2008-2009 HEARING & BALANCE RESEARCH GRANT RECIPIENTS

Each year since its inception, the Deafness Research Foundation (DRF) has funded promising research in the field of hearing and balance science. This research, which most likely would not have happened without DRF funding, has led to dramatic innovations that increase options for those living with hearing and balance loss as well as protect those at risk.

DRF continues to live up to its well-established reputation as the leading source of private funding for research in hearing and balance science in the United States.

DRF FIRST YEAR HEARING & BALANCE RESEARCH GRANT RECIPIENTS
Funded July 1, 2008 through June 30, 2009

R. Michael Burger, Ph.D., Lehigh University
Efferent Loizounction in Sound Localization Processing

Auditory processing relies on precise coding of acoustic features to build an accurate internal representation of the environment. Sensory systems build this representation through faithful encoding of sensory stimuli at the level of sensory organs. This neural signaling is enhanced by active feed back on sensory neurons from higher central processing centers. These "efferent" pathways have been characterized for the cochlea and to some extent, in the midbrain. There is little data efferent function in the early stages of auditory processing in structures that process sound location information. This may be due, in part, to the complexity of this system in mammalian circuits. The bird auditory system is a major model for human sound localization processing. In deed, birds process ascending circuitry that is strikingly similar to mammals in structure and function, but with efferent circuitry that is appealingly simple. My aim is to investigate this elegant efferent brain stem circuit in birds to build comprehensive model of its function within this functionally understood auditory circuit. These studies will both characterize the neurons responsible for this feedback, and examine their impact on their targets. The long-term objective is to build a mechanistic understanding of sound localization circuitry in vertebrate systems.

Snezana Levic, Ph.D., University of California, Davis
Mechanism of Hair Cell Development and Regeneration

Hair cells (HCs) convert sound signals into electrical impulses in the cochlea with remarkable precision and sensitivity. Our long-term goals are to stimulate HC regeneration in human inner ears in a controlled fashion, and to enable the functional innervation of the regenerated HC's by spiral ganglion neurons (SGNs). To do this, the functional mechanisms of the development of HCs must be understood. In the developing systems, there is temporal overlap between ion channel development, spontaneous activity and activity-dependent development. Therefore, APs could serve both intrinsic (ion channel expression) and extrinsic (neuronal refinement) development roles. We have identified differential patterning of APs in the developing cochlear axis that reflects differences in expression of ionic conductances. We will test the prediction that the activity of HCs influenced that of its neighbors, including the preservation of the synaptic transmission between auditory nerve and HCs. The proposed study will increase our understanding of the activity dependent development in auditory system.

Geng-Lin Li, Ph.D., Oregon Health & Science University
Auditory signal coding at the hair cell ribbon synapses

The sense of hearing starts at hair cells, which connect to afferent fibers via ribbon synapses. Across these synapses, auditory signals contained in graded potentials on hair cells are transformed into all-or-none spikes on afferent fibers. Therefore, these synapses face the tremendous challenge of continuous coding of auditory signals over a remarkable dynamic range. It is not well understood how these specialized synapses achieve their extraordinary ability to release continuously transmitter. This has greatly impaired our ability to treat hearing loss. The long-term objective of this study is to investigate mechanisms of synaptic transmission and strategies for auditory signal coding at this very first chemical synapse along the auditory pathway. In 2 years, the specific aims are: 1) To study multivesicular release and its mechanisms; 2) To determine how the release of vesicles is transformed to spikes on afferent fibers; 3) To investigate short-term plasticity and how it helps the coding of auditory signals.

Kathleen McNerney, Ph.D., State University of New York at Buffalo
The Vestibular Evoked Myogenic Potential - Unanswered Questions Regarding Stimulus and Recording Parameters

The vestibular evoked myogenic potential (VEMP) is a response that can be recorded from the sternocleidomastoid (SCM) muscle as well as other neck muscles such as the trapezius. It is believed to be generated by the saccule, which is a part of our vestibular system that is normally responsible for our sense of balance. Recent studies have shown that it is also responsive to sound. Three types of stimuli that are used to elicit the VEMP are air-conducted (AC) stimuli, bone-conducted (BC) stimuli, and galvanic (electrical) stimuli. Although there are several universal findings that have held true throughout previous studies, there are several questions which remain unanswered. The present study will attempt to address these issues by making a direct comparison between the three types of stimuli listed above, within the same subjects. In addition, input/output functions will be defined for all three types of stimuli. Finally, we will be looking at the repeatability of the three types of stimuli across subject, as well as address the inconsistencies that have been found between monaural and binaural stimulation. This study will not only provide us with a better understanding of the VEMP, it will also enhance its clinical utility.

Christian N. Paxton, Ph.D., University of Utah

The Role of Fgf4 in Otic Placode Induction

Development and patterning of the inner ear is a complex process that is mediated by several signaling molecules, including members of the Fibroblast growth factor (FGF) family. We recently found that Fgf4 is expressed in the earforming region just prior to the induction of ear development. Fgf4 has not previously been described in the induction or formation of the inner ear. Based on its temporal and spatial pattern of expression we hypothesize that Fgf4 is involved in the early processes of ear development and propose to investigate its role(s) in these processes by determining whether it is sufficient and/or required to induce the early stages of inner ear development. We also will examine the signals responsible for localizing Fgf4 expression to the otic forming domain.

Iris Schrijver, M.D., Stanford University of Medicine

The functional impact of single and dual expression of GJB2 missense variants V271 and E114G: An exploration of pathogenic effects on hearing

It is difficult to predict the consequences of DNA alterations the result in replacement of one protein building block by another. Yet, an important aspect of genetic testing is to predict whether a DNA change is harmful or not. With this project, we will solve this dilemma for two relatively common variants in the connexin 26 gene. Interestingly, it appears that these two variants do not contribute to hearing loss when opposite of a disease causing change separately, but when they occur together opposite such a change, there is hearing loss as if these changes have an additive deleterious effect. By using techniques in which we are experienced, we plan to continue our research in hearing loss and directly observe whether and how these variants affect the connexin 26 protein functions within the cell. We will determine the effect on the amount of connexin 26 proteins, on localization and transport within the cell, and on function by establishing whether the essential communication channels between cells are still formed. This work will enable the correct clinical interpretation of these commonly observed changes, and can help begin to link DNA changes to protein effects and clinical symptoms in patients with hearing loss.

Yu-chi Shen, Ph.D., University of Michigan

The role of MIF in zebrafish inner ear development

We hypothesis that MIF plays a major role in otic development, specifically in neurite outgrowth and survival of the developing SAG neurons in the inner ear. We have found that MIF is expressed in the mammalian (mouse) inner ear, the chick inner ear and in the model system we have chosen to study, the zebrafish inner ear. Zebrafish auditory system development recapitulates many aspects of early mammalian inner ear development and neurogenesis. More importantly, the same molecules that are active in the mammal are active in the zebrafish and the zebrafish inner ear development is both easier to study and is extremely rapid. We will examine early survival and maturation factors for the SAG and use advanced imaging to trace SAG precursor cell movements from the otic vesicle to the ganglion. Preliminary studies using in situ hybridization demonstrated that MIF and MIF-like genes and their receptors are expressed in zebrafish SAG and developing inner ear. Our experiments will examine whether MIF and MIFlike gene loss-of-function using morpholino antisense oligonucleotides (MOs) affects SAG development and whether this loss of function can be "rescued" by introducing MIF RNA.

Chin-Tuan Tan, Ph.D., New York University, School of Medicine

Measuring and Predicting the Quality of Nonlinearly Distorted Music and Speech as Perceived by Hearing-Impaired People

The first objective of the proposed research is to conduct listening tests to determine how hearingimpaired listeners evaluate the perceived quality of distorted speech and music. The sounds to be studied mimic different kinds of distortion that are inherent to various hearing aid designs: clipping, AGC, output limiting, and many others. The second objective of the proposed research is to develop a computational model for predicting perceived quality judgments made by hearing-impaired listeners- in other words, to predict the data obtained in the first part of the project. The PI has developed a similar model to predict the effect of distorted sounds, but on quality judgments made by normal-hearing listeners. If the outcomes of the present listening tests with hearing-impaired listeners show a similar pattern to those for normal-hearing listeners, then we will adapt our existing model for normal-

hearing listeners to the hearing-impaired data. If the pattern is very different, then we will attempt to develop an entirely new model, based on the perceptual characteristics of the impaired auditory system. The third objective of the proposed research is to test, and if necessary to refine, the developed models using recordings of speech and music replayed via existing assistive hearing devices.

Kathleen T. Yee, Ph.D, Tufts University School of Medicine

A Role for Pax6 in Cochlear Nucleus Development

We are interested in how information-transmitting cells in the brain (neurons) obtain their identity and acquire characteristics that allow them to perform very specific functions. To address these questions, we study a region of the brain that forms the cochlear nucleus, the first and only direct target for cochlear input. While a large body of data exists on features of mature cochlear nucleus neurons, studies are only beginning to examine the role of genes during development. Our preliminary data shows that the molecule, Pax6, a transcription factor, is expressed in the developing cochlear nucleus. The Pax6 gene has long been known to produce deficits in eye development, often manifesting as aniridia, type II (AN2) in humans. Only recently has it been recognized that these patients also have hearing deficits. Studies have implicated higher order brain centers as the sites of aberrant auditory processing. Our data demonstrating Pax6 gene expression in the cochlear nucleus suggests that AN2 patients may hearing problems much earlier in the primary auditory pathway. Our r data indicate that there are structural changes in the cochlear nucleus in heterozygous mouse mutants. This proposal will examine the extent of anatomical changes and how these changes affect function.

DRF SECOND YEAR HEARING & BALANCE RESEARCH GRANT RECIPIENT
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Tamara Alliston, Ph.D., University of California, San Francisco

The role of cochlear capsule bone remodeling in hearing loss

Cochlear bone differs from bone in the rest of the body – presumably due to its unique role in hearing. Special features include its hardness and its protection from normal bone remodeling. How these unique features contribute to hearing – or how their loss causes deafness - remains unclear. Our recent studies on bone disease-associated hearing loss have shown that cochlear bone hardness is critical for hearing. Loss of cochlear bone hardness likely contributes to hearing loss in cleidocranial dysplasia. We reached this conclusion by a novel combination of auditory physiology with tools from materials science and genetic animal models. Using a similar approach, we are poised to investigate how protection of cochlear bone from remodeling is important in hearing and how its disruption in diseases such as otosclerosis causes deafness. We hypothesize that abnormal remodeling in the ear bone causes hearing loss by damaging the hardness of cochlear bone matrix. Specific bone remodeling pathways will be blocked using genetic model systems and bisphosphonates to evaluate the role of bone remodeling in cochlear bone hardness and hearing. Understanding bisphosphonate action in the ear is clinically important because this drug is commonly used to treat osteoporosis and bone disease-associated hearing loss.

Mirna Mustapha-Chaib, Ph.D., University of Michigan

Determine the functional role of the unique amino terminus of Myo15 in hearing using genetically engineered mice.

Myosins are molecular motors that bind cytoskeletal actin and hydrolyze ATP to create force and movement. They are involved in processes including muscle contraction, cytokinesis, vesicle transport, and neurite outgrowth. Many of these diverse functions are accomplished through unique protein interaction domains in the C-terminal tails. Rare myosins have unique domains N-terminal to the motor: MYOVIIIa, NinaC, and MYOXV. The functions of the N-terminal domains of MYOVIIIa and NinaC were predicted by known protein motifs to encode a protein interaction domain and kinase activity necessary for vision, respectively. The N-terminus of MYOXV is essential for human hearing, but there is no homology to known motifs, leaving the function of this very large portion of the protein unresolved. Mouse mutants revealed the vital role of both the motor and tail regions of MYOXV for extension of the stereocilia on neurosensory hair cells of the cochlea. We propose to generate a mouse model with loss of function of the N-terminus of MYOXV and determine the consequences on morphological development and signal transduction within the cochlear hair cells. These studies will reveal the functional role of MYOXV in mammalian hearing and may lead to discovery of interacting proteins critical for hearing.

Irina Calin-Jageman, PhD, University of Illionois

Cortical Synaptic Plasticity in a Mouse Model ofModerate Sensorineural Hearing Loss

Usher syndrome is the leading cause of hereditary deafness and of combined deafness and blindness. The most severe form of the disease, Usher syndrome type 1 (USH1), has been linked to mutations in genes encoding myosin VIIa, cadherin 23, protocadherin 15, sans and harmonin. These USH1 proteins are localized in hair cells,

which are the specialized sensory cells of the cochlea. A prevailing hypothesis is that USH1 proteins may participate in a macromolecular complex necessary for signal transduction and development of cochlear morphology. A key member of this complex is harmonin (USH1C), a protein containing PDZ domains, which are motifs mediating protein-protein interactions in a wide variety of molecules. Harmonin, via direct interactions with other USH1 proteins, may serve as a molecular scaffold for organizing critical signaling molecules in hair cells. Therefore, clues to understanding how genetic alterations in harmonin cause Usher's syndrome may be revealed in analyses of its protein-protein interactions. We have characterized a novel interaction between harmonin and Ca√1.3 (L-type) Ca2+ channels, the primary voltage-gated Ca2+ channels in hair cells. Preliminary data indicate that harmonin directly interacts with Ca√1.3 , and that these two proteins may be colocalized in the apical membrane of cochlear hair cells. Given the importance of PDZ domain-containing proteins for targeting and clustering ion channels, the proposed research will test the hypothesis that the interaction between harmonin and Ca√1.3 is essential for the proper targeting and localization of apical Ca√1.3 channels in cochlear hair cells. That genetic disruption of the genes encoding Ca√1.3 and harmonin independently lead to deafness in mice provides compelling rationale for the proposed research. The specific aims are to further characterize this interaction between Ca√1.3 and harmonin utilizing biochemical and immunocytochemical techniques. A major goal is to develop a theoretical and experimental foundation on which to build an independent research plan focusing on the molecular and genetic alterations that can lead to cochlear dysfunction and hearing impairment.

Patricia Loomis, Ph.D., Rosalind Franklin University of Medicine and Science

Splicing Regulation of Pre-mRNA Generated From the Deafness-Associated Espin Gene

Espins are present in parallel actin bundles within cochlear and vestibular hair cell stereocilia and are produced in multiple isoforms from a single gene through differential start site selection and alternative splicing. Espins are expressed in an isoform specific spatiotemporal fashion during stereociliogenesis and maturation and their absence due to mutation results in loss of stereocilia and hair cell degeneration. This intricate developmental expression pattern suggests that specific espin isoforms function preferentially during discrete phases of stereociliary actin bundle formation. To elucidate the process by which Espin gene expression is controlled at the level of RNA processing, we will investigate how regulatory sequences in the pre-mRNA and the proteins that bind them function to modulate alternative splicing. The process of alternative splicing greatly expands the complexity of the proteome through the generation of both structurally and functionally distinct proteins from a single pre-mRNA. This project will for the first time identify RNA elements and their trans-acting proteins that function to regulate pre-mRNA splicing reactions that result in seven of the nine identified Espin isoforms. These studies will provide insight into how sensory epithelium of the inner ear uses alternative splicing to control the expression of Espins during stereociliogenesis and maturation.

Ania Majewska, Ph.D., University of Rochester

Cortical synaptic plasticity in a mouse model of moderate sensorineural hearing loss

Changes in hearing that occur as a result of defects in sensation at the cochlea likely affect the development of higher brain areas which process auditory information. Currently very little is known about how these higher order areas are influenced by early disruptions in auditory function. Our research will explore changes in the neural networks that process auditory stimuli in the cortex in a mouse model where prestin, a protein crucial for outer hair cell electromotile function is absent during development. We will address this question by looking at synaptic sites which link individual neurons into networks and compare their density, distribution and dynamic remodeling in control and prestin-null mice. We hypothesize that chages in both static and dynamic synaptic structure will be present in the auditory cortex of prestin-null mice, suggesting that cortical auditory networks are altered by degraded hearing during development. This work will shed light on synaptic mechanisms and possible treatments of developmentally acquired hearing loss.

Sonya Pyott, Ph.D., University of North Carolina at Wilmington

Enhancement of the Efferent-Hair Cell synapse by metabotropic Glutamate Receptors

Sensory hair cells of the cochlea communicate with the brain at specialized sites called synapses. Inner hair cells have numerous afferent synapses that relay information about sound from the hair cell to the brain. In contrast, outer hair cells are characterized by efferent synapses from the brain that regulate hair cell activity. Although these efferent and afferent synapses are normally considered to be independent from one another, experiments studying immature inner hair cells suggest that glutamate, the neurotransmitter required for transmission at the afferent synapse, may also modify the response of the efferent synapse. Specifically, activators of the metabotropic glutamate receptors enhance the response of the efferent synapse. The established role of metabotropic glutamate receptors in the central nervous system in combination with these preliminary data suggest that these receptors may be involved in a feedback loop between the hair cell and the central nervous system. That is, glutamate released from the hair cell activates metabotropic glutamate receptors, enhancing the efferent synaptic response. This enhanced efferent activity, in turn, inhibits the hair cells. A molecular understanding of this mechanism would provide novel insight into the mechanisms regulating hair cell activity. This research will address three specific aims to test the hypothesis that metabotropic glutamate receptors mediate such a feedback loop in the cochlea.

Valeriy Shafiro, Ph.D., Rush University

Perception of Environmental Sounds and Speech in Patients with Cochlear Implants

Environmental sound perception is an important component of daily living and a significant concern for cochlear implant patients. Environmental sounds can alert listeners to dangers (e.g., approaching cars, gun shots, alarms) as well as contribute to one's sense of aesthetic satisfaction and well-being. However, this topic has been only marginally addressed in previous studies. There are no published results in environmental sound perception for the implant models developed in recent years, despite significant advances in implant design and signal procession. In addition, based on preliminary data, perception of environmental sounds may also be related to speech perception abilities in cochlear implant patients. In this population, unlike in normal listeners, perception of speech and environmental sounds also appears to correlate with basic auditory abilities. However, their involvement in mediating the relationship between speech and environmental sound perception is not known in present-day implant patients. To address these issues, environmental sound will be assessed in 20 patients with Clarion II implants using a new more comprehensive test of environmental sound perception that overcomes the limitations of earlier materials. It will be compared with the results of speech tests (HINT, CNC, vowels, and consonants) and tests of basic auditory abilities.

Lisa Urness, Ph.D., University of Utah

FGF-regulated hearing loss genes:fast tracking to functional analysis

The inner ear is derived from a small patch of cells in the embryonic head, known as the otic placode. Fibroblast Growth Factors (FGFs) are required to specify the cells that will become the placode and these signals play additional roles at later stages of otic development. These include shaping the inner ear and controlling the numbers of sensory hair cells and the differentiation of supporting cells. The FGF signals required for placode induction specify the appropriate patterns of gene expression within the target tissue, but our understanding of the transcriptional response to FGF signaling in the ear is limited. We plan to compare the genetic profiles of normal and FGF-deficient otic placodes to identify genes that are required for otic development. We will exploit a new technology, a "gene-trap microarray", such that the FGF target genes we identify will already be mutated in embryonic stem cells and ready for mutant mouse production. Selected mouse strains will be analyzed for hearing and balance abnormalities.

Ilse Wambacq, Ph.D., Montclair State University

Neurophysiological and psychoacoustic indices of binaural processing in adults

Separating speech and noise signals spatially, an important skill needed for speech recognition in difficult listening environments, depends primarily on binaural processing using interaural differences in intensity (IIDs) and time (ITDs). Listeners with impaired hearing often perform more poorly than listeners with normal hearing on psychoacoustic tests of binaural hearing. However, binaural processing ability cannot be predicted based on the degree and configuration of hearing loss. We aim to investigate this relationship by obtaining neurophysiological and psychoacoustic responses to IIDs in adults with and without sensorineural hearing loss. IID detection will be compared between the two groups using cortical auditory evoked potentials (AEPs) that have been shown to reflect the detection of acoustic change. The N1-P2 complex will be recorded in response to periodically introduced IIDs in a continuous stream of diotic clicks. We will also test the ability of adults with and without sensorineural hearing loss to perceive IIDs in a psychoacoustic measure of IID just-noticeable differences (JNDs). Finally, we will evaluate the relationship between these psychoacoustic and neurophysiological responses.