic stimulation. The contralateral stimulus level Ls was decreased from 70 down to 10 dB SPL in 10 dB steps.

Ls at which significant CSUP occurred ($p<0.05$) was different for the different stimuli being 20 dB for BB, 40 dB for NB, and 70 dB for the 2 kHz-tone, respectively. CSUP exponentially increased with increasing Ls from 0.7 to 4.9 dB for BB and from 0.5 to 2.5 dB for NB. Significant CSUP to BB and NB at Ls well below the reflex threshold of middle ear muscles suggests the middle ear muscles not to be involved in DPOAE suppression. Thus, DPOAEs provide a non-invasive window on cochlear-efferent system under contralateral acoustic stimulation in humans. Due to the high CSUP threshold for the 2kHz-tone bilateral DPOAE recording in clinical application is recommended.

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765 Contralateral Suppression and Onset Adaptation of DPOAEs in the Rat

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Last year we presented preliminary evidence showing that onset adaptation and contralateral suppression of DPOAE's in the rat were the result of activation of the middle-ear reflex. The methods used in those studies included sectioning the middle-ear muscles, and injection of gentamicin or pancuronium. We will present the results of a completed study that relied only on sectioning the middle-ear muscles. Our original conclusion stands that both effects, which can be as large as 15 dB in the rat, are mostly eliminated by sectioning both middle-ear muscles. However, in the new, more complete data set, we also see residual effects that have a magnitude of 0 to 3 dB. The latter is most likely the result of activation of the MOC reflex. We have also examined in detail the time course of the magnitude and phase of the DPOAE during contralateral suppression and onset adaptation. It was found that the size and direction of these effects for both the amplitude and phase of the DPOAE were correlated with the shape of the DPOAE input/output function. When the input/output function was non-monotonic with a local minimum, drastic changes in the temporal responses could take place over small changes in primary intensities. The wide variety of the responses we observed in this region of the input/output function emphasizes how difficult it might be to discriminate between causation by the middle-ear reflex and/or the MOC reflex using only the responses themselves, particularly if one only examines a narrow range of intensities. For instance, while after sectioning the middle-ear muscles phase changes associated with residual suppression or onset adaptation were largely absent, conditions could be found that resulted in nearly zero phase shifts (with measurable magnitude changes) prior to sectioning as well.

766 Relative contributions of tensor tympani and the stapedius muscle in the middle ear reflex in the rat, as measured by contralateral suppression of DPOAEs.

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In the rat, the contralateral suppression of DPAOEs is mostly mediated by the middle-ear reflex (Relkin et al., 2001; Woods et al., 2001) with a minor contribution from the MOC reflex. Within the middle-ear reflex, the question of the relative contribution to suppression of DPOAEs by the stapedius muscle and by tensor tympani exists. There is apparent interspecies variability in the degree to which the stapedius muscle's involvement predominates. In the rat, evidence exists that the tensor tympani contributes little to the middle-ear reflex (Murata et al, 1986).

In one series of experiments, we record contralateral suppression of DPOAEs at several DP frequencies. At each DP frequency the intensities of the primary tones are varied. Subsequently the tympanic bulla is exposed and opened via a postauricular approach. The tensor tympani is cut, and the contralateral suppression is again measured over the range of DP frequencies and primary tone intensities as in the intact condition. Finally the stapedial tendon is cut and the paradigm repeated. In a second series of experiments the order of the muscle cutting is reversed, allowing for observation of tensor tympani function without

the stapedius muscle in place and comparison to the condition where both muscles are cut.

Tensor tympani and the stapedius muscle are compared with regard to the amount of contralateral suppression produced, the DP frequency range over which they are active, the onset and offset characteristics of suppression, latency and phase shift. Preliminary data point to tensor tympani function that is significantly greater than that previously attributed to it in the rat.

767 A detailed analysis of the effects of primary levels on DPOAE components

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Recent modeling and experimental efforts have established the presence of two spatially and mechanistically distinct sources of distortion product otoacoustic emissions (DPOAEs) in humans (e.g. Talmadge et al., 1997,1998,1999; Shera & Guinan, 1999; Knight & Kemp 1999,2000,2001; Konrad-Martin et al., 2001, Kalluri & Shera 2001). As a result, research is now focused on the specific characteristics of the two sources. Preliminary results that have already been published show that both primary level and frequency ratio play significant roles in determining the absolute and relative contributions from the two sources to the signal recorded in the ear canal (e.g., Knight & Kemp, 2000, 2001, Konrad-Martin et al., 2001). Most studies to date have used either suppression or inverse-FFT methods to separate the components of DPOAEs.

In this paper we present a detailed analysis of the role of primary levels in determining the contribution from the different sources. Data recorded with a wide range of primary levels and several primary frequency ratios will be presented. The two primary components are obtained using an inverse-FFT paradigm. The data is evaluated in the framework of the two-source interference model (Talmadge et al., 1997,1998,1999).

768 Mechanisms of OAE Production in Humans

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It is evident that there are two distinct sources of DPOAE in humans (Heitmann et al., 1998; Brown et al., 1996; Brown & Gaskill, 1996), with a theoretical framework arguing also for two distinct mechanisms of OAE production i.e., i. a retrograde traveling wave produced by the amplifier induced pressure gradient across the basilar membrane, and ii. linear coherent reflections (encompassing intra-cochlear standing waves) that are level-dependent, being less significant at high stimulus levels (Zweig & Shera, 1995; Talmadge et al., 1998, 1999; Shera & Guinan, 1999). Available evidence argues for two distinct mechanisms (Shera & Guinan, 1999), with DPOAE fine structure being explained by the rapid phase rotation of the CF place component relative to the component arising from the f2 region. The level-dependency of the mechanism of production of the CF place component has not been examined to date, and the extent of published data on DPOAE fine structure is limited. Here we present DPOAE fine structure data at a number of stimulus levels (L2 = 30 to 70dB SPL), suppressed and unsuppressed, examining whether the mechanism of production of the CF place component is level-dependent and whether there can be a shift in mechanism of production of the CF place component that is place-dependent.

769 Using DPOAEs to Measure Forward and Reverse Middle-Ear Transmission Noninvasively

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The interpretations of physiological and psychophysical measurements of hearing are often confounded by incomplete knowledge of the transmission characteristics of the middle ear. Although middle-ear transfer functions have been measured invasively in human cadavers and in laboratory animals, the substantial variability among ears from the same species limits the power of mean data to predict the characteristics of individual middle ears. Building on the work of Keefe (*Assoc. Res. Otolaryngol. Abs.*, 2001), we describe a promising method for using distortion-product otoacoustic emissions (DPOAEs) to measure the frequency dependence of forward and reverse middle-ear transmission noninvasively. As with the procedure outlined by Keefe, the method depends on the scaling properties of DPOAE generation in the cochlea (Shera and Guinan, 1999, *J. Acoust. Soc. Am.* **105**:782). We discuss experimental tests of the method's assumptions and validate the method in the scaling region using simulated middle-ear transfer functions and a simple model for DPOAE generation. We compare our method with that of Keefe and use model results to explore the systematic errors inherent in his procedure. Finally, we discuss extensions to the method---including the use of DPOAE unmixing (Kalluri and Shera, 2001, *J. Acoust. Soc. Am.* **109**:622) to mitigate the effects of DPOAE microstructure---necessary for applying the method in individual subjects.

770 Level-dependence of optimal stimulus-level difference for evoking DPOAEs in the gerbil

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Distortion product otoacoustic emissions (DPOAE) are produced by nonlinear mechanical amplification in the cochlea and strongly depend on functioning outer hair cells. To maximize DPOAE levels and hence to increase the sensitivity of DPOAE measurements, the level separation of the two primary stimuli has been shown to play a crucial role. In contrast to the conventionally used paradigm L1 = L2 + 10 dB, Kummer et al. (2000) found a variable level separation L1-L2 to be optimal for evoking maximal DPOAE levels in humans. They described this optimal level separation by the equation L1= 0.4L2 + 39 dB and used it to record threshold curves of mechanical auditory sensitivity. To obtain an adequate animal model for determination of auditory sensitivity and its pathologies our aim was to measure corresponding optimum level differences in the gerbil Meriones unguiculatus.

DPOAEs were recorded at two different test frequencies f2= 3 and 10 kHz and three different frequency ratios f2/f1 for each f2-frequency. Both stimulus levels L1 and L2 were varied from 15 up to 70 dB SPL in 5 dB steps resulting in 144 L1 x L2 level combinations.

The results showed that, as in humans, a variable level separation L1-L2 is optimal for generating maximal DPOAEs in the gerbil. With decreasing L2 the optimal level separation increased up to maximal 19.5 dB at L2= 15 dB SPL. The corresponding equations differed significantly between the tested frequency ratios (1.2, 1.28, 1.36) and between the tested f2 frequencies and ranged between L1opt= 0.5 to 0.78L2 + 12.3 to 26.7 dB. This indicates that in the gerbil the corresponding DPOAE growth functions are slightly steeper than in humans. Despite such smaller quantitative differences the gerbil proves to be a good animal model for DPOAE measurements using the new stimulus paradigm as employed in clinical research.

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771 Distortion Product Otoacoustic Emissions from the Basilar Papilla of the Tree Frog, Hyla cinerea.

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For the generation of distortion product otoacoustic emissions (DPOAE) in the cochlea, currently a disparate-place two-source model is accepted (Talmadge et al. '99). One source originates from the place of generation in the overlap region, and the other source originates from the distortion product tonotopic site. In these models, the interaction between the two cochlear sources gives rise to the DPOAE fine structure, and can explain the distinct notch in input-output functions of DPOAE (Mills '97). The frog inner ear lacks a cochlea but contains two hearing papillae, the amphibian papilla (AP) and the basilar papilla (BP), respectively. The BP is a simple auditory receptor mainly tuned to one specific frequency in each individual. Furthermore, neither papilla in the frog inner ear is over a basilar membrane. Here we report on DPOAE emitted from the BP of the tree frog. The primary tone frequencies (f_1 and f_2, respectively) for which the BP elicited maximum cubic distortion tone (CDT) were determined. Next, for this optimum set of stimulus frequencies, the level of the CDT was measured as function of tone levels L_1 and L_2. The levels were varied independently between 35 and 85 dB SPL with a 5 dB step interval. Preliminary results are in qualitative agreement with the model and experimental results in mammals presented by Mills (Mills '97). The results presented here are in support of the idea that DPOAE from the BP are generated in the same way as in the cochlea. Because of the anatomical properties of the BP it is hard to see how the two-source model is applicable in the frog inner ear.

Mills, DM (1997). Interpretation of distortion product otoacoustic emission measurements. I. Two stimulus tones: JASA 102(1), 413-429.

Talmadge, CL, Long, GR, Tubis, A, and Dhar, S (1999). Experimental confirmation of the two-source interference model for the fine structure of distortion-product otoacoustic emissions: JASA 105(1), 275-292.

772 Changes in Evoked Otoacoustic Emissions and Hearing Thresholds after a Six-month Deployment on an Aircraft Carrier

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Evoked otoacoustic emissions and hearing thresholds were measured in 339 sailors from the USS Dwight D. Eisenhower aircraft carrier before and after a six-month deployment to the Mediterranean. Sailors from the Air, Reactor, and Engineering departments were targeted because they were considered most at risk for noise-induced hearing loss. At pre-deployment and post-deployment testing, hearing thresholds (0.5 to 6 kHz) were measured using a modified Hughson-Westlake procedure and normal middle-ear pressure was established. Transient-evoked otoacoustic emissions (non-linear click stimulus at 74 dB pSPL) and distortion-product otoacoustic emissions (f2/f1=1.22, at four stimulus levels) were then measured using the Otodynamics ILO292 Echoport. There was no consistent change in average hearing thresholds for the group; however, some individuals showed significant threshold shifts. Temporary threshold shifts were confirmed for two sailors (two ears) and permanent threshold shifts were confirmed for fifteen sailors (eighteen ears), based on their noise history and a confirmatory audiogram. Some additional significant threshold shifts were unable to be confirmed. Preliminary group results indicated that after deployment there was a decrease in average distortion-product and transient-evoked otoacoustic-emission amplitudes. Changes in otoacoustic-emission amplitudes might be a more sensitive indicator of noise-induced damage to the inner ear than changes in hearing thresholds.

773 Correlation of DPOAE level shifts and ABR threshold shifts with noise-induced histopathological changes in chinchillas: Some surprises

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DPOAEs are thought to be produced by OHC. However, comparison with noise-induced pathology suggests that their origin is much more complex. DPOAE levels (f1&f2 at 55-75dB, f2=1.22 f1, 6 points/octave, 0.5-20kHz) & ABR thresholds were determined pre-exposure. Noise was a 4kHz OBN at 108dB (1.75h, n=6) or 85dB (24h, n=5). DPOAE level shifts (LS) & ABR threshold shifts (TS) were determined at 0 & up to 30 days post-exposure. The cochleae were fixed with OsO4, embedded in plastic & dissected into flat preparations. The length of the organ of Corti (OC) was measured; missing IHC & OHC counted; stereocilia damage graded; & regions of OC, nerve fiber & stria loss determined. Cytocochleograms were made showing loss/damage by % distance from the apex (& frequency) with the LS & TS overlaid. The best correlation of LS with pathology required plotting at f1. The best correlation of TS was with IHC & nerve fiber loss. Wide regions of up to 40% scattered apical OHC loss showed little LS. In 2 cases, LS occurred with OHC abnormalities but not loss. In 3 cases, there were R/L LS asymmetries with symmetric pathology; in 2 cases, LSs were symmetric with asymmetric pathology. At all recovery times, the largest LS occurred at 3f1-2f2 for mid-frequencies (MF, 4-12kHz) & at 2f1-f2 below & above that. With 108 dB, there was up to 40% DPOAE recovery at MF in 3/6 cases where there was 80-100% OHC loss in the basal half of the OC. Partial recovery at MF also occurred in regions where the OC was missing. With 85 dB, there was no LS at small focal lesions (100% loss of OHC over 0.4 mm) when f1 or f2 was within the lesion but not both. There was no correlation of LS with stereocilia damage. In 2/5 cases with a stria lesion, the only pathological correlate with LS was this damage. These results suggest that either noise-induced DPOAE LSs at MF include a component from the basilar membrane or they are augmented from someplace other than f1 or f2, possibly the basal 20%.

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774 Audiometric Threshold Estimation in Cochlear Hearing Loss Ears by Means of Weighted Extrapolated DPOAE I/O-Functions

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DPOAE threshold and audiometric threshold have been shown to be closely related when estimating DPOAE threshold by simply extrapolating DPOAE I/O-functions using linear regression analysis (Boege and Janssen 2001). The purpose of this study was to improve and to evaluate this new method for clinical application.

DPOAE I/O-functions were recorded in 826 sensorineural hearing loss ears at up to 50 frequencies between 500 Hz and 8 kHz in a wide level range from $L_2=20$ to $L_2=65$ dB SPL at up to 10 levels ($L_1=0.4\ L_2+39$). DPOAEs were accepted as valid for signal-to-noise ratios (SNR) exceeding 6 dB. For estimating DPOAE threshold an extrapolation of the DPOAE pressure I/O-function was performed by determining the point of intersection of the extrapolated regression line with the L_2 coordinate. In the linear fit $p_{DP}\ (L_2)=a+bL_2$, a and b give the threshold and the slope of the DPOAE growth, respectively, representing estimates of sensitivity and compression of the cochlear amplifier.

According to Boege and Janssen, in 54% of the DPOAE I/O-functions a linear dependency of the DPOAE sound pressure p_{DP} on the primary tone level L_2 was found. However, when adapting the proposed

acceptance criterions and using weighted LMSE regression with two independent weighting factors $Min\sum w_1 w_2 (p_{DP} (L_2)-p(L_2))^2$

with $w_1=70-L_2$ and $w_2=SNR(L_2)/6dB$, 74% of the I/O-functions (n=12165) can be used for the linear fit. High correlation and small differences between DPOAE-threshold and pure-tone threshold (r=0.52, Mean 3.8 dB, SD 12.7 dB) were found, which proves weighted extrapolated DPOAE I/O-functions to be a valuable clinical tool for estimating quantitatively cochlear hearing loss.

775 Evaluation of Auditory Status Using Both 2f2-f1 and 2f1-f2 Distortion-Product Otoacoustic Emissions

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Although several distortion-product otoacoustic emissions (DPOAEs) may be detected in the ear canal in response to two pure tone stimuli (f1, f2), the majority of clinical studies have focused exclusively on the DPOAE with the largest amplitude, 2f1-f2. Research has demonstrated that 2f1-f2 amplitude or signal-to noise ratio (SNR) can be used to predict whether an ear is normal-hearing or hearing-impaired at a given f2 frequency with the most accurate prediction for f2 frequencies of 2000 Hz and higher. At test frequencies below 2000 Hz, poorer test performance has been attributable to higher noise floors. Although the 2f2-f1 DPOAE is generally smaller in amplitude than the 2f1-f2 DPOAE, the 2f2-f1 SNR may be larger in some individuals due to the lower noise floors surrounding this DPOAE at low test frequencies (Gorga, Nelson, Davis, Dorn, & Neely, 2000). The present study evaluated whether measuring 2f2-f1 either alone or in combination with 2f1-f2 improved the ability to predict auditory status at low f2 frequencies. DPOAE testing was performed on 74 ears of 42 participants with normal hearing and from 76 ears of 44 participants with varying degrees of sensorineural hearing loss. Two DPgrams were recorded from each ear for f2 frequencies of 700, 1000, 1500, and 2000 Hz. The first DPgram was recorded using the parameters traditionally used in clinical testing with 2f1-f2: f2/f1 = 1.22, L1 = 65 dB SPL and L2 = 55 dB SPL. The second DPgram was recorded using parameters found to be optimal for recording 2f2-f1 in a previous experiment: f2/f1 = 1.073, L1 = L2 = 65 dB SPL (Fitzgerald & Prieve (2001), ARO Abst. 24:9). When test performance of a single DPOAE was evaluated, 2f1-f2 measured using the traditional parameters most accurately predicted auditory status at all four f2 frequencies. Combination of data from both DPOAEs resulted in only slightly better test performance at all four f2 frequencies.

776 Ipsilateral DPOAE (2f1-f2) Suppression in Children with Hearing Loss: Preliminary Data

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Ipsilateral DPOAE suppression is a non-invasive means of studying human cochlear function. Suppression measurements are made by introducing a third tone (fs) simultaneously with the two primary tones (f1, f2) and then observing the changes in DPOAE amplitude as fs is increased in level. By plotting the suppressor level required to achieve a criterion amplitude reduction as a function of suppressor frequency, a DPOAE suppression tuning curve (STC) can be recorded. The DPOAE suppression tuning paradigm has been effectively applied in the past to study questions of cochlear maturation and cochlear amplifier function. The purpose of the present investigation is to study of the effects of cochlear pathology on cochlear function as measured by DPOAE suppression tuning. DPOAE suppression measurements were recorded in children with mild to moderate SNHL and in children with normal hearing. DPOAEs were generated for f2 frequencies of: 1500, 3000 and 6000 Hz using optimal recording parameters. Twelve to 15 suppressor tones, ranging from one octave below f2 to 1/4 octave above, were presented to generate each STC. DPOAE STCs were analyzed for width (Q10), slope and tip characteristics. Additionally, growth of suppression was examined for representative suppressor tones. Data collection is currently ongoing. Preliminary results suggest that DPOAE suppression tuning curves from children with hearing loss are abnormal in morphology, reflecting dysfunction of the cochlear amplifier

777 Longitudinal DPOAE Suppression Data from Premature Neonates

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Previous DPOAE investigations from our laboratory indicate that the human cochlea has subtle immaturities in function prior to term birth: (1) DPOAE suppression tuning curves (STC) from premature neonates are narrower than adult STCs; (2) growth of suppression is more gradual in premature neonates than adults and (3) the DPOAE growth function of premature neonates appears to be more monotonic (less saturating) than the adult function. Cross-sectional group data have been used in past investigations to study human cochlear maturation, even though premature neonates represent a heterogeneous group with significant inter-subject variability. In the present study, longitudinal DPOAE data were collected from seven premature neonates tested weekly over a 5-7 week period. Testing was initiated between 31 and 33 weeks post-conceptional (PCA) age and continued weekly until each neonate reached 38-40 weeks PCA. Each test session included measurement of a DPOAE growth function and DPOAE ipsilateral suppression for f2 = 6000 Hz, as well as a DP-gram. Preliminary results have replicated previously reported findings of narrower STCs and a steeper low-frequency flank for premature neonates (versus adults), as well as significantly more shallow suppression growth in premature neonates for suppressor tones lower than f2. Thus far, the only within-subject change in DPOAEs over the course of the testing period is that DPOAE threshold (derived from the amplitude growth function) decreases notably as the neonates mature. It is hoped that continued data collection and more complete analysis of the results will help elucidate the time course and form of cochlear maturation in humans and give insight into cochlear amplifier function.

778 Intraoperative Monitoring of the Cochlear Function Using Distortion Product Otoacoustic Emissions (DPOAEs) in Patients With Cerebello-Pontine Angle Tumors

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The aim of this study was to investigate the utility of DPOAEs in monitoring cochlear status intraoperatively during removal of cerebello-pontine angle tumors (CPAT).

Methods: Twenty patients with CPAT as diagnosed by computed tomography and magnetic resonance imaging were included in this study. According to standard audiological procedure, in all of them, different degrees of retrocochlear hearing loss were diagnosed as measured by audiometric tests, auditory brainstem responses, and otoacoustic emissions. The patients selected for intraoperative monitoring had preserved DPOAEs. Depending on the amplitude and frequency band at which DPOAEs were preserved, the adequate option of intraoperative monitoring was applied: primary tone level ranging from 60 to 70 dB SPL, and frequency from 2.0 to 6.0 kHz.

Results: This study revealed that the most dangerous procedures during CPAT removal affecting reversibly or irreversibly inner ear function as monitored by DPOAEs were: (1) microcoagulation of the small bleeding vessels in the operation field, (2) maneuvers during tumor

removal such as mass reduction and internal auditory canal structure tension/compression, and (3) direct manipulation on the VIII nerve. DPOAE amplitude fluctuations as effects of the above were characterized by a 5-20 second delay. The effect of partial deficit in the cochlear blood supply on DPOAEs was different for various frequencies; high frequency DPOAEs were found to be reduced faster than middle and low frequency DPOAEs.

Conclusions: DPOAEs were found to be a sensitive method of monitoring the cochlear function intraoperatively, although the operating room noise limits its utility, especially in people with preserved DPOAEs only at middle and low frequencies.

779 Signal Detection and Estimation in Distortion-product Otoacoustic Emissions: Comparison of the Multitaper F-test with Direct Spectral Estimates

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Distortion-product otoacoustic emissions (DPOAEs) represent an important nonbehavioral frequency-specific measure of cochlear function. The development of more robust statistical algorithms for the detection of low amplitude DPOAEs in noise should improve its clinical application. Current protocols utilize direct spectral estimates based on a single window (taper). This direct spectral estimate has only 2 degrees of freedom and its variance does not approach zero asymptotically. Further, direct spectral estimates are logarithmically transformed resulting in a noise estimate which is highly non-Gaussian. Multitaper spectral estimation utilizes orthogonal optimally time- and band-limited prolate spheroidal wavefunctions, which span a limited tile of the time-frequency plane (Thomson, 1982). The orthogonality of the wavefunctions and their frequency-localized properties provide estimates with multiple degrees of freedom and approximate the best linear unbiased estimate of the amplitude of a line component under Gauss-Markov theorem (Miller, 1973). The multitaper framework was applied to the detection of DPOAEs and compared against direct spectral estimates. Comparisons were conducted analytically, through Monte Carlo simulations, and in guinea pig DPOAE data. Monte Carlo simulations showed that the multitaper estimate outperformed currently utilized clinical protocols at typically observed SNRs both in hit rate and number of time averages needed for detection. Further, the multitaper method achieved its nominal false alarm rate while the direct spectral estimate did not. In the guinea pig data, input/output functions revealed that the multitaper method detected the cubic distortion product at an average of 10 dB lower than the direct spectral estimate.

780 DPOAE during Salicylate Intoxication in the Mongolian Gerbil

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Salicylate causes temporary sensorineural hearing loss and tinnitus. This effect is thought to be related to changes in the outer hair cell (OHC) motility. To monitor the outer hair cell function during salicylate treatment distortion products otoacoustic emissions (DPOAE) were measured in gerbils using various salicylate doses.

Methods:

DPOAE input-output (I/O)-functions were recorded at different frequencies (f2 = 2-8 kHz, 4 measurements per octave; f2/f1 = 1.2) at various primary tone levels (L2 = 60 dB SPL to 20 dB; L1=0.786L2 + 12.285 dB) before and after salicylate application (i.p.). Additionaly, the levels of DPOAE at f2 = 3 kHz and L2=30 dB SPL were determined continuously within 4 h after salicylate administration with various doses (150 mg/kg body weight (b.w.); 200 mg/kg b.w.; 250 mg/kg b.w.).

Results:

typical compressive behavior. After salicylate intoxication DPOAE-I/O-functions changed to a linear behavior at all frequencies tested until no DPOAE could be measured.

The continuous measurement of DPOAE at f2 = 3 kHz revealed constant levels at about 5 dB SPL with a signal to noise ratio of 30 dB SPL. After salicylate administration a dose dependent decrease of DPOAE could be assesed. 1 h after salicylate application of 250 mg/kg b.w no DPOAE could be measured. A similar effect was seen after 2 h with 200 mg/kg b.w. A dose of 150 mg/kg b.w. exhibited a decrease of DPOAE levels to -15 dB SPL which remained unchanged during the measurement. No increase of DPOAE could be seen within 4 h after salicylate application independently of the salicylate doses.

The present results suggest an influence of salicylate on OHC function in a dose dependent manner.

781 Reliability of Distortion Product Otoacoustic Emissions (DPOAEs) in Broiler Chickens

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The long-term goal of this research is to determine if DPOAEs can be used to predict the amount of anatomical damage in the inner ear of broiler chickens. DPOAEs are thought to result from the micromechanics of cochlear hair cells and thus should vary predictably with anatomical damage to these hair cells. Prior to this research, however, we must first determine the within-animal reliability of DPOAEs measured in our laboratory.

DPOAEs were measured in nine broiler chicks on three separate testing sessions, over a period of time ranging from three to 17 days. Animals were anesthetized with ketamine and xylazine prior to recording emissions. DPOAE input-output functions at 2F1-F2 were measured using a F2/F1 ratio of 1.2. The levels of the two primary tones were equal as the signal level was decreased from 80 dB SPL to 20 dB SPL in increments of 5 dB SPL. Responses were recorded at 500 Hz, 1000 Hz, 2000 Hz, 3000 Hz and 4000 Hz. All data acquisition was done with Tucker-Davis Technologies hardware and software.

The within-subject variability was determined by calculating the standard deviation (SD) for each animal across the three sessions at each frequency and signal level above threshold. At frequencies greater than 500 Hz, within-subject variability was greatest at levels near threshold, and least when the signal level was between 50 dB SPL and 75 dB SPL. In general, DPOAEs at 500 Hz showed the most within-subject variability (mean SD at 50 dB SPL = 5.9 dB SPL.) The least amount of within-subject variability was observed at 3000 Hz (mean SD at 75 dB SPL was 2.9 dB SPL.) These findings indicate an adequate degree of reliability for DPOAEs as a non-invasive test for repeated measures.

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782 Postulated Action of the Auditory Efferent System on Cochlear Microphonic (CM) in Awake Guinea pig, after Noise Exposure and Gentamicin

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The efferent system was proposed as a modulator of the cochlear potentials, e.g.,habituation (1), visual attention (2), benzodiazepines (3), sleep (4). Moreover, high intensity noise exposure provokes amplitude

changes in CM potential (5). Our work was aimed to investigate the origin of the amplitude shifts after noise, i.e., are they due to intrinsic cochlear phenomena or to efferent system actions? Trying to establish this, a noise exposure and gentamicin experimental design was developed. Awake guinea pigs, with controlled behavior (electrocorticogram and electromyogram) were used. 1) The CM in response to a best frequency tone burst (50ms, 5ms rise and fall,60dB SPL) was recorded during 30 minutes (30s recording every minute) to measure the CM amplitude variability. 2) After the exposure to 30s 90dB SPL white noise - repeating the previous paradigm during the following 30 minutes of recording- CM presented a reduction in its amplitude variation in the first 5 minutes. 3) Because gentamicin at low doses produces a reversible suppression of the efferent system action (6), it could be used to separate peripheral and central actions. Under gentamicin action, the exposure to the white noise CM exhibited amplitude variability closer to the control condition, even in the first 5 minutes. These preliminary data suggest that the CM amplitude shift after high intensity noise exposure is partially due to actions on the auditory efferent system.

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783 Bilateral Deafness Results in Differential Gene Expression in the Central Auditory System of the Rat

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Deafness results in numerous functional, morphological, neurochemical and molecular changes in the adult central auditory pathways. In the inferior colliculus (IC) these include reduced inhibitory responses, changes in the tonotopic map, changes in neurotransmitter release, and changes in the subunit composition of receptors as well as receptor binding. In the cochlear nucleus (CN) these include changes in neurotransmitter uptake and release. We therefore used Clontech DNA microarrays to examine differential expression of 1176 genes in both the IC and CN 7 days after bilateral deafening by intrascalar perfusion of 10% neomycin. Three independent RNA samples were tested, each isolated from the IC or CN of 2-3 normal or deafened rats. Approximately 15% and 25% of the genes in the CN and IC, respectively, were differentially expressed. Many GABA related genes changed, consistent with previous reports of reduced GABA release, decreased inhibition, and changes in receptor composition with deafness or aging. In general, genes related to the role of GABA in presynaptic events (e.g. GAD 67) decreased, whereas genes related to post-synaptic processing (e.g. alpha 2 & 3, gamma 2 subunits of the GABA-A receptor) increased. A decrease was seen in GABA-B receptor 1a/1b that may reflect a potential pre-synaptic distribution. Similarly, expression of acetylcholinesterase increased and expression of acetycholine receptor beta 2 decreased. There was, however, a large increase in somatastatin expression. The expression of several potassium channels decreased. Changes in synapse related gene expression included increases in synapsin 1A/B and syntaxin B and a decrease in SNAP 25. RT-PCR is being used to confirm our results. Gene microarrays therefore provide a powerful screening tool to identify changes in gene expression with deafness and may also help identify patterns among these genes.

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784 Gabaergic Innervation of Neurons in High and Low Frequency Regions of the Rat Inferior Colliculus

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GABA is a major inhibitory neurotransmitter found within the central auditory system. The central nucleus of the inferior colliculus (CIC) receives considerable GABAergic input from numerous nuclei and serves as a locus for processing auditory information. Deafness due to exposure to agents such as ototoxic drugs result in changes in the auditory GABAergic system. As a first step towards examining changes in GABAergic input to the CIC following deafness, we have generated baseline data on GABAergic input into high and low tonotopic regions of the rat CIC. Five female rats were perfused transcardially with a mixed aldehyde fixative. Vibratome (150µm) sections through the CIC were then embedded in plastic. A comparable region from a single vibratome section was taken from each animal and post-embedding ICC for GABA was applied to every tenth semi-thin (1µm) plastic section. Digital images were acquired using a Spot camera and analyzed using MetaMorph (Univ. Imaging) software. Sections suitable for analysis were identified using anatomical landmarks consistent with those known to be present in sections through the mid-rostrocaudal portion of the CIC. In agreement with previous studies, large numbers of GABAergic puncta as well as GABA-IR neurons were seen throughout the CIC. GABAergic neurons were divided into two populations (labeled and unlabeled) based upon staining intensity using a frequency distribution histogram. GABA-IR puncta were seen on non-GABA-IR neurons as well as both populations of GABA-IR cells. GABA-IR puncta and both labeled and unlabeled GABA-IR neurons were found in both high and low frequency reference regions. In both high and low frequency reference areas, more GABA-IR puncta were observed on unlabeled neurons when compared to puncta on darkly labeled GABA-IR neurons. Future studies will examine changes in staining intensity of GABA-IR cells and puncta as a consequence of deafness.

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785 No Inhibition of Sound-Induced c-Fos Expression in the PKC-gamma Knockout Mouse

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Our laboratory has been studying the role of protein kinase C [PKC] isoforms in central auditory pathways, using the immediate-early gene product c-Fos as a marker for sound-induced activation of auditory neurons. We have previously reported that sound-induced c-Fos is colocalized in a subset of these neurons with several PKC isoforms, including PKC-gamma. We have also reported that pretreatment of animals with an inhibitor of PKC will attenuate sound-induced c-Fos expression. Because PKC antagonists that discriminate between the 12 isoforms of PKC do not exist, the availability of isoform-specific knockout mice presents an attractive experimental paradigm to test the role of specific isoforms in the auditory system. Using a commercially-available mouse with a disruption in the gene encoding PKC-gamma [Jackson Laboratories; "Pkcc" mouse], we tested the hypothesis that the absence of PKC-gamma would result in an inhibition of sound-induced c-Fos expression. Pkcc mice or strain-matched controls were placed in a sound-attenuated chamber overnight. The following morning, the mice were placed in individual cages and exposed to sound [80 dB, 10 kHz pulses] for 45 minutes, with sacrifice 2 hours after the onset of sound. Animals were perfusion-fixed with 3% buffered paraformaldehyde and brains were analyzed for expression of c-Fos and PKC-gamma using immunocytochemistry. No expression of PKC-

gamma was seen in the brains of the Pkcc mice, but normal patterns of expression were visualized in the control strain. Levels of sound-induced c-Fos expression were not decreased in the brains of Pkcc mice when compared to controls; however, in some brain regions, c-Fos levels were greater in the Pkcc mice, including the small cell shell of the cochlear nucleus. These findings suggest a complex role for PKC in auditory information processing, which may be isoform-specific and may involve both inhibitory and excitatory neurotransmission.

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786 Peripheral Cell Loss related to Calcium Binding Protein Immunoreactivity in the Cochlear Nucleus in C57BL/6J Mice during Aging

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The influence of cochlear hair cells and spiral ganglion neuron loss on calcium binding protein immunoreactivity (parvalbumin, calbindin, calretinin) in the dorsal and posteroventral cochlear nuclei (DCN and PVCN) in C57BL/6J (C57) during aging (1-30 month old) was determined. These calcium binding proteins play an important role in central auditory physiology, and have been shown to undergo age-related changes in some central nervous system structures. The C57 mouse demonstrates progressive cochlear sensorineural pathology and hearing loss early in their life. The unbiased quantitative stereological method, the optical fractionator was used for determining the total number of neurons and calcium binding immunopositive neurons in the DCN and PVCN. A statistically significant age-related decrease of the total number of neurons was demonstrated in the DCN and PVCN. A correlation between loss in the auditory periphery and calcium binding protein expression in the DCN was found for parvalbumin and calretinin, and for calbindin in the PVCN. These findings imply that the degenerative changes in the auditory periphery might influence the neuronal homeostasis and immunoreactivity of these calcium binding proteins in the DCN and PVCN.

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787 Effects of Hearing Loss on the Electrophysiology of Ventral Cochlear Nucleus Neurons

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The electrophysiological properties 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 20 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. Within a small sample population, we found that type II cells from high frequency regions in older DBA mice have higher input resistance and shorter membrane time constants compared to those from low frequency regions. Additionally, action potentials take longer to reach their peak in high frequency cells. In contrast, type II cells from both high and low frequency regions in good hearing CBA mice were not different. Interestingly, the measurements from CBA type II cells were comparable to those of low frequency type II cells in DBA mice. These results are consistent with some of the changes observed in AVCN neurons following cochlear ablation and suggest a physiological change in cochlear nucleus neurons associated with hearing loss.

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788 Loud sound exposure induced [14C]-2-deoxyglucose uptake in the auditory pathways in hamsters: data related to tinnitus

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We evaluated neural activity along the auditory pathways using [14C]-2-deoxyglucose autoradiography (2-DG) in hamsters whose left ears were exposed to a 10kHz tone at 127dB SPL for 4h. Animals with 23 days of postexposure recovery times were tested behaviorally for tinnitus. The results suggested that sound exposure caused tinnitus. Subsequent to the behavioral study, these animals were used to conduct 2-DG experiment. The results showed that 2-DG uptake after exposure was distributed more asymmetrically in the lower auditory centers and less asymmetrical in the higher auditory centers. As compared to the controls, 2-DG uptake levels appeared lower in the cochlear nuclei (CN) ipsilateral to the exposed ears, and lower in the lateral lemniscus (LL) and the central nucleus of inferior colliculus (CIC) contralateral to the exposed ears. At thalamic and cortical levels, a slightly greater 2-DG uptake was found on the ipsilateral side to the exposed ears. The asymmetrical distribution in auditory centers following cochlear trauma is possibly due to deprivation of peripheral input into the auditory pathways. A band-like area of 2-DG uptake was occasionally observed in the contralateral dorsal cochlear nucleus, CIC and auditory cortex, which may indicate that a reorganization process is taking place. However, measurement of 2-DG levels relative to a reference structure (spinal trigeminal tract) showed that sound exposure induced increase in 2-DG uptake in most auditory centers except for the ipsilateral CN and contralateral LL and CIC which have direct projections from the traumatized ear. These preliminary results suggest that sound exposure causes a decrease in activity in the lower auditory centers, but may elevate metabolic rate in some brain areas especially at thalamic and cortical levels.

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789 Modulation of D-[^{3}H]Aspartate Release In The Auditory Brain Stem By cAMP-Dependent Protein Kinase

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The release of certain transmitters in brain auditory nuclei was altered after hearing loss or the destruction of the cochlear nerve (Exp Neurol, 148: 222, 1997; 151: 273, 1998). Such lesions alter the excitation of auditory neurons and this change may be transduced by signaling pathways that use protein kinases to regulate transmitter release. Since cAMP-dependent protein kinase (PKA) is an important signaling molecule, we determined if it could regulate transmitter release in the major subdivisions of the cochlear nucleus (CN) and in three nuclei of the superior olivary complex (SOC). These tissues were microdissected

from young adult guinea pigs and used to measure the electrically evoked release of D-[^{3}H]aspartate, a poorly metabolized marker of transmitter glutamate. The PKA activator, dibutyryl cAMP (DBcAMP), elevated the evoked release in each nucleus in a concentration-dependent manner. Pretreatment with 0.2 uM H-89, a PKA inhibitor, did not alter the evoked release but completely blocked the stimulatory effect of 0.2 mM DBcAMP. This suggests that PKA can positively regulate transmitter release from glutamatergic presynaptic endings in the CN and the SOC. Seven days after unilateral cochlear ablation, when cochlear nerve endings had degenerated in the ipsilateral CN, 0.2 mM DBcAMP elevated the evoked release in many of the nuclei, implying that PKA can regulate glutamatergic release in most non-cochlear pathways remaining in the ipsilateral CN and in the other pathways after unilateral hearing loss. However, the postlesion stimulatory action of DBcAMP was greater in the ipsilateral posteroventral CN and medial trapezoid nucleus, suggesting that cochlear ablation increased the regulatory capacity of PKA in these nuclei.

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790 Factors Influencing 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 two types of experimental manipulations on the survival and differentiation of cochlear nucleus (CN) neurons grown in primary cell culture. The first series of experiments investigated the effects of growing CN neurons on several extracellular matrix components. The second series of experiments compared CN cells grown under minimal conditions with those co-cultured with inner ear or cerebellar cells. Cultures were prepared from postnatal mice and processed using microtubule-associated protein 2 (MAP2) immunohistochemistry. CN neurons were successfully grown on laminin, collagen and fibronectin, but neuronal survival was significantly decreased on all three substrates relative to controls. In addition, analysis of dendrite organization patterns indicated that CN neurons branched more extensively when grown on poly-D-lysine than on any of the ECM components tested. Collagen was a particularly poor substrate. Although dendrite growth has been shown to depend strongly on the presence of ECM components in other neuronal systems, the data from these experiments do not support the hypothesis that laminin, collagen or fibronectin is necessary for the survival and differentiation of CN neurons. Survival of CN neurons was significantly increased when the cells were co-cultured with cerebellar neurons but not with inner ear cells. Both the cerebellar and inner cultures contained MAP2-positive cells. Although CN neurons in the cerebellar co-cultures tended to be smaller and have fewer primary dendrites than control cultures or those grown in the inner ear co-cultures, there were no significant differences in dendrite organization and morphology among the control and co-cultured CN neurons. These results suggest that there are no diffusible substances secreted by cerebellar neurons or inner ear cells that greatly facilitate the survival and differentiation of CN neurons.

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791 DCN Hyperactivity Induced by Previous Intense Sound Exposure: Origin with respect to Cell Layer

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Intense sound exposure causes chronic hyperactivity in the dorsal cochlear nucleus (DCN), a condition thought to be an important neural correlate of noise-induced tinnitus. The cell population from which this hyperactivity originates is not known. In the present study, we sought to determine the histological layer of the DCN from which hyperactivity originates by quantifying changes in spontaneous rate, induced by previous sound exposure, as a function of depth below the DCN surface. Multiunit recordings were performed in animals that were previously exposed to intense sound and in unexposed control animals. In each animal, spontaneous activity was measured in 3 rows of sites located along the medial-lateral (tonotopic) axis of the DCN. In exposed animals, sites showing evidence of hyperactivity were selected for depth studies. At each of these sites, spontaneous activity was recorded at 20 um depth intervals until a total of 20 sites had been studied in each penetration. Similar measures were obtained at topographically corresponding sites in control animals. Mean differences between spontaneous rates in exposed and control animals were computed for each depth, yielding a profile of the degree of hyperactivity (i.e., change in spontaneous rate) as a function of depth. The results show that although hyperactivity induced by previous sound exposure was evident across the entire DCN thickness, by far the greatest increases in activity were found between 100 and 250 um below the DCN surface. Peak hyperactivity occurred at a depth of 200 um. This range corresponds to the DCN fusiform cell layer. The results are consistent with the view that the fusiform cell layer is the origin of the induced hyperactivity. Studies are now in progress to identify the subpopulation of cells producing this condition.

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792 Repeated Sound Exposure and Immediate-Early Gene Expression in the Rat Cochlear Nuclei

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Repeated exposure to loud sounds can induce neural plasticity of central auditory systems, possibly underlying deafness or tinnitus. To determine if this plasticity may be linked to alterations in expression of immediate-early genes (IEGs), we exposed rats to one of the following conditions, after overnight housing in a sound-attenuated chamber: a) no sound (controls); b) exposure to sound (80 dB, 10 kHz pulses) for 45 minutes, with sacrifice 2 hours after initiation of sound; c) exposure to sound, with sacrifice 8 hours later; d) exposure to sound, with re-exposure 6 hours later, and sacrifice 2 hours later; e) exposure to sound, with sacrifice 26 hours later; f) exposure to sound, with re-exposure 24 hours later, and sacrifice 2 hours later; g) exposure to sound, with sacrifice 1 week later; h) exposure to sound, with re-exposure 1 week later, and sacrifice 2 hours later. Rats were anesthetized and perfused for immunocytochemical localization of c-Fos, Fos-B, and the Fos-related antigens Fra-1 and Fra-2. In the dorsal (DCN) and ventral (VCN) cochlear nuclei, acute exposure to sound induced c-Fos expression, which returned essentially to baseline by 8 hours. Each re-exposure to sound induced a similar level of c-Fos expression. Thus, there were no long-term changes in the c-Fos response. Acute sound exposure did not induce Fos-B at 2 hours, but an induction was seen at 8 hours. Re-exposure to sound at 24 hours and 1 week induced Fos-B expression 2 hours later, suggesting a sensitization of the Fos-B response. Acute exposure to sound induced expression of Fra-2 in the DCN. However, re-exposure at 6 or 24 hours, or at 1 week failed to induce expression, suggesting a desensitization of the response. These results suggest that alterations in expression of IEGs may contribute to the neural plasticity induced by repeated exposure to sound.

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793 Neurotrophin Receptor TrkB in the Guinea Pig Cochlear Nucleus After Unilateral Cochlear Ablation

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Altered cell signaling activity may contribute to plasticities in adult brain auditory pathways after hearing loss due to unilateral cochlear

ablation. Postlesion changes in extracellular neurotrophin levels, detected by tyrosine kinase (Trk) receptors in the plasma membrane, may modulate the ERK cascade, alter gene expression and induce plasticity. Since the TrkB receptor is involved in such responses, we determined if its levels change in the young adult guinea pig cochlear nucleus after left cochlear ablation. Western blots were used to quantify TrkB levels in whole tissue lysates of the AVCN, PVCN and DCN. Compared to levels on the intact (contralateral) side, TrkB levels in the AVCN on the ablated (ipsilateral) side were elevated by 34% at 7 postlesion days, coinciding with a period of synaptogenesis in the ipsilateral AVCN (Synapse 1997 25: 243-257). By 30 days TrkB levels were depressed by 22% before returning to levels evident contralaterally at 60 days. The deficit at 30 days coincided with deficient glycine receptor activity in the ipsilateral AVCN (Exp Neurol 1998 154: 473-488); the recovery at 60 days coincided with recovering transmitter release from glutamatergic synaptic endings (Exp Neurol 1997 148: 222-235). In the ipsilateral PVCN, TrkB remained near contralateral levels, except for an elevation of 32% that took 30 days to develop, which preceded the recovery of transmitter release from glutamatergic synaptic endings evident at 60 days (Exp Neurol 1997 148: 222-235). In the ipsilateral DCN, TrkB also remained near contralateral levels, except for a severe decline of 56% at 30 days, which preceded deficits of glycinergic receptor and transmitter release activities evident at 60 days (Exp Neurol 1998 151: 273-288; 154: 473-488). Thus, the present findings suggest that the TrkB receptor and its regulation may contribute to several of the plasticities observed in the CN after cochlear ablation.

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794 Central Synaptic Maturity in Congenital Deafness: A Time Course of Endbulb Development in the Neonatal *shaker-2* Mouse

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Endbulbs of Held are distinctive, axosomatic endings that arise from myelinated auditory nerve fibers and terminate in the cochlear nucleus. They possess structural specializations necessary for maintaining the temporal relationship of spike trains to environmental sound streams. We have previously shown that mature *shaker-2 (sh2)* mice, deaf from birth, exhibit dramatic synaptic alterations at the endbulbs compared with normal hearing heterozygotes. These findings were consistent with our published observations in the congenitally deaf white cat (Ryugo et al, JCN, 1997), indicating that the synaptic changes are due to deafness and not a syndrome. The impact of congenital deafness on early synaptic development, however, has not been described. The *sh2* mouse is a well-characterized model of non-syndromic recessive hearing loss. Homozygous *sh2* mice *(sh2/sh2)* have mutations of *Myo15* and exhibit stubby inner ear stereocilia and profound deafness. Our current ultrastructural analysis examined the time course of endbulb development in *sh2* mice. Normal hearing heterozygous and deaf *sh2* littermates were studied at 1 day, 1 week, 2 weeks, 1 month, and 2 months of age. Endbulbs were analyzed through serial section electron microscopy, and reconstructed using 3-D imaging software. No significant differences in nuclear to cytoplasmic ratio, ending morphology, or PSD area were seen in normal hearing or deaf mice up to 2 months of age, although at older ages, there was a non-significant trend for PSD surface area to be larger. These data reveal that the onset of deafness-induced synaptic abnormalities in the cochlear nucleus occurs between 2 and 7 months for these mice, and provides a narrower time window in which to look for a developmental "critical period." Our goal is to identify the mechanisms and time course of deafness-induced synaptic changes in the cochlear nucleus.

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795 Behavioral assessment of function of the central olivocochlear pathway in knockout mice lacking α9 cochlear acetylcholine receptors and in CBA/J mice

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Olivocochlear (OC) efferent neurons link the auditory brainstem to the inner ear. Animals with OC lesions often show deficits in their ability to process sound in noise. The α9 knockout mouse, derived from stem cells of the 129/SvEv strain (Vetter et al., 1999, Neuron 23: 93-103), has intact OC pathways, but is functionally "de-efferented" because no cochlear α9 ACh receptor subunits remain on outer hair cells. Last year (these meetings), we reported that α9 knockout mice (homozygous for gene deletion) and α9 controls (heterozygous or homozygous for gene presence) had similar detection and discrimination abilites in quiet and in noise. These contrary results suggested either that central pathways can mask peripheral deficits, or that the 129/SvEv mouse strain was an unfortunate choice for behavioral studies because these mice may suffer an age-related hearing loss. This report addresses those hypotheses.

Subjects included CBA/J mice, which have excellent hearing throughout their lifespan, and α9 mice. Behavioral assessments, including absolute thresholds and intensity difference limens (IDLs), were determined in quiet and in noise. We also measured ABR thresholds from 11-12 month old CBA/J and 129/SvEv subjects. Behavioral findings indicated that while α9 mice were less sensitive to CBA/J mice in some listening conditions, all subjects had comparable IDLs in quiet and in noise. ABR thresholds in 129/SvEv mice corresponded well to CBA/J baselines, showing no signs of age-related hearing loss at frequencies used in behavioral testing.

OC neurons that innervate the auditory periphery also send collateral projections to the cochlear nucleus. Our data support the existence of these pathways for the preservation of hearing in noise.

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796 Tinnitus in Hamsters Following Exposure to Intense Sound

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Hamsters were trained with a conditioned suppression/avoidance procedure to drink in the presence of a broadband noise and/or a tone and to stop drinking in the absence of sound. A variety of tones and loudspeaker locations were used during training so that the animals would respond to a sound regardless of its frequency or location. Four groups of animals then had their left ears exposed to a 10-kHz tone at 124 or 127 dB for 0.5, 1, 2 or 4 hrs. The animals were then tested by determining if they were more likely to continue drinking during silent intervals than unexposed animals, a result which would suggest that the exposed animals perceived a sound when no external sound was present, i.e., that they had tinnitus. The groups exposed for 2 and 4 hrs tested positive for tinnitus whereas those exposed for 0.5 and 1 hrs did not. The degree of hearing loss produced by the tone exposure was assessed using behavioral and auditory brainstem response (ABR) procedures. A partial dissociation was found between the hearing loss, as estimated by the ABR, and the tinnitus test results in that animals exposed for 1 hr had the same hearing loss as the 2- and 4-hr animals, but did not appear to have tinnitus. This result suggests that the hearing loss caused by the tone exposure does not account for positive scores on the tinnitus test.

797 Hearing in the Greater Spear-nosed Bat, *Phyllostomus hastatus*: Audiogram, Temporal Integration, Sound Localization, and Use of Binaural Cues

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P. hastatus is a large, omnivorous New World bat that uses relatively low-intensity echolocation calls. As part of a survey of hearing in bats, the auditory abilities of 3 bats were determined using a conditioned suppression/avoidance procedure. **Audiogram**. Pure-tone thresholds were obtained from 1–110 kHz. At an intensity of 60 dB SPL, the animals could hear from 1.8 to 105 kHz with a best sensitivity of 1 dB at 20 kHz. **Detection of brief tones**. Because echolocation pulses are brief, we obtained absolute thresholds for pure tones as brief as 2 ms for six frequencies from 5.6 to 64 kHz. Reducing the duration from 400 to 2 ms increased thresholds by less than 25 dB at 25 kHz and higher, comparable to both lab rats and Egyptian fruit bats. However, for frequencies of 10 kHz and below, the 2-ms thresholds were raised more than 60 dB. A similar but less dramatic increase in threshold for brief low-frequency tones has been found in the Egyptian fruit bat, but was not observed in rats or other non-echolocating mammals. **Sound-localization acuity**. The bats were able to localize a 100-ms broadband noise burst with a threshold of 9°. Analysis of their retina indicates that this result is consistent with the positive relationship among mammals between the width of an animal's field of best vision and its sound localization acuity. **Use of binaural locus cues**. The ability to use binaural phase- and intensity-difference cues to localize sound was assessed by determining the bats' ability to localize 100-ms pure tones at a separation of 60°. The bats were able to localize frequencies of 12.5 kHz and higher, indicating that they can use the binaural intensity-difference cue. However, they performed at chance for frequencies of 8 kHz and below, even when the tones were amplitude modulated, indicating that they are unable to use interaural time differences in either the carrier tone or its envelope.

798 Spectral and Temporal Characterization of the Echo-Threshold in the Nectar-Feeding Bat, Glossophaga soricina

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The flower-visiting bat G. soricina uses its echolocation system for short range localization of individual flowers. Recently, it was shown that the vexillum of the flower of a bat-pollinated neotropical vine, Mucuna holtonii, serves as an acoustic guide for the sonar of its bat-pollinators. The echolocation calls of G. soricina are short, broadband, multiharmonic sweeps. The most prominent component is the second harmonic with its peak around 120 kHz and a bandwidth of approximately 50 kHz measured 20 dB below peak. In three bats, auditory sensitivity for echoes of emitted echolocation calls was measured as a function of echo spectral content and echo delay. Echolocation calls were recorded while the bats were visiting a central feeder in front of a microphone. Then echo spectral content was varied by band-pass filtering the calls with one of four different center frequencies (63, 80, 100, and 126 kHz) and a bandwidth equal to 20 % of the center frequency. Echo delay was varied between 2.4 and 6.4 ms. The filtered and delayed calls were replayed from one of two adjacent speakers with reward feeders. In this 2-AFC paradigm, psychometric functions for the detection of the manipulated echoes were obtained. Latest data indicate highest sensitivity for frequencies around 126 kHz. Sensitivity as a function of delay increases by no more than 8 dB. Results are discussed with respect to the spectral characteristics of the emitted call and regarding echo spectral content and echo delay as they occur in the bats' natural foraging behavior when approaching individual flowers.

799 Evaluation of Time/Frequency Biosonar Models using Psychophysical Results from FM Echolocating Bats

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Use of FM biosonar signals by bats led to early hypotheses about time/frequency methods for processing echoes (or else why bother with FM?). Bats might employ FM pulse-compression analogous to "chirp" radar, which historically was the first explicitly time/frequency echo-processing algorithm, predating modern use of numerically equivalent A-to-D sampling and serial DSP crosscorrelation techniques. Frequency-dependent delay dispersion for pulse-compression actually resembles what has since been discovered about auditory processing of echo delay by bats. Psychophysical measurements of integration-time for echo detection and delay resolution by big brown bats confirm the need for a time/frequency model. We have developed an advanced biomimetic model of biosonar that incorporates features of neural responses in multiple banks of wavelet-based parallel time/frequency filters for coarse delay accuracy and fine delay resolution. Performance of the model in simulations of psychophysical tests is comparable to that of bats for echo detection, delay acuity, and delay resolution at known SNRs. The model focuses attention on why FM bats have proportionally large numbers of auditory afferents (>50 per inner hair cell), on the need for measurements of low-pass smoothing in bat auditory-nerve fibers, and on physiological noise obscuring stimulus-driven response-latency distributions in the brainstem.

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800 Detection of Rustling Sounds in the Gleaning Bat, *Megaderma lyra*

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The gleaning bat Megaderma lyra hunts relatively large prey from the ground and listens for prey-generated rustling sounds. While echolocation is also used especially in the final phase of the attack, prey-generated sounds are essential to initiate a hunting behavior. In a natural situation, prey-generated rustling sounds masked by other natural rustling sounds generated by e.g. wind or water do not provide pronounced spectral cues because both the signal and the masker are broadband. Thus, temporal characteristics of both the signal and the masker are presumably very important. In a series of experiments, two M. lyra individuals were trained to detect a rustling sound recorded from prey moving in dried leaves. Exploratory experiments revealed that the degree of envelope modulation of the signal does not influence detection threshold: With a white noise masker, detection of the original rustling sound was no better than that of the phase-randomized rustling sound which lacks the pronounced envelope modulation. Detection of the original rustling sound was also no better than of an equal-energy, white-noise signal. These results indicate that with a white-noise masker, rustling-sound detection is based on intensity cues only. However, when the masker is comodulated, the rustling-sound detection improves with increasing degree of comodulation of the masker. Thus, M. lyra reveals comodulation masking release even for a comodulated, broadband signal. This result indicates that M. lyra in a hunting behavior would benefit strongly from comodulation in natural masking signals arising from wind or water. A simulation of the latter experiment based on decision cues related to fluctuation strengths of the peripheral auditory representation of the waveform provided a good first approximation of the experimental results. However, signal-masker confusion effects seen in the simulations but absent in the experimental data reveal the influence of additional, unknown decision cues.

801 Backward and simultaneous masking of short sounds by ferrets following ear plugging

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Deficits of auditory temporal processing have been associated with language impairments in children. Here we measured backward masking (BM), a test of temporal resolution, simultaneous masking (SM) and absolute thresholds (AT) in ferrets following ear plugging. For BM and SM, ferrets were trained to distinguish >tone+noise= trials from >noise= trials. On >tone+noise= trials, the tone (1 kHz) was presented immediately before or during a noise burst of fixed spectrum (0.6-1.2 kHz), level (68 dB SPL) and duration (300 ms). Tone level was varied adaptively and duration was 20 ms (BM, SM) or 200 ms (SM). For AT, the ferret discriminated between tone and silence, cued by a light. Ferrets were either (i) normally hearing, (ii) reared from P26-28 for 3-6 months with a unilateral plug that alternated monthly between the ears, (iii) received 3-6 months alternating monaural plugging in adulthood, or (iv) reared with bilateral ear plugs. Training on the task began 3 months after plugging started. The plug(s) were removed ~3 months later, before testing started. Unilaterally plugged ferrets performed as well as normally hearing ferrets on all tasks. However, bilaterally plugged (BP) ferrets were much poorer than normal on BM (0 ms delay). Mean BM tone thresholds in normal ferrets decreased from 75-69 dB SPL over 3 blocks of trials. BP ferret mean BM tone thresholds exceeded 90 dB in the first block of trials, and decreased to a threshold of 77 dB in the fifth trial block. SM thresholds for a short, but not for a long duration tone were also elevated. AT (20-200 ms tones) were generally normal in the BP ferrets. The results show that prolonged bilateral conductive hearing loss, resembling severe middle ear disease in humans, can impair the detection of short tones masked by noise. They are consistent with impaired auditory experience having a role in processing efficiency and temporal deficits (see Hartley and Moore, Hill and Moore, ARO 2002).

802 Measuring the Ferret Spectro-Temporal Modulation Transfer Function (MTF) using a Conditioned Avoidance Behavioral Task

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Previous psychophysical research in our laboratory on birds and humans has investigated detection thresholds for spectral and temporal modulations. In both studies, spectro-temporal modulation transfer functions (MTFs) were derived as a function of ripple peak density and drifting velocity. In humans, the MTFs exhibit a low-pass function with respect to both dimensions, with 50% bandwidths of about 16 Hz and 2 cycles/octave (Chi et al, 1999). Spectro-temporal MTFs in birds also exhibit similar trends and upper limits (Amagai et al, 1999).

We use a slightly modified version of the conditioned avoidance task (Heffner and Heffner, 1995) to study the ferret MTF. A thirsty ferret in a wire cage placed in an IAC chamber is given a steady trickle of water through a reward spout. Contact with the spout initiates a trial in which a sequence of identical acoustic stimuli are presented to the animal, followed by a different sound. If the ferret does not withdraw its tongue and break contact with the spout when the sound changes, it receives a mild electric shock. The animal learns to withdraw its tongue and avoid shock whenever a different stimulus is presented during the stimulus sequence in a trial. By recording hits, misses and false alarms, it is possible to determine the animal performance using signal detection theory.

In previous work (Kowalski et al, 1996; Depireux et al, 2001), we have used ripples as acoustic stimuli for neurophysiological studies in the ferret auditory cortex. In this study, we will present a comparison of the results of our behavioral studies with the MTFs derived from our physiological studies as a first step in developing a unified description of perceptual abilities and physiological responses in the ferret.

803 Behavioral Assessments of Comodulation Masking Release in Cats

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This poster describes the current status of our ongoing animal psychophysical studies on auditory signal detection in background noise. Our recent experiments have focused on spectrotemporal masking effects in cats that replicate characteristics of comodulation masking release (CMR) in humans. In contrast to our previous critical band experiments with random spectrum noise (Müller & May, Assoc. Res. Otolaryngol. Abs. 2001), we have found that the detection of a tone in coherent amplitude-modulated noise improves with increased masker bandwidth. In this paradigm, comodulation was achieved by multiplying the masking noise with low-pass noise, which is an established procedure for eliciting CMR in humans. Our validation of the cat as an appropriate behavioral model of CMR creates new opportunities for exploring the physiological mechanisms that enhance signal detection in noise. Our current behavioral studies are being directed by neural models based on physiological and psychoacoustic responses to amplitude transients, which have proposed an important role for neurons that respond best to the dynamics of amplitude envelopes, or 'auditory edges.' (Fishbach et al., J. Neurophysiol. 2001). The model predicts that the hypothetical edge-detector neurons will entrain to amplitude transients in comodulated masking noise. The disruption of this noise-driven activity by the presentation of an auditory signal may contribute to CMR-like masking release. Similar response patterns have been observed in the auditory cortex of anesthetized cats (Nelken et al., Nature 1999). Our behavioral results support predictions of the edge-detector model by demonstrating robust masking release in linear gated noise with sharp temporal edges. Planned experiments will evaluate the perceptual consequences of smoothing spectral edges in the masking noise. If our modeling analysis is correct, this masker condition will be a less effective source of CMR.

804 Effects of signal duration on Comodulation masking release (CMR) in the Mongolian gerbil (Meriones unguiculatus)

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Comodulation masking release reflects a mechanism developed in the evolution of hearing to improve signal detection in background noise. CMR describes the reduced masking of a tone when the components of a masker show correlated amplitude modulation in different frequency regions. In a band-narrowing paradigm (Klump et al., ARO 2001) and in a flanking-band paradigm (Wagner & Klump, 2001) gerbils showed a similar pattern and an even larger amount of masking release than humans (Schooneveldt & Moore 1987, 1989). In this study we investigated the effect of signal duration on CMR in a band-narrowing paradigm.

Three Mongolian gerbils were trained in an operant Go/NoGo procedure with food rewards to report a 2 kHz tone of 410, 200, 100 or 50 ms total duration (10 ms raised-cosine ramps) centered in a continuous masking noise of different bandwidth (spectrum level 40 dB SPL/Hz). Masked thresholds were determined for masker bandwidths of 200 and 1600 Hz. For a signal duration of 410 and 200 ms thresholds were also measured for maskers of 50, 800, and 3200 Hz bandwidth. All noise bands were generated digitally following procedures described

in Hall et al. 1984. CMR was defined as the threshold difference between the unmodulated and the comodulated condition.

The observed pattern in the gerbil is similar to the results described in humans: Masked thresholds (criterion d' =1.8) were increased for a signal duration below 200 ms. At any signal duration the CMR is improved with increasing masker bandwidth (-1 to 6.3 dB at 200 Hz, 9.4 to 15.3 dB at 1600 Hz). The amount of masking for equal masker bandwidth was reduced with shortening of the signal.

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805 Discrimination of Direction in Frequency-modulated Tones by Rats

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There is strong evidence that human speech and animal communication calls share common acoustic characteristics and general principles of processing and that, both in humans and animals, temporally changing acoustic cues are of critical importance. Especially frequency modulation (FM) is a critical parameter of acoustic communication signals. This importance triggered several studies in different mammalian species on the neural encoding of FM signals. Only few data, however, are available on the perception of FMs. These data indicate that in general the ability to discriminate the direction of FM tones is reduced at high modulation rates.

We tested the discrimination of direction in exponential FM tones in five rats in a 2AFC paradigm, in which animals were trained to select different response sites for rising and falling FM tones. The frequency range of FM tones in the initial experiments was between 1 and 32 kHz. Thresholds for the discrimination of FM direction depending on the modulation rate, as read from psychometric functions, ranged from 70 to 150 octaves/s.

In additional experiments we determined the discrimination threshold for the direction of FM stimuli of different spectral excursions and different center frequency using an adaptive method (PEST). Thresholds were shifted towards lower modulation rates when the frequency excursion was reduced down to 2 octaves. In addition, thresholds were shifted towards lower modulation rates when the center frequency of FMs was increased (e.g. threshold around 40 octaves/s for short FMs around 16 kHz).

These data indicate that discrimination of the direction of FM tones is dependent on the spectral excursion (or temporal duration) and, surprisingly, also strongly dependent on center frequency even for stimuli well inside the animal's hearing range.

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806 Masking by Harmonic Complexes in the Hearing Impaired Belgian Waterslager Canary

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Harmonic complexes created with component starting phases selected according to scaled modifications of the Schroeder (1970) algorithm are differentially effective as maskers in humans and several species of birds. Complexes which have peakier or more modulated envelopes are always less effective maskers than complexes with relatively flat envelopes in birds, but not always in humans. In the present study, we examined masking by such harmonic complexes in a strain of canary with a hereditary hearing impairment associated with abnormalities of the ear, the Belgian Waterslager canary. Thresholds for detecting tones embedded in harmonic maskers with envelopes that vary systematically between very peaky and very flat were measured using standard operant conditioning methods and the Method of Constant Stimuli. Belgian Waterslager canaries generally showed more overall masking compared to normal-hearing canaries and other birds. In particular, Belgian Waterslagers were much more susceptible to masking by complexes with peakier envelopes than were normal-hearing birds. These findings may reflect poor frequency selectivity resulting from the cochlear damage that underlies the hearing loss in the Belgian Waterslagers.

807 Modulation rate discrimination in the European starling (Sturnus vulgaris)

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The discrimination of a change in modulation rate is impaired in the presence of a spectrally remote masker modulated with the same frequency, but not with a temporally correlated envelope (Yost et al. 1989). Maskers with correlated envelope modulation improve the modulation rate discrimination (Buus & Pan 1994). Since in the starling modulation detection is not impaired in the presence of a modulated masker (Klump et al. 2001) it is interesting to see if similar patterns can be found in modulation rate discrimination.

Three starlings were trained in a Go/NoGo paradigm to report an increase in the modulation frequency of a sinusoidal amplitude modulated 4 kHz probe (500 ms duration, mod. freq. 10 and 40 Hz). There were four conditions: (1) probe alone, (2) probe plus a 1 kHz-unmodulated masker, (3) probe plus a modulated 1 kHz-masker with uncorrelated envelope, (4) probe plus a modulated masker with correlated envelope. The modulation depth of probe and masker was 100 %. The modulation frequency was varied in steps of 0.4 Hz according to the method of constant stimuli. Modulation-rate discrimination thresholds were determined using signal-detection theory (criterion d'=1.8).

The just noticeable increase in modulation frequency was similar for the probe alone (cond. 1) and the probe plus unmodulated masker (cond. 2). Adding the modulated uncorrelated masker (cond. 3) had no effect on the discrimination. In the presence of the modulated correlated masker (cond. 4) the thresholds were slightly but not significantly better than in the probe alone condition. Apparently European starlings, unlike humans, analyse modulations at one carrier frequency without interference of signals at remote frequencies.

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808 Paradoxical Perception of Directional Pitch Change in Japanese Monkeys

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In this research perception of directional pitch change in Japanese monkeys was investigated on an operant task with water reward. A pair of tone bursts and/or harmonically structured complex tone bursts were sequentially presented. They could always discriminate the direction of pitch change by using the fundamental frequency cue but in a specific case. Their performance was worse than a chance level, or they oppositely responded to the fundamental frequency cue in the specific case. The case was that the temporal sequence was made of a simple tone burst and a complex tone burst where the frequency of the simple tone burst was in between the fundamental and the second harmonic frequencies of the complex tone burst, and the fundamentals of the complex tone were lower than 500 Hz. In order to compare data obtained from monkeys to humans, human subjects were also used in the same paradigm except for the water reward. Some human subjects responded in the same way as monkeys while others perfectly judged by the fundamental frequency change. For the former human subjects effects of the duration of tones or interval between tones were tested. They showed amelioration in using the fundamental frequency cue as tone duration was prolonged or interval between two tones was

expanded. The monkeys showed similar tendency as humans. Results suggest that the music training could make a difference in judging what cue should be used in human subjects. So, untrained humans and monkeys might have similar sequential pitch perception.

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809 Spectro-Temporal Modulation Detection In Rhesus Macaques And Humans

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Monkeys have been found to be comparable to humans in detecting temporal modulations of sounds (Moody, 1994, JASA 95:3499). However, their sensitivity to spectral modulations was reported to be worse than that of humans (O'Connor et al, 2000, J Comp Physiol A 186: 903). Sensitivity to combined spectro-temporal modulations has been examined in humans by using dynamic rippled spectra (e.g. Chi et al, 1999, JASA 106:2719). Monkeys are expected to be worse than humans in the combined spectro-temporal task, assuming the spectro-temporal modulation transfer functions (MTFs) are linearly predicted on the basis of separate temporal and spectral MTFs (Chi et al). To test this prediction, we measured spectro-temporal MTFs in monkeys.

So far, one male rhesus macaque (Macaca mulatta) was trained to detect a change in a broad-band sound. A lever was pressed to initiate a trial and released upon detection of a change. The monkey was rewarded a drop of water for a correct response. Human subjects performed the same task. Sounds were presented in a sound- and echo-attenuating room at 1 m in front of the subject. The stimulus was a broadband complex with densely spaced components, consisting of two segments: a stationary spectrum with randomly varied durations (0.5-2.5 s) followed by a dynamic ripple (0.7 s). Ripple densities were 0 to 3.2 cycles/octave, ripple velocities 4 to 256 Hz, and modulation depths 10 to 70 %.

The results in humans were similar to those reported by Chi et al (1999). The thresholds from one monkey were roughly similar to those of the humans. These preliminary data seem to contrast with expectations on the basis of spectro-temporal separability and earlier findings in monkey.

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810 Microarray Expression Profiling Chick Cochlea and Utricle Sensory Epithilia

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Avian auditory hair cells, unlike those in the mammalian cochlea, can regenerate after damage. One hurdle in identifying the signals that regulate regeneration is the small numbers of cells in the cochlea. With micro-cDNA synthesis methods and custom cDNA microarrays, we are able to expression profile <1,000 cells. This allows us to measure gene expression differences in the sensory epithelia from normal chick cochleae and utricles, as well as changes during regeneration of hair cells after injury. We have employed two types of high density spotted microarrays in these studies. The first is an array of 7,500 sequence-verified human cDNA clones. Within this array are numerous genes that have been implicated in inner ear development or hearing loss. The second is an oligonucleotide array of 50mers designed to detect all known transcription factor genes. In a comparative analysis of chick cochlea (which is quiescent when undamaged) and utricle (which is in a constant process of apoptosis and regeneration), we have identified >3-fold increases in genes encoding tyrosinase related protein, WNT1-inducible signaling pathway protein 2, MET, cadherin-4, neuregulin-1, and connexin 46.6. In the cochlea, WNT inhibitory factor 1, neurofibromatosis type 2, dynein light chain, apoptotic protease activating factor 1, and retinoic acid receptor responder3 showed higher levels of expression (relative to the utricle). To complement this approach we are employing a novel cDNA subtraction method to identify genes expressed in the utricle but not in the cochlea. After three rounds of subtraction we have reduced highly abundant genes by several hundred-fold and enriched for utricle specific genes. Using these approaches, we hope to derive new insights into the genetic control of auditory hair cell regeneration.

811 Analysis of the Expression of Cell Proliferation and Apoptotic-Related Genes in the Organ of Corti

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Hearing loss is frequently a consequence of the destruction of hair cells of the organ of Corti. Also, as part of aging, mammalian cochlear hair cells are lost by apoptosis and are not replaced. Two potential therapeutic approaches to alleviate hearing loss, would be to inhibit cellular apoptosis prior to hair cell death, or to stimulate hair cell regeneration. A first step for either of these approaches would be to identify the apoptotic and proliferative pathways, if any, that are expressed in cells within the mature organ of Corti. To assess the repertoire of proliferative- and apoptotic-related genes expressed in the organ of Corti, we analyzed an EST database (Kachar, et al., this meeting) generated from a non-amplified cDNA library constructed from mouse organ of Corti tissue dissected from around the period of hearing onset (P5-15). Of 5387 unique genes we identified 23 that are known to be involved in apoptosis and 64 involved in cell proliferation. Additional clusters show various degrees of similarity to known genes (4 for apoptosis and 14 for cell proliferation) and may represent new isoforms. Highly expressed apoptosis-related genes include FLICE-like inhibitory protein (a caspase inhibitor associated with the extrinsic apoptotic pathway) and a sequence 88 % similar to TRAL (a TNF receptor-associated protein). As for the proliferation-associated genes, the most expressed were: insulin-like growth factor (IGF) binding proteins 2, 3, 4 and 5, cdc42, FGF4, and the cyclins ania-6b and 1. IGF binding protein 5 was among the most abundantly expressed genes in the library. The results demonstrate that components of both apoptotic and proliferative pathways continue to be expressed within the mature organ of Corti. Moreover, the results may provide valuable insights regarding specific points at which apoptotic or proliferative pathways might be regulated. In particular, the high representation of IGF binding proteins is intriguing in light of experimental evidence demonstrating that IGF is one of the most potent inducers of cellular proliferation within the mammalian utricle.

812 The loss of hair cells by apoptosis induces p27 downregulation in mouse vestibular epithelia

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Introduction> p27 is known to play a certain role in regulation of cell proliferation in the inner ear during development. Recently, involvement of p27 in degenerative process of chicken basilar papillae was reported. In this study, we examined the expression of p27 in healthy normal vestibular epithelia and those damaged by aminoglycosides. <Materials and Methods> ICR mice were used. A small hole was opened in the left posterior semi circular canal. Using a microsyringe, a 10 microliter of neomycin solution (400mg/ml) was

introduced to the endolymphatic space. Then the animal was decapitated and temporal bones were removed. The right temporal bone was used as the control. A crista ampullaris of the lateral semicircular canal was extracted and fixated in 4% paraformaldehyde. Frozen sections of cristae were prepared for immunohistochemistry. We performed immunostaining for p27 followed by counterstaining with DAPI. <Results> In healthy matured vestibular epithelia, p27 expression was found in supporting cell nuclei, not in hair cell nuclei. In damaged vestibular epithelia, apoptotic nuclei were observed in the hair cell layer. Some of supporting cells lacked p27 expression. The number of supporting cells lacking p27 expression increased in parallel with the loss of hair cells. <Conclusion> These findings indicate that p27 plays a certain role in the degenerative process of vestibular epithelium following the aminoglycoside treatment.

813 Structure and function of the inner ear of p27 kip1-/- mice.

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In mammals, lost auditory hair cells (HCs) can not regenerated. To induce HC regeneration, it is necessary to generate and differentiate the new cells into HCs. One way to induce generation of HCs is to manipulate cell cycle regulatory proteins. p27Kip1 (p27) prevents entry of cells into the cell cycle, and maintains the differentiated state. p27 deficient mice exhibit continual production of HCs leading to excessive number of cells in the organ of Corti. These mice provide a model for post-natal HC production. We studied the structure and function of the inner ear of another line of p27 deficient mice originating from the Memorial Sloan-Kettering Cancer Center. The deficiency in p27 expression in these mice is incomplete, as they retain expression of AA52-197. In this study we characterizd the inner ear phenotype of these mice and compare the data to that in the other p27 knock-outs (Chen P et al. Development 1999,and Lowenheim H.et al. PNAS 1999). We used a total of 21 mice divided into three genotypic groups: p27-/-, p27 and p27 at weaning age (three weeks old) and assessed their inner ears using SEM, TEM, phalloidin histochemistry and BrdU. The number of inner and outer HC is largest in homozygous mice, followed by heterozygous mice, which had a larger number of HCs than the wild types. Assessment of the vestibular epithelium revealed a mild increase in the number of HCs in the homozygous mice compared to wild types. BrdU label was found in cell in the sensory epithelium of the cochlea and the utricle. Auditory was severely impaired in the homozygous mice, moderately impaired in the heterozygotes and normal in the wild types. The ultrastructure of the sensory epithelium was only mildly pathological. The morphological data cannot by itself explain the severe loss of function in these mutants.

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814 Ongoing proliferation in the organ of Corti of mice lacking p27Kip1

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p27Kip1 inhibits cell cycle progression by acting as a cyclin dependent kinase inhibitor. Targeted deletion of p27Kip1 results in supporting cell proliferation in the cochlea of postnatal (age: postnatal day (Pnd) 7) and at a reduced level in adult mice (age: 4 months) (Loewenheim et al., Proc. Natl. Acad. Sci. USA, 96, pp. 4084-4088, 1999; Chen and Sigel, Development 126, 1581-90, 1999).

In the present study we examined the change of proliferation rate during postnatal development. Proliferation in the sensory region of the organ of Corti of p27 homozygous and heterozygous mice (129/Sv inbred background) was investigated at Pnd 7, 11, 15, 19 and 30. Proliferating events were assigned to the different types of supporting cells.

Mice were given daily injections of BrdU (30 mg/kg) for three consecutive days and were sacrificed 12h after the last injection. Cochleae were prepared as whole mounts and immunostained for BrdU.

In p27 homozygous cochleae BrdU positive cells were located mainly in the inner sulcus, the Hensen cell and the inner phalangeal cell region. From Pnd 7 proliferation rate decreased by a factor of 3 until Pnd 30. In p27 heterozygous animals only few proliferating cells were detected in the Hensen cell region of the apical turn in about one third of the 7 day old individuals. Other p27 heterozygous age groups showed no proliferation.

According to Loewenheim et al. 1999, only few cells were found to proliferate in animals at 4 month of age. It is interesting to note that cell proliferation proceeds long after onset of hearing. The results demonstrate that proliferation of cells in the organ of Corti can occur in juvenile stages at which the cochlea has reached normal hearing function. This implicates that disruption of p27Kip1 allows for continued proliferation in the mature organ of Corti after the onset of hearing.

815 Dorsal Root Ganglion and Stemcell Implantation Into the Cochlea and the Vestibulocochlear Nerve.

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Fetal DRG neurons have been shown to extend functional connections in the rat spinal cord (Kozlova et al, 1995). Embryonic stem cell (ES) and adult neural stem cells (ANSC) have also been shown to have the potential to differentiate into a variety of cell types, not limited to neurons (Arnhold et al; Clarke et al 2000). We implanted fetal DRG neurons, ES-cells and ANSC into the cochlea and vestibulocochlear nerve. All implanted animals received daily injections of cyclosporin (0.56mg/100g BW) and vibramycin (0.24mg/100g BW) for 3-4 weeks postoperatively. To determine whether the ES-cells developed into neurons we doublelabeled with a neuronal specific antibody Thy 1.3.

Adult guinea pig and rat scala tympani were implanted with E13 DRGs from guinea pig and mice, respectively. Three groups of guinea pigs were implanted: I) normal hearing, II) deafened via transtympanic neomycin and III) deafened plus intrascalar treatment with BDNF and CNTF. The rats were implanted with E13 DRGs from a transgenic mouse line that produces green fluorescent protein (GFP) or E13 lacZ DRGs. The distal vestibulocochlear nerve on adult guinea pigs and rats were implanted with E13 GFP DRGs or E13 lacZ DRGs. For the stem cell experiments adult guinea pig and rat vestibulocochlear nerves were implanted with either GFP-ES cells or lacZ neural stem cells.

The results from implanting adult guinea pigs show a high degree of DRG survival in the scala tympani. The implant was often attached close to the organ of Corti. The neurotrophin-treated animals showed a significantly higher neuronal survival. Deafening did not affect the number of surviving DRG neurons. The rats implanted with GFP- and lacZ DRGs had labeled DRGs after sacrifice. The stem cell results showed GFP and lacZ profiles at the site of injection. It was noted that

many GFP profiles were labeled with Thy 1.3. GFP-and lacZ profiles had migrated centrally into the vestibulocochlear nerve and in some instances reached the medulla.

816 Neural stem cell transplantation into the mouse inner ear

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Previous investigations indicate that regeneration of inner ear hair cells rarely occurs or not. Recently, neural stem cells have been reported to have a potential as a material for transplantation into the central nervous system and retina. Some of transplanted cells differentiate into the cellular components of these tissues. Thus, there is possibility that neural stem cells can survive in the inner ear and differentiate into cellular components of inner ear epithelia. Our previous study revealed that neural stem cells could survive in the endolymphatic space of the normal rat. However, few of them could intrude into the epithelium. These findings indicated that cell-cell junctions at the luminal surface and lack of space in the epithelium obstructed intrusion of neural stem cell in the epithelium. We, thus, applied aminoglycosides into the inner ear in order to produce disruption of the cell-cell junctions and a space in the epithelium. Degradation of E-cadherin, a major adherent molecule in the inner ear, and induction of hair cell apoptosis was observed in damaged epithelia 3 days after injection of neomycin into the posterior semicircular canal. At this timing, we introduced neural stem cells obtained from GFP expressing mice into the endolymphatic space. One-week later, neural stem cells were observed within the sensory epithelium. A few transplanted cells existed in the hair cell layer 2 weeks after transplantation, which indicates possibility that neural stem cells can differentiate into cellular components of inner ear epithelia. Consequently, this experimental model was thought to be suitable for evaluation of the differentiating ability of neural stem cells in the inner ear.

817 Potentials of neural stem cells for inner ear transplantation

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The recent advances in the field of neural stem cells have brought great expectation that severe CNS damages can be repaired by using stem or progenitor cells. It has been shown that transplanted neural progenitor cells, even heterotypical, can integrate with the host brain tissue and differentiate into appropriate neurons. Into the retina, neural stem cells have also been reported to be grafted where they have demonstrated specific neural differentiation. For the inner ear, we have previously reported that neural stem cells could survive in the rat cochlea and showed the possibility of utilization in the future treatment of sensorineural hearing disturbance. In this study, to estimate the effects of damage to the inner ear on the survival and integration of the grafted neural stem cells, we have transplanted neural stem cells into the aminoglycoside treated adult mouse inner ear in vivo. Neomycin were injected into the posterior canal of the adult mouse and neural stem cells prepared from the telencephalon of the E11.5 mouse embryos were transplanted into the cochlea second turn three days after the treatment. Many neural stem cells could survive in the inner ear, and within two weeks of grafting to the cochlea, some were integrated into the sensory epithelium of the inner ear. In addition, some of neural stem cells existed in the hair cell layer, which indicated the possibility that neural stem cells can be cellular components of the sensory epithelium in the damaged inner ear.

818 Spontaneous hair cell repair in the mouse vestibular epithelium

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The loss of cochlear hair cells after several ototoxic insults is considered to be irreversible in the mature mammalian cochlea. In the vestibular organ, however, hair cells destroyed by gentamicin can be replaced by new hair cells. The regeneration of vestibular hair cells has been demonstrated in several mammalian species including guinea pig, rat and chinchilla. In this study, we tested the regenerative capability in the vestibular epithelium of the mouse, an important laboratory animal model. Gentamicin (40μg) was introduced into the posterior semicircular canal of 3-4 week old CBA/J mice. In addition, the mice were given bromodeoxyuridine (BrdU) dissolved in the drinking water (2mg/ml) to detect cell proliferation. The progression of the lesion, the morphological recovery and the presence of BrdU-positive cells were assessed in the utricular macula 1, 2, 3 and 4 weeks after the treatment. The number of atypical hair cells (cells with immature appearance or short stereocilia) was counted using scanning electron microscopy. One week after the insult, a severe lesion with hair cell loss was evident in the utricle. Immature cells could first be seen 2 weeks after the treatment and cells with short bundles were visible at 3 weeks. The number of cells with small stereocilia increased with time. BrdU uptake was confirmed in the positive control (gut epithelium). No BrdU uptake was seen in the utricle, suggesting that the regeneration of the mouse vestibular hair cells is non-mitotic. It remains to be determined if the observed regeneration is via transdifferentiation (conversion), as previously reported in the avian basilar papilla, or via the repair of injured cells.

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819 Connexin42 Expression in the Normal and Regenerating Chick Cochlea.

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We have explored the expression of Connexin42 mRNA in the normal and regenerating chick cochlea utilizing whole mount in situ hybridization. Connexins are gap junction proteins that form transmembrane channels between neighboring cells and facilitate the exchange of ions and small metabolites. In the mammalian and avian cochlea gap junctions are present between supporting cells in the sensory epithelium and between cells in the ion-transporting epithelium. It has been proposed that connexins play an important role in potassium recycling during transduction. Recently, we have described the expression of connexins 31, 43, and 56 in the avian basilar papilla by in situ hybridization or immunocytochemistry. Each of these three connexins has a separate and distinct distribution in the basilar papilla. Moreover, we have shown that Cx31 and Cx56 are upregulated immediately following gentamicin treatment but that Cx43 is downregulated between 72h and 96h after gentamicin injection. We have now identified a fourth connexin isoform, Cx42, in the normal avian basilar papilla by in situ hybridization. It has a more widespread distribution than the three previously identified isoforms and is present throughout all but the proximal 20% of the basilar papilla. Surprisingly, its mRNA levels do not appear to be altered between 24h and 96h following a gentamicin injection. This preliminary study supports our previous assertion that the expression of connexin isoforms in the avian cochlea is complex and that each connexin isoform has a unique role in normal cochlear function and during hair cell regeneration.

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820 Parallel Apoptotic Pathways Employed by Gentamicin and Sound Damage in the Avian Cochlea

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We have explored the expression of a battery of proteins involved in the apoptotic pathway of hair cells dying from both gentamicin treatment and sound damage. The timing and location of proteins involved in early and late stages of apoptosis were examined in whole mount immunocytochemical preparations of the chick basilar papilla. TIAR, an early marker of apoptosis, can be identified 12-24h after a single injection of gentamicin (300 mg/kg). From studies using fluorescently labeled gentamicin (see Steyger et al, this meeting) it appears that gentamicin takes 6-9h to reach cochlear hair cells following systemic injection. Later events in the apoptotic cascade, such as cytochrome c release and activation of caspase-3 are first observed beginning 30-36h after the gentamicin injection, concurrent with the appearance of hair cell damage and ejection from the sensory epithelium. Using a 1500 Hz pure tone sound exposure at 120 dB SPL, TIAR translocation begins 3h after the onset of sound exposure, reaches a peak by 9h but is still prominent at 12h. Cytochrome c release from mitochondria and activation of caspase-3 are first seen 12h after the onset of sound exposure and reach peak levels by 15-18h. Hair cell ejection from the basilar papilla can initially be seen 12-15h after the onset of sound exposure and reaches a peak by 24h. Both gentamicin treatment and sound damage exhibit a similar cascade of events in the apoptotic pathway indicating that these traumatizing insults induce hair cell apoptosis by equivalent mechanisms. They both show the first steps in the cascade 3-6h after the stimulus reaches the hair cells but then gentamicin damage takes 18-24h to complete the cell death cascade, while sound damage reaches the terminal stages within half that time (9-12h). The reasons for this difference are not yet clear but it may be related to the mechanical component of sound damage, the continuous presence of the sound stimulus versus the diminishing drug concentration, or to some other mechanism within the hair cells.

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821 Time Course of Mitosis in the Avian Cochlea after Topical Application of Gentamicin

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Hair cells in the avian cochlea (basilar papilla) can regenerate after damage from ototoxic drugs or noise exposure. Recently we have developed a method for topical, unilateral application of gentamicin (Hearing Research 125:109-119, 1998). The extent of damage is dose-dependent and may include just basal, high frequency hair cells (low damage) or may extend to encompass the entire cochlea (total damage). The purpose of this study was to determine the time course of mitotic generation of hair cells and support cells in the basilar papilla after topical application of gentamicin. Ten day-old broiler birds were treated unilaterally with a Gelfoam pledget soaked with gentamicin (100mg/ml). The contralateral ear of each bird was untreated. At 3, 5, or 9 days after gentamicin treatment birds were given a single, subcutaneous injection (150 mg/kg) of bromodeoxyuridine (BrdU) and sacrificed the next day. Cochleae were perfused, dissected as whole mounts, and reacted for BrdU immunocytochemistry to reveal cochlear cells newly generated by mitosis. Cochleae were also stained with phalloidin to reveal the extent of hair cell damage. No BrdU-labeled cells were observed in the untreated ears in any animal. The greatest number of BrdU-labeled cells was seen in birds injected at 3 days. In cochleae sustaining low damage, labeled cells were scattered throughout the area of hair cell loss. In cochleae with total damage, labeled cells were concentrated in the apical region. In birds injected at 5 days, fewer labeled cells were observed in damaged regions, with cells often concentrated at the transition zone. In birds injected at 9 days, few labeled cells were observed. These results suggest that the majority of cells in the basilar papilla are generated within 5 days of topical gentamicin damage.

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822 Hair Cell Corpse Removal: Are Macrophages The Undertakers?

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Macrophages phagocytose dead cells and stimulate wound healing in many tissues, but whether they have a role in repair of inner ear sensory tissues is not clear. We therefore looked for macrophage recruitment during hair cell loss in mature and early postnatal mouse organ of Corti (OC), and in saccular maculae of an amphibian, the newt. Mice aged between 20 and 35 days were treated with a single kanamycin-bumetanide combination that produces loss of all outer hair cells (OHCs) within 72h. OHCs died by apoptosis and the cell bodies were retained within the epithelium. There was no evidence for the presence of macrophages within the OC over the period when OHCs were dying in either thin sections or frozen sections labelled with the macrophage antibody F4-80, but they were present in the tunnel of Corti after two weeks recovery. In cultured explants of early postnatal mouse OC exposed to gentamicin, time-lapse video microscopy, SEM and TEM showed entire cells, with apoptotic nuclei, extruded from the apical surface, but there was no evidence for macrophage recruitment. In newt saccular maculae exposed in vitro to gentamicin, hair cells died by apoptosis within the body of the epithelium and supporting cells contained apoptotic body-like structures. Labelling with Griffonia-lectin to identify macrophages, showed labelled cells in the epithelium lining the roof of the saccule but there was no evidence for macrophage migration into the epithelium or to the apical surface. The results suggest that macrophages are not involved in removal of dead hair cells in either mice or newts.

823 Wortmannin, a specific inhibitor of phosphatidylinositol 3-kinase influences neurotrophin-induced spiral ganglion growth.

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Phosphatidylinositol 3-kinase (PI3K) is considered to be an important enzyme in cell signaling, mediating certain aspects of neurotrophin signals from the cell surface receptor to the nucleus. The participation of PI3K in the mediation of neurotrophin-induced effects in the spiralganglion (SG) of neonatal rats was investigated in vitro. SG explants were stimulated with neurotrophin (NT)-3 and treated with Wortmannin, a specific inhibitor of PI3K. After fixation and immunhistochemical staining, the growth of the SG neurites was evaluated. Stimulation with NT-3 lead to a significant increases in number and length of neurites, when compared to non-stimulated controls. Treatment of NT-3 stimulated SG explants resulted in a dose-dependent reduction of both parameters, whereas the neurite growth of non-stimulated control explants was not significantly influenced by the incubation with Wortmannin. The results demonstrate that neurotrophin-induced neurite growth from SG explants can be modulated with the PI3K inhibitor Wortmannin and indicate that PI3K is a key enzyme in the mediation of NT-3 effects in cochlear neurons. These observations together with results of previous studies suggest that the activation of PI3K as well as Ras and MEK are essential for neurite growth in cochlear neurons. Further knowledge of cell signaling

mechanisms influencing SG neurite growth could lead to new therapeutical strategies for the treatment of inner ear diseases.

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824 Auditory Hair Cell Regeneration In Vitro; Birds Have Problems, Too!

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The avian auditory epithelium normally generates no new hair cells after birth. However, when auditory hair cells are killed experimentally in mature birds, mitotic activity among sensory epithelial progenitors is upregulated, and new functional hair cells are formed. In vitro methods provide an excellent opportunity to explore the cellular and molecular mechanisms underlying this renewed sensory cell production. We used two approaches to develop in vitro methods to study hair cell regeneration in the mature auditory epithelium (basilar papilla, BP). First, P7 chicks were treated with gentamicin (1x@400 mg/Kg or 2x@250 mg/Kg) to initiate hair cell death and early stages of regeneration in vivo. At different times after treatment, cochlear ducts (CDs) were cultured, and the ability of the BPs to continue the regenerative process in vitro was assessed. Second, CDs were dissected from control P7 chicks, placed in gentamicin (0.5-2 mM) for 1-2 hours, and hair cell death and regeneration were examined during the ensuing culture period. Similar culture conditions were used for each paradigm. We found striking differences in the regenerative capacity of the BP in vitro when hair cells were injured in vivo versus in vitro. Following in vivo damage, sensory progenitor division continued in culture up to 3 days after explantation. New cells that were formed in vivo prior to culturing continued to differentiate, and many new hair cells were formed via mitosis, in the dish. In contrast, despite moderate to severe hair cell loss throughout the BP, we detected very little progenitor cell proliferation in CDs cultured up to 96 hours after in vitro gentamicin exposure. Further, labeling with hair cell markers demonstrated no evidence for non-mitotic hair cell regeneration. Our current efforts explore molecules that may be necessary for hair cell regeneration in the avian BP after in vitro gentamicin treatment.

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825 Characterization of Leukocyte Subtypes in Avian Inner Ear Sensory Epithelia

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Leukocytes reside in undamaged sensory epithelia of the avian ear and increase in number after trauma, prior to hair cell progenitor proliferation. It has been hypothesized that leukocyte-produced growth factors may trigger hair cell regeneration. Several leukocyte morphologies, including a highly ramified cell type, are seen in normal and damaged avian inner ear epithelia, suggesting several leukocyte subtypes may be present. Macrophages are present (Warchol, 1997, J. Neurobiol. 33:724-734), but the identification of the leukocyte subtype with the ramified morphology is unclear. Candidate leukocyte subtypes known to exist with ramifications include γδ T cells, dendritic cells, macrophages, and microglia. Immunohistochemistry with a panel of antibodies to chicken leukocytes (Bu-1, CIa, 74.2, CD3, TCR1) was used to characterize leukocytes in normal and aminoglycoside-damaged chicken ears. Bu-1 labels B lymphocytes and a subset of the monocyte/macrophage lineage of cells. CIa functions as the avian major histocompatibility (MHC) class II cell surface antigen and is expressed on B lymphocytes, a subpopulation of monocyte/macrophage lineage of cells and mitogen-activated T lymphocytes. 74.2 labels mature tissue macrophages. CD3 labels T lymphocytes, and TCR1 labels γδ T cells. Birds were injected once with streptomycin (to damage vestibular organs) or twice with gentamicin (auditory organs) and allowed to recover 1, 2, or 3 days before immunohistochemical processing of the basilar papillas or utricles. Data indicate that T cells do not reside in normal ear sensory epithelia and are virtually absent from aminoglycoside-damaged epithelia. Bu-1, CIa, or 74.2 positive cells are seen in avian ear sensory epithelia and often have a ramified appearance. Findings suggest that the highly ramified leukocytes are not γδ T cells. They are of the myeloid lineage and are unlikely to be dendritic cells.

826 Actin Dynamics during Repair of Hair Bundles of Sea Anemones

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Hair bundle mechanoreceptors can be damaged by overstimulation or by exposure to calcium free buffers. Provided the trauma is slight, hair bundles recover although the subcellular mechanisms for such recovery are poorly understood. Hair bundle mechanoreceptors on tentacles of sea anemones are especially resilient in that they recover normal structure and function within 4 hr after severe trauma caused by 1 hr exposure to calcium free seawater. During this interval, large protein complexes are secreted named "repair proteins" that include replacement linkages for those lost during trauma. In the present study, we find that the repair process requires reorganization of the actin-based cytoskeleton in hair bundles. Confocal microscopy suggests that F-actin is first partially depolymerized and then repolymerized. Video microscopy indicates that stereocilia show considerable motility during repair. In the presence of exogenously supplied repair proteins, recovery of vibration sensitivity at the organismal level occurs within 8 min. Paradoxically, a full recovery of morphology of hair bundles requires approximately 45 min. Similarly, a full recovery of mechanoelectric responses to bundle deflection requires approximately 45 min. It appears that the recovery of normal responsiveness at the organismal level precedes a full recovery of hair bundle mechanoreceptors.

827 Analysis of changes in chicken Nucleus Magnocellularis neuron number during cochlear hair cell loss and regeneration: Is the tissue stain significant?

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Gentamicin-induced cochlear hair cell death creates a temporary loss of afferent input to chicken Nucleus Magnocellularis (NM) neurons. This loss of afferent input results in changes such as decreases in cell size, glucose metabolism, and cell number. During hair cell regeneration, these changes return to normal, including cell number. We have shown previous evidence for limited regeneration of these NM neurons via mitosis (Brain Research, submitted). The number of mitotically regenerated NM neurons is considerably less than the number of regenerated NM neurons revealed by profile counts of Nissl-stained tissue (Hearing Research 126:84-98). In the current study, we have repeated our previous study of profile counts using a different tissue stain to investigate whether the apparent regeneration of NM neurons during hair cell regeneration is repeatable. Chickens (P10) were given a single, systemic dose of gentamicin or saline and killed after 5 days (n=12 gentamicin and n=10 saline) or 28 days (n=11 gentamicin and n=12 saline). At 5 days the cochlear hair cells have died and at 28 days they have regenerated. Brains were cut and stained for cytochrome oxidase. Profile counts of NM neurons were performed in a blind manner using the nucleus to identify cells. Results are not in agreement with results from our previous study using thionin-stained tissue. We did not observe a significant decrease in the number of NM neurons at

the early survival time when cochlear hair cells have died. Possible reasons for the discrepancy will be addressed.

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828 Influence of Muscle Tone and Surfactant Replacement on Eustachian Tube Mechanics

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Eustachian tube (ET) dysfunction has been associated with the development of persistent otitis media with effusion (OME). Specifically, an impaired ability to open the collapsible ET results in fluid accumulation in the middle ear (ME) and subsequent infection and inflammation. The inability to clear ME fluid and perform other important physiological functions has been related to abnormal ET mechanical properties. These properties include the ET compliance, C, which is a measure of the tube's distensability or "floppiness" and the wall viscosity, μ_w, which is a measure of the tube's viscoelasticity. Recently, we have developed a novel testing technique (Ghadiali et. al., 2001, Auris Nasus Larynx, in press) that accurately determines C and μ_w by correlating pressure-flow measurements with a mathematical model of flow in a collapsible tube. The objective of this study is to use this testing technique to determine how manipulation of certain ET structural elements affects the mechanical properties of the ET. First, the influence of muscle tone will be investigated by experimentally paralyzing the left tensor veli palatini (TVP) muscle in 12 cynomolgus monkeys. Next, the influence of the mucosal surface condition will be investigated by washing out the normal mucous layer and then installing a replacement pulmonary surfactant (Infasurf©) in the right ET of 6 cynomolgus monkeys. Muscle paralysis resulted in a decrease in the pressure required to open the ET (P_{open}), an increase in C, and a decrease in μ_w. The removal of the normal mucosa did not significantly alter P_{open}, but did result in a decrease in C and μ_w. Treatment of the mucosa with Infasurf© was effective in reducing P_{open} and increasing C and μ_w to baseline values. Knowledge of how these specific components alter ET mechanics may lead to improved treatment strategies for OME.

829 Development of a Diagnostic Apparatus for Stapes Fixation

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It is important to evaluate the stapes mobility in tympanoplasty surgery, because it affects the prognosis of improvement of the hearing level. However, quantitative evaluation of stapes mobility has been difficult to date. The mobility of the ossicles has been investigated directly by the Mossbauer technique using a gamma-ray (Lynch et al., 1982; Gummer et al., 1989; Ruggero et al., 1990), a video measuring system (Gyo et al., 1987) and a laser Doppler vibrometer (Stasche et al., 1994; Kurokawa et al., 1995; Schon et al., 1999). Although movement of the ossicles can be measured at the nanometer level with these methods, the required equipment is too large to use in surgery. The above techniques measure dynamic impedance of the stapes at relatively high frequencies. However, the difference in stiffness is more detectable by measurement at low frequency than at high frequency because the stiffness component of the dynamic impedance is dominant at low frequencies. Therefore, to detect the variance of the stapes mobility based on the difference in annular ligament stiffness, low frequency or static measurement is advantageous. At the ARO meeting in 2000, we presented a sensor to measure the semi-static load and displacement of the stapes. In that report, the slope of the load-displacement curve was used as an index of the stapes mobility, and the significant difference was found between the mobility of the normal and fixed stapes. However, the size of the sensor was large and its shape was unsuited for a use during the surgery. In this study, a new apparatus for measuring the stapes mobility, i.e., the load-displacement curve, was developed by assembling a small-sized capacitive force sensor and a hydraulic micromanipulator, and its clinical applicability was evaluated by measuring the stapes mobility of guinea pigs.

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830 Activation of a Src-dependent Raf-MEK1/2-ERK Signaling Pathway is required for IL-1α-induced up-regulation of human β defensin 2 in human middle ear epithelial cells

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Otitis media is the most common cause of hearing impairment and the most frequent bacterial infectious disease in children. The molecular pathogenesis of otitis media, however, is not well understood. It is believed that the innate immunity plays a critical role in protecting the tubotympanum from being infected because the middle ear cavity is normally sterile despite of a paucity of immune cells. Among known antibacterial molecules, defensins have been shown to greatly contribute to innate immunity. β defensins, unlike α defensins, are produced by epithelial cells and serve as the first line of defense mechanism in urinary and respiratory tracts and skin. It is still unclear whether or not b-defensins are expressed in human middle ear mucosa. The molecular mechanisms underlying the cytokine-induced up-regulation of human defensin 2 (hBD-2) have yet to be defined. Here we show that hBD-2 is expressed at higher level in the diseased human middle ear mucosa and that IL-1α up-regulates hBD-2 transcription via activation of a Src-dependent Raf-MEK1/2-ERK signaling pathway. These studies may open up novel therapeutic targets for the treatment of patients with otitis media.

831 Nitric oxide synthase (NOS I) mediates osteoclast activity in vitro and in vivo.

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Cholesteatoma-induced bone resorption is an inflammatory osteolytic process resulting from the pathologic activation of osteoclasts. These bone-resorbing cells develop from hematopoetic precursors of the macrophage lineage and are recruited to the site of inflammation. Recent studies suggest that nitric oxide (NO) is involved in osteoclast development and activation. NO is a neutral free radical gas generated from L-arginine by the enzyme nitric oxide synthase (NOS), which has three known isoforms. NOS I, II, and III were first identified and characterized in neurons, macrophages and endothelial cells, respectively. Since osteoclasts and macrophages develop from a common hematopoetic cell lineage, we expected NOS II to play an important role in osteoclast function. However, we demonstrated the presence of NOS I, but not NOS II or NOS III, in unstimulated osteoclasts (although NOS II was inducible by cytokine stimulation). This study further determined the effect of NOS I deletion on osteoclast development and activity both in vitro and in vivo. In vitro, NOS I -/- osteoclasts had a profoundly increased spread area compared to wild

type. Additionally, in an in vivo model of cholesteatoma-induced bone resorption, NOS I -/- mice demonstrated an attenuated response. These results suggest that NOS I is necessary for inflammation-induced osteoclast development in vivo and the increase in osteoclast size observed in vitro may reflect a defect in osteoclast fusion or adhesion.

832 In vitro collagen expression potential of cells cultered from the human temporal bone

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A disordered synthesis of the interstitial collagens as reason for otosclerosis is discussed. The aim of our study was to investigate the collagen expression potential of primary bone cells from the middle ear.

Normal and otosclerotic human bone specimen were collected during stapedectomy and prepared for cell culture. Subcultures were obtained every third week. Cells from the fourth and last one were detached from the flasks by an initial short digestion to eliminate the less adhesive fibroblasts and a second longer incubation to remove the remaining osteoblasts. The primary cells were grown on chamber slides and fixed with 4% paraformaldeyd at different time points. Characterisation of the cells was done by polyclonal antibodies against osteocalcin (OC), the collagen-types (C) I, II, III and V and the staining for endogenous alkaline phosphatase (AP). Positive and negative cells were performed as controls.

The primary cells showed the expected expression of bone cells: OC+, CI+, CII-, CIII-, CV+ and AP+. The immunohistochemical investigation of collagen expression at different time points showed the steps of synthesis of the interstitial collagens. The specific chains of the various collagen types were chosen intracellular until day 7. In the second week the molecules were smuggled outside the cell membrane and after 19 days the matrix fibres were arranged while the cells were undergoing. Our results show, that primary bone cells from normal and otosclerotic bone have the same expression of extracellular matrix in vitro as osteoblasts in vivo.

Siggelkow H, Niedhart C, Kurre W, Ihbe A, Schulz A, Atkinson MJ, Hufner M. In vitro differentiation potential of a new human osteosarcoma cell line (HOS 58) Differentiation 1998 Jun;63(2):81-91

833 Ossicular Chain Reconstruction in a Temporal Bone Model Using Ionomeric Cement

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The erosion of the long process of the incus is a commonly encountered problem in ossicular reconstruction. Current methods relying on the placement of a PORP or Applebaum prosthesis often produce poor audiometric outcomes. We examined the use of ionomeric cement to repair this defect in a human temporal bone model. Five fresh cadaver temporal bones were used. The bony external ear canal was removed and a 2 cc coupler was fixed in its place. A mastoidectomy was then performed in preparation for laser Doppler vibrometry (LDV) measurements of the stapes footplate. An ER2 earphone was used to deliver a 100 dB SPL swept sinusoid to the coupler, and footplate displacement was measured in the intact chain with LDV. An ER10C microphone was then placed in the coupler and the time-averaged response to a series of eight chirps was used to calculate wideband energy reflectance (ER) and impedance (Keefe et al., 1992). Following these measurements, a 2 mm section of the long process of the incus was removed using an argon laser. LDV and ER were again performed. The chain was reconstructed using ionomeric cement, and the measures were repeated.

In the disarticulated chain, LDV measurements showed a transmission loss of 40 dB, which was limited by the LDV noise floor. There was a characteristic notch at 750 Hz in the ER pattern with an average decrease of 30%. In the repaired chain, average LDV measurements showed displacements to be 8 dB less than the intact chain at frequencies below 1000 Hz, and 6 dB greater above 1000 Hz. Average ER measurements in the repaired chain were within 10% of intact values.

This study supports the use of ionomeric cement to repair a defect in the long process of the incus. Wideband LDV and ER measurements were successfully used to monitor the effect of the lesion and the success of the repair.

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834 IL-1beta Stimulates Osteoclast Formation and Activity Independent of Osteoblasts and Mediates Inflammatory Bone Resorption

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Osteoclast activity is vital to normal bone development and is an integral component of many pathological processes. In inflammatory bone diseases, osteoclast recruitment and activation can cause significant bone loss. When this occurs in chronic otitis media with or without cholesteatoma, middle ear damage can occur. The specific components of inflammation that lead to osteolysis are currently under investigation. IL-1beta is a known inflammatory cytokine that has been shown to promote osteoclast differentiation and prolong survival in the presence of murine stromal cells or osteoblasts. Previous models have proposed that IL-1beta stimulates osteoblasts to express RANKL, an essential factor for osteoclastogenesis.

Using RANKL-supported osteoclast cultures, it is now possible to culture osteoclasts independent of osteoblasts. With this system, we found that IL-1beta directly stimulates osteoclast differentiation dose dependently. This IL-1beta dose response was not observed in RANKL-supported osteoclast cultures from IL-1 receptorI knock-out mice. In addition, IL-1beta directly stimulated osteoclast activity as measured by resorption pit cross-sectional analysis. These results indicated a direct effect of IL-1beta on osteoclasts in vitro, independent of osteoblasts. We also observed a decreased response to RANKL in IL-1RI KO cultures.

Mice infused with IL-1 receptor antagonist showed no significant change in osteoclast number as compared to saline-infused mice controls after keratin implantation, in vivo. However, IL-1RI KO keratin implanted mice were found to have fewer osteoclasts at sites of inflammation compared to wildtype mice. The results of these studies suggest that, in addition to the known stimulatory effect of IL-1beta on osteoblast/stromal cells, a direct interaction between IL-1beta and osteoclasts exists and may play a role in inflammatory bone diseases.

835 Presence of four major human herpesviruses in middle ear fluids from young children with acute otitis media

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Acute otitis media (AOM) is one of the most common diseases treated in the pediatrician office. Recently viruses have been shown to play a crucial role in the etiology and pathogenesis of AOM. The common viruses previously reported in patients with AOM include respiratory viruses. Infection with some herpesviruses such as cytomegalovirus (CMV) in infants and children is common. However, the role of

herpesviruses in middle ear disease is unknown. In this study, one sixty-nine middle ear fluid (MEF) samples collected by tympanocentesis from children aged 5 months to 6 years with AOM were examined by multiplex nested polymerase chain reaction (PCR) for the presence of the four major human herpesviruses: CMV, herpes simplex virus (HSV), Epstein-Barr virus (EBV), and varicella-zoster virus (VZV). CMV, HSV, EBV or VZV, alone or in combination with bacteria or other viruses, was isolated from 32 MEF samples (18.9%). CMV alone was detected in 4 MEF samples, HSV alone was present in 1 sample, EBV alone in 1, VZV alone in 2, and dual infections of HSV and VZV in 1. The patients associated with the presence of these herpes virus DNA in MEF have clinical signs of AOM despite negative bacterial culture and negative respiratory viral infection. These results suggest an etiologic role for four major human herpesviruses, CMV, HSV, EBV and VZV in AOM.

836 Effect of nitric oxide on concentration of mucin in experimental otitis media

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Otitis media (OM) is one of the most common diseases of childhood. Among types of otitis media, mucoid otitis media (MOM) tends to cause more sequelae such as hearing loss, retraction of the tympanic membrane, and bone destruction. Our previous studies showed that concentrations of nitric oxide (NO) metabolites were highest in MOM compared to serous OM or purulent OM and NO in middle ear causes sensorineural hearing loss. We suspect that NO has a role in inducing MOM. The purpose of this study is to determine the role of NO in the production of mucin in lipopolysaccharide (LPS) induced experimental OM.

Experimental OM was induced in chinchillas by injecting LPS with or without NO-producing compound SNAP (S-nitroso-N-acetylpenicillamine) to the left bullae. Five experimental groups were studied, 1, 2, 4 mg of SNAP alone, LPS (0.3 mg) alone, and LPS + 2 mg SNAP. The right bullae served as control. Samples of middle ear fluid were collected after 48 hours for the SNAP only groups and at 96 hours for the LPS and LPS+SNAP groups. At the end of each experiment the animals were euthanized and the temporal bones were harvested for histopathological studies. Concentration of mucin was assayed with the PAS method by modifying a commercially available kit. This is a calorimetric method measureing absorbance at 540 nm. Mucin analysis revealed that concentration of mucin (glycoprotein) increased with increasing levels of SNAP (1mg SNAP,109.27 μg/ml, 2mg SNAP, 214.82 μg/ml, 4mg SNAP, 196.55 μg/ml). Levels of mucin were highest in LPS + SNAP group (1161.51 μg/ml). Concentration of mucin in LPS alone group was 560.22 μg/ml. We demonstrated that addition of NO in LPS-induced OM increased the concentration of mucin in the middle ear effusion. This study suggests that NO has a role in inducing MOM.

837 Chronic Otitis Media with Effusion Sequelae in Children Treated with Tympanostomy Tubes

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Study objective was to determine incidence, prevalence of middle ear sequelae after tympanostomy tube (TT) treatment. The prospective study was conducted in a multi-specialty community clinic and academic medical center. Children with chronic otitis media with effusion (OME) (n= 140)were followed for 8 years after TT treatment. Mean age was 5.5 years, 61% were male, and 56% had >1 TT surgery by 3 years of follow-up. Annual incidence was typically greater between 4 to 6 years of follow-up than between 7 to 8 years; incidence ranged between 0 (perforation, tympanosclerosis, negative peak pressure(TPP), low static admittance(SA)/broad peaked tympanogram, year 8) and 18% (negative TPP, year 5). Monomer/atrophy, retraction pocket, and high SA/narrow peaked tympanogram showed the greatest increases in prevalence between 3 and 8 years: 25% to 55%, 12% to 41% and 23% to 64%, respectively, whereas low SA/broad peaked tympanogram exhibited the largest decrease in prevalence (37% to 8%). Individual sequela tended to be unilateral at year 3 and bilateral at year 8. Concordance of two sequelae in the same ear was not particularly striking (kappas .05-.42). Sequelae incidence decreased between 3 to 8 years of follow-up in this cohort, which parallels the decreasing incidence of OM and TT placement as children mature, demonstrating that sequelae are more likely to develop during the period of active acute and chronic OME. Some sequelae were common, however, occuring in >=50% of ears at 8 years of follow-up. In summary, in this cohort treated with TT for chronic OME, risk of OME developing new sequelae decreased with age. Nevertheless, functional and morphological sequelae were prevalent and may increase the risk of continuing middle ear problems during adolescence and adulthood.

838 Apoptosis And The Expression Of Fas Ligand In The Rat Model Of Acute Otitis Media

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Objectives/Hypothesis: The natural course of acute otitis media (AOM) involves the influx and eventual phagocytosis of inflammatory cells in the middle ear. Upregulation of several apoptosis-related genes, including Fas ligand (FasL), has been reported in a rat model for AOM. The goal of this study was to determine the extent and localization of apoptosis and of FasL expression, in AOM. *Study Design/Method:* 36 healthy rats were inoculated by a ventral neck approach with *S. pneumoniae* or placebo carrier and sacrificed at 12 and 48 hours for immunohistochemical and Western blot analysis. *Results:* Apoptosis by TUNEL staining was demonstrated predominantly among neutrophils and not in mucosal epithelia at 48 hours of infection. Concomitant with the increase in neutrophil apoptosis was the increase in FasL expression shown by Western blot. *Conclusions: S. pneumoniae*-induced AOM in the rat leads to neutrophil apoptosis and FasL expression after 48 hours. Altering the degree of FasL-mediated neutrophil apoptosis may influence the course of AOM.

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839 Eustachian Tube Function In Older Children, Adolescents And Adults With Chronic Otitis Media With Effusion

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By 3 years of age thirty-three percent of children have 3 or more episodes of acute otitis media (Teele etal., 1989). Rosenfeld (1999), in a meta-analysis of otitis media with effusion (OME) studies, found a spontaneous resolution of 60% within 4 years for children presenting with effusions persisting 3 months or longer. During the 1980's, studies began documenting the relationship between OME and poor eustachian tube (ET) function. While organic mechanical obstruction was an occasional observation, functional obstruction was more frequent. Primate models of functional obstruction, via debilitation of the TVP, established the causal relationship between ablation of active ET function and OM as evidenced by tymanometry and MRI. To ascertain the relationship between COME and ET dysfunction we performed the forced response test (FRT) of eustachian tube function on 22 females

and 16 males with COME. Their age distribution was: less than12 years of age-7 females:4 males, 12-17 y.o. 12:11 and greater than 17 y.o. 3:1. The FRT was performed at three flow rates (11, 22, 44 ml/min). Ninety-two percent of the subjects (72 % of ears) had abnormal ET opening pressures, 90% of these were abnormally high. A similar percentage of subjects and ears evidenced abnormally high active resistance. All of the subjects had one or the other of these functional abnormalities and 80% had both.

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840 Expression of Clara Cell Secretory Protein in Experimental Otitis Media of the Rat

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Clara cell secretory protein (CCSP) is an abundant 16-kDa homodimeric protein and secreted by non-ciliated secretory epithelial cells in the lung. It has an important protective role against intrapulmonary inflammatory process. The aim of this study was to investigate the expression of CCSP in endotoxin-induced OME of the rat. We instilled endotoxin and saline as a control into the middle ear cavity of the rat. Middle ear mucosa were taken at 0h, 1h, 3h, 6h, 12h, 1day, 3days, 7days, and 14days, and then the expression of CCSP mRNA and CCSP protein was evaluated using semi-quantitative RT-PCR, Western blotting and immunohistochemistry. RT-PCR revealed that CCSP was first identified at 1h after endotoxin instillation and was dramatically increased between 1h and 1day, with maximal expression at 1day and decreased after 3days. Expression patterns of CCSP protein identified by Western blotting were similar to their expression of mRNA. Expression of CCSP at both mRNA and protein levels were not detected in normal middle ear mucosa and saline-instilled middle ear mucosa. On the immunohistochemistry, granulated cells were stained. These results suggest that CCSP may be up-regulated in OME and have a protective role in middle ear mucosa.

841 Genomic Sequence of an Otitis Media Isolate of Nontypeable *Haemophilus influenzae*

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In 1995, TIGR completed the genome sequence of a rough derivative of *H. influenzae* serotype d, strain KW20. This sequence has been extremely useful in understanding the basic biology of *H. influenzae* but has not provided significant insight into our understanding of disease caused by this group of microorganisms due to the fact that serotype d strains are generally not pathogens. In contrast, nontypeable *H. influenzae* (NTHI) is the primary pathogen of chronic otitis media in children. Thus, we hypothesized that NTHI would contain genes not found in the genome of strain KW20 and that this information would provide insight into the pathogenic mechanisms of *H. influenzae*.

Previously, we used subtractive hybridization to identify genes present in a low-passage, virulent NTHI isolate (86-028NP). Sequences with homology to the HMW adhesin gene clusters as well as gene fragments with homology to DNA methyltransferases, a Tn*3*-like transposase and genes in the recently identified bacteriophage HP2 were identified. However, to more comprehensively evaluate the differences between the genomes of *H. influenzae* KW20 and NTHI strain 86-028NP, a shotgun sequencing approach was used. Strain 86-028NP genomic DNA was sheared to an average size of 2-4 kb and cloned into the high copy number vector pUC18. Genomic sequence at 3-fold coverage was obtained and provided a partial assembly of the genome. This has allowed us to identify essentially all of the genes in this otitis media isolate of NTHI and compare them with those of strain KW20 and other known pathogens. These data have provided important new information that will complement and extend our ongoing analysis of NTHI virulence determinants and the identification of potential novel vaccine candidates.

842 Effects of Pneumococcal Peptidoglycan-Polysaccharides (PG-PS) on the Middle Ear and NL-20 Epithelial Cells

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A hallmark of otitis media is proliferation of the middle ear epithelial cells; a condition that contributes to hypersecretory activity in the middle ear. Recent studies suggest that pneumococcal cell wall components PG-PS play an important role in the proliferation of middle ear epithelial cells, including mucous cells. To test whether PG-PS is responsible for the proliferation of middle ear epithelial cells, we purified PG-PS from pneumococcus 6A and incubated them with NL-20 tracheal epithelial cells or cultured primary middle ear epithelial cells in a dose (0.25, 0.5, and 1.0 μg/mL) and time (8, 24, and 48 hours) dependent manner. Proliferation of the NL-20 cells was determined by thymidine incorporation, cell counts, and cell cycle analysis. Proliferating cell nuclear antigen (PCNA) analysis was done on primary culture of middle ear epithelial cells. The results of thymidine incorporation demonstrated that (1) PG-PS significantly increased thymidine incorporation from 0.25-1.0 μg/mL within 8 hours; (2) PG-PS significantly increased thymidine incorporation at 0.5 and 1.0 μg/mL within 24 hours; and (3) PG-PS significantly reduced thymidine incorporation at 0.5-1.0 μg/mL, but not at 0.25 μg/mL, within 48 hours. Cell counts showed the same results as those of thymidine incorporation and the PCNA data was consistent with those of the cell counts and thymidine incorporation. Cell cycle data demonstrated that the apoptotic cells decreased at 0.25 μg/mL within 8 hours and increased at 0.5 and 1.0 μg/mL within 48 hours. It is tentatively concluded that PG-PS has both proliferative and apoptotic effects on the NL-20 cells; at a low dose (0.25 μg/mL) the proliferative effect on the NL-20 cells is dominant, but at higher doses (0.5-1.0 μg/mL), apoptosis dominates.

843 Expression of Mucins in Human Mucoid Otitis Media

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A hallmark of mucoid otitis media (MOM) is mucus accumulation in the middle ear cavity, a condition that impairs transduction of sounds in the ear and cause hearing loss. The mucin identities of mucus and the underlying mechanism for the production of mucins in MOM are poorly understood. In this study, we demonstrated that the MUC5B and MUC4 were major mucins in MOM that formed distinct tree-like polymers (mucus strands). The MUC5B and MUC4 mRNAs in the middle ear mucosa with MOM were up-regulated 5-fold and 6-fold, compared to the controls, which was accompanied by the extensive proliferation of the MUC5B and MUC4 producing cells in the middle ear epithelium. Further study indicated that the mucin hyperproduction was significantly linked to CD4+ and CD8+ T cells and/or CD68+ monocyte-macrophages. We found that TNF-α, one of the above cell products, was present in the effusion of MOM. Results in the study of rats demonstrated that TNF-α inoculation into the middle ear increased mucin production in a dose-dependent manner. Furthermore, TNF-α inoculation plus Eustachian tube obstruction significantly induced mucus accumulation in the middle ear. We tentatively concluded that TNF-α plays an important role in the pathogenesis of MOM.

844 Synergistic up-regulation of MUC5AC mucin transcription by Nontypeable Haemophilus influenzae and PMA

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Nontypeable Haemophilus influenzae (NTHi) is a major human pathogen in children and adults. In children, it causes otitis media with effusion (OME), the leading cause of conductive hearing loss in the United States. In adults, it causes exacerbation of chronic obstructive pulmonary disease (COPD), the fourth leading cause of patient death in the United States. Mucin overproduction, a hallmark of both diseases, has been shown to directly cause conductive hearing loss in OME and airway obstruction in COPD. The molecular mechanisms underlying mucin overproduction remain largely unknown. Here, we show that MUC5AC mucin gene transcription is synergistically up-regulated by NTHi and phorbol 12-myristate 13-acetate (PMA), a potent activator of protein kinase C (PKC). This synergistic up-regulation is greatly inhibited by both bisindolymaleimide and calphostin C, potent inhibitors for PKC activity. Moreover, SB203580, a specific inhibitor for p38 MAP kinase, also blocks the synergistic up-regulation of MUC5AC induced by NTHi and PMA, suggesting the involvement of p38 MAP kinase. Future studies will focus on understanding the detailed signalling mechanisms underlying the synergistic regulation of MUC5AC. These studies may lead to novel therapeutic intervention for OME and COPD.

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845 Detection of various respiratory virus genomic sequences in middle ear fluids in children with acute otitis media and its relationship to the prognosis

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Acute otitis media (AOM) is among the most common childhood infections.Although the disease is considered a bacterial infection and generally treated with antibiotics, the rate of unresponsiveness to antibiotic therapy, recurrences, and relapses are high. Recently respiratory viruses have been shown to be important etiologic agents in AOM, either alone or in combination with bacteria. The aim of this study is to know whether respiratory viruses may affect the prognosis of AOM . We examine 180 middle ear fluid (MEF) samples from 91 children with AOM for the presence of various respiratory viruses, using a new molecular biologic technique, a multiplex reverse transcription-polymerase chain reaction (RT-PCR) assay. Virus RNA was detected in total of 72 MEF sample (40 %). Respiratory syncytial virus (RSV) is detected in 47 samples, adenovirus is detected in 12, influenza A (H3N2) is detected in 4, and rhinovirus is detected in 3. Dual viral infections of RSV and adenovirus were detected in 6. Bacteria pathogen were detected in 141 MEF samples (78%). Viral RNA was detected in 14 (36%) MEF of 39 bacteria-negative and in 58 (41%) of 141 bacteria-positive MEF samples. No significant differences in the risk of occurrence of recurrent otitis media (ROM) were noted between children with virus-positive and virus-negative MEF samples. However, in the group Streptococcus pneumoniae or Haemophilus influenzae infected, RSV infection had significantly higher risk of developing ROM than those without RSV infection. These findings suggest that co-infection of RSV with Streptococcus pneumoniae or Haemophilus influenzae may cause higher risk of developing ROM in young children with AOM.

846 Synergistic Activation of NF-kB by Nontypeable Haemophilus influenzae and Streptococcus pneumoniae

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Otitis media (OM) represents the most common diagnosis of the pediatric population. Inflammation of the middle ear is the hallmark of OM. The molecular mechanisms underlying the inflammation are largely unknown. It is believed that bacteria play an important role in initiating the inflammatory process. Streptococcus pneumoniae and Nontypeable Haemophilus influenzae (NTHi) represent the most common bacterial pathogens causing otitis media as well as respiratory infections in the setting of chronic obstructive pulmonary diseases (COPD). Our recent study demonstrated that NTHi activates NF-kB, a key transcription factor involved in the inflammatory response, via IkBa-dependent and –independent signaling pathways. Given the clinical fact that up to 36% of patients diagnosed with OM have infections with a combination of S. pneumonia and NTHi, the molecular mechanisms underlying mixed infections, however, remain totally unknown. Here, we show that NTHi alone induces potent activation of NF-kB as expected. S. pneumoniae, however, does not induce a strong response of NF-kB as assessed by luciferase assay and immunoflourescence staining. Surprisingly, both pathogens together synergistically activate NF-kB. Moreover, the synergistic response seen by both pathogens is inhibited by overexpression of a dominant-negative mutant of IKKb, suggesting the important role of IKKb signaling. Future studies will focus on identifying the signaling pathways involved in the synergistic activation of NF-kB. These studies will provide new insights into the molecular pathogenesis of synergistic infections and lead to new therapeutic options for these diseases.

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847 Changing Gene Expression Patterns in Biofilm-Forming *P. aeruginosa*, a Major 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 this chronic infection is *P. aeruginosa (PA)*. The new paradigm that chronic infections are caused by bacterial biofilms prompted us to study the relationship between bacterial pathogenicity, biofilm formation and bacterial communal cooperation. It is essential to determine the gene sets which are 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 global changes in the expressome during biofilm development in vitro and in a chinchilla model system. The genomic DNA of twelve clinical *PA* isolates was used to construct a genomic library, comprised of 250,000 clones. This library has an ~1.4 kb average insert size and better than 3x redundancy for each of the 12 genomes. Our sequencing effort (~2 million nucleotides to date) reveals that a large number of genes (89 clones) are not represented in the genome of the reference strain PAO1, yet they are present in one or more of the clinical strains as demonstrated by PCR. Our data also shows that wild-type phage or plasmid infections appear to be frequent in clinical isolates. To test the changes in gene expression between planktonic- and biofilm-grown *PA* we printed the *PA* library on nylon membranes (about 10,000 clones per membrane). RNAs isolated from these two envirovars were used to probe these

arrays. This comparative hybridization uncovered sets of clones with significantly different levels of expression and we are working on the identification and analysis of these clones, as well as evaluation of their potential involvement in biofilm formation and pathogenicity.

848 Bactericidal activity of antimicrobial peptides from the human middle ear

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We recently demonstrated that innate immune molecules such as lysozyme, lactoferrin, and defensins are produced by tubotympanal epithelial cells and act synergistically against nontypeable H. influenzae (NTHi) in a colony forming assay (Lim et al., 2001, Vaccine 19: S17-25; Lee et al., 2001 ARO midwinter meeting #741). In order to confirm and extend these observations, we examined the role of lysozyme, lactoferrin, b-defensin-1 and b-defensin-2, individually, on the growth of four OM pathogens - NTHi strain 12, M. catarrhalis strain 035E, and S. pneumoniae serotypes 3, and 6B - using the more robust radial assay method. Results of the growth inhibition experiments demonstrated that of the molecules tested, b-defensin-2 was the most potent and could inhibit the growth of all four pathogens. Lysozyme was shown to be partially bacteriostatic/bactericidal against M. catarrhalis strain 035E, and S. pneumoniae serotype 6B, while b-defensin-1 was shown to be active against M. catarrhalis strain 035E alone. Lactoferrin showed no growth inhibition and appeared to enhance the growth of the pathogens. All four molecules showed salt sensitivity and were not as effective in 100 mM NaCl. Ultrastructural analysis showed that incubation of OM pathogens with lysozyme and b-defensin-2 results in damage to the bacterial membranes and the extrusion of cytoplasmic contents. We also analyzed the expression of the innate immune molecules in normal and inflamed middle ear tissue. Real-time PCR analysis of human middle ear mucosa showed that the transcripts for b-defensin-1, b-defensin-2 lysozyme and lactoferrin were expressed in this tissue and that the mRNAs for the latter three were present at higher levels in the inflamed mucosa. The levels of b-defensin -1 mRNA, on the other hand, showed little change. Together, our data suggest that the molecules of innate immunity are expressed in the middle ear and may constitute the first line of defense against pathogens that cause otitis media.

849 Suppression of Sodium Channel, Sodium Pump, And Sodium-Hydrogen Exchanger mRNAs During Pneumococcal Acute Otitis Media in the Rat

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The pathogenesis of acute otitis media, no doubt, involves multiple cellular signaling pathways. Until recently, it was not feasible to conduct genome-wide screening for molecular variations that play key roles in the pathogenesis of otitis media other than focusing on only a few mediators or genes. Microarray technology was used to profile differential gene expression pattern from rat middle ear mucosa at 12 and 48 hours after S. pneumoniae challenge (Swarts et al., ARO 2001). Real-time RT-PCR was performed for independent verification of microarray results. Three ion transport mRNAs were consistently down-regulated with time, including 1) Na,K-ATPase alpha-1subunit (SPATPa1) (4.7-fold decrease at 12h, and 3.7-fold decrease at 48h); 2) sodium-hydrogen exchange protein-isoform 2 subunit (NHE2) (4.2-fold decrease at 12h, and 9.6-fold decrease at 48h); and 3) sodium channel beta 2 subunit (SCHB2) (4.2-fold decrease at 12h, and 6.96-fold decrease at 48h). The SPATPa1 is an integral membrane protein responsible for establishing and maintaining the electrochemical gradients of Na and K ions across the plasma membrane. The NHE2 belongs to a family of transport proteins involved in the intracellular pH regulation and sodium transport across various epithelial tissues. Interestingly, the function of the SCHB2 increases the plasma membrane surface area and its folding into microvilli because of its extracellular NH2-terminal domain containing an immunoglobulin-like fold. The SCHB2 may be not only an important regulator of sodium channel expression but also a crucial factor for the formation of the middle ear mucosal microvilli. Our results provide, at the molecular level, strong evidence that suppression of ion-transport mRNAs is integrally associated with defective ion transport and mucociliary clearance and subsequent middle ear effusion during acute otitis media.

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850 Mucin Gene Expression in Cultured Middle Ear Epithelium

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Otitis media is the most common diagnosis in pediatric patients who visit physicians for illness in the United States. Mucin production in response to otitis media causes significant sequelae including hearing loss and the need for surgical intervention. Because 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 gene expression of cultured middle ear epithelium(MEE) in response to IL-1β stimulation. Primary cultures of chinchilla MEE were established and exposed to 0, 50, 100, and 200 ng/ml concentrations of IL-1β in growth media for 16 hours after exposure to 5 μCi/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-1β exposure. This study demonstrated the ability of IL-1β to up-regulate mucin expression in cultured middle ear epithelial cells. This investigation and future studies may lead to novel and efficacious treatments for otitis media through cytokine modulation.

851 Sites And Numbers Of Epidermoid Formations In The Developing Ear

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At the last meeting of the ARO it was shown that epidermoid formations (EFs) are present only after 15w gestation, that they arise following close apposition of the external ear epidermis to the tympanic membrane and that enlargement of EFs takes place with growth of the

ear. Although the general impression was confirmed that the EF was usually present in the anterosuperior region of the tympanic membrane, there was no detailed knowledge of the frequency of its appearance at other sites or of the actual quantity of epidermoid tissue that may reach the middle ear during normal development. To address these problems the location of EFs in relation to the tympanic membrane was mapped in serial histological sections of 22 temporal bones from subjects aged from 16 gestational weeks to 8 months post partum.To do this in each temporal bone the position of each EF was inserted into a scaled diagrammatic reconstruction of the lateral tympanic membrane. 116 EFs, confirmed to be of epidermoid tissue by immunohistohemical analysis, were found. Their numbers in any one bone varied from 1 to 23, the latter number being found in a fetus aged 17 gestational weeks. The numbers of EFs decreased significantly with increasing age. 82 EFs (70.6%) were found in the anterosuperior lateral wall of the middle ears, 5 (19.8%) anteroinferiorly, 1 (0.9%) on the malleus, 8 (6.9%) posteriosuperiorly and 2 (1.7%) posteroinferiorly. Most EFs were located around the edge of tympanic membrane. The unexpectedly large total amount of epidermoid tissue that we have shown may be present in the middle ear during development further confirms the likelihood of origin of congenital cholesteatoma from the EF, and the wide variety of sites of the latter explains the different primary locations of congenital cholesteatoma that have recently been described

852 Vitamin D Stimulates Osteoclast Activation And Recruitment In The RANKL-Osteoclast Culture.

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Vitamin d is known to be a potent stimulator of bone resorption both in vivo and in vitro. The ability of vitamin d to resorb bone has been attributed to its indirect action on osteoclasts through osteoblasts. This theory was based on previous studies from the 1980¡¯s, which failed to demonstrate the presence of a vitamin d receptor (VDR) in osteoclasts. Flaws in these studies include: 1) the use of chick osteoclasts which are unlike mammalian osteoclasts (e.g. they do not contain calcitonin receptors), and 2) the use of low sensitivity techniques. (e.g. immunohistochemistry to detect VDR.). Recent studies using in situ hybridization and RT-PCR have demonstrated the presence of VDR in osteoclasts. In addition, monocytes (precursors to osteoclasts) have been shown to produce the active form of vitamin d. These observations strongly suggest a direct role for vitamin d in the recruitment and activation of osteoclasts. To further elucidate the role of vitamin d on osteoclast recruitment the RANKL osteoclast development and resorption assay was used. Briefly osteoclasts were grown on dentine and plastic in the presence of the active vitamin d metabolite (1,25D) and/or its precursor (25D). Osteoclasts were exposed to concentrations ranging from 10-11 to 10-9 After 9 days their effects on osteoclast number and pit resorptive area were measured. The wells were then stained for tartrate-resistant acid phosphatase and counterstained with toludine blue. Cell number and resorptive area were quantified. Both 25D and 1,25D increased osteoclast number relative to control by 110% and 90% respectively. Osteoclast resorptive area also appeared to be increased. This study shows a direct role for vitamin d in both osteoclast activation and recruitment.

853 Inhibition of the JNK/c-Jun Pathway Arrests Oxidative Stress Induced Apoptosis of Rat Auditory Neurons in Vitro

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Oxidative stress induces apoptosis of P4 rat auditory neurons. An apoptosis cascade is activated in response to an array of insults which include cisplatin (CDDP), 4-hydroxynonenol (HNE), ischemia, hypoxia, and neurotrophin withdrawal. Our studies with P4 rat auditory neurons identify the c-Jun cascade as a major contributor to programmed cell death. In our system, oxidative stress activates upstream mediators that trigger MEKK1 to phosphorylate JNKK1. Once activated, JNKK1 phosphorylates JNK facilitating its entry into the nucleus. Nuclear JNK can now phosphorylate c-Jun which dimerizes into an AP1 complex. Our group has determined that Apoptosis Specific Protein (ASP), a downstream product of the c-Jun pathway, serves as a reliable marker of cell death. We have been successful in arresting apoptosis by inhibiting key upstream regulators in the JNK/c-Jun pathway, as well as c-Jun itself. Curcumin effectively inhibits c-Jun expression at the same level or upstream of MAPKKK. PD09850, a specific MEK1 inhibitor, successfully inhibits the c-Jun apoptotic pathway in our system. We have shown that these inhibitors decrease Jun expression and arrest apoptosis. Our results confirm both a decrease in ASP expression and an increase in neuronal survival. To test the importance of c-Jun expression in our cell death activating pathway, we utilize c-Jun antisense oligonucleotide (AS) to interrupt the translation of c-Jun mRNA. We demonstrate that c-Jun AS treatment of P4 rat auditory neurons exposed to CDDP for 48 hours are spared from apoptosis. In contrast, c-Jun scrambled AS treated neurons do not survive exposure to CDDP toxicity. Better understanding of these pathways will assist in devising otoprotective therapies aimed at various steps along the apoptotic cascade to prevent auditory sensory cell loss due to oxidative stress induced apoptosis.

854 Intracochlear Administration of Thiourea Protects Against Cisplatin Induced Ototoxicity in the Guinea Pig.

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Reduction of cisplatin ototoxicity is of significant clinical value. Systemic administration of a protective agent may reduce the anti-tumour effect and protection by local administration has therefore been explored with some success. Clinically local protection may be more difficult, as the human inner ear is more shielded from the middle ear. A small, neutral and highly potent molecules, such as Thiourea (TU) with a molecular weight of 76, can reach inner ear targets when administered in the middle ear. As a first step, we assessed the protective effect of this agent when administered intracochlearly.

Pigmented guinea pigs, with normal ABR response, were implanted with Alzet miniosmotic pumps, delivering 0.5 iL/hr of TU, 27mg/ml (Tu, n=6) or artificial perilymh (AP, n=8) directly to the scala tympani of the left ear. Three days after pump placement, a second ABR was performed, to ensure normal thresholds following surgery. Cisplatin (8 mg/kg) was then administered intravenously. Subcutaneous saline was given to protect from renal toxicity and chloramfenicol as wound infection prophylaxis. Four days after cisplatin administration the cochleae were fixed and processed for phalloidin surface preparations.

The overall loss of outer hair cells (OHC) was significantly greater in the AP group and the right untreated ears of the Tu group than the left Tu ears. There was no significant difference in OHC loss between ears in the AP group or between these ears and the right ears of the Tu group. Thus TU offers protection from cisplatin induced ototoxicity. Further studies, using middle ear application, are warranted as TU appears to possess unique properties and high reactivity to cisplatin.

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855 Protective Role of L-carnitine on the Ototoxic Effects of Cisplatin in Newborn Guinea Pigs.

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Among children, premature neonates have the highest prevalence rate of sensory-neural hearing loss, which is strongly associated with perinatal exposure to ototoxic agents. The primary site of action of several ototoxic drugs has been linked to the mitochondria. L-carnitine (LCAR), an endogenously produced antioxidant and fatty acid carrier, is required for normal mitochondrial function. Carnitine deficiency is frequently observed during the perinatal period, however, the potential otoprotective effect of LCAR in newborns has not been investigated. We used auditory brainstem response (ABR) and confocal microscopy to evaluate whether LCAR supplementation during pregnancy exerts a protective effect against the potent ototoxic effect of cisplatin in newborn guinea pigs. Pregnant guinea pigs (n=12) were divided in three groups. Animals from group 1, received a supplementary dose of LCAR (100 mg/kg/day) in their drinking water during the second half of the pregnancy and the immediate postnatal period. Newborn guinea pigs from groups 1 and 2 received two intraperitoneal injections of cisplatin (4 mg/kg) at 7 and 8 days of age, respectively. Newborns from group 3 (control) were injected with saline solution. The experiment was evaluated 2 weeks later. ABR showed a significant cisplatin-induced threshold shift in non-supplemented animals respect to controls (46±4 vs. 21±2 dB, P*0.001). In contrast, no differences were observed between controls and those newborns from mothers supplemented with LCAR (28±2 dB, P*0.13). In addition, confocal images showed that outer hair cell damage associated with cisplatin treatment was reduced in LCAR-supplemented animals. Altogether, these results suggest a protective role of LCAR on cisplatin-induced hearing loss and cochlear damage in newborn guinea pigs, and may provide the basis for the development of simple intervention strategies aimed at preventing ototoxic drug-induced sensorineural hearing loss in humans.

856 Resveratrol decreases noise-induced cycloxygenase-2 expression in the cochlea

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Resveratrol is a grape constituent, noted for its antioxidant and anti-inflammatory properties. By decreasing the activity of complex III of mitochondrial oxidative phosphoryration chain, it both supresses the production and scavenges reactive oxygen species (ROS). Our previous studies have demonstrated the efficacy of resveratrol to reduce temporary threshold shifts and decrease cochlear hair cell damage following noise exposure. The current study was designed to identify the potential protective mechanism of resveratrol by measuring cyclooxygenase-2 (Cox-2) expression.

F344 rats (n=10, 2-3 months old) were exposed to 24h of 105 dB SPL of broad band noise. Cochleae were harvested at 0, 4, 8, 16 and 24h and Cox-2 protein expression was measured by immunoblotting. Additional groups of F344 rats were randomly assigned as a control group (no treatment, n=4) or one of two experimental groups (noise group, n=4; resveratrol and noise group, n=4). The subjects in experimental groups were pretreated with 5 mg/kg/day of resveratrol for 3 days by gavage or vehicle of equivalent volume. Animals were then exposed to a 24h of 105 dB SPL of broad band noise.

The results showed the progressive upregulation of Cox-2, beginning at 8 hours and reaching a maximum at 24 hours. Cochleae were harvested and Cox-2 protein expression was examined by immunostaining and western blot analysis. The results showed that Cox-2 protein expression in the noise with vehicle-treated group was significantly increased as compared to the control group. Cox-2 expression in the noise with resveratrol-treated group was significantly inhibited as compared to the noise with vehicle-treated group. In the control group, little or no Cox-2 expression was found. These data indicate Cox-2 levels can be induced dramatically following noise exposure and that this increase can be inhibited by resveratrol. These findings suggest Cox-2 is a potential mediator in the protective action by resveratrol.

857 Inhibition of the small GTPase Rac / cdc42 protects protects hair cells from ototoxicity

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The hair cells (HCs) are the most vulnerable elements in the cochlea and damage to them is the most common cause of sensorineural hearing loss. Understanding the intracellular events that lead to death of HCs is a key to developing protective strategies. During the past several years intracellular events that mediate aspects of damage to HCs have been discovered. We are evaluating signal transduction events involved in

aminoglycoside (AGC) ototoxicity. Mitogen activated protein kinases (MAPKs) are important signal transducing enzymes that are involved in many facets of cellular regulation (Chang et al.,Nature 410:37, 2001). They often connect cell surface receptors to critical regulatory targets within the cell. Recently it has been shown that inhibition of members of the MAPK group, the c-Jun N-terminal kinases (JNKs) can rescue HCs from ototoxicity (Pirvola et al.,The Journal of Neurosciences 20(1):43, 2000).

The small GTPase Rac / cdc42 is an efficient activator of a cascade leading to JNK activation (Minden et al.,Cell 81:1147, 1995). We tested an inhibitor of Rac/cdc 42 for its ability to protect HCs from AGC toxicity.

Corti explants from p5 rat cochleas containing the basal turn were maintained in tissue culture and treated with the inhibitor for 24 hours. They were then treated with the inhibitor plus gentamicin (30uM) for 72 hours. Results were compared to cultures treated with either the inhibitor or gentamacin alone.

We observed significantly less hair cell death in the gentamicin with inhibitor group, compared to the group with gentamicin alone. The inhibitor alone had no effect on HCs. This supports a role for Rac/ cdc42 proteins in ototoxicity signaling, perhaps as upstream activators of a pathway leading to JNK activation.

858 Ebselen has a protective effect on noise-induced hearing loss

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Ebselen(2-phenyl-1, 2-benzisoselenazol-3 (2H)-one), an anti-inflammatory seleno-organic compound, mimics glutathione peroxidase activities and scavenges peroxynitrite. Among the antioxidants that have been tested, ebselen is promising for human use, although its therapeutic efficacy needs to be confirmed by controlled clinical trials. It has been reported that ebselen protects against gentamicin and cisplatin-induced ototoxicity. We have investigated if ebselen can provide the protection of the auditory hair cells against noise-induced hearing loss. Male pigmented guinea pigs (250-350g) were evaluated with the baseline ABR to confirm normal hearing , and then exposed to 115 dB SPL octave band noise, centered at 4 kHz for 5 hours. Ebselen was administered orally by gavage an hour before noise exposure and 24 hours later. The protective effect of ebselen was evaluated by means of ABR and hair cell counting. Ebselen very effectively reduced permanent threshold shifts and outer hair cell loss. Recent studies have suggested, in the inner ear, reactive oxygen species and nitric oxide can

give rise to certain pathological conditions. Peroxynitrite formed in vivo from superoxide and nitric oxide can damage normal tissue. The results in the current study suggest that ebselen can prevent noise-induced hearing loss, presumably by scavenging peroxynitrite. Enhanced production of both nitric oxide and superoxide anion, with consequent formation of peroxynitrite, are therefore considered an important factor responsible for noise-induced injury to the inner ear.

859 Chinese Herbal Medicine Reduces Aminoglycoside-induced Free Radical Formation In Vitro And Ototoxicity In Vivo

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Antioxidant therapy can protect against aminoglycoside-induced ototoxicity in animal models. The requirements for a clinically suitable antioxidant include no effect on the therapeutic efficacy of the aminoglycosides and no side effects. In addition, the treatment should be inexpensive and convenient if it is to be implemented in developing countries where the use of aminoglycosides is most common. Chinese traditional medicine utilizes a wide variety of herbal extracts some of which are free radical scavengers. Salviae miltiorrhizae (Danshen) extracts contain different ketone and alcohol derivatives with antioxidant properties and are used to treat disorders such as ischemic injuries and renal failure.

We combined in-vitro and in-vivo approaches to investigate the effect of commercial Danshen on aminoglycoside-induced free radical generation and ototoxicity. In vitro, Danshen inhibited gentamicin-induced lipid peroxidation (formation of conjugated dienes from arachidonic acid), as well as the gentamicin-catalyzed formation of superoxide (in a chemiluminescence assay with lucigenin) and hydroxyl radicals (oxidation of the indicator NDMA). Danshen (6 g/kg body weight bid) was then administered to adult CBA mice receiving concurrent treatment with kanamycin (700 mg/kg body weight bid × 15 days). Threshold shifts induced by kanamycin (approx. 50 dB) were significantly attenuated by injections of the herbal extract.

"Traditional" medications may be source of antidotes against aminoglycoside ototoxicity. Such traditional medicines are still in wide-spread use in many developing countries and could therefore become an easily accepted and inexpensive protective therapy.

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860 Antioxidant N-L-acetylcysteine (NAC) can protect cochlea from impulse noise trauma

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Noise-induced hearing loss is one of the most common causes in our society to hearing disability and at present there is no effective cure. Impulse noise such as firearm and industrial equipment can generate very high level impulse noise, which causes sensorineural hearing loss. It has been shown that antioxidants such as N-L-acetylcysteine (NAC) can protect the inner ear from chemical-induced trauma. The present study investigates whether NAC (i.p) can protect the cochlea from impulse noise trauma. The impulse noise level was 160 dB SPL. Guinea pigs, rats and mice were exposed to 160 dB impulse noise from 50 to 400 times. Animals exposed to impulse noise without treatment of NAC had larger threshold shifts than those of animals injected with NAC from 4 kHz to 40 kHz. We found that NAC could protect the cochlea from impulse noise trauma for both temporary threshold shift and permanent threshold shift in rodent species and guinea pigs. Morphology was also analysed using surface preparation.

861 Low dose methionine with N-acetyl-L-cysteine reduces noise-induced threshold shift in the chinchilla

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It is now believed that excessive stimulation of the inner ear leads to the generation of reactive oxygen species [(ROS); Ohlemiller, 1999], which may damage hair cells. We previously have demonstrated that low dose salicylate (Kopke, 1999) and the combination of N-acetyl-L-cysteine (LNAC)and salicylate (Kopke, 2000), and recently methionine (MET),and acetyl-L-carnitine (Kopke, 2001) were all useful in diminishing the amount of NIHL in chinchillas after loud sound. Here we report that a low dose of the combination of MET/LNAC reduced noise-induced PTS. The ABR thresholds shifts at week three were 2.5, 12.9, 18.8, and 19.6 for low dose MET/LNAC compared to saline injected noise exposed controls which were 12.5, 30, 34.6, and 31 above average pre-exposure baseline at 2, 4, 6, 8 kHz respectively. We investigated if the effects were due to one of the compounds in particular or to a synergism of the two agents. Animals were injected with MET or LNAC (I.P.; 12.5mg/kg each group) 48 hrs. before and after sound exposure to an octave band noise centered at 4 kHz, 105 dB SPL for 6 hrs. The ABR thresholds were determined pre-noise, immediately following noise and 1, 2, 3, weeks post noise. Noise exposed saline injected animals at week three post-noise measured ABR thresholds of 12.5, 30, 34.6, and 31 dB SPL and the LNAC group measured 16.7, 25.8, 40, 38.7 dB SPL above average pre-exposure baseline at 2, 4, 6, 8 kHz respectively. MET treated animals recovered to 5.8, 10.8, 22.9, and 20.8, dB SPL above average pre-exposure baseline at 2, 4, 6, 8 kHz respectively. These results indicate that the attenuation of PTS by the low dose MET/LNAC combination was similar to low dose MET.(Supported by ONR, Naval Medical Center SD, US Army)

862 Post-noise administration of methionine attenuates noise-induced hearing loss in the chinchilla

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We have been investigating strategies for ameliorating noise induced hearing loss (NIHL) due to loud continuous noise exposure. Loud sound can cause increases in reactive oxygen species (ROS)which can damage the inner ear. A recent publication demonstrated nearly a 4-fold increase in ROS 1-2h post-noise exposure when compared to controls (Ohlemiller, 1999). Several investigations from this laboratory (Kopke, 1999, 2000, 2001) have demonstrated that animals exposed to loud sound and pre and post-treated with low dose salicylate and N-L-acetylcysteine (LNAC)/salicylate, acetyl-L-carnitine, methionine (MET), and MET/LNAC all resulted in significant reductions in hair cell loss and PTS. These findings demonstrated the feasibility of the reduction of NIHL using clinically available antioxidant compounds. This study extended these findings in exploring the effectiveness of MET in attenuating PTS. Animals were injected (I.P.; 200mg/kg) with MET 1 hr. after sound exposure to an octave band noise centered at 4 kHz, 105 dB SPL for 6 hrs and again with MET twice a day for a further 48 hrs. post-noise(PN). The ABR thresholds were determined pre-noise, immediately following noise, 11, and 21 days PN. Noise exposed saline injected animals at week three measured ABR thresholds of 15.6, 31.1, 35, and 31.7 dB SPL while MET treated animals recovered to 4.6, 19.1, 30.4, and 22.9, dB SPL above average pre-exposure baseline at 2, 4, 6, 8 kHz respectively ($p < 0.05$ at 2, 4, 8 kHz). Previously we reported that animals treated 48 hrs. before and after with MET recovered to 4, 8, 11, 12 dB SPL above average pre-exposure baseline at 2, 4, 6, 8 kHz respectively. This is an encouraging

result towards our long-term objective of developing agents for oral administration in clinical populations.

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863 Protection against noise trauma by the infusion of acidic fibroblast growth factorProtection against noise trauma by the infusion of acidic fibroblast growth factor

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Recent studies have suggested that acidic fibroblast growth factor (aFGF) and FGF receptor 3 are upregulated in the cochlea after acoustic overstimulation. This study was designed to investigate that aFGF plays a role in the protection of cochlear function in response to the noise trauma.

Hartley guinea pigs were used in the present study. We administered aFGF (aFGF treated group) and saline (control group) to the perilymph of the guinea pig cochlea using osmotic pumps (Alza Co., Palo Alto, CA, USA). After exposure to moderately intense sound (120 dB SPL, 5 h), we assessed auditory brainstem response (ABR) thresholds to evaluate cochlear function. And the cochlear surface structure was observed under a confocal laser scanning microscopy (CLSM).

The ABR threshold showed an increase of approximately 50 dB SPL that recovered after 14 days. Cochlear function in aFGF treated group recovered more quickly than that in control group. Under CLSM essentially normal hair cells were visible in the second and third turns of the cochleae in all groups 14 days after noise exposure.

This study demonstrates that the ABR thresholds in the aFGF-treated ear showed faster recovery than did the thresholds in the saline-treated ears. This evidence indicates that aFGF has a neurotrophic effect in recovery from acoustic trauma.

864 The effects of glucocorticoid on protection against aminoglycoside ototoxicity in the guinea pigs

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Steroid hormones are used for the treatment of various inner ear diseases. 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. Dex was administered using implanted osmotic pumps (ALZET 2ML2) filled with 0.1, 1 and 10ng/ml Dex delivered via a catheter to the left scala tympani. ABR recordings (at 4,8,12,16 and 20 kHz) 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. These results suggest that the Dex can protect the cochlea both auditory physiologically and morphologically against aminoglycoside ototoxicity.

865 Increased Resistance to Noise After Unilateral Ablation of the Superior Cervical Ganglion

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In 1966, sympathetic fibers (SF) were identified in the cochlea, terminating on the cochlear artery and near the auditory nerve fibers at the level of the habenula perforata (Spoendlin and Lichtensteiger, 1966). The cochlear SF originate in the Stellate Ganglion and the Superior Cervical Ganglion (SCG). The SCG fibers have been implicated in modulating noise-induced hearing loss (NIHL) (Borg, 1982; Hildesheimer et al., 1991; Horner et al., 2001). Ablation of the SCG generally leads to protection from NIHL, but the previous studies do not separate the effects of SCG ablation on temporary and permanent threshold shift (TTS and PTS), and there is some question about whether the effect is unilateral or bilateral. In the current study, we explore the effects of unilateral SCG ablation on NIHL resulting from a standard noise exposure. The left SCG was isolated at the level of the bifurcation of the carotid artery and removed in nine animals. All animals had hearing thresholds within the normal range before and after the surgery in both the ipsilateral and contralateral ears as assessed by evoked potentials from chronically implanted electrodes in the inferior colliculi. The animals were exposed to a 4 kHz octave band noise for six hours at 105 dBSPL. Hearing thresholds were measured one day, one week, and fifteen days after the noise. TTS and PTS were calculated. No differences in hearing loss were detected between the ipsilateral and contralateral ears of SCG-ablated animals, but both sets of ears showed 10-20 dB less TTS one week after the exposure, and 10-20 dB less PTS at the frequencies of 2, 4, and 8 kHz than controls that underwent no surgery. The finding of protection in the ear contralateral to the unilateral SCG ablation is consistent with findings by Horner, et al. (2001). The findings also corroborate earlier findings of protection from NIHL by ablation of the SCG.

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866 Effects of retinoic acid administered concurrently with gentamicin on zebrafish ears

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Recent studies have shown that application of exogenous retinoic acid (RA) to aminoglycoside-treated ears can potentially protect hair cells from damage and/or restore lost hair cell function. In this study we examined the effects of exogenous RA administered concurrently with a dose of gentamicin known to produce inner ear damage in adult zebrafish. The fish were treated with 0.75% solution of gentamicin, or a combination of 0.75% gentamicin and 10^{-8}M RA (all trans-retinol palmitate) via aquarium water for a duration of 6 hours. Control fish that received no treatment were handled in a similar fashion. Twenty-four hours after the exposure, the fish were overanesthesized in 0.03% MS222 and perfused transcardially with 0.7% saline followed by 2% paraformaldehyde and 3% glutaraldehyde (in 0.1M phosphate buffer, pH 7.4). The lagenar and saccular epithelia were dissected away from the otic capsules, treated with 1% osmium tetraoxide and embedded in polybed 812. Semi-thin and thin sections cut in a transverse plane were examined via light and transmission electron microscopy. The results showed disintegrating hair cells with swollen afferent terminals in gentamicin-treated fish as compared to normal appearing hair cells and afferent terminals in the control animals. Epithelia of fish treated with gentamicin plus RA showed the presence of several vacuoles in hair cells, blebs on apical surfaces of hair cells and disintegrating afferent nerve terminals. These findings indicate that concurrent administration

of gentamicin and RA can produce a synergistic effect and potentially exacerbate the ototoxic effects of aminoglycosides.

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867 Can ethacrynic acid rescue cochlear hair cells from gentamicin ototoxicity?

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A single co-administration of gentamicin (GM) and ethacrynic acid (EA) causes significantly more hearing loss and hair cell loss than administration of GM alone. EA may potentiate GM ototoxicity by damaging the stria at a time when the concentration of GM in the blood is high. One consequence of strial damage may be a rapid efflux of GM from the strial blood supply into the fluids of the cochlea. This interpretation is consistent with results showing that hearing loss and hair cell loss are greatly reduced when EA is given 12-15 h after a single GM treatment, when levels of GM in the blood are low. When GM is administered over a period of days, GM levels gradually increase in the cochlea. We hypothesized that administration of EA during the time when GM levels in the cochlea are high and GM levels in the blood are low (i.e, 12-15 h after the last GM injection), might actually rescue hair cells from damage by permitting efflux of GM from the cochlear fluids into the blood stream. To test this hypothesis, guinea pigs were injected with GM (125 mg/kg) twice a day for 7 or 10 days. A single injection of EA (40 mg/kg) was given to the experimental group, but not the control group, 15 hours after the last GM injection. In guinea pigs that received GM for 7 days (14 injections), outer hair cell (OHC) loss was 15% in the control group versus 0% in the group treated with EA. In guinea pigs that received GM for 10 days (20 injections), OHC loss was 67% in the control group compared to 52% in the group treated with EA. These results suggest that EA can reduce GM ototoxicity if it is administered at a time when GM levels are high in the cochlea and low in the blood stream. The results may have implications for the clinical management of aminoglycoside ototoxicity in humans.

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868 Inflammatory damage of the round window membrane and cochlear connective tissues caused by Streptococcus pneumoniae

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Purified bacteria inoculated into the middle ear, passed into the round window membrane. Bacterial invasion induced damage of all three layers of the round window membrane: an outer epithelium lining the middle ear, a middle layer of connective tissue, and an inner epithelium bordering the inner ear. The leukocytes and other inflammatory cells are likely to play major role in the injury of the round window membrane epithelium. Interactions of inflammatory cells with epithelium were characterized by their adhesion to the epithelium and by detachment of epithelium cells. This process was accompanied by bacterial penetration into epithelium and connective tissue cells of the round window membrane.

After bacteria passed through the round window membrane, they invaded the scala tympani of the cochlea. The invasion was accompanied by penetration of bacterial cells between bundles of collagen fibrils around myelinated nerve fibres, the vas spirale and spiral ligament facing the scala tympani. Bacteria were extensively associated in microcolonies via thin protrusions over the bacterial polysaccharide capsule. No visible, ultrastructural damage of collagen fibrils in the cochlear connective tissues was observed, however, the extracellular matrix of the basilar membrane containing fibronectin fibrils was severely injured in the presence of polymorphonuclear leukocytes. Polymorphonuclear leukocytes appear to release proteases, which induce proteolytic cleavage of fibronectin fibrils accompanied with detachment of mesothelial cells from the basilar membrane.

869 Expression of heme oxygenase-1 in the guinea pig cochlea under the inflammatory condition

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Heme oxygenase (HO) is the first and rate-limiting enzyme in the catabolism of heme. Three genetic isoforms of HO, designated HO-1, HO-2 and HO-3, have been characterized. HO-1 is inducible by a variety of oxidant stresses and acts against oxidant tissue injury. Endotoxin (lipopolysaccharide, LPS) cause the induction of HO-1 in the rat lung (M.S.Carraway et al., Am.J..Physiol. 275:L583-L592, 1998). We examined whether HO-1 was induced in the guinea pig cochlea after instilling LPS into the tympanic cavity or saline as control. Reverse transcriptase-polymerase chain reaction (RT-PCR) was performed to detect HO-1 mRNA in the LPS treated and saline treated cochlea. Furthermore, the localization of HO-1 was determined immunohistochemically in paraffin sections of the LPS treated cochlea.

HO-1 expression was examined at different times after LPS instillation. A single band was generated in the agarose gel electrophoresis of the RT-PCR product (12h, 24h), which was coincident with the PCR product using spleen cDNA, a positive control, as a template. On the other hand, no band was obtained in the case of other times (0h, 6h, 48h) and saline instillation. Immunohistochemical study revealed that initiated expression of HO-1 in the LPS treated cochlea (24h) was observed in the stria vascularis, HensenÕs cells and the outer hair cells in the organ of Corti.

These results suggest that HO-1 exerts its function in the cochlea under the inflammatory condition and has a possible role for the protection against the cochlear injury.

870 Changes of cochlear blood flow due to endotoxin-induced otitis media

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Acute otitis media can be accompanied by inner ear disturbances which cause sensorineural hearing loss. The purpose of this study is to elucidate the influence of the otitis media on cochlear blood flow. We also investigated effects of local application of prostaglandin (PGE1) on damaged blood flow. Lipopolysaccharide (LPS) inoculation was performed into the right middle ear cavity of rats through the tympanic membrane. Animals were examined 1day, 7 days and 14 days following LPS inoculation. Cochlear blood flow (CBF) was measured through a 1.0mm laser Doppler probe positioned on the basal turn of the cochlea. Ratio of normalized CBF (nCBF) in the inoculated ear to that in untreated ear was computed in each animal. PGE1 was applied to the round window niche of inoculated ear and changes of nCBF were examined. After CBF measurement, temporal bones were removed and prepared for morphological examination. HE staining revealed acute inflammatory reactions in the middle ears of rats sacrificed 1 days after inoculation. The inner ears showed no distinctive pathological changes. At 1 day after inoculation, nCBF in treated ears decreased significantly (0.74 in average), and this decrease was improved gradually at 7 and 14

days. In normal rats, nCBF increased significantly after application of PGE1, however, this effect of the drug was not found in the ears measured 1 day after inoculation. In ears of rats examined at 7 and14 days, significant changes of nCBF after PGE1 application were observed. There was no difference in the thickness of RWM in rats of three groups. Disturbances of cochlear blood flow due to bacterial otitis media may be reversible to some extent. Therapeutic or prophylactic use of drugs provides important aspects concerning damaged cochlear blood flow following otitis media.

871 CTL2 Expression in Human and Guinea Pig Inner Ear Tissue

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The KHRI-3 monoclonal antibody binds to an inner ear supporting cell antigen (IESCA) in guinea pigs (s) and causes hair cell damage and hearing loss. In western blots of GP inner ear extracts KHRI-3 binds to a protein doublet of 68 and 72 kDa. Sera from patients suspected to have autoimmune hearing loss contain antibodies that bind to the 72 kDa band precipitated from GP inner ear by KHRI-3. IESCA was affinity purified on a KHRI-3 antibody column and subjected to MS/MS sequencing. Ten amino acid sequences identical to sequences in CTL2, a member of the choline-like transporter family, were found. To verify the expression of CTL2 in the inner ear, four primer sets spanning the length of the CTL2 protein were created within the identified peptides. RNA extracted from human and guinea pig inner ear tissue, converted to cDNA with reverse transcriptase (RT) and amplified by PCR. All four primers created products from human inner ear, all were sequenced and this verified that they are identical to the CTL2 gene. Two of the four primer sets also produced products with identity to CTL2. Alternative base use in guinea pig codons is suspected to be the cause of poor amplification from the other primer sets. The RT-PCR results demonstrate that CTL2 is expressed in the inner ear. We also screened human cell lines and found strong expression of CTL2 in UM-SCC-11A, a squamous cell carcinoma cell line raised in our laboratory. PCR products identical to CTL2 were obtained with all four primer sets from UM-SCC-11A cDNA. Thus CTL2 is expressed in human and guinea pig inner ear tissue as well as in a tumor cell line. We hypothesize that CTL2 provides choline for membrane biosynthesis in the inner ear and in the tumor cells.

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872 Role of nitric oxide in focal microcirculation disorder of guinea pig cochlea

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This study was designed to evaluate the role of endogenous nitric oxide (NO) in focal microcirculation disorder of the guinea pig cochlea. Focal microcirculation disorder was induced by a photochemical reaction at the lateral wall of the second cochlear turn. Saline or NG-nitro-L-arginine methyl ester (L-NAME) was administered before the onset of photochemical reaction. Cochlear blood flow (CBF) was measured at the focal lesion (ischemic core), 1 mm from the lesion in the apical and basal direction (ischemic border zone) by using a novel non-contact laser blood flowmeter. NO synthase activities were measured by radioenzymeassay. In the saline pretreatment group, CBF was significantly decreased to 58.8±4.4% of the baseline at ischemic core 30 min after the onset of photochemical reaction ($P<0.01$), while CBF showed no significant change at the ischemic border zone . In L-NAME pretreatment group, CBF was significantly decreased not only at the focal lesion (48.3±6.5%, $P<0.01$), but also at the ischemic border zone (apical, 49.3±2.3%, $P<0.05$; basal, 58.7±7.1%, $P<0.05$, respectively). NO synthase III activity of cochlea was increased significantly ($P<0.01$) 15 min after microcirculation disorder. These findings suggest that formation of endogenous NO plays a key role in the maintenance of CBF in acute focal cochlear microcirculation disorder.

873 Hypercapnic hypoxia selectively inhibits endothelium-dependent hyperpolarization and vasodilation by acetylcholine in guinea pig in vitro spiral modiolar artery

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Ischemia/reperfusion is a common clinical condition. It may also involve in noise-induced hearing losses. The vascular pathophysiology of ischemia/reperfusion appears complex and could be site-heterogeneous. In the smooth muscle cell (SMC), KATP & KCa channels are activated and L-type of Ca+-channel inhibited in cerebral vasorelaxation to hypoxia and acidosis but different findings are also reported. Using in vitro spiral modiolar artery (SMA) and intracellular recording methods, we previously demonstrated that the cells in the SMA are bi-stable at low (~-40 mV) and high (~-75mV) resting potentials (RP) and, in low RP state, acetylcholine (ACh) produces hyperpolarization via activation of KCa in the endothelial cells (ECs), high K+-induces hyperpolarization via activation of Kir in SMCs, and nitric oxide (NO) induces hyperpolarization by activation of KATP in both SMCs and ECs. Here we explored the membrane potential and vasomotion effects in the SMA of an acidic hypoxic Krebs solution (pO2 0 mmHg, pCO2 150 mmHg, pH 6.5). We found that the 1 h acidic hypoxic treatment caused: 1) vasodilation and hyperpolarization or a low-to-high RP shift of the resting potential (RP) in the majority of the low RP cells in the second half hour of treatment, resulting in a significant increase in the high/low RP cells ratio, 2) an inhibition on NO-induced dilation (40-100%) and hyperpolarization whereas an enhancement on L-NAME-induced depolarization in low RP cells, 3) an inhibition on high K+-induced vasodilation but without effect on 10 mM K+-induced hyperpolarization, 4) a partially reversible inhibition on ACh-induced dilation (20-80%) and hyperpolarization (20-100%). We conclude that the hypercapnic hypoxia induces a vasodilation that is at least partially related to an enhanced release of NO; and the treatment induces damage to the endothelium-dependent KCa-mediated vasodilation.

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874 Bilateral Cochlear Potentials In Chinchillas Following Unilateral Superior Cervical Ganglionectomy

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The sympathetic innervation of the ear is composed of two branches, perivascular and non-perivascular. Whereas the perivascular fibers are shown to modulate the cochlear blood flow, the role of the non-perivascular fibers is unknown. The present study aimed to examine whether the perivascular branch of the sympathetic supply is necessary for the maintenance of normal electrocochleography.

Surgical ablation of the left superior cervical ganglion was performed in 5 adult chinchillas under deep anesthesia. During the surgery, the entire superior cervical ganglion was visualized, sectioned, and examined under microscope. Post-operative signs of Horner's syndrome in the animals also confirmed successful ablation. Chronic round window electrodes (silver wire, 0.05 mm in diameter) were then implanted to measure cochlear potentials. The response amplitudes of cochlear microphonics and summating potentials showed a trend of enhancement in the left ears (ipsilateral to the superior cervical ganglion ablation). The differences between the right control ear and the left experimental

ear in the same animals, however, were not statistically significant. Both peaks (N1 and N2) of the cochlear compound action potentials were analyzed. The response thresholds were similar for the control and the experimental ears. Similarly, there were no substantial differences in either response amplitudes or latencies of these two peaks.

Because of the close relationship between the terminal adrenergic plexus and the afferent fibers, it is conceivable that the sympathetic fibers may control the sensitivity of the sensory organ, or modulate the response of the afferent fibers. The current data, however, provide no evidence to support such a role of the sympathetic fibers.

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875 Different vulnerability of outer hair cells (OHCs) against photochemically induced cochlea damage.

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We made two spots of ischemia at the stria vascularis (SV) of second turn of guinea pig cochlea by using a photochemical reaction. After 3 days, 7 days and 14 days, we observed morphological change using Scanning Electron Microscope (SEM). Although the strial cells between two ischemic lesion remained intact, the OHCs facing those intact strial cells revealed damaged. Moreover, 1st and 2nd rows of OHCs were much more damaged than 3rd row of OHCs. OHCs of 1st and 2nd rows seems more vulnerable against free radical induced ischemic damage than those of 3rd row.

876 Apparent Recovery of Vestibulo-Ocular Function After Intratympanic Gentamicin Treatment for Ménière's Disease

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We have found that intratympanic gentamicin causes a marked reduction in the human angular vestibulo-ocular reflex (AVOR) gains for head thrusts exciting semicircular canals on the treated side. This reduction in gain typically is not as great as that seen after surgical unilateral vestibular destruction. We sought to determine if these ipsilateral AVOR gains increased over time after gentamicin treatment.

Quantitative magnetic search-coil testing of the horizontal angular vestibulo-ocular reflex (AVOR) during head thrust testing was performed in four subjects prior to, immediately following, and 6-18 months following gentamicin treatment. Each subject had good initial control of vertigo, and horizontal AVOR gains markedly decreased immediately after gentamicin treatment. However, gains increased ($p<0.05$) between early and long-term followup tests, from 0.42±0.20 to 0.65±0.26.

This apparent recovery of AVOR function could arise peripherally from recovery of hair cell function and afferent modulation and/or centrally from amplification of small remaining signals from the treated labyrinth. The preservation of baseline afferent firing may also enable the CNS to better adapt to unilateral vestibular hypofunction.

877 Comparison of vestibular responses in pediatric cochlear implant candidates and age matched controls.

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Hearing impaired children who are candidates for cochlear implantation are at risk for vestibular dysfunction. We used a battery of peripheral and central vestibular tests to prospectively assess the vestibular function of such children prior to cochlear implantation, and compared their performance with that of age matched controls. Children ranging from 2-10 years of age were given a set of age appropriate measures including ENG or VNG (with calorics if tolerated), sinusoidal and velocity step vertical-axis rotary chair, head impulse, post head shake nystagmus, dynamic visual acuity, sensory-organization, motor-control, adaptation and visual-vestibular conflict platform tests, and VEMP. 40% of the cochlear implant candidates displayed vestibular loss with residual function, often unilaterally. This suggests that pediatric vestibular testing could be a useful adjunct to audiometric testing in cochlear implantation.

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878 Tilt perception and memory at unilateral defective vestibular mechanisms

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Space orientation is mainly supported by the vertically oriented semicircular canal systems and otolith organs which are involved in spatial orientation, through multi-sensorial interaction with visual, tactile and proprioceptive cues. Otolith organs are sensitive to linear acceleration during head displacement. Normal vestibular function can set to the postural vertical with extraordinary accuracy in absence of visual cues. This study compares the effect of immediate or prolonged body tilt with varying speed, on the perception of the postural vertical and postural tilt in normal and unilateral vestibular loss, concerning the function of the otolith in the perception of the postural vertical. Subjects applied motorized gimbal padded chair with the occluded eyes and restrained head and thorax. The gimbal executed cycles of 5º tilt to either sides around the vertical at 0.5º and 1º/s in roll plane respectively. 8 settings to the upright were made from 5º tilt with 0.5º/s and 1º/s speeds, alternating right and left tilts, immediate and after three minutes. Statistical analyzing shows significant results at: tilt perception & memory of patients & controls (0.011), tilt perception of patients & controls (0.008), tilt memory of patients & controls (0.017), tilt perception & memory in patients (0.023). Also averaging control data shows undershoot to target point as well as the averaging patient data shows overshoot to target point.

After 8th cranial nerve section, Unilateral vestibular loss causes a vestibular perception of an erroneous tilt that is probably caused by an imbalance of otolith signals and apparently never fully compensates. As we observe in patients the length of sector toward the lesion side enlarged. It seems the compensatory mechanisms in subjective postural vertical at both perception and memory is not completed in roll plane as well as disturbance of the inhibitory process to prevent patients from target points.

879 Postural Sensory Organization Tests in Patients with Central and Peripheral Visual Field Loss

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This study investigates how central or peripheral visual field defects impair postural stability. So far, 3 patients with glaucoma and good central vision (ages 57, 73, 85 years), 1 patient with glaucoma and slightly impaired central vision (78 years) and 2 patients with macular degeneration (86, 87 years) have undergone ophthalmologic, vestibular and postural testing. Each patient had similar visual impairment in each eye. All patients had normal vestibulo-ocular reflex gains and phases in response to yaw-axis rotations. One macular degeneration patient had spontaneous right-beating nystagmus. Composite scores for the postural sensory organization tests (SOT, Equitest system) were normal (80, 80, 82) for patients with glaucoma and good central vision, borderline (70) for the patient with glaucoma and impaired central vision and abnormal (62, 63) for patients with macular degeneration. All patients had normal SOT scores for SOT1, 2, 3 (fixed support surface) and for SOT4 (sway-referenced support surface, fixed visual background). SOT5 (sway-referenced support surface, eyes closed) and SOT6 (sway-referenced support surface, sway-referenced visual background) were normal in patients with glaucoma and good macular vision. The 2 patients with macular degeneration fell on one of three SOT5 and SOT6 trials. The patient with glaucoma and impaired central vision fell on one SOT5 trial. Five patients were more stable during SOT4 than during SOT5. The sixth patient was the most stable during SOT5 (mean score of 80) indicating that vestibular cues were sufficient for this subject to reach a high level of postural stability.

These preliminary results suggest that vision can be used successfully in patients with glaucoma or macular degeneration during challenging tasks of postural control. Patients with macular visual deficits had abnormal scores during SOT5 and/or SOT6. This may be due to age-related vestibular (otolith?) deficiencies.

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880 Abstract: Optokinetic Testing of Patients with Traumatic Brain Injury Compared to Normal Subjects

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Individuals suffering from traumatic brain injury (TBI) often complain of dizziness or unsteadiness. As the function of the Optokinetic Reflex is to keep a moving image focused on the retina, this reflex may play an important role in these complaints. Results of optokinetic testing will be presented.

Methods: A study was conducted at the Mayo Clinic, Rochester, Minnesota to assess a group of 10 TBI patients compared to 10 normal subjects. The Optokinetic Reflex was tested with the subject in a rotary chair room with the light source rotating at 20, 40, and 60 deg./sec. Two parameters were assessed: (1) measurement of the slow-phase eye velocity (SPEV) of the optokinetic nystagmus generated by the rotating sphere, and (2) subjects were asked to report if they perceived whether the light spots were rotating around them (egocentric motion perception), or if they felt that they were rotating (circularvection). Most normal individuals perceive that initially the light spots seem to be rotating. Then they begin to sense that the spots appear to slow down, stop, and ultimately the subject begins to feel they are rotating in a direction opposite to that of the lights.

Results: Mean (SPEV) for all three speeds and both directions were always slightly higher for controls compared to TBI patients. However, a two-factor ANOVA with repeat measures on two factors showed that group differences were not statistically significant p=0.490. An intriguing finding, however, was that 4/10 TBI subjects never experienced circularvection, whereas, all 10 normal subjects experienced circularvection.

Conclusion: Patients with TBI appear to have normal optokinetic nystagmus. However, 4 of the 10 TBI patients appeared to have had their neural perception of circularvection altered. In other words, they correctly perceived that the light spots were moving, rather than themselves. Further study is required to determine how this affects their overall dizziness.

881 Cortical processing of vestibular sensation and its habituation: a PET study

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We applied hot and cold air irrigation to the external auditory canal of 12 normal subjects, and monitored eye movement by an infrared CCD video system. Changes in regional cerebral blood flow (rCBF) during vestibular stimulation were measured by a PET (positron emission tomography) scanner, and the data were analyzed by SPM (Statistical Parametric Mapping).

Brain regions that exhibited rCBF increase significantly correlated with the slow-phase eye velocity (SPV) of caloric nystagmus were the left middle insular region, the right posterior insular region, the right inferior parietal lobule (Brodmann□fs area (BA) 40), the right precuneus (BA7), the right visual area (BA19) and the right and the left cerebellar hemispheres. Among these regions, peri-insular regions were activated depending on the direction of the nystagmus, while the right inferior parietal lobule was activated irrespective of the direction of the nystagmus

The average SPV during caloric stimulation were; 7.4 degree/sec for the 1st irrigation, 6.3 degree/sec for the 2nd irrigation, 4.5 degree/sec for the 3rd irrigation and 4.3 degree/sec for the 4th irrigation. The SPV decreased along with the repetition of caloric stimulation, and the SPV during the 3rd and the 4th irrigation was significantly lower than that during the first irrigation. We compared rCBF between the 1st and the 4th measurement. The regions that exhibited higher rCBF at the 4th caloric stimulation were the left anterior cingulate gyrus (BA24, 32), the right anterior cingulate gyrus (BA32), the right cingulate gyrus (BA29) and the right parahippocampal gyrus and the bilateral visual cortices (BA17, 18), which might be related to vestibular habituation.

882 Attention Influences Balance in Patients with Vestibular Disorders

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This study investigated the relationship between standing postural control and attention through a dual task paradigm in patients with unilateral vestibular disorders. Eleven patients with unilateral vestibular loss and age-matched healthy controls have been studied to date. Subjects stood on a posture platform while concurrently

performing information processing tasks. Balance was challenged with different postural tasks: a) seated, b) standing on a fixed floor, c) sway referenced floor, and d) continuous sum-of-sines antero-posterior translations (translating floor). The information processing tasks were: 1) No task (control), 2) Simple reaction time (SRT) response to an auditory stimulus, and 3) an auditory forced choice reaction time (FCT) task. The dependent variable was reaction time. The results showed that patients with unilateral vestibular reduction have increased reaction times in SRT and FCT tasks compared to controls. Both populations had greater reaction times during the sum-of-sines translations compared to the other conditions. Patients showed a greater impact of this postural challenge on reaction times in both SRT and FCT. Patients also showed an increase in FCT reaction time during sway referenced platform movements compared to a fixed platform whereas controls did not. The results of this study suggest that attention is utilized in postural control in both control subjects and patients with vestibular loss. However, patients appear to have a greater requirement of attention when posture is sufficiently challenged.

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883 Visual-otolith Interaction in Older Humans

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Previous studies of vestibulo-ocular function in older adults have assessed the semicircular canal-ocular reflex, visual-semicircular canal interaction, and otolith-ocular function. The purpose of this study was to assess visual-otolith interaction in older subjects using off-vertical axis rotation (OVAR). Data are reported from 28 individuals between the ages of 21 and 30 (14 females and 14 males) with a mean age of 23.5 ± 2.9 years and 28 individuals (14 females and 14 males) between the ages of 65 and 75 with a mean age of 68.8 ± 3.0 years. Rotational conditions included sinusoidal earth-vertical axis rotation (EVAR), constant velocity off-vertical axis rotation (OVAR), and sinusoidal OVAR. These conditions stimulated the semicircular canals, the otolith organs, and both the semicircular canals and otolith organs, respectively. Visual conditions included rotation in darkness (VOR), rotation with a lighted visual surround (VVOR), rotation with a head-fixed target (VOR-fix), and moving stripes with the subject stationary (OKN). Eye movements were measured with DC-coupled electro-oculography. Response measures included gain and phase of the sinusoidal VOR, gain of sinusoidal VOR-fix, VVOR, and OKN, and modulation and bias components for VOR, VOR-fix, and VVOR. Results indicated that the modulation component during VVOR was larger in the older subjects. For sinusoidal stimuli, visual-vestibular interaction did not differ between the older and young subjects. These data lend further support to the idea that visual-vestibular interaction for semicircular canal stimulation and visual-vestibular interaction for otolith organ stimulation differ, and that there are some differences between visual-vestibular responses of older as compared to younger subjects.

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884 Vestibular Rehabilitation Outcomes in Patients with Cervicogenic Dizziness

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The purpose of this retrospective case series was to explore the outcomes following vestibular rehabilitation in patients with cervicogenic dizziness. Twenty-one patients with a diagnosis of cervicogenic dizziness were identified (mean age 51.6±11.37 years; 8 females, 10 males). Three patients were excluded because of inadequate follow-up. Duration of symptoms before therapy varied from 1 to 36 months (median 7.5 months). Patients were treated with a custom rehabilitation program for a mean of 4.8 visits over 3.8 months. Patients completed the Dizziness Handicap Inventory (DHI), the Activities-specific Balance Confidence Scale (ABC), falls in the last 4 weeks and rated the severity of dizziness and neck pain on an analog scale of 0-100. The Dynamic Gait Index (DGI), Timed "Up & Go" (TUG), gait speed, and Five Times Sit to Stand (FTSTS) were used as measures of physical function. Data were analyzed using paired t-tests and the Wilcoxin-Sign test. Significant differences were seen pre- vs post-therapy in the DHI, ABC, DGI, number of falls, and symptoms of dizziness and neck pain. Significant differences were not seen pre-vs post- therapy in FTSTS, TUG or gait speed. The average decrease in DHI was 14 points, with 53% of the patients demonstrating a decrease equal to or greater than 18 points. ABC scores increased an average of 23 points. Subjects increased their DGI an average of 3 points. At the initial evaluation 22 % of the patients reported more than one fall during the prior 4 weeks. Following rehabilitation only 11% of the subjects reported more than one fall. Symptoms of dizziness decreased an average of 23 points. Symptoms of neck pain decreased an average of 30 points. Patients with cervicogenic dizziness demonstrate improvement in both physical performance measures and self-perceived abilities following vestibular rehabilitation.

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885 Vibratory Induced Nystagmus

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Vibration-Induced Nystagmus (VIN) is elicited after applying a customary vibration stimulator on different bony points of the skull (vertex and mastoids) and muscular regions of the neck (posterior left and right cervical region). A consistent nystagmus is found in 6% of normals, and 60-90% of subjects with unilateral vestibular pathology when stimulating on mastoids. It has been stated that VIN is equivalent to a unilateral vestibular weakness rather than directional prepondrance. However VIN when stimulating dorsal neck is neither a specific nor a sensitive test to detect a unilateral vestibular loss when compared to caloric testing.

We study 120 subjects with known peripheral pathology in order to characterize the frequency of VIN, its intensity and direction considering the stimulation of the mastoid or dorsal neck of the lesioned or the normal side. In order to define the side of the lesion, clinical data, caloric testing results (canal paresis), and the result after the head-thrust test were collected. Other parameters, the caloric directional preponderance and the head-shaking induced nystagmus, were used to analyze the direction of VIN.

When VIN is found its intensity is higher when the mastoid of the normal side is stimulated, specially when canal paresis is <29% but other signs (clinical, audiometry) allowed to define the side of the lesion. However when, independently of the amount of canal paresis, there is head-shaking nystagmus, VIN is more intense when the stimulus was applied to the side of the lesion. No specific or significant relation was found when stimulating the dorsal neck.

886 Afternystagmus following earth axis horizontal optokinetic stimulation of patients with unilateral vestibular lesions

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Humans with intact vestibular function have a position-dependant, up-down asymmetric response of putative otolith origin after optokinetic stimulation about an earth-horizontal axis. The present study was designed to test the hypothesis that this positional optokinetic afternystagmus (pOKAN) would show additional asymmetries in subjects with unilateral vestibular lesions. Eight subjects, who had undergone surgery for vestibular schwannoma, were studied by using an earth horizontal axis optokinetic stimulation device. Subject mean age was 51 years (range 31-61). The unilateral vestibular lesion was verified by electronystagmography and vertical axis rotation. Subjects were placed in 4 static orientations (nose-up, nose-down, right or left ear down) with respect to gravity. Each test run consisted of 60o/s constant velocity optokinetic stimulus for 60 sec, followed by 60 sec of complete darkness. We processed the slow component eye velocity (SCV) and calculated the slow cumulative eye position (SCEP). Good optokinetic nystagmus was seen in most subjects, but optokinetic their afternystagmus was notably less than in normal subjects and intersubject variability was greater. Preliminary analysis suggests there may be a reduced response in the lesioned-ear-down position, but this will require further analysis, which is ongoing.

887 Vestibular Assessment Profiles in Adolescent Idiopathic Scoliosis

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Scoliosis affects approximately 3-6% of the school age population. Although there are several causes of scoliosis, in over 80% of cases the cause is unknown. The latest theories behind the etiology of adolescent idiopathic scoliosis (AIS) have focused on the pathology of cerebral asymmetry, asymmetrical otolith vestibulo-ocular reflex function and decreased proprioception causing impaired vibratory sensation. Many AIS cases may be secondary to vestibulospinal dysfunction resulting in chronic muscular imbalance and subsequent spinal deformity. In order to predict scoliotic curve progression we studied and compaired Scoliosis Research Society parameters of age, gender, menarche status, neurologic findings, scoliometer value, Risser Sign and Cobb Angle to clinical measures of vestibular-ocular reflex function from vertical axis rotation (VAR). Otolithic function measurements from eccentric vertical axis rotation (EcVAR) were also completed. Routine computerized oculomotor (OM) testing, computerized dynamic posturographyn (CDP) and qualitative clinical tests of vestibular spinal reflex function completed the comprehensive vestibular-balance assessment. Profiles of the comprehensive vestibular assessment and correlation results between the vestibular and orthopaedic measures will be presented from twenty AIS subjects and ten adolescent normal control subjects. Adolescent (ages 11-17) normative data from the rotation chair using ISCAN video eye tracking will also be compared with young adult normative data (ages 18- 30).

888 Comparison of Static and Dynamic Balance

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Static balance assessments are typically performed on patients with dizziness and/or balance dysfunctions. However, it is questionable whether the performance on static tests accurately reflects the dynamic type of balance needed for the performance of activities of daily living. The purpose of this study was to examine the relationship between tests of static and dynamic balance in young and dizzy subjects. Eighteen healthy subjects (4 males, 14 females) with a mean age of 23.4 years and 15 dizzy subjects (2 males, 13 females) with a mean age of 59.3 years underwent tests of static balance including timed tandem stance (TS) and the sensory organization test on the Equitest (SOT), and dynamic balance. evaluated using the Dynamic Gait Index (DGI), the Tinetti Balance and Mobility Scale, total score (TTS), balance score (TBS), and gait score (TGS), Timed Up and Go (TUG) and the Berg Scale (BS). The order of presentation was randomized for each subject. Independent t-tests used to determine the differences in the performance of the two subject groups indicated that there was a statistically significant difference between the groups on all of the static and dynamic tests, with the dizzy group exhibiting poorer balance performance, which is consistent with previous literature. Pearson correlations used to determine the relationship between the two types of balance tests indicated that there was a significant correlation between static TS and the dynamic tests of TBS, TGS, TTS and DGI. Significant correlations were also observed between EQ scores and TGS, TTS, and DGI. There was no correlation between the two types of balance in the younger group, suggesting that in healthy individuals static and dynamic tests measure different aspects of balance. The significant correlations between the static/dynamic tests in the dizzy subjects suggests that in dizzy subjects where balance is likely more impaired either test could be used to measure functional balance abilities.

889 A New Clinical Rotation Test for Identification of Asymmetric Vestibular Function.

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The medical evaluation of patients with balance complaints often includes an assessment of their vestibular function. Standard clinical tests (caloric, conventional rotation, head thrust, autorotation) are sometimes unable to detect an abnormality or, if an abnormality is detected, to provide a detailed assessment of severity and/or side of lesion. Therefore, we were motivated to develop a new type of quantitative test that overcomes the limitations of conventional clinical tests of peripheral vestibular function.

We have begun evaluation of a new rotational stimulus that is designed to isolate and test vestibular semicircular canal function in each ear. The stimulus consists of 2 components. The purpose of one component, the "bias" component, is to generate a large cyclic shift in the discharge rate of afferents innervating one semicircular canal pair using a low frequency (~ 0.1 Hz), high amplitude rotation (up to 250°/s). The purpose of the second component, the "probe" component, is to test the ability of the canals to accurately encode rotational motion during different portions of the bias component period using a high frequency, low amplitude sinusoid (~ 1 Hz, 20°/s). For example, consider a subject with absent right-side vestibular function rotated about a vertical axis with their horizontal canals oriented perpendicular to the axis of rotation. If the bias component velocity is large enough, rotation toward the right will silence neural activity from the left horizontal canal. Since right side function is absent, the vestibular system will not encode the probe component motion during the portion of the bias component cycle when left side activity is silenced, and there will be no

vestibulo-ocular reflex eye movements related to the probe component of the stimulus. Data from normals and unilateral vestibular loss subjects are presented that demonstrate the utility of this method.

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890 Oscillatory Body Sway Following Support Surface Transitions: A Reflection of Adapting Sensory Gain in Postural Control

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Proprioceptive cues make an important contribution to human postural control, particularly during eyes closed stance on a fixed support surface (SS). However, if the SS is altered so that it moves with the subject (i.e. a "sway-referenced" (SR) support surface), proprioception no longer provides reliable information about body orientation in space. In this condition, it is generally believed that subjects maintain balance by increasing reliance on vestibular cues. We developed a simple adaptive gain feedback control model to test this control strategy. We found that the model made the intriguing prediction that a transient body oscillation of about 1 Hz should occur following a transition from a SR to a fixed SS condition. This oscillation is caused by an overproduction of corrective torque due to the sudden return of reliable proprioceptive feedback, causing a transient oscillation until vestibular and proprioceptive contributions are readjusted.

To test this model prediction, experiments were performed on 9 healthy adult subjects. Body sway velocity was recorded while subjects performed 180 second trials consisting of 60 s stance on a fixed SS, 60 s SR, and 60 s fixed. Tests were performed with eyes closed using a backboard assembly that only allowed A/P sway about the ankles. Although subjects showed a wider range of responses than those predicted by our feedback control model, 5 subjects showed strong oscillatory sway (0.82 – 1.09 Hz) after the transition from SR to fixed SS. The oscillation decreased over time, suggesting that subjects adjusted their use of sensory cues to restore stable stance control.

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891 Unilateral otolith function testing using eccentric rotation

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The utricle plays a crucial role in the detection of orientation with respect to gravity and its malfunctioning is believed to provoke e.g. major space motion sickness. The unilateral otolith function test using eccentric rotation is a method to test each utricular system separately. We used a paradigm during which the subject was rotated about an earth vertical axis at a velocity of 400 degrees per second until the semicircular canal response ceased. Then, during the ongoing rotation, the subjects were translated at a speed of 1mm/s along an interaural axis 4 cm to the right and left. When the axis of rotation is positioned through one utricular system, only the contralateral, eccentric utricle is stimulated and is subjected to a centrifugal force of 0.4g, corresponding to a gravito-inertial acceleration (GIA) tilt of 21 degrees. Utricular stimulation induces ocular counterrolling (OCR), that was measured on-line using validated three-dimensional video-oculography during the entire translation phase.

Results were analyzed using a linear relationship between the OCR and the GIA tilt. The function of the right and left utricles was assumed to be additive during the unilateral otolith function test. The slope of the linear regression is a measure of the degree of responsiveness of both utricles and thus equivalent to a gain, whereas the intercept is a measure of lateralization of the utricular response.

Our preliminary experimental data in 16 normal subjects indicate an average gain of 0.15 which is in accordance with Clarke et al. 1996. Data from acoustic neuroma patients indicate a decreased slope and a non-zero intercept, as predicted by the model.

Unilateral otolith function testing using eccentric rotation can be used to investigate the effect of pathology, compensation and medications on utricular function. Such testing may also be useful in the assessment of pharmacological countermeasures for space motion sickness

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892 A New Look On The Subjective Visual Vertical (SVV): The Initial Orientation Of The Light Bar Affects The Outcome

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Introduction

The subjective visual vertical (SVV) test is a method to evaluate the perception of the head position relative to gravity. Moreover, the SVV is a sensitive measure of otolith and especially utricular function. The essence of the test consists of the alignment of a dimly illuminated bar to the gravitational vertical in a totally darkened room.

Study set-up

In our experiment, the SVV was measured in 16 healthy subjects with the head upright, as in the standard procedure, but also with the head laterally flexed to the right and to the left. The initial orientation of the laser beam was alternately set clockwise and counterclockwise with respect to the earth vertical.

Parallel and anti-parallel paradigms

This study shows a so far uncovered phenomenon with respect to the initial orientation of the light bar. Apparently, this orientation affects the outcome of the subjective visual vertical test when the head is laterally flexed. Two paradigms are distinguished according to the initial orientation of the laser beam. First, in the so called "parallel" paradigm, the initial orientation of the laser line is relatively parallel to the length axis of the tilted head. Second, in the "anti-parallel" paradigm, the initial orientation of the line is relatively perpendicular to the length axis of the tilted head.

Results & conclusions

We found that subjects adjust the laser beam much closer to the real vertical in the parallel paradigm (average SVV = 1.3 ± 0.5°) than in the anti-parallel paradigm (average SVV = 5.3 ± 0.5°). The difference of 4° is significant ($p<0.001$). In both conditions the head position is kept constant with respect to the earth.

We suggest that the main explanation may lie in the antagonistic interaction between visual and utricular information.

893 Galvanic Vestibular Stimulation Reveals Sensory Reweighting in Human Postural Control.

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A subject standing with eyes closed on a tilting support surface (SS) orients to that surface if the tilt amplitude is small, suggesting that proprioceptive cues are the primary source of orientation information in this situation. However, with large tilt amplitudes, subjects are less influenced by the tilt, and tend to orient to earth vertical. This suggests that a reweighting of sensory cues occurs in this situation, with an increased contribution from vestibular cues. If such a reweighting occurs, we hypothesized that body tilt evoked by galvanic vestibular stimulation (GVS) would increase as SS tilt amplitude increases.

To test this hypothesis, subjects stood eyes closed on a SS that tilted in a medio-lateral direction according to a pseudorandom motion profile. The peak-to-peak SS tilt varied from 1° to 8° in different trials. Pulsed bilateral, bipolar GVS was simultaneously delivered through electrodes placed over the mastoid processes. The GVS and SS stimulus were mathematically uncorrelated, allowing body sway responses to these 2 stimuli to be measured separately. GVS was also presented with the SS sway-referenced – a condition where vestibular cues provide nearly all of the sensory information used for stance control.

A model-based analysis of responses to SS tilt provided one set of vestibular sensory weight estimates, and a second set was obtained from GVS data by normalizing the GVS response during a given pseudorandom SS stimulus by the GVS response obtained from a SS sway-referenced trial. Both sets of vestibular weight estimates increased with increasing SS amplitude, and there was nearly perfect correlation between these two estimates. These results support the idea that the human postural control system can alter its source of sensory orientation information in different conditions.

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894 Postural Stability in Spinocerebellar Ataxia 5, 6, and 8

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The autosomal dominant cerebellar ataxias are a group of adult- or juvenile-onset neurodegenerative diseases characterized by progressive incoordination and postural instability due to degeneration of cerebellar and brainstem neurons. Recent advances have established that there are more than 16 genetically distinct subtypes designated as spinocerebellar ataxia (SCA). The aims of the present work were to study postural stability in the SCAs to a) identify abnormalities that might be useful for characterizing a given SCA subtype and b) provide a greater understanding of the underlying pathophysiology.

Patients representing the genetic subtypes, SCA 5, 6, and 8, were selected for an initial study because clinically they have signs and symptoms of cerebellar dysfunction and show little evidence of other CNS involvement. The patients (9, 12, and 9 with SCA5, 6, and 8, respectively) were classified clinically as having either early/mild or moderate/severe symptoms. They were tested with the Equitest (Neurocom) posturography protocol and a MANOVA and univariate F statistics were performed. The patients classified as severe in all the subtypes had low scores for test conditions 5 and 6, indicating a significant vestibulo-spinal deficit. However, there were some differences across the SCA subtypes for conditions 2 and 4, indicating varying degrees of somatosensory and visual deficits.

By extending the analysis to oculomotor tests and including other SCA subtypes, it might be possible to identify patterns that would be diagnostic for the different genetic subtypes. Furthermore, the results might indicate the selective vulnerability of neurons controlling eye movements and postural stability.

895 Vestibular Evoked Myogenic Potentials Using Tonal Stimuli

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Vestibular evoked myogenic potentials (VEMP) are presumed to originate in the saccule and have been proposed as a clinical test of saccular and/or inferior vestibular nerve function. While most investigators have recorded VEMPs in response to broadband click stimuli, animal studies suggest that the saccular nerve fibers are most sensitive to low frequency stimuli. This study was designed to determine the threshold, amplitude, and latency of the VEMP at each tone burst frequency in order to identify the ideal stimulus to evoke the response in a clinical setting. VEMPs were recorded in ten subjects with normal hearing and no history of vestibular or neurological disease. Subjects were seated upright and asked to turn their heads to one side (away from the stimulus ear) to unilaterally activate the SCM muscle. A two-channel recording of the myogenic response was obtained with noninverting electrodes at the midpoint of the SCM muscle on each side of the neck and the inverting electrodes at the sternoclavicular junctions. Input-output functions were obtained in response to tone burst stimuli delivered via insert earphones. Tone burst frequencies included 250, 500, 750, 1000, 1500, and 2000 Hz. The stimulus level ranged from 95 to 120 dB pSPL. The response amplitudes varied with stimulus frequency, and the largest amplitude VEMP was obtained with 500 Hz tone bursts. Response latency was inversely related to stimulus frequency. VEMP thresholds ranged from 100 to 120dB pSPL with the lowest thresholds at 500 and 750 Hz and the highest thresholds at 2000 Hz. The results of this study are consistent with the neurophysiological findings that acoustically responsive afferent fibers in the mammalian inferior vestibular nerve have broad, V-shaped tuning curves with best frequencies between 500 and 1000 Hz (McCue & Guinan, 1995, 1997).

896 Vestibular Evoked Myogenic Potential (VEMP) Dynamics Are Altered in Meniere's Syndrome.

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Objective: Acoustical stimulation of the saccule gives rise to a vestibulocolic reflex whose output can be measured in the neck as inhibition of activity in the ipsilateral sternocleidomastoid muscle. This vestibular evoked myogenic potential (VEMP) has been promoted as a means of assessing integrity of saccular function. In this study we test the hypothesis that the cochleosaccular hydrops of Meniere's syndrome leads to alterations in saccular motion that change the dynamics of the VEMP.

Methods: VEMP was recorded to ipsilateral clicks and tonebursts (70-100 dB SPL; 13/s) in normal (250-4000 Hz) and unilateral Meniere subjects (250 & 500 Hz). Subjects were seated upright with chin turned over contralateral shoulder. Response presence, threshold, amplitude and latency were evaluated as functions of frequency, level, and ear (normal/nonsuspect or suspect).

Results: VEMP was present across the frequency range tested in all normal/nonsuspect ears (n=22; 9 normal subjects, 13 Meniere subjects). In normal subjects, interaural amplitude symmetry was equivalent to intra-aural test-retest repeatability. Amplitude was greatest for 250 and 500 Hz tonebursts and decreased with increasing frequency. VEMP threshold was lower for low frequency tonebursts. VEMP latency was inversely related to toneburst frequency. VEMPs in the nonsuspect ear of Meniere subjects were indistinguishable from normal subjects' VEMPs. VEMP was absent in 3/13 (23%) Meniere ears. In 4/10 (40%) Meniere ears with intact VEMP, the threshold was greater and/or latency longer than in the contralateral nonsuspect ear.

Conclusions: Meniere's syndrome is associated with loss of detectable VEMP in some cases. Other Meniere ears display alterations in VEMP threshold or latency that may indicate altered motion arising from saccular hydrops. If confirmed by further study, these findings could be the basis for a means of earlier diagnosis of Meniere's syndrome.

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897 Towards explaining distortion products in basilar-membrane motion

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A stimulus consisting of two tones, presented to the ear at a sufficiently high level, can give rise to a host of distortion products (DPs). We measured the response of the basilar membrane (in the basal turn of the guinea pig cochlea) to such stimuli in great detail. We report data taken at 70 dB SPL and higher for the two primary tones. The frequency ratio ranged from 1.01 to 1.25. In all conditions a few to many DPS were observed, they have amplitudes of 10 or more dB below the level of the primaries.

In earlier work we developed a nonlinear cochlear model [JASA 107, 1497-1507 (2000)] to explain the effect of stimulus level of noise stimuli on the basilar-membrane response. In that model outer hair cells (OHCs) are assumed to have a memoryless nonlinear transduction characteristic, and these cells are embedded in a structure of which the best frequency varies with location. To explain our present results the model has been extended to handle waveforms of the relevant signals, input and output of OHCs. Over the length of the model the OHCs receive input signals of different spectral composition. Each OHC can generate distortion products and all generated components are included in the model computations. After a number of iterations, for each component done separately, a stable solution is usually found. It is shown that with the extended - yet preliminary - model the data from any of our experiments can be explained to within errors of the order of 10 dB. An advantage of the model is that its response is not computed in the time domain, and this allows us to get insight in a very direct way because the effect of each contributing factor can be inspected separately. Using this property we will develop the model further.

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898 A New, Realistic, Multi-Mode Theory of the Cochlear Amplifier

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We have discovered a new explanation for the cochlear amplifier while investigating a multi-compartment model of the cochlea. The hypothesis results from the observation that, when realistic parameter values are used, there are two distinct modes of wave propagation, which vary competitively in their relative magnitudes. The transverse mode (TrM), which is excited by pressure difference between scala vestibule and scala tympani, is a relatively fast-traveling wave that moves the organ of Corti as a whole. The organ of Corti mode (OCM) is a slow-traveling mode that is amplified by outer hair cells and causes the organ of Corti to change in cross section. In the model, the TrM dominates in a region basal to the characteristic place along the length of the cochlea; but approaching the characteristic place, there is a transition to OCM dominance. Thus the phase angle of the wave on the basilar membrane (BM) first accumulates slowly and then accumulates rapidly in the amplification region. The OCM soon dies and the TrM again dominates further down the cochlea. By the time this location is reached, the TrM is small, but is still traveling at a relatively fast velocity. This second modal transition is denoted by the unwrapped phase angle of BM motion "switching" from the phase of the OCM to the phase of the TrM. Among several testable measures that may support or disprove the theory is the prediction that at the characteristic frequency the reticular lamina and BM move out of phase at low sound levels and in phase at high sound levels.

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899 A Comprehensive Three-dimensional Computational Model of the Cochlea.

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We use the immersed boundary method to model the mechanics of the mammalian cochlea. We have constructed a realistic, three-dimensional, curved model of the cochlear anatomy. The elastic properties of the basilar membrane are modeled using partial differential equations of shell theory. Similarly the oval and the round windows are modeled as an elastic plate and an elastic membrane, respectively. The viscous fluid of the cochlea is described by the non-linear Navier-Stokes equations. The immersed boundary simulation code, computing the fluid-structure interactions, is numerically compute intensive, and for the full cochlea model requires the use of a supercomputer. Numerical experiments have been carried out on a 32-processor shared memory HP SuperDome installed at Caltech's Center for Advanced Computing Research. In order to study the active mechanism in the cochlea the present model will be extended to include the micro-structural effects of the organ of Corti.

Numerical experiments introducing sinusoidal input at the stapes have been carried out. They indicate that even in the absence of an active mechanism the traveling wave in the basilar membrane possesses an envelope which is sharply localized, though not narrow.

These preliminary experiments demonstrate the potential of large scale immersed boundary computations to model the effects of cochlear geometry and the non-linear aspects of cochlear mechanics.

900 A new micromechanical model for the radial vibrational modes in the mammalian cochlea

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We develop a new finite element micromechanical model for the radial vibrational modes in the mammalian cochlea, where the cross section of the cochlea is divided into coupled 2D fluid and elastic domains. We treat the organ of Corti (OC) as an elastic body whose inhomogeneities are due to discrete cellular elements including inner and outer hair cells (IHCs and OHCs), Deiter's cells and tunnel pillar cells. Thus the cross section has a material property map with each subdomain having the Young's modulus and Poisson's ratio of the corresponding cell structures. The tunnel of Corti is modeled as a fluid domain. The cochlear fluid is viscous and incompressible, with viscous effect confined to oscillatory boundary layers and the thin gap between the reticular lamina (RL) and the lower surface of the tectorial membrane (TM). The hair bundle stiffness of the OHCs is incorporated in the calculation of the tangential force of the gap. The TM is modeled as an isotropic elastic body. A clamped beam represents the basilar membrane (BM). We have computed the interactions between the BM, TM, OC and the cochlear fluid to find the complex-valued wavenumber-frequency relation and vibrational modes. Assuming no axial elastic coupling, 3D flow is solved in 2D domains with interactions with 2D elastic domains. The details of the flow and displacements are calculated. Multiphasic radial modes of RL and TM are found that play a key role for the rotation direction of the stereocilia: the three rows of OHCs seem to be differentially activated by a radial wave traveling on the RL in the direction from IHCs to OHCs. We have also modeled the effect of a detached TM induced by the targeted deletion of alpha-tectorin. We found the TM/BM displacement ratio is much smaller in

this "knockout model" than in the "wild-type" model. We also found that radial waves traveled in both directions on the RL of the "knockout model" .

901 Structurally and Functionally Correct Cochlear Feedback Model

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The model is expressed in terms of an analog electrical transmission line whose elements correspond to mechanically relevant anatomical parts of the cochlea, including in particular the tectorial membrane with its visco-elastic attachment to the spiral limbus and the OHC stereocilia that couple the tectorial membrane to the organ of Corti and, through it, to the basilar membrane. According to prevailing evidence, the cochlear active feedback is assumed to reside in the electromotility of the OHC oscillating parallel to their longitudinal axes. In the model, the OHCs are considered as high impedance sources of the feedback current that is directly proportional to the stereocilia deflection and whose strength depends on the magnitude of cochlear excitation. The feedback effect is described in terms of a set of 3 algebraic equations. The numerical values of the model parameters have been adapted to the conditions in the human cochlea in vivo. They have been derived either from measurements on the human cochlea post mortem, or, by analogy, from measurements on live animal models. The model makes it possible to simulate intensity series of empirical magnitude and phase gain functions at the level of the basilar membrane relative to the stapes oscillation as well as at the level of the shear motion between the reticular lamina and the tectorial membrane. The latter is assumed to be directly proportional to the alternating potentials recorded in the OHCs or Hensen's cells. Furthermore, it makes it possible to simulate intensity series of response characteristics (called "transfer functions") at these locations and to derive from them input/output functions. Finally, effects of the resistance of the tectorial membrane attachment to the limbus are studied. They suggest that the resistance increases with the magnitude of the tectorial membrane oscillation. In all the aspects studied, the model characteristics are in close agreement with the empirical ones.

902 Interpreting Experimental Measurements of Local Mechanical Response and Acoustic Emisions Due to Electrical Stimulation

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Electrical stimulation is used to investigate *in vivo* basilar membrane (BM) velocity response and acoustic emission. We have shown that round window electrical stimulation of the guinea pig cochlea gives rise to a broad frequency electrical evoked otoacoustic emission (EEOAE) response, from 100 Hz to 40kHz. Placing bipolar electrodes very close to the BM near the first turn in the scala vestibuli and scala typmani gives rise to a much narrower frequency range of EEOAE, limited to around 20kHz. A gold-coated glass bead 20 micrometer diameter was placed on the BM in the area of the voltage field. Local BM velocity measurements were made using a laser Doppler velocimeter. The character of the local velocity response can be divided into three frequency regions. The low frequency region (up to just above BF at 18 kHz) has the appearance of a conventional mechanical tuning curve. The second region, from just above 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 _A, RMS) with an apparent resonance at 40-60 kHz. Predictions using a three dimensional fluid model in conjunction with a simple model for outer hair cell (OHC) activity are used to interpret the experimental results. Predictions show that the high frequency limit of the acoustic emission is determined by the spatial extent of the current stimulus (also called the current spread) while the global peaks in the EEOAE spectra are interpreted as constructive interference between forward and backward traveling waves.

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903 A new approach for optical flow analysis of cochlear motions

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The optical flow technique is often used to estimate instantaneous velocity fields that represent the motion in successive video images. The method has been applied to motion analysis in the hemicochlea preparation. While the method appears promising it is clear that improvement in its implementation would be very useful. In its usual implementation the method is mathematically ill-posed: the scalar equation of conservation of intensity contains more than one unknown velocity component. Usually the problem is regularized using optimization techniques. However, the problem can be made well-posed by neglecting out-of plane motions, which are evidently small in the cochlea, and introducing a 2D in-plane incompressibility constraint. While such a regularization has been previously proposed as part of an optimization scheme, our treatment of the regularized system is novel. In our scheme we compute the optical flow field via the direct solution of the new system, which reduces to a single 1st order p.d.e. We employ a geometric transformation algorithm, in which the image coordinate system is transformed into a new curvilinear coordinate system characterized by an initial data curve and base characteristic curves. The initial data curve requires an estimate of the motion of a single edge, e.g. the lower surface of the basilar membrane. The base characteristic curves are determined by filtered spatio- temporal derivatives of the image intensity. A stream function is determined by integration of uncoupled o.d.e.s along different base characteristics. Streamlines are determined as constant values of the stream function. The velocity vectors are tangent to the stream lines. We have validated our method using pairs of images generated from our calculations of the vibrational deformation in a radial section of the organ of Corti in the mammalian cochlea.

904 Effects of viscous and non-linear terms in NS equation on wave propagation

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Two dimensional macro mechanical cochlea model was solved numerically using the marker and cell(MAC) method with the aid of SOR method in calculating the Poisson's equation. The unrolled 2-d model used consists of two rooms filled with viscous fluid and one elastic membrane, divided into 16(along y axis)x286(along x axis) cells. Special attention was paid to two terms in the Navier-Stokes equation, i.e., the viscous term and quadratic term of fluid velocity, usually ignored for the sake of simplicity. The velocity and pressure distributions in the fluid at each cell point were obtained.

Another feature of the present method is the applicability to transient response problem; response of an arbitrary wave form with any time duration can be obtained. Dumping of the traveling wave along the BM was observed at viscosity values higher than 10 poise, which is 100 times larger than that of pure water. It was demonstrated that

dissipation through the viscous effect is not the main part of acoustical energy loss. Effect of nonlinear term on the wave propagation was also examined. Along with the calculation we have studied the wave propagation using a rectangular cochlea model with a dimension, 20x20x70mm. The response of a partition membrane(BM) following one cycle of sinusoidal stimulus was recorded and compared with the calculation.

905 Three Dimensional, in vivo Measurements of the Tectorial Membrane's Vibratory Responses to Sound

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Previous studies of tectorial membrane (TM) motion in response to sound have had mixed results. One study showed the TM to move in simple, almost rectilinear paths which were oriented almost perpendicular to the reticular lamina, while two others have claimed that the TM moves in a more complex manner, with the major axes of its elliptical orbits lying almost parallel to the reticular lamina at certain frequencies. All of these studies have been performed in vitro, and it is not clear which set of observations is more likely to reflect the behavior of the TM in vivo. The aim of the present study is to ascertain how the TM moves in vivo.

Reflective microbeads were deposited on the TM in the apical turn of the guinea-pig cochlea. The guinea-pigs were deeply anesthetized, and were killed humanely at the end of the in vivo measurements to permit comparisons of in vivo and post-mortem data. The sound-evoked vibrations of the beads were recorded from multiple viewing angles using a displacement-sensitive heterodyne laser interferometer. Three dimensional (3D) reconstructions of the TM's motion were then made using a modified version of the technique described by Decraemer et al. (1994). The acoustic stimuli were short tone pips ranging from 50Hz to 1kHz and from 60 to 80 dB SPL.

The transfer functions for the TM resembled those of high-order low-pass filters. In general, the amplitudes of the transfer functions changed significantly with the viewing angle, but the phases did not. The basic patterns of the transfer functions changed very little for several hours post-mortem. The 3D reconstructions showed that: (1) below the transfer function's cutoff frequency of ~600Hz, the TM vibrates around thin elliptical pathways whose major axes are directed more perpendicular to the reticular lamina than parallel to it. (2) above the cutoff frequency, the motion appears more complex (i.e. less rectilinear).

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906 Tectorial Membrane Stiffness at Multiple Longitudinal Locations

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The stiffness of the tectorial membrane as a function of distance from the cochlear base provides clues as to whether and how quickly any resonance of this structure would be expected to change with longitudinal position. Moreover, the stiffness can be used to examine the effectiveness of stimulation of the stereociliary bundles, at least for frequencies below resonance. We report on measurements of stiffness of the gerbil tectorial membrane for multiple distances from the base of the cochlea. These measurements have been made in the hemicochlea tissue preparation (Hu et al., 1995) using a piezoelectric-based stiffness sensor. Radial and transverse stiffness data were obtained with and without attachment of the tectorial membrane to the organ of Corti / stereociliary complex. Using a simple spring-mass model, we examine the stiffness data in conjunction with estimated changes of mass (based on measured changes of cross-sectional area) to explore the existence and magnitude of any gradients of tectorial membrane resonance frequency along the length of the cochlea.

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907 The Hemicochlea as a Tool for Measurement of Mechanics in the Passive Cochlea

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The hemicochlea (Hu et al., 1995) is an *in vitro* tissue preparation that has been used to measure mechanical response properties of the mammalian cochlea. A hemicochlea is prepared by cutting a cochlea in half along a plane containing its modiolar axis. Although it provides a clear cross-sectional view of the tissue structures and allows for relatively easy placement of measurement probes, there are valid potential concerns with the use of this preparation. These concerns arise from some known fundamental differences between a hemicochlea and an *in vivo* cochlea: the hemicochlea is not in a live animal, the hemicochlea has a gross cut through the tissue, and in the hemicochlea all of the fluid spaces are filled with a modified artificial perilymph solution. It is understood that the hemicochlea provides information on no more than the passive and localized behavior of the cochlea. At issue, however, is whether the preparation of a hemicochlea results in further detrimental effects on the tissue response. In order to address these concerns, a series of stiffness data were obtained *in situ* in an anesthetized gerbil preparation and were compared to stiffness data obtained in the hemicochlea preparation. Using stiffness measured at the basilar membrane as an indicator of mechanical integrity, we demonstrate that the response in the hemicochlea is similar to the response of a passive cochlea *in situ*. This result further legitimizes the hemicochlea with respect to the types of measurements for which it was intended to be used: localized motion and material properties at multiple locations in a passive cochlear preparation.

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908 Resolution of a Laser-Feedback-Interferrometry Based Vibrometer

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We report on the resolution of a Laser-Feedback-Interferrometer-based system for measuring the *in vivo* vibration amplitude and phase of structures within the organ of Corti. The system is composed of a He-Ne laser followed by a beam expander and a long-working-length objective. Light returning from the target is made parallel by the infinity-corrected objective. The beam expander reduces the diameter of the returning beam and passes it on to the laser. Out-of focus light is not parallel and so cannot interfere efficiently in the laser cavity. Thus, the system is inherently confocal. The intensity profile of a sweep through a planar target, using a lens with a numerical aperture of 0.42, has a theoretical Full Width at Half Maximum (FWHM) of three microns. Bench-top measurements using a cover-slip target show that aberrations due to the quality and alignment of the optics, as well as those inherent in the objective lens, limit the achievable resolution.

We have used a ~500 micron glass bead as a second target behind the cover slip in order to investigate the effect of a second reflective surface near the first. Coherent addition of light reflected from both targets, vibrating at the same frequency, imposes resolution limits on both amplitude and phase measurements. We found that a target separation on the order of the FWHM significantly affects both measurements.

We have also investigated the effect of placing a small aperture between the objective and the target. This ‘pinhole’ mimics the laboratory

condition that measurements in sensitive cochlea must be made through a small hole (< 500 microns) in the cochlear wall. Varying the size and axial position of the pinhole such that the numerical aperture of the system is significantly reduced decreases both resolution and signal intensity.

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909 A Two-Mode Model of Motion of the Alligator Lizard Basilar Papilla

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Most cochlear mechanical models assume the cochlea has multiple modes of motion, but the number and nature of modes has not been determined. We present such a characterization for the alligator lizard basilar papilla. Early measurements of basilar papilla motion revealed a simple rocking motion (Frishkopf and DeRosier, 1983; Holton and Hudspeth, 1983), which has been modeled as a single mode (Weiss and Leong, 1985; Freeman and Weiss, 1990). We have recently shown that the basilar papilla has a resonant peak near 5 kHz (Aranyosi and Freeman, 2000), and that each location moves elliptically. These results cannot be explained by single-mode models, but a functional interpretation of anatomy leads to a two-mode model. The basilar papilla rests off-center on the basilar membrane, between two regions of different thicknesses. This arrangement allows both a translational and a rotational mode of motion. At each longitudinal position, the magnitude and phase of both modes and the center of rotation of the rotational mode were determined by finding the least-squares fit of the sum of modes to the measured motions. The rotational mode has a center of rotation that runs roughly parallel to the longitudinal axis on the neural side of the papilla. The translational mode is perpendicular to the basilar membrane. At low frequencies, the magnitude and phase of both modal fits are independent of position, and the two modes are in phase. These results imply that the basilar papilla moves as a rigid body at these frequencies. The peak in motion at 5 kHz is reflected in both the translational and rotational modes. Above 5 kHz, both modes show a relative phase lag at the basal end, suggesting that the basilar papilla is both twisting and bending. Since both modes drive deflection of hair bundles, we conclude that multi-modal motions are significant to the function of the alligator lizard cochlea.

910 Brainstem Stimulation Causes Long-lasting Enhancement of Cochlear Neural Activity.

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All reported peripheral effects of the olivocochlear (OC) efferent system appear to be mediated by the myelinated fiber component, the medial (M)OC fibers projecting to outer hair cells. In the absence of masking noise, MOC activation reduces cochlear sensitivity to acoustic stimulation and firing rates in the auditory nerve. The peripheral effects of activating the unmyelinated OC component, the lateral L(OC) efferents, are unknown.

The present study attempts to activate the unmyelinated lateral L(OC) efferents, (1) indirectly, by stimulating inferior colliculus (IC) neurons that may project to LOC cells, and (2) directly, by delivering shock trains, designed to activate unmyelinated fibers, to LOC cell bodies in the lateral superior olive (LSO). In anesthetized and curarized guinea pigs, bipolar stimulating electrodes are positioned in the IC, the LSO, or the OC bundle (OCB) at the midline floor of the IVth ventricle, while measuring cochlear compound action potentials (CAPs) and distortion product otoacoustic emissions bilaterally.

Electric shocks to the IC or LSO can cause reversible, long-lasting (minutes) enhancement of the CAP, an effect never seen when stimulating the OCB. CAP enhancements from IC shocks are seen only in the contralateral cochlea, and are only elicited from some IC stimulation sites; other IC sites elicited only fast inhibitory effects similar to those seen when shocking the OCB. CAP enhancements from LSO shocks are restricted to the ipsilateral cochlea; only suppression is seen contralaterally. These preliminary results are consistent with the hypothesis that LOC efferents enhance type-I afferent firing, and can be activated with electrical shocks.

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911 High frequency vibrational modes in the organ of Corti

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The radial vibration pattern of the guinea pig organ of Corti (OoC) was measured during AC electrical stimulation. The preparation consisted of one half turn of the modiolar bone, the attached basilar membrane (BM) and the overlaying OoC. The experimental chamber was filled with Hank's balanced salt solution. The preparation was placed on a support in a way that the BM was supported from below. This results in a strong attenuation of BM motion. The tectorial membrane (TM) was removed. This arrangement allows measurement of the relative motion of the OoC during electrical stimulation of the outer hair cells (OHC), without influence of BM or TM. Electrical stimulation was provided via platinum electrodes above and below the sample. The frequency range of the stimulation was 480 Hz to 70 kHz. Velocity of the reticular lamina (RL) was measured by a laser heterodyne interferometer. No beads or the like were needed to enhance reflectivity of the sample. Radial locations were between inner hair cells (IHC) and Hensen cells (HC). Longitudinal locations were between 3 and 13 mm from the base of the cochlea.

Displacement amplitudes were constant up to 10 kHz, after which they dropped with -14.4 ± 5.6 dB/oct on the OHCs and -17.3 ± 3.4 dB/oct on the IHCs. Phase shifts between 480 Hz and 70 kHz were -210° ± 96° on OHCs and -180° ± 41° on IHCs. Moving along the radial direction (at one frequency) phase reversals appeared between inner pillar cells (iPC) and outer pillar cells (oPC) and between 3rd row OHCs and HCs. Measured frequency responses were quantitatively evaluated by fitting model equations to both amplitude and phase simultaneously. The results from this investigation show that local OoC micromechanics is not as simple as it is commonly assumed in mathematical models.

912 Pattern of Basilar Membrane Vibration in Sensitive Gerbil Cochlea

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The cochlear traveling wave has been considered the fundamental mechanism for analysis of sounds in the cochlea since von Békésy discovered it in human cadavers. Because of technical challenges, the cochlear traveling wave in the sensitive living cochlea has not been measured directly. In this study, the magnitude and phase of the velocity response of the basilar membrane (BM) to tones were measured as a function of the longitudinal location in the gerbil cochlea with a scanning laser interferometer. The entire waveform of the traveling wave was calculated from the velocity magnitude- and phase-longitudinal location data. It was found that a low-level sound of 16 kHz induces a traveling wave over a very restricted range (~500 μm) along the basilar membrane. Vibration amplitude falls to the noise floor at the apical and basal ends of the observed area (~800 μm), and an approximately 6π phase delay accumulates over this region. The data indicate that sound propagation in the living cochlea differs from the

widely assumed von Békésy's traveling wave. Sound from the stapes footplate does not initiate a traveling wave at the base of the cochlear partition, which gradually propagates along the BM to its characteristic frequency (CF) location. Instead, it reaches its resonant location instantaneously through a cochlear fluid wave, which then starts a traveling wave locally.

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913 The "Traveling Wave is to be Taken Simply as a Temporal Pattern of Motion": the Effect of Sound

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The quote in the title is from the text of Wever, Lawrence and Békésy (1954. Proc. Nat. Acad. of Sci. 40:508-512. p512). Wever and Lawrence preferred the fluid hypothesis with the energy for the membrane vibration coming from the fluid. I have enhanced their hypothesis by proposing that basilar membrane (BM) vibrations are brought about by the energy in the cochlear sound waves. The oval window and stapes complex may be compared to a speaker that projects sound into the perilymph of the cochlea. Results have shown that: 1) sound pressure waves were recorded throughout the cochlea (Dancer, 1994. Audiology 33:131-142) and 2) that there was less than a 100 ? sec latency before the apical BM vibrations began (Cooper and Rhode, 1996. Aud. Neurosci. 2:289-299). With the fluid hypothesis, it is necessary to explain the basis of the increasing time-delays in apical cochlear activity that has been part of the basis of the classical slow-moving wave of BM vibration. These time-delays may be the result of the restricted speed of the sound wave, the increasing wavelengths with decreasing frequency of the stimulus and the response of the BM to the vibrational energy. Sounds with lower frequencies have longer wavelengths and longer rise-times. Apical regions where the BM is tuned to the low frequencies have responses with longer rise-times and longer delay-times. When summated with the slowed speed of the sound waves, these longer rise-times tend to explain much of the recorded delays. However, the actual delays are longer than can be accounted for by the physical stimulus. Additional delays may be contributed by the time needed for the BM to begin vibrating at its tuned frequency. The round window and secondary cochlear openings influence the movement of sound energy. Sound vector components are probably at times the adequate stimulus for hair cells in the cochlea.

914 Effects of f2/f1 Ratio and L1-L2 Difference on 2f1-f2 DPOAE Amplitude

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Objective: Stimulus parameters play a significant role in DPOAE production. In this study, 2f1-f2 amplitude input/output functions were measured for f2=1001 Hz in human ear with various f2/f1 ratios and L1-L2 differences to observe their effects on 2f1-f2 amplitudes. **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 of 2f1-f2 DPOAE was plotted as a function of f2 intensity and f2/f1 ratio or L1-L2 difference. **Results:** Increasing intensities of primary tones resulted in systematic increase of 2f1-f2 DPOAE level. Varying f2/f1 ratio and L1-L2 difference caused complex changes in 2f1-f2 amplitude input/output functions. When f2/f1 was set at 1.19 and L1-L2 at 3 dB, patterns of 2f1-f2 input/output functions were less variable than other stimulus parameter combinations. **Conclusion:** These results re-emphasize the complexity of relations between DPOAE amplitudes and stimulus parameters. The complex patterns of DPOAE amplitude changes in response to parameter variation may reflect non-linear interactions between energies of primary tones along the basilar membrane.

915 Tension-Dependence of the Active and Passive Modes of Energy Generated in the Outer Hair Cell Wall

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Analysis of different modes of energy generated in the outer hair cell (OHC) wall is important for understanding the cell's physiology. We discuss the active and passive strain and energy generated in the OHC wall. We consider a model problem where the cell is under the action of an electric field and an axial force. The active energy is the energy produced by the resultants on the active strain. The passive energy is the energy produced by the resultants on the passive strain. At the subcellular level, the active and passive characteristics are associated, respectively, with the molecular motors in the plasma membrane and the passive rest of the cell wall. To describe the tension-dependence of the active properties, we use a new approach. The tension-dependence is determined by two coefficients corresponding to the components of the resultant in the Boltzmann function. We derive two equations for these coefficients. One of them is based on the observed shift of the electromotile curves in response to changes in turgor pressure. The other is a symmetry condition resulting from the piezoelectric relationships for the outer hair cell wall (Spector, 2000). By using the pressure-related shift (Kakehata and Santos-Sacchi, 1995) we estimated the sought coefficients as 4.7 nm2 and -1.4 nm2. The substitution of the shift measured by Gale and Ashmore (1994) gave similar results. Previous estimates of the coefficients (Iwasa, 1993, 1994, 2000) obtained by a different method do not satisfy the proposed conditions. The tension effect is determined by the axial force and reactive pressure. We plot the active and passive strain and energy vs. voltage for different values of the axial force. The application of the axial force shifts the graphs along the vertical and horizontal axes. The force-dependence is nonlinear, e.g. the passive energies that correspond to the same voltages and to equal but opposite axial forces are different.

916 Measuring the Equilibrium Stess/Strain Relationship of the Isolated Tectorial Membrane

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Recent studies have shown that defects of the tectorial membrane (TM) underlie a number of important genetic disorders of hearing (Petit 1996; Steel and Kros 2001). Mouse models of these mutations show hearing loss and abnormalities in molecular architecture of the TM (Simmler et al 2000). To understand the physiological implications of these changes in molecular architecture, we are developing physiological measurements of the TM that can be related back to molecular architecture. We measure the relation between stress and strain of isolated TMs by varying the osmotic pressure in the bath using polyethylene glycol (PEG). At low osmotic pressures, the TM volume decreases in proportion to the osmotic pressure exerted by the PEG. At higher osmotic pressures, the ratio of strain to stress decreases and the volume reaches an asymptotic limit. These results are consistent with a simple interpretation of the molecular architecture of the TM. The TM consists of a matrix of cross-linked macromolecules that exhibit both mechanical and electrical properties. Normally, the TM matrix is in tension because of electrostatic repulsion of charge groups fixed to these macromolecules. Small amounts of PEG in the bath generate small osmotic forces that reduce mechanical tension in the macromolecules. We can characterize the linear relation between stress

and strain by the bulk modulus. In three experiments, we have found the bulk modulus to be 33±12 kPa. As the osmotic pressure is increased, tension goes to zero and further osmotic increases are resisted by electrostatic repulsion due to fixed charge. Based on results at high osmotic pressures, we estimate the fixed charge concentration to be 30±10 mmol/L. Our method provides a new tool for measuring physiological properties of the TM. This tool will enable direct characterization of physiological consequences of changes in molecular architecture between normal and mutant TMs.

917 Constructing a cochlear transduction curve from DPOAEs

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Applying a nonlinear systems identification technique to the cochlear microphonic (CM), we have characterized inner ear mechano-electric transduction (MET) in gerbils. This technique provided a third-order polynomial model of MET and physiologic indices that were sensitive and specific to different cochlear insults (Bian & Chertoff, 2001, JASA, 109:671). The invasive nature of measuring the CM, however, limits the application of this technique in humans. Here, we introduce a new method to obtain a cochlear transducer function from distortion product otoacoustic emissions (DPOAEs). Distortion products are generated by the nonlinearity of cochlear transduction. The cubic difference tone (CDT) is produced from the odd-order terms of a power series that approximates a nonlinear function (f_{NL}) (Engebretson & Eldredge, 1968, JASA, 44:548). Mathematical exploration showed that the CDT amplitude is proportional to the third derivative of an f_{NL} when the primary levels are sufficiently small. By using a bias tone to position a two-tone signal at different places along an f_{NL}, the CDT amplitudes can be used to construct the third-derivative of the f_{NL}. To test the validity of the method, DPOAEs were measured from nine gerbils. In the experiment, a two-tone signal (f_1=3968, f_2=5120Hz, L_1=L_2) was presented to the animals along with a low-frequency bias tone (25 Hz) of various magnitudes. A plot of the CDT amplitudes as a function of the bias levels demonstrated a shape similar to the absolute value of the third-derivative of a Boltzmann function. Curve fitting revealed the parameters of the Boltzmann function representing cochlear transduction. The r^2 was highest (.95) at primary levels of 55 dB SPL. This indicates that the low-frequency modulated DPOAEs can be used to estimate a cochlear transducer function.

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918 A Putative Autoregulatory Feedback System at the Hair Cell Afferent Synapse in the *Xenopus laevis* Lateral Line Organ

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Afferent fibers in the lateral line organ discharge spontaneously due to neurotransmitter release from hair cells. We monitored this discharge following stimulation of the nerve fibers with pulse trains through the recording electrode. At voltages consistent with activation of afferent nerve fibers, we observed a heretofore undescribed suppression of spontaneous discharge with a half-time of recovery of ~35 ms. This suppression occurs at stimulation levels lower than those required to stimulate efferent fibers, lasts much too long to be due to collision of antidromic and afferent spikes, and is not affected by agents that block acetylcholine, catecholamines, serotonin, glycine, or GABA.

The antidromically-mediated suppression can be manipulated with agents acting on nitric oxide (NO) metabolism or on glutamate receptors. Sodium nitroprusside (which releases NO), and the glutamate receptor antagonists, CNQX and kynurenic acid, enhance the suppression and produce an after-excitation. Glutamate receptor agonists, NMDA and kainate, block the suppression.

The antidromically-mediated suppression could act as an autoregulatory feedback system. Since each afferent fiber innervates several synapses, more active synapses could suppress less active synapses through antidromic spike invasion. Glutamate receptor activation could prevent self-inhibition at the synapse initiating the spike.

In the presence of CNQX, kynurenic acid or sodium nitroprusside, the antidromically-mediated effect is remarkably similar to the effect of stimulating the cholinergic efferent fibers – both in that it has the same effects on spontaneous discharge (inhibition followed by after excitation), and the same time course. They can be differentiated because the antidromic effect is not blocked by anticholinergics and is generated at lower stimulation voltages. However, the similarity of the two effects suggests a common final mechanism.

919 Neurophysiological Evidence for Increased Vesicle Pool Size in Hair Cells after Intense Sound Stimulation

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From capacitance measurements, it has been shown that chick tall hair cells possess at least two releasable vesicle pools that deplete with exponential time courses. Such synaptic depression theoretically forms the basis of spike-rate adaptation in peri-stimulus-time histograms of single unit activity in the chick cochlear ganglion. Adaptation functions of single units in response to 100 ms pure tone bursts were characterized in both the normal and acoustically over-stimulated chick cochlear nerve and had time courses that were best fit by single-exponent equations with time constants in the range of ~5 to ~50 ms. This compares well to the time constant for depletion of a small, readily releasable pool of neurotransmitter vesicles identified by capacitance measurements (<50 ms). The acoustic over-stimulation (0.9 kHz @ 120 dB SPL for 48 hours) had no effect on adaptation time constants, but the percent adaptation, which measures the relationship between the peak response and the asymptotic level of activity, was elevated after the exposure. This occurred through an increase in peak response, as opposed to a decrease in asymptotic firing rate, and was accompanied by a concomitant increase in the number of spikes in the adapting component. If adaptation results from vesicle pool depletion, the number of spikes in the adapting component should be proportional to the initial size of the depleting vesicle pool. Thus, a possible interpretation of these results is that the number of vesicles contributing to the small, readily releasable pool is increased by intense sound stimulation. Since the content of this pool correlates well with the number of vesicles tethered to hair cell dense bodies, the results presented here lead to the testable hypotheses that both the size of the readily releasable pool and the number of vesicles tethered to dense bodies are augmented by acoustic over-stimulation.

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920 Diversity of Auditory-Nerve Rate-Level Functions explained by a Statistical Model of Vesicle Release by Inner Hair Cells

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Firing rate versus SPL functions (rate-level functions) of auditory-nerve (AN) fibers range in shape from sigmoidal or "flat saturation" via "sloping-saturation" to "straight". In mammals, the shape of the function obtained with characteristic frequency (CF) tones covaries with

spontaneous rate and threshold (e.g., Sachs and Abbas, JASA 56: 1835-1847, 1974; Winter et al., Hear Res 45: 191-202, 1990). The prevailing interpretation of the diversity of shapes and its covariation with threshold is that the rate-level function results from the combination of the inner hair cell (IHC) to AN fiber synaptic function with the transition from linear to non-linear compressive growth of the basilar membrane (BM) vibration that occurs beyond a particular SPL for a given frequency (e.g. Yates et al. TINS 15: 57-61, 1992). The non-linearity is thought to arise from partial saturation of the cochlear amplifier in outer hair cells and, because it is thought to act via the BM, should act similarly on IHCs of similar CF. While most experimental data are well described by this mechanical model, some observations appear at variance with it (Palmer and Evans, Hear Res 2: 319-326, 1980; Müller and Robertson, Hear Res 57: 71-78, 1991). Thus, there might be an alternative unifying explanation for the diversity of the shapes of AN fiber rate-level functions. Here we present a statistical model of synaptic vesicle release from IHCs that is based on our finding that the first spike of AN fibers is triggered by temporal integration of pressure (Heil and Neubauer, J Neurosci 21: 7404-7415, 2001). Our statistical model describes all rate-level functions recorded from cat AN fibers at CF very well, at least as good as the mechanical model, without requiring non-linear BM vibration growth.

921 The Spatial Distributions of Nodes of Ranvier and "Units" Recorded Using Microelectrodes in the Eighth Nerve

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Silicon technology enables fabrication of implantable nerve recording and stimulating devices with a hundred or more extracellular microelectrodes. Fully exploiting this ability requires knowledge of the spatial distribution of signal sources in the neural tissue under study. We used morphological measurements on nerve fiber internodes to estimate the spatial density of signal sources (nodes of Ranvier) in bullfrog eighth nerve. Combining this with a model of the extracellular potential field generated by each node and an estimate of electrode noise, we estimated the number of recordable axons encountered by a static set of microelectrodes (as on an implanted device) or a needle microelectrode as it advances through a nerve.

Internode diameter and length were measured using microscopy of dye-filled internodes in whole-mount nerves. These data provided a relation to predict internodal length based on fiber diameter. This relation was applied to a fiber diameter distribution (measured by microscopy of eighth nerve cross sections) in a Monte Carlo simulation to compute an estimate of the mean spatial density of nodes of Ranvier. This estimate, about 0.0475 nodes/1000 μm^3, fit observations using planar silicon microelectrodes. The model was also used to predict the number of extracellular units recorded using tungsten needle microelectrodes passed through bullfrog eighth nerve. Again, model predictions (3.4 units/100 μm of track) agreed fairly well with observations (1.7 units/100 μm).

Although these results are quantitatively specific to the electrodes used and to bullfrog eighth nerve, the approach is generally applicable to design of extracellular microelectrodes and to estimation of the number of nodes and electrophysiologic "units" in any nerve.

922 Modeling Physiological and Psychophysical Responses to Tonal Amplitude Modulation

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Making quantitative connections between physiology and psychophysics is an important step in understanding sensory perception. This study applies statistical decision theory to auditory-nerve (AN) model discharge patterns in response to sinusoidally amplitude modulated (AM) tones. Simulated AN patterns are produced by a nonlinear computational model of the auditory periphery developed by Zhang et al. (J. Acoust. Soc. Am. 109, 648-670, 2001). In order to verify that the AN model responses were realistic for AM stimuli, individual model-fiber firing patterns were compared to physiological AN responses (Joris and Yin, J. Acoust. Soc. Am. 91, 215-232, 1992). Like empirical AN recordings, the model simulations phase-lock to the envelope of a high-frequency carrier modulated by a low-frequency sinusoid. Average firing rates are generally not affected by changes in the modulation frequency, whereas psychophysical performance can be strongly influenced by the same parameter.

These and other observations motivate an attempt to determine which features of the AN discharge patterns are necessary to explain human perception of AM stimuli. Different criteria can be used to quantify the amount of information in a given model discharge pattern. We are studying predictions based on differences in the average rate across intervals, as well as those assuming that the precise timing of spikes is necessary to explain human performance. Additionally, a monaural, cross-frequency coincidence counting mechanism can be applied to population responses as a more physiologically realistic means to include both rate and timing information. These predictions allow us to test hypotheses concerning the mechanisms used by the brain to process AM stimuli, and this strategy can be used to study complex sounds in general.

923 Modelling the temporal representation of single- and double- vowels in the guinea-pig auditory nerve using a non-linear filterbank

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We have recently developed a non-linear filterbank model of guinea-pig auditory periphery (BSA abstracts, 2001, P17). It is intended as a computational tool for investigating auditory nerve coding, and as an input to models of the auditory brainstem. The model is implemented on the Development System for Auditory Modelling (DSAM; available from www.essex.ac.uk/psychology/hearinglab/dsam). It incorporates a dual resonance non-linear (DRNL) filter architecture (Meddis et. al., 2001, JASA, 109:2852-2861) together with a recently proposed IHC model (Sumner et. al., JASA, under revision). The DRNL filter consists of two parallel bandpass pathways, one of which is compressive. The IHC model incorporates a biophysical model of the receptor potential, calcium controlled neuro-transmitter release, and quantal-stochastic transmitter re-cycling. The composite model reproduces the characteristics of different spontaneous rate classes of auditory nerve fiber. Filter parameters are a logarithmic function of best frequency and reproduce the variation in tuning and non-linearity across best frequency (BF). The model was originally fitted to the responses of pure tone stimuli alone. Here we test the responses of the model for complex stimuli, by comparing the temporal responses of the auditory nerve model with those of the guinea-pig, for single- and double- vowels (Palmer et. al., 1986, JASA 79:100-113; Palmer, 1990, JASA 88:1412-1426). The model responses are analysed in the form of Period Histograms, FFTs, synchronisation to different harmonics and of the dominant components in the temporal response. The model reproduces many of the temporal features of vowels across the range of filterbank BFs for both single and double vowels. The results support the approach of using pure tone stimuli to fit the model, and demonstrate that the model is adequate as a computational tool for investigating the spectro-temporal coding of complex stimuli in the auditory nerve.

924 Effects of Selective Demyelination of the Auditory Nerve Following Intraneural Injection of Doxorubicin (Adriamycin)

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Demyelinating diseases often affect the central auditory pathway but are less likely to affect the auditory nerve. Recent clinical studies, however, suggest that demyelination of the auditory nerve may be the primary pathological mechanism underlying auditory neuropathy. The goal of this study is to explore the anatomical and physiological changes resulting from selective auditory nerve demyelination produced by an intraneural injection of doxorubicin. It has been shown that doxorubicin induces selective and segmental demyelination of peripheral nerves. Two groups of chinchillas were used as subjects. Each group received the drug in a dose of either 0.19 or 0.38 μg through microscopic intraneural injection into the auditory nerve bundle of one ear. The cochlear microphonic (CM), compound action potential (CAP), and inferior colliculus evoked potential (IC-EVP) were recorded before and after drug injection through electrodes chronically implanted at the round window and IC. Preliminary results show that IC-EVP thresholds were elevated (10 to 60 dB) or absent depending on the test frequency and the dosage received. Likewise, IC-EVP amplitudes decreased to 10% of the pre-injection value and latencies increased by as much as 1.2 ms. Although CAP thresholds did not change appreciably, CAP amplitudes decreased and latencies increased. CM measures showed essentially no change after injection. Animals were sacrificed five days after injection, and the cochleas with auditory nerve bundles were prepared for histological study. When comparing the injected and normal cochleas, no significant hair cell loss was detected for either ear. Similarly, no significant difference in the number of myelinated fibers in the habenula perforata or spiral ganglion cells was found between ears. Morphological changes in the distal nerve fibers are still under analysis to complete the description of this potential animal model of auditory neuropathy.

925 Inhibiting metabotropic glutamate receptors in the cochlea reduces temporal threshold shift after noise exposures

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Evidences have accumulated over the years supporting glutamate as the primary neurotransmitter released by hair cells. However, few experiments have examined the role of metabotropic glutamate receptors (mGluRs) played in cochlear neurotransmission. In a companion abstract presented at this meeting, we reported the presence of multiple subtypes of mGluRs in the cochlea as detected by RT-PCR, immunolabeling and Ca ratio imaging. In this work the role of mGluRs in cochlear neurotransmission was examined by testing the hypothesis that mGluRs are involved in generating temporary threshold shift (TTS) in the guinea pig.

Normal hearing adult guinea pigs (pigmented, 250-300 g) were exposed to 110 dB SPL white-band noise for 30 minutes, and their hearing thresholds were measured by auditory brainstem responses (ABRs). Animals showed a threshold elevation of 30+4.6 dB 20 minutes after noise exposure terminated, which gradually recovered in the next few weeks. After locally applying drugs (10 mM) on the round window membrane (RWM), we test the threshold shift caused by the same noise exposure protocol. Compared to artificial perilymph (APL), a broad spectrum mGluR antagonist (LY341495) reduced the threshold elevation by 12.5+4.3 dB ($n=4$, $t<0.05$) as tested by click ABR. Another group I specific mGluR antagonist, AIDA, also reduced the threshold elevation by 13.8+4.7 dB ($n=4$, $t<0.05$). When either APL, LY341495 or AIDA were applied to the RWM without subsequent noise exposures, these drugs alone did not cause any appreciable threshold changes.

Our results are consistent with a hypothesis that mGluRs were not involved in the AMPA-type fast cochlear neurotransmission, but participating in modulating synaptic responses in the hair cell synapses. Excessive activation of mGluRs in the cochlea may be one of the mechanisms partially responsible for generating TTS.

926 Regrowth of Auditory Nerve Peripheral Processes Following Deafness with Chronic Cochlear Electrical Stimulation

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Studies have shown neurotrophic factors both enhance the survival of spiral ganglion cells (SGC) following inner hair cell loss, and induce the regrowth of SGC peripheral processes. Since chronic electrical stimulation of the auditory nerve following deafness can also enhance SGC survival, the present study examined whether peripheral process regrowth might occur under this condition as well. Guinea pigs were bilaterally deafened and received a unilateral cochlear implant on day 4 following deafening; and on day 7 began chronic stimulation, at 100μA, for 14 days. Animals were then anesthetized and fixed in 4% paraformaldehyde. After local fixation, the otic capsule, stria vascularis, Reissner's and tectorial membranes were removed and the remaining cochlea immunoreacted with pan-trk antibody to selectively immunostain peripheral processes. Cochleae were decalcified, processed into methacrylate and sectioned in a paramodiolar plane. Sections revealed large numbers of remaining SGC and myelinated fibers within Rosenthal's canal and the osseous spiral lamina, compared to cochleae from unstimulated deafened animals. In the stimulated ears, many pan-trk labeled processes were seen emerging from the habenula perferota and traveling into the scar tissue replacing the organ of Corti. This was seen in all turns of the cochlea, though greatest in more basal turns. Interestingly, in some of the animals receiving chronic cochlear electrical stimulation, a few labeled processes were also observed in basal turns of the contralateral, non-stimulated cochlea. Such regrowth of peripheral process, as well as a "contralateral effect," has been observed with cochlear infusion of neurotrophic factors or gene vectors. This is the first report of such findings with electrical stimulation.

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927 Regeneration in the adult vertebrate n.VIII: Behavioral and genetic responses

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The adult bullfrog (*Rana catesbeiana*) can regenerate the injured eighth cranial nerve (nVIII) in the absence of exogenous treatment. We correlated behavioral recovery of function after unilateral or bilateral crush of nVIII with expression of gene products at various time points after damage. During recovery (2 hours to 90 days), we quantified the animals' degree of passive head tilt and active response to rotation. Frogs with unilateral nerve crush show a pronounced head tilt towards the operated side within 2 hours after surgery. The degree of tilt rapidly recovers over the initial 2 weeks post-surgery, but most animals still maintain a slight measurable head tilt up to 60 days later. Control animals receiving sham operations exhibit a stereotypical turning response to rotation. Lesioned animals react abnormally to rotation, showing flaccid paralysis and no compensatory head movement. Animals recovered for 10 days or more gain limited head movement, and after 30 days continue to approach normal. The technique of differential display was employed to explore genetic factors expressed following n.VIII lesion. At set time points, n.VIII, including medullary

targets, was processed, and differentially regulated gene products were cloned and sequenced. At 24 hours after lesion, three cDNAs are observed. Two are previously undescribed, and a third exhibits 91% coding region homology with a human brain protein. Study of the molecular bases of the regenerative capacity of the bullfrog nVIII may elucidate factors permissive of recovery of function in the vertebrate nervous system.

928 Towards an Interpretation of Individual FMRI-Activation in the Auditory Cortex of Patients with Psychoacoustic Deficits

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Functional magnetic resonance imaging is developing into a diagnostic tool for neurological impairments but in most instances does not yet allow a reliable diagnosis of individual patients. As steps to overcome this problem it is necessary to develop fMRI-paradigms suitable to test for activation changes concomitant with an impairment and to relate fMRI-activation to the psychophysical performance of each individual.

The fMRI-paradigm used in this single case study tested for the ability to discriminate the direction (rising vs. falling) of frequency modulated tones (FM) as an acoustic model for prosody processing. The basis for this study was the finding of a previous group study with healthy volunteers showing that the activation of the auditory cortex territory T3 in the right hemisphere correlated with the performance of discriminating the direction of FM (Brechmann & Scheich (2000) Neuroimage 11: S799). The volunteer studied here showed significant deficits in the ability to discriminate the direction of FM of short duration (400 and 600 ms) but not of FM with the same speed of modulation which had longer duration (700 and 1000 ms). Results of the repeated fMRI-study in several sessions revealed strongly left lateralized activation of the auditory cortex territory T3 on planum temporale.

These preliminary results suggest that this volunteer might rely chiefly on the left auditory cortex to discriminate FM-direction which could entail the pronounced deficit. Chronic inflammation of the left middle ear in the volunteers childhood might be responsible for these results.

929 Temporal Dynamics of fMRI Responses in Human Auditory Cortex: Primary vs. Non-primary Areas

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We have previously shown that the temporal envelope characteristics of sound are strongly represented in the fMRI response waveshapes of human auditory cortex. Continuous or high-rate stimuli (e.g. high rate noise bursts) produce a phasic response characterized by distinct peaks at sound onset and offset. In contrast, discontinuous or low-rate stimuli produce sustained responses. Animal work suggests that the representation of sound temporal envelope differs between cortical areas. The present study tested for such differences in humans by comparing fMRI responses between two cortical regions: Heschl's gyrus (HG), the site of primary auditory cortex, and the superior temporal gyrus (STG), a site of non-primary areas.

Responses were measured in HG and STG for a variety of 30s-long stimuli: trains of noise bursts (2 – 35/s), tone bursts (2, 35/s) or clicks (35, 100/s), continuous noise, orchestral music and running speech. The data for each structure consisted of 174 responses, each corresponding to a particular stimulus, subject and imaging session. Response waveshape was quantified by an index that ranged from zero for the most sustained responses to one for the most phasic.

Looking across all stimuli, responses were usually more phasic in STG than in HG (122 of 174 cases). Moreover, this trend occurred particularly often for certain stimuli. For instance, the waveshape index was almost always greater in STG for continuous noise (24/27) and 10/s noise bursts (7/7), but was greater for music in only 21 of 38 cases.

Our results indicate that a functional distinction between primary and non-primary auditory cortex exists in fMRI response waveshape, and that certain sounds are particularly effective in showing this distinction. The findings suggest underlying differences in how the temporal envelope of sound is coded in population neural activity of primary vs. non-primary areas in human auditory cortex.

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930 Representation of Sound Bandwidth in the Human Auditory System Using fMRI

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Bandwidth is a fundamental feature of sound, yet its representation in the neural activity of the human central auditory system is largely unknown. The present study examined the representation of bandwidth using fMRI at two stages of the human auditory pathway: inferior colliculus and auditory cortex.

Stimuli were bursts of: 500 Hz tones, third- or two-octave noise centered at 500 Hz, and broadband noise. They were (1) equal in energy or, (2) equal in spectrum level (energy increases with increasing bandwidth). A single 6mm thick plane that passed through the inferior colliculi and auditory cortex was functionally imaged.

Inferior colliculus activation increased with increasing bandwidth for equal spectrum level stimuli, but remained constant for equal energy stimuli. Previously, it has been shown that inferior colliculus activation increases with increasing sound intensity and repetition rate. The present and previous data together indicate that sound energy is a major factor in determining the magnitude of population neural activity in the inferior colliculus.

In contrast to the inferior colliculus, auditory cortex activation varied non-monotonically with bandwidth for equal energy stimuli. For equal spectrum level stimuli, broadband noise produced greater activation than narrower-band stimuli, a trend that is similar to that for the inferior colliculus.

Bandwidth dependencies in the inferior colliculus, as shown with fMRI, fit with a simple view in which a majority of neurons have a single best frequency (BF) and respond increasingly with increasing sound level. However, the dependencies in auditory cortex indicate a preponderance of neurons with more complex frequency tuning properties such as off-BF inhibition, which can result in a complex interplay between sound level and bandwidth.

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931 Growth of activation in Heschl's gyrus with increasing level of low and high frequency tones

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Demonstration of tonotopicity in the human auditory cortex by fMRI and PET has proved to be difficult. Likely reasons are insufficient resolution compounded by the wide spread of activation that has been repeatedly reported in physiological studies as the sound level is increased and the inability to distinguish excitation and inhibition. However, physiological studies have shown that differences in tuning and in the non-monotonicity of rate-level functions lead to different growth of primary auditory cortical activation at different frequencies [e.g., Phillips et al. (1994) Exp Brain Res 102: 210-226]. Here we use fMRI to investigate whether the growth in activation of the human

auditory cortex with increasing sound level is discernibly different for high- and low-frequency tones.

Ten volunteers were scanned whilst listening to sequences of low-frequency (0.30 kHz) tones at levels between 42 and 96 dB SPL, and 10 whilst listening to high-frequency (4.75 kHz) tones at the same levels. Extent of auditory activation (number of activated voxels) was calculated for each subject in a region of interest on Heschl's gyrus, which includes human primary auditory cortex.

The mean trend indicated that the extent of auditory activation produced by 0.30 kHz tones did not change much with intensity until, at around 70 dB SPL, there was a sharp increase in the number of activated voxels, which continued up to the highest level studied (96 dB SPL). In contrast, increasing the intensity of 4.75 kHz tones produced a steady growth in activation across the range of levels studied. These results are consistent with existing physiological evidence suggesting that recruitment of primary auditory cortical neurones may be different at high and low frequency.

932 Regions Outside Primary Auditory Cortex In Humans Are More Activated By Modulated Than By Static Stimuli.

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Hall et al. (2001) recently showed that pulsed FM tones generate high activation in non-primary auditory regions, immediately posterior and lateral to Heschl's gyrus (HG). In the present study, we explore the type of modulation necessary (using frequency or amplitude modulated tones) to evoke the higher activation of these non-primary areas. Carrier signals were either a single (300 Hz) or a harmonic-complex (300, 600, 900 and 1200 Hz) tone, modulated at a rate of 5 Hz either in frequency (depth 50 Hz), or in amplitude (depth 100%), to create 6 stimulus conditions (static, FM, AM). Stimuli were presented at equal loudness (using a model of loudness summation) continuously for 6.8 s, during 3T fMRI scanning on 12 volunteers. Coronal brain images were acquired every 9 s, at the transition between stimulus conditions. Using SPM99, images were motion corrected and transformed into a standard brain space for a random-effects group analysis. All stimuli generated bilateral auditory activation relative to a silent baseline. There was no significant difference between carrier signals. However, both AM and FM tones generated significantly greater activation than the static tones ($P<0.001$) both in lateral HG and immediately posterior and lateral to it. The activations by AM and FM tones were mostly overlapping, but that due to AM extended more laterally. For both types of modulation, activation peaks occurred laterally in HG and in the posterolateral region. Comparing AM and FM tones, AM was a more potent stimulus than FM, particularly in the posterolateral region. These data indicate that, while both types of modulation strongly activated lateral parts of HG and posterolateral regions, greater activation was evoked by the variation in amplitude, than in frequency. Thus, the activation reported by Hall et al. (2001) may have been influenced by the pulsed nature of the FM tones as well as by their frequency modulation per se. Hall DA et al., (2001) Cerebral Cortex, in press.

933 PET and fMRI Studies of the Analysis of Sound-Source Motion by the Human Brain

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The functional anatomy of human sound motion processing was studied using the same virtual acoustic space paradigm in parallel, whole brain positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) experiments. Amplitude-modulated broadband noise was convolved with a generic head-related transfer function to simulate a virtual sound source in external space, which either remained stationary or rotated horizontally around the head, with fixed (first-order) or changing (second-order) angular velocity. The PET and fMRI studies both show a bilateral, posterior contiguous network of activation, excluding Heschl's gyrus and involving planum temporale (PT), parieto-temporal operculum and inferior parietal lobule, common to both first- and second-order motion conditions. We propose that a posteriorly directed, temporo-parietal pathway including PT mediates obligatory, perceptual sound motion processing in humans, perhaps analogous to the posterior stream in the macaque. The fMRI group data further suggest functional subspecialisation of medial PT for sound motion processing. Activation of superior parietal and frontal areas is variable, and may reflect a spatial attentional function.

934 Activation of Human Auditory Cortex Territories During a Non-Matching to Sample Experiment with Comparison of FM-Direction (up vs. down): A fMRI Study

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The focus of the present study was to test the influence of memory load on fMRI auditory cortex activation during a nonmatching to sample task with a list of auditory patterns. Specifically, we studied the influence of three time-delays (short, middle, long) between pairs of items using mixed rising and falling frequency modulated tones (FM) with different spectral content comparable to those of a prevoius study (Brechmann & Scheich (2000) Neuroimage 11: S799). It was expected that increasing memory load from short to long delays would lead to increased activation of those auditory cortex territories which are specifically involved in memory processing of the items. Conversely, a decreasing number of FM-stimuli, would lead to decreasing activation strength of all auditory territories not involved in the memory aspect.

18 subjects (9 female and 9 male) had to compare the direction of FM-pairs in a one-back memory task. The results showed a strong activation of the territory T3 on planum temporale and the territory T2 which were previously implicated in the processing of FM (Brechmann & Scheich, J Neurophysiol, in press). With increasing delay the activation of these two territories decreased. On the other hand activation of territory T1a (anterior of Heschl's gyrus) and BA 44 increased in activation strength and thus might be involved in short term memory during the non-matching to sample task.

935 Cognitive Brain Activity During Auditory Tasks Changes with Minor Modification of Temporal Sound Characteristics

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Auditory masking experiments have shown deficits in the perception of subtle temporal features of non-speech signals in children with a specific language impairment, SLI (Wright et al., 1997). In particular, a backward masking tasks proved to be difficult for SLI-children, while simultaneous masking was performed normally. We investigated whether backward masking requires specific brain resources.

Eight adults performed a two-alternative forced choice masking experiment, while their brain activity was monitored using functional MRI. Stimuli consisted of a 20-ms 1500-Hz tone pulse, and a 300-ms noise masker with 1000 Hz bandwidth centered at 1500 Hz. The tone pulse started either 20 ms prior to the noise (backward masking), or 200 ms after onset of the noise (simultaneous masking). Whole brain MR images were acquired in a sparse-sampling block design, in which non-stimulus intervals were alternated with the backward or the simultaneous masking task.

Backward masking gave significantly more activity than simultaneous masking in several non primary-auditory regions: [1] bilaterally in the anterior temporal lobe, which presumably play a role in language processing, and [2] the frontal cingulate gyrus, to which executive functions have been attributed. We conclude that the baskward masking task requires specific cognitive brain resources.

Wright, B.A., Lombardino, L.J., King, W.M., Puranik, C.S., Leonard, C.M., and Merzenich, M.M. (1997) Deficits in auditory temporal and spectral resolution in language-impaired children. Nature 387, 176–178.

936 Music Alters Brain Activity Associated with Processing an Arithmetic Task

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Behavioral observations suggest that music affects performance of arithmetic tasks (Abikoff et al., 1996). Cortical activation to music presentation occurs predominantly in the right temporal lobe (Kanwal et al, 2000), whereas neural activity due to arithmetic tasks is localized within the left inferior frontal cortex and the parietal lobes (Dehaene et al, 1999). Thus, for music to have an effect on arithmetic task processing, neural activity due to music must affect areas that lie outside of classical auditory processing streams. To examine a neurobiological basis for this effect we measured BOLD contrast signal changes due to arithmetic task processing in the absence of and during the simultaneous presentation of music. Five right-handed male subjects performed a task involving counting tones presented to the right ear as multiples of 6, 7, and 8 (right-multiply task). This task was performed independently and during the simultaneous presentation of music to the left ear. Twenty transverse T2-weighted gradient-echo EPI images were obtained using a 1.5T Siemens MRI scanner. Control scans consisted of either presentation of tones in the right ear, or tones together with music in the left ear but without performance of any task. The right-multiply task alone yielded significant activity ($p<0.001$) in the left inferior frontal and inferior parietal gyri (Z-score: 8.16). During this task performance with music, regions that were significantly activated in the previous condition became insignificant ($p>0.05$), e.g., average Z-scores in the inferior frontal gyrus declined by 2.8 in contrast to a change of 1.2 in the cerebellum (a control region). At the same time, a significant increase in activity was observed in the left anterior cingulate, middle frontal gyrus and right superior parietal cortex (Z-score: 6.05). Our results suggest that even in the absence of an overlap in their spatial representations, music alters the neural substrate of arithmetic task processing.

937 Control of subcellular distribution of Prestin protein in outer hair cells

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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 which is referred to as electromotility. The molecular motor of outer hair cells has been identified (Zheng, J. et al. 2000 Nature 405 149-155). We recently suggested thyroid hormone (TH) as one of the transcriptional regulators of the prestin gene in rat cochleae (Weber et al.). During these studies we noted a developmental redistribution of prestin proteins: while prestin proteins are distributed along the whole outer hair cell membrane prior to hearing onset prestin became redistributed to the lateral membrane during the onset of hearing. In the absence of TH the developmental redistribution of prestin to the lateral membrane failed, indicating TH as a direct or indirect regulator of the subcellular distribution of prestin proteins. Our aim is to investigate the molecular link between TH and the subcellular redistribution of prestin proteins in outer hair cells. First data towards this aim, indicating a role of OHCs` ion channels, will be presented.

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938 Muscle-specific Kinase (MuSK): A Potential Role for MuSK in Inner Ear Development.

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Nicotinic acetylcholine receptors (nAChRs) mediate synaptic transmission between cochlear hair cells and olivocochlear (OC) axons in the mammalian inner ear. 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, muscle-specific kinase (MuSK), rapsyn, and other synaptic molecules form a multimeric scaffold that is essential for the clustering and stabilization of nAChRs. In this study, we have considered the possibility that rapsyn and MuSK play a role in the clustering and stabilization of nAChRs in the mammalian inner ear.

Partial transcripts of rapsyn and MuSK were isolated from rat cochlear RNA using gene-specific primers and RT-PCR. Preliminary data suggest that rapsyn is expressed at a relatively constant level during the first two weeks of postnatal development. In contrast, MuSK expression appears to peak after the first week of postnatal development and then persists at slightly lower levels during the second postnatal week. Immunofluorescent investigations show that MuSK is localized to the hair cell synaptic area, during the second week of postnatal development (P15). Our data also suggest that MuSK expression is greater in apical regions of the cochlea than in the middle or basal turns. Using growth-associated protein (GAP-43) as a marker for growing efferent axons, we found both MuSK and GAP-43 are localized at the synaptic pole of IHCs. Our results raise the possibility that the mechanism of clustering or organizing nAChRs proposed for the NMJ may be similar for nAChRs in hair cells.

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939 Differential Patterns of α and β Parvalbumin Expression during Cochlear Development in the Rat.

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Two isoforms of parvalbumin (PV) are present in mammals. While αPV is found in inner hair cells (IHCs), βPV (oncomodulin, OM) is found only in outer hair cells (OHCs) of the adult cochlea. The rapid motile response of the OHCs is modulated by efferent stimulation and mediated by Ca^{2+}. During development, efferent olivocochlear (OC) axons innervate OHCs exclusively only after transient connections with IHCs. Using RT-PCR and immunofluorescence methods, we investigated the relationship between efferent innervation and OM expression during development.

While αPV mRNA is expressed in embryonic and postnatal (P) tissues, OM expression begins around P4, correlating with the efferent innervation of the OHCs. Before birth, αPV immunoreactivity is present in IHCs and OHCs, increasing with age in IHCs and decreasing in OHCs. In contrast, OM immunoreactivity is not detected until P4 and is restricted to OHCs. Moreover, OM immunoreactivity is first seen in the mid-basal cochlear regions. To test the relation between the onset of OM expression and efferent innervation, cochleae of different ages were cultured for 2 to 7 days in vitro. At the end of the culture period, the organs were fixed and labeled for OM, phalloidin, and/or growth-associated protein-43 (GAP-43). For controls, age-matched cochleae were fixed immediately after dissection. Phalloidin labels the stereocilia of both IHCs and OHCs while GAP-43 labels growing efferent axons. Within cultured organs, OM is uniquely expressed in OHCs and demonstrates a basal to apical gradient. Specific OM expression and GAP-43 labeled fibers were detectable only in cultures that are P4 or older. Our data are consistent with the idea that OM expression coincides with efferent synaptogenesis and provide evidence that OM expression is limited to OHCs during development.

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940 Transient Expression of Tenascin-C Co-localizes with GAP-43 Immunoreactivity during period of Efferent Synaptogenesis.

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Extracellular matrix (ECM) molecules may have either attractive or repulsive interactions on growing axons. In the inner ear, previous studies have not directly compared the expression of ECM with afferent or efferent innervation. To investigate whether tenascin-C might be involved in the guidance of efferent axons in the inner ear, the expression of these molecules was investigated in E16 through adult rats. Antibodies against growth-associated protein 43 (GAP-43), synapsin and neurofilament proteins were used to investigate efferent and afferent innervation, respectively.

Using both RT-PCR and immunofluorescence methods, we find a transient peak of tenascin-C expression between embryonic day (E) 16 and postnatal day (P) 10 in the inner ear. At E18, tenascin-C immunoreactivity (IR) is strongly expressed below vestibular hair cells. In some animals at E18, tenascin-C IR is also found on the modiolar side of inner hair cells (IHCs) in basal regions of the cochlea. At E21, tenascin-C IR is unchanged below vestibular hair cells. In the cochlea, tenascin-C IR is now present on both modiolar and pillar sides of IHCs in basal regions and is present on the modiolar side of IHCs in apical regions. By birth (P0), tenascin-C IR is expressed throughout the inner ear: below vestibular hair cells and in all cochlear turns below IHCs and OHCs. The robust expression of tenascin-C IR below vestibular hair cells extends through P5. However after P2, tenascin-C IR is significantly reduced below IHCs and OHCs. By P10, tenascin-C IR is decreased below vestibular hair cells and is absent below cochlear hair cells. Regions of GAP43 IR show extensive overlap with regions of tenascin-C IR both spatially and temporally. However, synapsin IR comes after the onset of tenascin-C expression and neurofilament IR precedes tenascin-C expression. Within the cochlea, the spatial and temporal colocalization of tenascin-C IR with GAP-43 IR occurs between E18 and P5. Our results suggest that tenascin-C expression may play a role in the guidance and synaptogenesis of efferent axons.

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941 Cochlear β Tubulin Isotype Reorganization During And After Post-Mitotic Microtubule Elaboration

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Previous studies have shown that mammalian β tubulin isotypes are selectively expressed in cells of the adult cochlea and vestibular periphery (Hallworth & Ludueña, Hear. Res., 2000; Perry et al., ARO abstr., 2001). In the adult cochlea, inner hair cells (IHCs) express β_I and β_{II}, while outer hair cells (OHCs) express β_I and β_{IV}. The β_{II} and β_{IV} isotypes are manifested in the pillar cells, while β_I, β_{II}, and β_{IV} are present in the Deiters cells. Adult vestibular hair cells express β_I and β_{IV}, while supporting cells express β_I, β_{II}, and β_{IV}. In post-mitotic cochlea development, microtubules are elaborated in a specific developmental pattern beginning with hair cells, then pillar cells, and finally Deiters cells. We have now identified several post-natal changes in β tubulin isotype distribution in the developing gerbil cochlea, using isotype-specific antibodies and indirect immunofluorescence. IHCs express all four isotypes at post natal day 0 (P0) and maintain β_I and β_{II} throughout development but lose β_{III} and β_{IV} by P6. OHCs similarly initially express all four isotypes, but lose β_{II} around P12 and β_{III} by P6, while maintaining β_I and β_{IV}. Beginning at P3, both inner and outer pillar cells manifest β_I, β_{II} and β_{IV}. The β_I isotype is not lost until after P20, and is lost earlier in inner pillar cells than in outer pillar cells, while β_{II} and β_{IV} are maintained. When Deiters cell microtubules are elaborated (P6), they express β_I, β_{II}, and β_{IV}, the same β tubulin isotypes as in the adult. Changes in isotype expression in hair cells were found to occur during the period of extensive post-mitotic changes in cellular morphology (P3 to P12). It is interesting that pillar cells lose β_I much later, well after any known morphological maturation.

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942 Development Of Hair Cell Stereovilli Bundle Abnormalities In Belgian Waterslager Canary

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Adult Belgian Waterslager canaries (BWS) show an average 30% reduction in hair cell number despite continuous hair cell regeneration. Many of the hair cells that are present have severe stereovilli bundle (SVB) abnormalities. Preliminary evidence indicated that hair cells and stereovilli bundles had developed adult-like abnormalities by 3 months of age. The purpose of the present study was: 1) to quantify the development of hair cell loss and SVB abnormalities and 2) to describe the development of SVB abnormalities as a function of hair cell position and type.

Canaries (n=36) were sacrificed at 2,12,31,66,90 or > 180 days following hatching. Their basilar papillae were fixed and prepared for analysis by SEM. There was no significant difference in hair cell number in BWS and non-BWS canaries between 2 and 31 days of age. By 66 days of age total hair cell number had decreased 16%. Most hair cell loss was observed in the apical half of the papilla.

SVB abnormalities were first seen as multiple bundles on tall hair cells located on the neural edge in the mid-basal region of the papilla. SVB abnormalities progressed from neural to abneural cells and toward the apex. Multiple SVB were first seen at 2 days of age and progressed to abbreviated bundles eccentrically located on the edges of the cuticular plate in both tall and short hair cells by 31 days of age. Quantitative analysis of the development of stereovilli bundle abnormalities revealed a progression in the number of cells with abnormalities from 4% at 2 days of age to 44% by 66 days of age.

These results provide the first evidence of developmental abnormalities in stereovilli bundle formation in a bird and are reminiscent of developmental abnormalities in stereovilli bundle formation described in several mouse models of deafness.

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943 Localization of Kinesin and Dynein in the Gerbil Organ of Corti During Development

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In the organ of Corti, cells have a distinct shape and organization. Their unique properties related to cell polarity, motility and specialized function are all highly dependent on a system of intracellular transport that carries needed materials along a complex cytoskeleton to precise locations in the cell. Alterations in the components of this system through noise exposure, ototoxicity and other forms of trauma may account for hearing loss. Kinesins and dyneins are mechanochemical ATPases which transport materials along microtubules. We have shown previously that, in the adult gerbil organ of Corti, these motor proteins were localized in the Deiters' cells. In order to better understand intracellular transport in the cochlea, we have used western blot analysis to search for motor proteins during cochlear development. Whole cochleae from 8 day old gerbils *(Meriones unguiculatus)* were run on an SDS polyacrylamide gel, transferred to nitrocellulose and immunoblotted. A monoclonal antibody to α-tubulin was used to detect a 55 kDa band in the tissue. Monoclonal anti-kinesin heavy chain detected a 130 kDa band. Monoclonal anti-dynein heavy chain detected a 440 kDa band.

We then used immunocytochemistry to localize the motor proteins in the organ of Corti at 1, 5, 8, and 15 days after birth (dab). Immunocytochemical results on the organ of Corti showed both anti-kinesin and anti-dynein antibodies label supporting cells during development. Pillar cells seem to be labeled at 1 dab while Deiters' cells are labeled by 15 dab. Our findings suggest that kinesin and dynein are present in our tissue samples; they also suggest that the localization patterns of these motor proteins change during development.

944 Postnatal Maturation of Ion Transport Activity in Cochlear Lateral Wall Fibrocytes

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The functional maturation of subtypes of fibrocytes in the cochlear lateral wall was appraised by immunohistochemical assessment of the developmental expression of several ion transport mediators in the gerbil inner ear. Na,K-ATPase and Na-K-Cl cotransporter (NKCC) were first detected in suprastrial (type V) fibrocytes at 10-12 days after birth (DAB). Thereafter, staining for Na,K-ATPase and NKCC appeared and increased in intensity in type II and type IV fibrocytes, nearing adult levels at 16 DAB. In contrast, the expression of carbonic anhydrase (CA) and creatine kinase isozyme BB (CK) developed more slowly in the cochlear lateral wall, reaching an adult level at around 24 DAB. These data indicate the type V fibrocytes gain functional maturity at 10-12 DAB, whereas type II and IV and type I and III fibrocytes mature at around 16 and 24 DAB, respectively. A comparison of the expression of each ion transporting enzyme among similar types of cochlear and vestibular fibrocytes revealed that cochlear fibrocytes reached adult staining levels earlier than their vestibular counterparts. This finding suggests that ion transport capacities mature earlier in the cochlea than the vestibular system. These observations offer new insights into the role of inner ear fibrocytes by showing that the rate of maturation of their ion transport capacity differs apparently with relation to biologic function.

945 Onset of Strial Marginal Cell Replacement During the Maturation of Hearing

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A double staining technique, which allows detection of cell proliferation and apoptosis in the same cochlear section, has been used in these studies. In the stria vascularis of adult rats, the percentages of the total marginal, intermediate, or basal cells which were apoptotic were found to be 32.9% ± 3.7, 22.5% ± 2.8, and 23.8% ± 2.8, respectively. In these same sections, BrdU incorporation was not detectable in marginal cells and was 29.0% ± 3.6% and 43.4% ± 4.0 of the total intermediate and basal cells, respectively. Since none of the marginal cell nuclei had incorporated BrdU, it is likely that their replacements did not arise from marginal cell division. The possibility of cell migration into the marginal layer was examined by determining the appearance of BrdU-positive nuclei in the marginal strial cell layer. This was accomplished using a single i.p. injection of BrdU followed by a pulse of dT after 2 hr and determining the locations of BrdU positive nuclei at 2, 24, 48, and 72 hours post BrdU administration. In the adult rat stria, BrdU incorporation after 2 hr was found primarily in basal and to a lesser extent in intermediate cells. By 24 hr post injection, cells with nuclear incorporation of BrdU had increased 50% in intermediate cells and were now detectable in marginal cells. This trend continued in subsequent days suggesting that underlying strial cells migrate to the strial margin where they eventually undergo cell death. During the onset of hearing in rats (10-23 days after birth), very little incorporation of BrdU in any strial cell at 10 days postpartum was noted, suggesting that the strial cell replacement process had not developed. By 16-17 days after birth, the onset of this process was evident. By 30 days post partum, the process had reached the levels found in adult rats. Strial cell movement apparently matures during the onset of hearing and could be an important aspect in maintenance of the function of the stria vascularis.

946 Cochlear Development in the Deaf White Cat: Structure and Hearing Thresholds

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Postnatal (PN) cochlear development in the cat appears nearly complete by the 4th week. During this time, the tunnel of Corti develops its characteristic shape, the basilar artery shrinks, and cells of the organ of Corti assume their adult-like shapes (Pujol and Marty, JCN, 1970). The congenitally deaf white cat (DWC) represents a model for the Scheibe deformity, a form of human hereditary deafness. Classically, the DWC

was reported to have early onset, progressive cochleosaccular degeneration resulting in eventual obliteration of the cochlear duct and complete deafness (Mair, Acta Oto,1973). In our DWC colony, however, we observed a population of non-deaf adult white cats (Ryugo et al., JCN, 1998). This conflict prompted us to re-investigate the "progressive" nature of deafness in DWCs. We collected ABRs from the same animals from the time of ear canal opening (PN22-26 days) until 1 year of age in 30-day intervals in pigmented and white cats. ABR thresholds, regardless of sensitivity, did not change for individual cats across age. Cochleae were harvested for histologic analysis at different ages, beginning with postnatal day PN0. Morphological differences between the deaf and hearing kittens were already evident at PN0: Reissner's membrane of DWCs was convoluted, not straight, the tectorial membrane was abnormally thin, and receptor and support cells appeared swollen. By PN10, two distinct cochlear patterns were evident in DWCs. One exhibited the collapse of Reissner's membrane onto the organ of Corti. The other featured a thickened yet still highly convoluted Reissner's membrane and enlarged cells along the basilar membrane. Both abnormalities accompanied absent ABR thresholds. These findings indicate that hearing loss in our white cat colony is not progressive and that congenital deafness in the same litter can result from two separate types of cochlear abnormalities.

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947 Development of auditory sensitivity in Belgian Waterslager (BWS) canaries

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Canaries have been selectively bred for their song and plumage for over a century. One strain, the Belgian Waterslager (BWS) canary, has become noted for its loud, low-pitched song, and its high-frequency hearing loss, which results from missing and/or damaged hair cells on the basilar papilla. Recent experiments have shown that hatchling BWS canaries have a normal complement of hair cells and stereovilli. This study tracked the development of auditory sensitivity in nestling BWS and non-BWS canaries using the auditory brain stem response (ABR). ABRs were recorded in adult BWS and non-BWS canaries as well.

Non-BWS adult canary ABR audiograms were similar in shape to behavioral audiograms but were elevated by 20-30 dB. BWS adult canary ABR audiograms were flatter than behavioral curves and elevated by 25-50 dB. Reliable responses were obtained for P5 in non-BWS and P8 in BWS nestlings for almost all frequencies tested. Thresholds for all frequencies were above 80-90 dB SPL but improved over the next 10 days in both strains. Non-BWS canaries were within 1SD of the adult average by P20. Thresholds for BWS canaries improved until about P17 and then gradually worsened from P19-38. By P51, thresholds were within 1SD of the BWS adult average for all frequencies except 0.5 kHz.

Experimental crosses between BWS and non-BWS canaries indicate a sex-linked, recessive inheritance pattern. Anatomical findings of similar cell numbers in NM and NL of adult BWS and non-BWS canaries also suggests a later developing hearing loss since, typically, cell loss in brainstem auditory nuclei only occurs with very early onset auditory deprivation.

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948 Developmental expression of the B1-subunit of H+-ATPase (ATP6B1) and the effects of loss of its function on the mouse inner ear

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Abstract: Studies of a large kindred affected by distal renal tubular acidosis revealed a sensorineural hearing loss phenotype in individuals homozygous for mutation of the B1-subunit of a H^+-ATPase (*ATP6B1*). To examine the potential roles of *ATP6B1* in inner ear development and function, we defined the temporal and spatial expression patterns of the murine *ATP6B1* homolog in the developing mouse inner ear and examined the morphologic and physiologic effects of loss of *ATP6B1* function on the mouse inner ear. *ATP6B1* was first detected weakly at embryonic day 11.5 (E11.5) in the endolymphatic sac epithelia. *ATP6B1* expression was highly specific and heterogeneous in the developing endolymphatic sac through E18.5. In the cochlea, *ATP6B1* was observed from E15.5 in the interdental cells of the spiral limbus. Mice lacking *ATP6B1* showed normal a Preyer's reflex and no obvious vestibular abnormalities (e.g. circling behaviors). Histologic analysis did not reveal any gross malformations or abnormalities of early postnatal mouse inner ears. Interestingly, Auditory Brainstem Response (ABR) and Endocochlear Potential (EP) testing of *ATP6B1* null mutant mice revealed normal hearing and a normal EP. This suggests redundant proton pumping mechanisms in the inner ear that maintain a functional environment despite loss of function of this specific H+-ATPase subunit.

949 The Effects of Hearing Loss on Binaural Performance of Older Listeners

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This study was designed to explore the effects of hearing loss on binaural processing in older adults. Performance was measured on three types of binaural tasks, detection, sound source localization, and speech intelligibility gain.

Binaural performance was measured for listeners over the age of 60 years with and without hearing loss. Following a complete audiological evaluation, the performance of each subject was measured on several binaural tasks. Binaural detection was measured using both a virtual detection task and a masking-level difference task. Virtual speech intelligibility gain was measured in an anechoic and reverberant environment. The speech was presented from straight ahead (0°) and the noise was presented from either straight ahead, near the right ear (+90°) or near the left ear (-90°). Virtual localization ability was measured in quiet and with background interference in an anechoic and a reverberant environment. The target was presented from nine locations in the horizontal plane from +90° to –90°, in 22.5°steps.

Results obtained for older adults with and without hearing loss on these binaural tasks suggest that there is an effect of hearing loss for some binaural tasks, but not for all binaural tasks. Masking-level differences are comparable for listeners with and without hearing loss. Similarly, speech intelligibility gain measured in both an anechoic and reverberant environment is not affected by hearing loss in these older adults. However, on the localization task, performance measured in quiet and in noise, in an anechoic and a reverberant environment, is poorer for the older adults with hearing loss than those with normal hearing. The

older impaired hearing group has root mean square errors that are consistently larger than the normal-hearing group.

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950 Effects of age and frequency region on gap discrimination

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The ability to accurately process temporal information in speech is important for successful communication. Previous studies in our lab and others have shown that: (1) perception of temporal gaps between markers of similar (static) or different (frequency-dynamic) spectral characteristics deteriorates with age, (2) gap perception of all listeners deteriorates as the frequency disparity between markers increases, and (3) gap perception deteriorates with frequency disparity more rapidly for older listeners than young listeners, regardless of hearing status. It has been suggested that temporal gap perception requires different levels of processing depending upon the frequency relationship of the markers bounding the silent gap. The frequency difference at which the processing strategy changes has been estimated at ½ an octave for young listeners with normal hearing (e.g., Formby et al., 1998) but has not been firmly established. The effects of frequency disparity and frequency region on gap discrimination was measured for two groups of listeners with normal hearing: (1) 20-40 yrs and (2) 50-70 yrs. Stimuli were 350 ms sinusoids that were paired and separated by a silent gap of variable or fixed duration. Leading sinusoids were fixed at one of three frequencies (500, 2000, 4000 Hz). Trailing sinusoids were fixed at the same frequency as the lead or varied adaptively above and below the leading frequency. A broadband noise was simultaneously presented to mask gating transients. The study was designed to address three important questions: (1) At what minimum frequency disparity (MFD) between markers does the gap discrimination task require across-channel processing? (2) How does the frequency composition of the markers affect gap difference limens (GDLs) and MFDs? and (3) How are GDLs and MFDs affected by listener age? Preliminary results show larger GDLs for older listeners than for younger listeners, regardless of the frequency region of the markers.

951 The Effects of Aging and Age-Related Hearing Loss on the Neural Representation of Speech Cues.

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Age-related deficits in speech understanding are well documented. Because speech is a complex signal, composed of multiple time-varying acoustic cues, many studies propose that aging adversely affects the ability to process temporal cues. Recently, animal and human data revealed that certain time-varying speech cues, such as voice-onset-time (VOT), are partly dependent on neural responses patterns generated in primary auditory cortex. Therefore, in this study, we examined the effects of aging, and age-related high-frequency hearing loss on the neural representation of VOT.

N1 and P2 late cortical responses were recorded from younger (YNH) and older (ONH) normal-hearing adults, as well as older adults with high frequency sensorineural hearing loss (OHI). N1 responses were evoked by synthetic stimulus tokens from a /ba/-/pa/ VOT continuum. There were significant shifts in N1 and P2 latency with each 10 ms shift in VOT. However, significant age-effects were found. In comparison with younger participants, N1 and P2 latencies were prolonged for older adults with (OHI) and without hearing loss (ONH). Furthermore, response amplitudes were significantly larger for the older group with hearing loss (OHI), than for younger and older listeners with normal-hearing. In addition, older adults (ONH and OHI) had more difficulty than younger adults identifying and discriminating the same VOT contrasts used during electrophysiology testing. Together, these results suggest that aging and peripheral pathology alter temporal response properties in the central auditory system. Furthermore, some of the speech perception difficulties described by aging adults may be due to underlying age-related changes involving structural and/or neurochemical mechanisms regulating excitatory and inhibitory processes.

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952 Discrimination of temporal intervals in complex tone sequences by young and elderly listeners

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The study investigated age effects on auditory temporal processing by comparing the ability of younger and older listeners to discriminate changes of intervals within tone sequences of varying spectral and temporal complexity. The interaction of hearing loss and age on discrimination was also examined. The stimulus sequences were comprised of five 50-ms tones separated by silent intervals, with tone interonset intervals (IOI) that were either uniform in size (200 or 600ms), or non-uniform within a range of values centered at 200 or 600ms. Also, tone frequencies within stimulus sequences were either fixed at 4000Hz, or varied within a range from 2000-4000Hz. Stimuli were presented via earphone at 85 dB SPL, and for each stimulus condition, a duration difference limen (DL) was measured for increments of one or more target IOIs within a sequence. Listeners in the study were younger (18-44 yrs) and older (65-76 yrs) individuals with either normal hearing or sloping sensorineural hearing losses. Results indicate that the measured DLs for all listeners are small for target intervals within uniform-rate sequences and substantially larger for targets within variable-rate sequences. Effects of spectral complexity on discrimination are evident, but less pronounced than those of temporal complexity. The DLs of older listeners were larger than those of younger listeners in each condition, with the greatest age effects observed for variable-rate stimulus sequences. The effects of hearing loss on discrimination performance were minimal.

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953 Longitudinal Study of Vestibular Contributions to Postural Stability in Aging Normal Subjects.

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INTRODUCTION: Is postural stability with increasing age due to aging per se, or due to pathological processes? METHODS: Sway during sensory Organization Test (SOT) conditions was measured in 47 normal subjects 10 years apart and analyzed statistically.

RESULTS: Averages of SOT 5 and 6 peak-to-peak sway amplitudes were clearly larger 10 years after the first test. Sway during SOT 5 increased about 50% ($t=4.86$, 35 df, $P=0.000024$) and SOT 6 increased over 50% ($t=4.59$, 35 df, $P=0.000055$). Fifty percent changes as seen in SOT 5 and SOT 6 would be detectable with a power exceeding 90%. The slopes of the linear regression of SOTs against age were not significantly different from zero for pooled data. For SOT 6 the maximum estimated decline of 0.51 in 10 years is small (about 33%) relative to the standard deviation. Quadratic polynomials were fitted to the data. SOT 6 which previously seemed to have a pronounced minimum did not have statistically significant coefficients ($F(2,69)=0.54$, $P=0.58$). However, when males and females were analyzed separately, the female postural instability increased approximately linearly with age compared to males who showed little

or no change through age 80 years. Sway during SOTs 1-4 did not change.

CONCLUSION: Our findings suggest that the loss of vestibular contribution to postural control with increasing age is a cumulative effect of disease and that females are affected more than males.

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954 Preliminary Evidence for Pharmacological Improvement of Impaired Gap Detection in Gerbils.

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Increasing evidence demonstrates that central auditory processing in the temporal domain is frequently impaired in elderly humans. To test the hypothesis whether this may be partially due to a decline of inhibitory processing in old subjects (e.g., Caspary, Milbrandt and Helfert, 1995, Exp Gerontol, 30:349-360), we evaluated the effect of a drug on auditory temporal resolution. Sabril (Hoechst) increases the levels of GABA in the brain by inhibiting the degradation of GABA released from presynaptic terminals.

Gap-detection thresholds were behaviorally determined in gerbils for stimuli presented at 10dB HL before, during and (if possible) after Sabril treatment (50mg/kg/day). Mean gap-detection thresholds decreased significantly from 13.3 to 9.5ms during Sabril (N=10 gerbils, t-test, $p<0.05$). In a sub-sample of 6 gerbils in which gap detection was measured before, during and after the treatment, thresholds decreased from 11.1ms pre-drug to 8.6ms during Sabril and increased again significantly to 12.4ms ($p<0.05$) after Sabril treatment. Gap detection was little affected by Sabril in those 4 animals that had initial gap-detection thresholds below 10ms (pre drug 6.8ms versus 7.7ms during Sabril). In contrast, mean gap-detection thresholds in the 6 gerbils that initially had elevated thresholds above 10ms decreased from 17.7ms pre-drug to 10.7ms during Sabril. These data support the notion that a decline in the GABAergic system can lead to impaired temporal auditory processing and that a pharmacological boost of the GABAergic system may ameliorate such deficits suggesting new therapeutic strategies for some forms of central auditory processing disorders.

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955 Temporal Resolution in the Aging Auditory System: Gap Detection in Young and Old Mongolian Gerbils (*Meriones unguiculatus*)

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A decline of temporal processing in the auditory system with increasing age has been observed in several studies in humans. To understand the underlying mechanisms, an animal model exhibiting this effect is needed. Age-dependent peripheral hearing loss has been extensively studied in gerbils. Here we present psychoacoustic data comparing temporal resolution of young and old gerbils. We found no significant increase in auditory thresholds for the broad-band noise in subjects up to 3 years of age suggesting that gap thresholds in older gerbils are not affected by a substantial peripheral hearing loss.

The minimum audible gap in a broad-band-noise stimulus was determined at 10dB and 30dB above the individual's threshold for broad-band noise (10dB SL, N=12 and 30dB SL, N=21, respectively) and at a fixed presentation level of 50dB SPL (N=23). For each of these presentation levels, the average gap-detection threshold was approximately 40 to 60% higher in the old (>30 months) as compared to the young gerbils (<20 months). The difference between young and old gerbils was significant at a presentation level of 50dB SPL. Over 90% of the young animals had gap-detection thresholds below 3ms. In contrast, the distribution of gap-detection thresholds in old animals were more variable and extended towards higher values. The majority (60%) of old animals had thresholds over 3ms. The overlap of data from young and old gerbils and the increased variability of gap-detection thresholds in old gerbils resembles observations that compared gap detection in young and aged human listeners (Snell and Frisina, 2000 J. Acoust. Soc. Am. 101:2214-2220) suggesting that gerbils provide a suitable model to further study age-dependent deficits of temporal processing. Supported by the DFG (Str. 275/4-1).

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956 Topographic Auditory Diagnosis Of Patients With Menière's Disease: Selective Damage Of The Outer Hair Cells

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Menière's disease (MD) is a clinical entity with manifestations suggestive of an inner ear disorder. Advances in medical technology, with the recent application of otoacoustic emissions (OAEs), have enabled more precise clinical definition of this condition.

This study describes the findings in 92 patients with MD, attending a neuro-otology clinic over a period of one year. They underwent detailed auditory assessment, including standard pure tone audiometry (PTA), tympanometry, stapedial reflexes (SRs), OAEs; brainstem evoked response (ABR) and CT and/or MRI for exclusion of retrocochlear pathology. In addition, 20 patients underwent cortical evoked audiometry (CERA).

PTA showed elevated thresholds, at two or more frequency (0.5-2 kHz range): 30-40 dB in 23%, 40-70 dB in 68% and >70dB in 9% of the patients. SRs were obtained in 90% of the patients. OAEs were recorded in 61% of the patients, 73% of whom had PTA thresholds >40dBHL. CERA confirmed the presence of a hearing loss, except in one patient. However, dissociation between PTA and CERA was observed in more than a half of the patients.

The findings of a mild/moderate hearing loss and present SRs (which indicate preserved IHCs) in the overwhelming majority of the patients, is suggestive of a selective lesion of the outer hair cells (OHCs). This is supported by previous findings that a selective OHC lesion reduces hearing sensitivity by ~ 40 dB and that MD becomes stable when hearing thresholds reach ~ 50-60dB level. The presence of OAEs in a significant number of patients with reduced hearing sensitivity implies some form of functional cochlear abnormality.

In summary, this study provides the evidence suggesting that a selective OHC lesion, functional or structural, is the typical pathophysiological feature of MD. The loss of the non-linearity, for which OHCs are assumed to be responsible, may explain a number of auditory symptoms in MD.

957 The Correlation Between Pure-tone Audiometric and Cortical Evoked Thresholds in Patients with Menière's Disease

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Traditionally, auditory sensitivity in patients with Menière's disease (MD) has been evaluated by pure-tone audiometry (PTA). More recently, otoacoustic emissions (OAEs) have allowed direct evaluation

of the outer hair cells. Despite the general view that OAEs are not recordable if PTA thresholds exceed 35 dBHL, in a considerable number of patients with MD, OAEs are recorded even when thresholds are greater than 40 dBHL, suggesting the possibility of some form of functional cochlear abnormality. The perceptual ability of these patients might also be reduced by different auditory symptoms. Therefore, it is of importance to establish the relationship between behavioural (PTA) and objective (cortical evoked) responses, which, according to previous studies, correspond very closely (within 10 dB).

Standard PTA and cortical audiometry (CERA, at 0.5, 1 and 4 kHz) were performed in 19 patients with MD: 10 with and 9 without recordable OAEs; 9 with "active" and 10 with "stable" disease. In all patients, PTA thresholds were 40 dBHL or worse at least at two frequencies in the range 0.25-2 kHz.

The results showed a difference of 15-55 dB between PTA and CERA in 46% cases, of which 82% ($p<0.002$) had "active" disease and 62% had recordable OAEs. The remaining 54% cases displayed a difference between PTA and CERA of within 10 dB.

This discrepancy suggests that perception of pure tones in nearly one half of MD patients in this study does not reflect the electrical activity in the auditory cortex. The presented tones are likely to be distorted and may compete with tinnitus, especially in patients with "active" disease. However, it would be difficult to accept that auditory symptoms affect behavioural thresholds more than 10-15 dB. It may be possible that some unknown pathophysiological mechanism underlying MD leads to different sound processing compared with patients suffering from sensorineural loss of other aetiologies.

958 Hearing Loss as a Prognostic Factor for Unsucessfull Result after Gentamycin Treatment for Ménière's Disease

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The use of intratympanically administered gentamycin is an accepted method of treatment in patients with incapacitating Ménière's disease.

This is a retrospective study of 92 patients treated with gentamycin as a therapy for unilateral Ménière's disease that did not respond to medical treatment.

An assessment of the level of hearing was performed both before and after therapy. The pure-tone thresholds, decibel (dB) hearing level and findings were reported in terms of pure-tone average (mean thresholds at 500, 1000, 2000, and 3000 Hz). The speech discrimination score was assesed using phonetically balanced words (PB) correctly repeated at 30 dB sensation level or the most comfortable sensation level. The treatment protocol we used consisted in weekly transtympanic injections of gentamycin (27mg/ml). Patients returned for weekly follow-up evaluations during the course of the treatment.Special attention was given to the appearance of spontaneous and horizontal head-shaking nystagmus and, a hypometric response to the head-thrust manoeuver. An audiogram was performed. Titration of gentamycin was determined to be complete when any of the bedside tests that previously proved to be negative, gave a positive result at the time of the follow-up visit. We considered a second course of injections when any of the patients referred any syntom of vertigo after the first course. All the patients have been monitored for at least 2 years after the termination of the treatment.

When we compared those patients who reached control of vertigo with a single course of injections, to patients who needed two courses, we found significant differences in the pure tone average values of both groups. Those patients whom we performed an only course of injections, had a PTA loss of 12 dB in the first week of treatment. The second group of patients ,had an initial PTA loss of 6 dB, during the first course of injections, and needed further on, a second course of injections.

959 Auditory sensitivity in individuals with history of noise exposure and opiate abuse

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The current report is based on auditory thresholds obtained from 23 male individuals with a history of opiate abuse. Participants consisted of patients admitted to Stanley Street Treatment and Resource of Rhode Island (SSTAR of RI), an alcohol and drug detoxification center. Twelve of the individuals reported history of noise exposure due to hobbies and 7 individuals reported occupational noise exposure. Four individuals reported no history of noise exposure. Auditory thresholds were determined in the frequency range from 250 to 8000 Hz. Multivariate Analyses of Variance was performed to detect differences in auditory thresholds between the three noise exposure types (no noise exposure, occupational noise exposure and hobby-related noise exposure), ear (left and right) and test-frequencies. No main effects or interactions were apparent except for a significant interaction between the noise exposure type and frequency. Post-hoc analyses with the LSD test revealed that occupation noise exposure resulted in significantly higher auditory thresholds at 0.5, 2, 3, 6 and 8 kHz when compared to no noise exposure. In addition, hobby related noise exposure resulted in significantly higher auditory thresholds at 8 kHz when compared to noise exposure. Auditory thresholds obtained from individuals exposed to occupational noise exposure were also significantly higher at 0.25, 1, 2, 3 and 6 kHz when compared to those obtained from individuals with hobby related noise exposure. Seventeen of the individuals had auditory thresholds above 20 dBHL at least at one frequency in at least one ear, suggesting the presence of a hearing loss in 74% of the individuals. Similarly three of the 4 individuals not exposed to noise had a hearing loss. Detailed data will be presented.

960 Hearing Levels of Firefighters

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Although much is known about occupational noise induced hearing losses (ONIHL)in "typical" industrial environments, where individuals work 8 hours per day in more or less constant noise, less is known about ONIHL from "nontypical" exposures, such as those experienced by firefighters. Firefighters work longer shifts, are exposed to short duration, high level sounds from emergency warning signals and rescue equipment, and often work in environments contaminated by other toxic agents, such as smoke. We evaluated the results of hearing tests, obtained from company-wide hearing conservation programs of firefighters working in two large metropolitan communities. A total of 1432 male subjects were selected for the study who had at least seven annual audiometric tests. Differences in hearing between the first and last test, and comparisons of the data with age-matched control data obtained from national and international standards were evaluated. It was found that the changes in firefighters' hearing did not exceed changes expected on the basis of age alone. These data are consistent with published studies of occupational exposures, which suggest that although firefighters are occasionally exposed to high level noise, their daily dose does not exceed that considered hazardous by the Occupational Safety and Health Administration.

961 Characteristics of Auditory and Brain Cognitive Function in Aging of China

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An Auditory,Brain Cognitive Function Research work were performed in elder group(Ge),age ranged from 60-74,95cases;Yong group(Gy),age ranged from 21-30,85 cases,as a control group.The test item include:pure tone Audiometry(GAI-16,0.25-8.0KHz);Middle ear analysis(GSI-33);Acoustic Brainstem Evoked Response(SANYO-7S11A);Brain Cognitive function test, include Mental Arithmetric Index,Numeric Span,Pairing of Symbol with Numeric,Elimilate test,and Visual,Auditory Signal Processing Speed test,introduce man-machine interaction;P300 Acoustic Evoked Potential record from Cz point of skull,introduced auditory oddball paradigm.The testing results showed that in Ge group,the sensitivity for PTA were decreased from 14.28-61.84dB,as Gy group were 6.67dB.The discrimination Score of Chinese Word-PBmax for Ge group were decreased from 92.8-62.3%,but for the Gy group were 95.6%.The threshold of contralateral stapedial reflex in Ge group elevated from 90.94-98.75dB,but for the Gy group were 76.16. The percentage of recruitment ear (1KHz tone) increased from 6.3-100%,but for Gy group were 0%.On ABR records, the 1-5 interpeak latency for Ge group were in normal range(4.18-4.19ms),as the Gy group were 4.11ms. All brain cognitive function test score of Ge group were diminished from 38.40 to 51.37,as compared with Gy group were 53.26 to 59.10.The information processing speed(IPS)measured from visual and auditory signal processing speed test of Ge group were 2.25,but for the Gy group were 2.68.The P300 wave latencies of Ge group prolonged(327.75ms) as compared with Gy group (317.88ms),but the amplitude of P300 for Ge group were significantly decreased to 7.28uV,as compared for Gy group(14.14uV).The conclution was:The characteristic of auditory and brain function in aging presented mild to severe senso-neural hearing loss,and it showed a wide range of degeneration from the ending(cochlear)to central part of auditory system;So it must be pay attention to it for the auditory rehabilitation.

962 Microdefects in Cochlea as a Possible Cause for Obscure Auditory Dysfunction

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The main characteristic of obscure auditory dysfunction (King-Kopetzky syndrome) is discrimination problems in listening situations in background noise while the audiogram shows normal or slight subnormal pure tone thresholds. It has been proposed that the syndrome is caused by a central defect such as e.g. auditory neuropathy. However, since the syndrome causes similar discrimination problems as those experienced by almost all patients with well-documented cochlear defects we have searched for cochlear defects in patients with obscure auditory dysfunction.

We tested eight patients referred consecutively to our department. All patients reported difficulties in understanding speech in slight background noise, whereas they were having absolutely no problems in quiet surroundings, even if the person they were talking to was whispering.

Audiograms measured in the clinic all showed normal thresholds (better or equal to 20 dB HL) apart from a slight symmetrical loss at 6 and 8 kHz for 3 patients. We performed fine-scaled adaptive audiometry testing (1/6 octave frequency steps) to search for cochlear microdefects not showing up in standard audiogram examination. All our patients showed a marked increase in threshold ranging from 4 to 8 dB in a frequency band of 400 to 800 Hz. The band was typically located in the frequency area between 1 and 3 kHz. Also, psychophysical tuning curves (1/6 octave steps, sinusoidal masker) showed a localized defect in relatively narrow frequency range. In conclusion, fine-scaled audiometric measurement suggest that cochlear defects may be the cause of some of the cases of obscure auditory dysfunction.

963 Specific Language Impairment and Auditory Processing Skills

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Children with Specific Language Impairment (SLI) and those diagnosed with Auditory Processing Disorder (APD) share characteristics like deficits in language learning and listening comprehension. Reported research suggests that children diagosed with SLI demonstrate deficits in auditory processing skills. This project investigated auditory processing skills of twelve 7-8 year-old children. Six children were placed in the SLI group based on: age-appropriate academic skills as determined by their teacher; normal intelligence as measured by the Kaufman Brief Intelligence Test; below appropriate age-level score on the Test of Language Development Primary 3rd edition; absence of emotional/psychiatric disorder; absence of neurological impairment. The control group consisted of six children matched for academic skills and intelligence, with no history of language disability. All subjects had normal hearing sensitivity at the time of testing. The test battery included the Staggered Spondee Word (SSW), the Duration Pattern Sequence (DPS), the Pitch Pattern Sequence (PPS), and Auditory Brainstem, Middle, and Late Responses (ABR, MLR, LAER). The SLI group scored lower on average on all of the behavioral auditory processing tests with significantly lower scores on the PPS test. This study showed that children with SLI tend to score lower on verbal and non-verbal auditory processing tests. Therefore, auditory processing measures may be a valuable addition to the differential diagnosis and treatment of SLI.

964 Self-perceived Dizziness Disability/Handicap Correlates of "Compensated" and "Uncompensated" Unilateral Peripheral Vestibular System Impairment

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The objective of the present project was to determine whether conventional definitions of "compensated" and "uncompensated" peripheral vestibular system disease can be validated by measures of self-perceived dizziness disability/handicap.

Test results from 115 consecutive patients were reviewed retrospectively. Subjects underwent electronystagmography (ENG) and rotational testing and completed a Dizziness Handicap Inventory (DHI; Jacobson and Newman, 1990). Patients were placed into four groups based upon the following ENG and rotary chair test results: Group 1 - Normal caloric examination, no slow phase velocity (SPV) phase leads, no SPV gain reductions or asymmetries; Group 2 - Significant UW, SPV phase lead at .01 Hz, SPV gains normal .01-.32 Hz, no SPV asymmetry; Group 3 - Significant UW, SPV phase leads at 3 or more adjacent frequencies from .01-.32 Hz, SPV gain normal, no SPV asymmetry; Group 4 - Significant UW, SPV phase leads present at 1 or more adjacent frequencies .01-.32 Hz, SPV gain normal, SPV asymmetry present at 3 or more adjacent frequencies .01-.32 Hz. The data were subjected to an ANOVA. The dependent variables were, separately, DHI total score, and functional, emotional and physical subscale scores. The different patterns of ENG and rotary chair test results (Groups 1-4 described above) served as the grouping factor. Results indicated a systematic increase in DHI total and subscale scores for Groups1-4. These findings are discussed in the context of common physiological definitions of vestibular system compensation.

965 Sound Levels Used in Tinnitus Retraining Therapy

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Sound therapy is an integral part of Tinnitus Retraining Therapy (TRT). While TRT can be implemented without any instrumentation, wearable broad-band sound generators are frequently used. The sound used in TRT should not suppress ("mask") tinnitus, while it should be above level of potential range of stochastic resonance. There is no information about sound levels actually used by patients.

A modified utilization of Real Ear Measurement allowed assessment of the sound level and its spectrum in the ear canal of a patient in 4 different situations: 1) background environmental sound, 2) output of sound generators at patient's hearing threshold, 3) output at the beginning of partial suppression ("mixing point"), or maximum level acceptable for long term use in case of patients with dominant hyperacusis, 4) maximum tolerable level, or maximum output available from the instruments. Measurements were performed for each ear separately.

Evaluation of results from 90 patients and 258 measurements revealed that they are using sound from the threshold up to 30 dB SL, with the mean of 10.7 dB SL. 66.3% uses sound within the range of 7 to 20 dB SL, which is considered to be optimal for TRT, with 26.4% of cases with sound set at the level which potentially might evoke stochastic resonance effect, and 7.4% set above 20 dB SL, being in the range which might evoke annoyance, interfere with speech perception, or cause partial suppression of tinnitus.

These results point out the need of systematic evaluation of sound used by patients by Real Ear Measurements.

966 Tinnitus Retraining Therapy in Treating Tinnitus and Hyperacusis in Children

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Tinnitus and hyperacusis are recognized, but under-reported in pediatric population. These complaints are frequently ignored and children are considered as having behavioral, psychological or psychiatric problems. Recent data indicate that while children rarely report this symptom on their own, tinnitus is actually quite common, particularly in children with hearing loss. When children complain of tinnitus, it is most likely problematic, and should be taken seriously. In the past the management of tinnitus and hyperacusis in children was focused mostly on underlying pathologies, depressive and psychological components.

Tinnitus Retraining Therapy (TRT) was adapted to treat childhood tinnitus and hyperacusis. Specifically, the counseling process was modified, the language and examples adjusted to be age appropriate, and a separate part was added to simultaneously counsel parents. In sound therapy, more stress was put on the enrichment of the background sound, considering school environment. Contrary to popular expectations, a significant proportion of children expressed willingness to wear and use sound generators or hearing aids, and reported regular use during follow up contacts.

Ten children, age 5 to 16 responded very successfully to TRT, with time needed for recovery less than 12 months, even in the most extreme cases. The comparison of specific elements of TRT and the treatment outcome between pediatric and adult groups will be presented. It appears that on the average children tend to have faster recovery and higher probability of success.

967 Intracellular Anions as the Voltage-Sensor of Prestin, the Outer Hair Cell Motor Protein

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Outer hair cells (OHCs) of the mammalian cochlea actively change their cell length in response to changes in membrane potential. This electromotility, thought to be the basis of cochlear amplification, is mediated by a voltage-sensitive motor molecule recently identified as the membrane protein prestin. Structural rearrangements of the protein are driven by the movement of a charged voltage sensor through the electric field, giving rise to the characteristic voltage dependent charge movement or non-linear capacitance of the OHC. We attempted to identify this voltage sensor, a key element in electromechanical transduction by prestin.

Mutational neutralization of candidate charged amino acid residues of prestin failed to eliminate charge movement and thus to identify a protein domain as the voltage sensor. In contrast, we show that voltage-sensitivity is conferred to prestin by the intracellular anions chloride and bicarbonate. Removal of these anions abolished non-linear capacitance as well as fast voltage-dependent motility. Upon removal of intracellular Cl^-, prestin adopted its contracted conformation, independent of the membrane voltage. Moreover, the characteristics of voltage-dependent charge movement were dependent on the anion species present at the cytoplasmic side of the membrane. In inside-out patches from OHCs, the amount of translocated charge systematically varied with different monovalent anions ($I^- > Br^- > NO_3^- > Cl^- > HCO_3^- > F^-$) and the steepness of voltage dependence decreased with increasing size of the anion (formate > acetate > propionate > butyrate). No charge movement occurred with the divalent sulfate.

The results support a model in which anions act as extrinsic voltage-sensors, that bind to the prestin molecule and thus trigger the conformational changes required for motility of OHCs.

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968 C-terminus Deletion Mutants of the Prestin Molecule

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Amplification in the mammalian cochlea is attributed to the activity of outer hair cells. These specialized neuroepithelial cells possess electromotility that results from the concerted action of molecular motors driven by changes in transmembrane potential. The gene encoding this motor has been identified as *Prestin*. Electrical stimulation produces voltage-dependent gating currents that result in a nonlinear bell shaped capacitance-voltage function, commonly used to evaluate the activity of the motors. Although it belongs to a family of anion transporters called SLC26, only prestin displays this unique nonlinear capacitance (NLC). Hydrophobicity plots and antibody labeling have revealed the intracytoplasmatic location of the N and C-termini. To understand which portion of the molecule is responsible for its activity, different portions of the C-terminus were deleted and expressed in TSA 201 cells. Our results show that deletion of amino acid residues 590-744 completely eliminates NLC. Immunoflourescence experiments confirmed expression of the mutant form of the protein and its localization to the plasma membrane. To test this mutant further, wild-type (WT) prestin was cotransfected with Del 590-744, and its appropriate expression and localization were confirmed. This procedure resulted in a significant decrease in the value

of Qmax, the maximum charge transfer, from the WT control value. No other parameters in the capacitance function were significantly affected. Previous studies examined mutations of the different charged intramembranous residues (Oliver, et. al., 2001). This project has demonstrated that the C-terminus has an important role in the electrophysiology of prestin.

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969 Tension-dependent Chloride Current affects OHC Capacitance

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The mammalian cochlear amplifier is likely driven by a single protein, prestin, expressed exclusively in OHCs. Recently, Oliver et al, (2001) determined that Cl- ions, on the intracellular side, work as voltage sensors for this protein. We studied the effects of Cl- concentration and conductance on the electrical signature of prestin's voltage sensor, viz., its nonlinear capacitance (Cm), in intact OHCs under voltage clamp.

In the presence of 140 mM extracellular Cl- (all other ions impermeant), peak nonlinear Cm decreases by only 1/3 after patching with Cl- free pipette solutions. Reducing extracellular Cl-, which we simultaneously monitor as a decrease in Cl- current, further reduces nonlinear Cm; however, even at zero Cl- outside, substantial nonlinear Cm remains, and is indicative of either an intracellular store (subsurface cisternae), or a restricted compartment (space between subsurface cisternae and lateral plasmalemma).

The Cl- current has a sigmoidal I-V curve, though it is maximally activated at hyperpolarized potentials. We evaluated the effects of membrane tension in the presence or absence of intracellular and/or extracellular Cl-. In the absence of Cl- inside and out, we find virtually no change in the I-V function of OHCs in response to membrane tension. Under these same conditions, a small residual nonlinear peak Cm of several pF remains that is susceptible to changes in turgor pressure, i.e., shifts in Vpkcm are evident. In the presence of Cl- outside only, an increase in stretch activated current is observed with a corresponding increase in nonlinear Cm.

Our data confirm that Cl- has profound effects on prestin-generated Cm, but we find that, in the intact OHC, extracellular Cl-, and possibly an intracellular store play important roles. The existence of a stretch-activated, current driven control of the OHC motor may be very important for high frequency activity of the cochlear amplifier.

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970 Effect of the Membrane Piezoelectric Properties on the Outer Hair Cell Receptor Potential under High-Frequency Conditions

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Equivalent circuit analysis of the outer hair cell (OHC) based on conventional electrical properties of the stereocilia and the cell membrane predicts a low-pass frequency response that prevents its role as the cochlear amplifier at high frequencies. We have found that the band pass characteristics can be improved by introducing the piezoelectric properties of the cell membrane. This introduces an additional, strain-dependent, capacitive-type current in the cell membrane. In our analysis, we have included the membrane longitudinal and circumferential piezoelectric coefficients, which we have previously estimated, together with the electrical properties of the stereocilia and the cell membrane. We have considered a cell strain that the OHC would experience in situ for a low-level acoustic signal. To estimate the membrane strain, we developed a model of the OHC deformation in the organ of Corti where an individual cell interacts with two planes representing the vibrating cochlear partitions. The OHC is inclined in the longitudinal and transverse directions. Because of this, the strain caused by axial loading is accompanied by a bending-related component. A roll-off was still observed but, in contrast to conventional analyses, the receptor potential did not tend to zero and at any frequency was greater than a limiting value. The limiting value of the receptor potential depends on the ratio of the typical strain in the membrane to the membrane capacitance. We have found that for the original (low-frequency) value about 2-3 mV and the strain level about 0.1% the receptor potential is greater than 0.2-0.3 mV throughout the whole frequency range. In addition, the phase shift between the transduction current and receptor potential tends to zero. These results suggest that the membrane piezoelectricity can contribute to high frequency receptor potentials in the outer hair cells.

971 Effect of cytoskeleton modulators on outer hair cell lateral wall membrane mobility and motility

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Outer hair cell (OHC) electro-mechanical force generation is distributed along the cylindrical cell's lateral wall. The lateral wall consists of the plasma membrane (PM), cortical lattice (CL), and subsurface cisterna (SSC). The CL is a highly organized 3D structure with circumferential F-actin filaments, spectrin in an approximately longitudinal orientation parallel to the cell's long axis, and radially oriented pillars that connect the PM to the actin filaments. We have reported that cell signaling pathways are involved in the ballooning deformation and recovery of local damage to the CL. H-7, a protein kinase C inhibitor, enhanced the damage but slowed cytoskeletal self-repair, while H-89, a protein kinase A inhibitor, accelerated and enhanced the damage. In contrast, jasplakinolide (JASPA), an F-actin stabilizer, prevented the ballooning deformation but did not eliminate changes in cell length. In this experiment, the effects of these CL modulators on PM lateral diffusion and OHC electromotility were investigated. Lateral diffusion was measured using fluorescence recovery after photo bleaching. H-7 decreased the lateral diffusion of the PM (diffusion coefficient, D= 2.42±0.15, n=48 vs 3.04±0.16 in normal control) while H-89 increased the diffusion (D= 3.47±0.31, n= 47). However, H7 and H89 together dramatically decreased the diffusion coefficient (D=1.93±0.27, n=12). JASPA did not alter the lateral diffusion (D=3.03±0.79, n=11) and OHC electromotility measured by voltage-dependent nonlinear capacitance with whole cell patch recording. However, 3 μM of JASPA either in bath solution or in patch pipette resisted the spontaneous negative shift of the peak capacitance voltage in whole cell recording. The data demonstrate interactions between the PM and the CL.

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972 Ca^{2+}-dependent Slow Motility of Cochlear Outer Hair Cells is a By-Product of Axial Stiffness Regulation

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Two Ca^{2+}-dependent mechanisms regulating the mechanical properties of cochlear outer hair cells (OHCs) have been proposed. One proposes that the efferent neurotransmitter, acetylcholine, decreases OHC axial stiffness [Dallos et al, 1997, J.Neurosci. 17: 2212-26] without an appreciable effect on OHC electromotile mechanism [Frolenkov et al., 2000, J. Neurosci. 20: 5940-8]. The other suggests that elevation of intracellular free Ca^{2+} triggers an active contractile processes resulting in the elongation of OHC, known as "Ca^{2+}-dependent slow motility" [Dulon et al., 1990, J. Neurosci. 10: 1388-97]. Both phenomena may significantly affect the operation of the cochlear amplifier that is based presumably on the active cycle-by-cycle movements of OHCs. Here we provide evidence that both phenomena may share the same mechanism.

We observed elongation of OHCs following different procedures producing the increase of free intracellular Ca^{2+} concentration: extracellular application of Ca^{2+}-ionophore ionomycin, release of caged Ca^{2+} by UV photolysis, and stimulation of intracellular Ca^{2+}-stores with flash-photolysis of caged inositol 1,4,5-triphosphate. In all these cases, elongation of OHC occurred in the whole-cell patch-clamp conditions when the intracellular potential was fixed, suggesting that cell hyperpolarization via activation of Ca^{2+}-dependent K^{+}-channels cannot be responsible for Ca^{2+}-induced OHC elongation. Meanwhile, the elevation of intracellular Ca^{2+} resulted in the increase of voltage-driven OHC motile responses and did not alter voltage-dependent capacitance of OHC. Both effects are similar to that of acetylcholine and they are usually associated with the decreased axial stiffness of OHC. In OHCs partially collapsed by applying small negative pressure via patch pipette, elevation of intracellular Ca^{2+} was not able to restore the cylindrical shape of OHC, which is expected if Ca^{2+}-induced OHC elongation would be driven by circumferential contraction of the lateral wall. The same cell became cylindrical during voltage-driven motile responses. We concluded that unlike the active voltage-driven motile responses of OHCs, Ca^{2+}-induced elongation is most likely a passive reaction of the turgid cell to a decrease of axial stiffness.

973 Local Mechanical Properties of the Outer Hair Cell Lateral Wall Measured by Atomic Force Microscopy

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In vivo, the OHCs are located between the reticular lamina and Deiters' cells on the basilar membrane, and the force produced by the OHC, which accompanies its motility, is considered to amplify the motion of the basilar membrane. As a result, the force production of the OHC presumably leads to the fine tuning of the mammalian cochlea. Since the force production of the OHC is known to be related to their mechanical properties, it is important to understand these properties. The longitudinal stiffness of the whole cell has been reported to be 3.0 mN/m based on measurement of the change in length of the OHC in response to the external stimulus (Zenner et al., Acta Otolaryngol. (Stockh.) 112: 248-253, 1992). Hallworth (J. Neurophysiol. 74: 2319-2329, 1995) showed the axial stiffness of the OHC to be in the range of 0.8 – 25 mN/m and Ulfendahl et al. (Pflügers Arch. Eur. J. Physiol. 436: 9-15, 1998) reported that the mean axial stiffness is 1.1 mN/m. However, local mechanical properties have not been clarified. In this study, therefore, an attempt was made to elucidate the local mechanical properties of the OHC using an atomic force microscope. Results revealed that Young's modulus in the apical part of the OHC was larger than that in the other parts and that Young's modulus of the OHC was inversely proportional to the natural length of the OHC.

974 Relationship between the Local Stiffness of the Outer Hair Cell along the Cell Axis and its Ultrastructure Observed by Atomic Force Microscopy

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Recent investigations have shown that the isolated outer hair cell (OHC) can elongate and contract in response to an electrical stimulation. This electromotility is suggested to produce the force and magnify the deformation of the organ of Corti. As a result, the high sensitivity and the sharp tuning of our auditory system are made possible. As the electromotility would arise from a conformational change of the molecules' "protein motors" which might be distributed along the OHC lateral wall, the force generated by the OHC electromotility would be related not only to the conformational change of the protein motors but also to the mechanical properties of the lateral wall. Therefore, a detailed understanding of the mechanical properties of the OHC lateral wall is important. In our previous paper (Wada et al., 2001), for the purpose of understanding the difference in the stiffness along the cell axis, the local deformation of the OHC in response to hypotonic stimulation was analyzed by measuring the displacement of microspheres attached randomly to the lateral wall of the cell. The investigation revealed that the stiffness of the cell in the apical region was higher than that in other regions, where the stiffness is constant. In this study, the ultrastructure of the OHC lateral wall was investigated by atomic force microscopy (AFM), and the relationship between the stiffness along the cell axis and the ultrastructure that was observed by the AFM imaging was analyzed. From the analysis, it is concluded that the circumferential filaments observed in the AFM tapping mode would be actins which are part of the cortical lattice, and that the difference between the intervals of the circumferential filaments in the apical region and those in other regions is one factor that causes the high stiffness in the apical region.

975 Depolarization And ATP Induced Movements of The Stalks of Isolated Deiters' Cells.

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ATP receptor agonists and antagonists placed in perilymph change cochlear mechanics as monitored by distortion product otoacoustic emissions (e.g., Chen et al., Hear. Res. 118, 47-61, 1998). ATP receptors are present on the perilymph exposed surfaces of cells in the cochlea, including Deiters' cells. Deiters' cells are positioned to affect cochlear mechanics by changing the load they apply to the OHCs. The hypothesis that application of ATP can change the mechanical properties of Deiters' cells was examined.

Deiters' cells were isolated from guinea pig cochlea and bathed in artificial perilymph (MHBS). Drugs were applied to Deiters' cells by iontophoresis and by pressure from micropipettes. The motions of the stalks of the Deiters' cells were monitored with an inverted microscope and video camera. Measurements were made from the video tapes.

The stalks of many Deiters' cells slowly moved away from the cell body (uncurled) or towards the cell body (curled) without a stimulus being applied. Only stable cells were tested. Iontophoretic application of Cl^{-}, a control, and ATP^{-} by anodal current (10 to 50 nA applied in concurrent one min steps) induced a movement of Deiters' cell stalks. There was no difference between the response to Cl and ATP, suggesting that depolarization of the cell induced the movement. Pressure ejection of ATP (100 μM) but not the drug vehicle MHBS onto Deiters' cells induced a movement of their stalks. The movements were several microns (2.7 ± 0.4 μm; mean ± s.e.m.; n=7), occurring after a latency of 1- 4 min, peaking at 7 min, recovering at 18 min, and curling or uncurling in an equal number of cells.

Results support the hypothesis that ATP released onto Deiters' cells modulates cochlear mechanics by affecting the mechanical properties of Deiters' cells.

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NOTES

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