2007-2008 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, 2007 through June 30, 2008

Tamara Alliston, Ph.D., University of California at San Francisco
The Role of Cochlear Capsule Bone Remodeling in Hearing Loss

Although several bone diseases cause sensorineural hearing loss, the mechanism by which bony defects impair auditory function remains unclear. The long term goal of this research is to better understand the role of bone in the sensorineural function of the ear – with the objective of identifying bone targets that might be therapeutically effective in the prevention or reversal of hearing loss. The goal of this proposal is to test the hypothesis that abnormal remodeling of the cochlear capsule results in hearing loss by damaging the material quality of cochlear bone matrix. Our recent studies on bone disease-associated hearing loss have shown that cochlear bone hardness is critical for hearing. Understanding bisphosphonate action in the ear is clinically important because drugs are commonly used to treat osteoporosis and bone disease-associated hearing loss.

Dwight E. Bergles, PhD, Johns Hopkins University
Connexin Involvement in Spontaneous Activity in the Developing Cochlea

Our recent studies indicate that spontaneous activity in the developing auditory nerve is initiated by the release of ATP from supporting cells in the organ of Corti. The goal of these studies is to evaluate the role of connexins in triggering ATP release from supporting cells. We propose to use electrophysiological and imaging methods in whole-mount preparations of pre-hearing cochleas to probe the sensitivity of spontaneous activity to manipulations that inhibit gap junction/hemichannel activity. We will extend these studies by testing whether expression of connexin 26 mutants associated with congenital hearing loss (R75W, W44C) alters this spontaneous activity. The studies outlined in this proposal seek to test the hypothesis that connexins play an essential role in the propagation of Ca2+ waves through the support cell network, and are responsible for the release of ATP in the developing organ of Corti.

Kristin Hamre, Ph.D., University of Tennessee Health Science Center
Evaluation of stereocilia morphology in genotypically Math 1-null cells in chimeric mice

Takako Kondo, PhD, Indiana University School of Medicine
Role of T1x3 Signaling in Inner Ear Sensory Neuron Development

The primary goal of this study is to elucidate novel functions of the Y1x3-class homeobox gene 3 (T1x3) in the development of inner ear sensory neurons. The specific aims in this study are: (1) To test whether *T1x3* is required for normal development of inner ear sensory neurons, and (2) To test whether *T1x3* is sufficient for multipotent progenitor cells in the early embryonic ear to become competent to commit to a glutamatergic neural subtype. The long-term goal of this study is to clearly understand the molecular mechanisms underlying specification of auditory and vestibular neurons.

Patricia A. Loomis, Ph.D., Rosalind Franklin University of Medicine and Science
Splicing Regulation of Pre-mRNA Generated From the Deafness-Associated Espin Gene

The goal of this proposal is to determine how Espin gene expression is controlled at the level of RNA processing. Loss of function mutational analysis will identify RNA sequences on the Espin pre-mRNA that are essential for alternative splicing reactions. Proteins that bind the regulatory RNA sequences will be identified by UV-cross-linking, Western blotting and immunoprecipitation. Correlation of the *in vitro* analysis with *in vivo* activity will be accomplished

through modulating by RNAi and overexpression the levels of these proteins in HeLa cells transfected with Espin mini-gene constructs containing genomic sequence corresponding to the alternatively spliced exon and flanking introns.

Anna Majewska, PhD, University of Rochester

Cortical Synaptic Plasticity in a Mouse Model of Moderate Sensorineural Hearing Loss

The development of cortical networks is exquisitely sensitive to patterned activity elicited through sensory stimulation. Although much is known about somatosensory and visual cortical development, very little is known about the development of auditory cortex network connectivity. 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. 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 changes 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.

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

Determine the Functional Role of the Unique Amino Terminus of Myo15 in Hearing Using Genetically Engineered Mce.

Assessing the role of the N-terminus of MYO15 in structural development of hair cells and in the neurosensory process of hearing is expected to provide basic information about the process of hearing at the molecular level. Long term, we expect proteins that interact with the N-terminus of MYO15 will also be defective in some forms of hearing loss. Models similar to the one we propose have been used as proof of principle for gene therapy. Mutations in humans indicate that the N-terminal portion of MYO15 is required in some way for hearing. Using our resources and experience in genetically engineered mice will advance the understanding of the specific molecular function of the N-terminus of Myo15 in mammalian hearing and determine the consequences on morphological development and signal transduction within the cochlear hair cells. Thus, these studies will immediately make a contribution to the rapidly advancing field of molecular hearing research. The next step will be to identify the proteins that interact with the N-terminus, screen pedigrees for mutations in these genes and work towards therapeutic intervention for genes that are common causes of deafness.

Tatjana Piotrowski, PhD, University of Utah Medical School

Molecular Analysis of Hair Cell Regeneration in the Zebrafish Lateral Line

We are aiming to elucidate the genetic pathways underlying hair cell regeneration in zebrafish with the long-term goal of activating these pathways in mammals. Our lab is taking a two-fold approach to identify genes involved in hair cell regeneration. We are performing gene expression analyses from mantle cells of control larvae and from larvae in which mantle cells are proliferating to regenerate killed hair cells (as proposed in this application). As a second approach we are performing a mutagenesis screen for zebrafish mutants which are not able to regenerate hair cells, and thus carry mutations in regeneration-specific genes. A prominent cause of deafness is loss of hair cells due to age, noise or antibiotic treatments. In contrast to mammalian hair cells, fish, bird and amphibian hair cells turn over frequently and regenerate following hair cell death. Little is known why lower vertebrates are
able to regenerate hair cells but humans do not. This is partly due to the relative inaccessibility of inner ear hair cells to direct observation and manipulation. Our aim is to take advantage of the lateral line of zebrafish to define and characterize the molecular and cellular interactions occurring during hair cell regeneration. If successful, our results will set the stage for testing whether hair cell regeneration can be activated in humans.

Sonja Pyott, Ph.D., University of North Carolina Wilmington

Enhancement of the Efferent-Hair Cell Synapse by Metabotropic Glutamate Receptors

This proposal aims to improve our understanding of the molecular mechanisms regulating synapses in the cochlea and will specifically characterize how a class of molecules, metabotropic glutamate receptors (mGluRs), regulates the efferent-hair cell synapses. 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. Efferent innervation of the cochlea is thought to protect against noise-induced hearing loss. Considering that noise-induced hearing loss accounts for one-third of all cases of deafness, understanding the mechanisms regulating efferent synapses is of

special clinical relevance. This project will investigate this hypothesis and should uncover novel pharmaceutical targets to modulate the efferent synaptic response to either dampen hair cell activity and prevent noise-induced hearing or boost hair cell activity and combat deafness.

Valeriy Shafiro, PhD, Rush University Medical Center

Perception of Environmental Sounds and Speech in Patients with Cochlear Implants

This project will assess the ability of patients with contemporary cochlear implants to perceive environmental sounds using a new test of environmental sound perception. It will further examine the relationships between perception of environmental sounds and speech. A close association between these abilities would open an exciting possibility of developing a language-independent instrument for estimating speech perception abilities based on environmental sound tests (e.g., when speech materials are not available for some languages for potential candidates). Such a test would have highly useful clinical applications in large urban clinics or in developing countries with fledgling implant programs.

Lisa D. Urness, PhD, University of Utah

FGF-Regulated Hearing Loss Genes: Fast-tracking to Functional Analysis

With the myriad roles of FGFs in multiple stages of ear development, it is not surprising that some human hearing loss syndromes are caused by mutations affecting FGFs and their receptors. However, little is known about the genes that are controlled by FGFs. Because FGF signals are reused during later stages of otic innervation, morphogenesis, and sensory cell differentiation, the FGF target genes we identify during placodogenesis may also be targets of later FGF signaling events and could provide many new candidates for hearing and/or balance disorders, thereby impacting diagnosis. Importantly, elucidating the functions of these genes may suggest potential therapeutic interventions. Fibroblast Growth Factors (FGFs) are required to initiate otic development and are subsequently reused during morphogenesis and sensory development. Our long-term objective is to identify FGF effector genes and to determine their function and relevance to human deafness by analyzing mouse mutants. Specifically, we propose to isolate RNA from pre-otic ectoderm of control and FGF-deficient embryos and perform an expression profiling experiment utilizing a "gene-trap microarray." This will identify embryonic stem cell lines that carry mutations in FGF target genes. Selected cell lines will be used to generate the corresponding mutant mouse strains for functional studies of hearing and balance.

Ilse Wambacq, PhD, Montclair State University

Neurophysiological and Psychoacoustic Indices of Binaural Processing in Adults.

The overall goal of the proposed research is to investigate neurophysiological and psychoacoustic indices of binaural processing in adults with normal and impaired hearing. In order to develop and implement effective remediation for individuals with sensorineural hearing loss, it is essential to determine a straightforward means to identify binaural processing problems. It is particularly important to ascertain the relationship between neurophysiological and psychoacoustic measures because there are many individuals for whom it is difficult to obtain behavioral responses. In the proposed study we will evaluate the effect of sensorineural hearing loss on processing of IIDs and determine the relationship between neurophysiological and behavioral measures of sensitivity to IIDs. Results will provide the information necessary to assess binaural processing of IIDs and to develop remediation strategies for individuals with sensorineural hearing loss.

Julian R A Wooltorton, PhD, University of Pennsylvania

Probing the Inner Hair Cell Bundle Displacement-quantal Synaptic Response Transfer Function

How do sub-micron displacements of hair bundles on inner hair cells lead to a neural code perceived as sound? This proposal investigates the critical relationship (or transfer function) between hair bundle displacements and afferent fiber bouton responses in the gerbil cochlea. Understanding how we encode the acoustic wave into sound is vital to hearing research. By investigating the relationship between the response to acoustic waves of sensory cells in the cochlea and the resulting postsynaptic neuronal response, we will provide vital information on how the first synapse in the auditory pathway works. This is the basic step carried out by cochlear prostheses. Further insight into the biological details of this encoding step promise new insight in to how to improve the design and performance of cochlear prostheses, and help to further ameliorate hearing loss and deafness. Mechanical energy of an acoustic wave enters the ear *en route* to the cochlea where it is translated into the electrical signals of the auditory nerve. This process involves numerous steps dependent upon the unique architecture of the mammalian ear and various specialized cellular processes to maintain fidelity in reporting frequency, amplitude, timing, and range of auditory stimuli. The inner hair cell processes acoustic waves in the cochlea. A hair bundle atop this cell senses acoustic stimulus and allows current to flow into the hair cell. This ultimately results in neurotransmitter release onto an afferent fiber bouton and subsequent sound perception. One of the true wonders of the biological world is the ability of the auditory system to detect the nearly molecular scale displacements of the hair bundle that result from acoustic wave

stimulation. How these tiny displacements lead to a neural code that we perceive as sound is poorly understood. In this application, we propose to define the quantitative relationship (transfer function) between inner hair cell bundle displacement and the quantal response in the afferent fiber bouton.

DRF SECOND YEAR HEARING & BALANCE RESEARCH GRANT RECIPIENT
Funded July 1, 2007 through June 30, 2008

Gregory J. Basura, MD, PhD, University of North Carolina at Chapel Hill

Synaptic Organization and Plasticity in the Auditory Cortex Following Cochlear Ablation: Role of Serotonin Neurotransmission

Hearing loss has become an identified growing problem. Hearing loss during early development may cause deficiencies in speech development, sound discrimination, and cognitive function. Clinically, this may lead to permanent loss of auditory perceptual skills and impaired language acquisition. To date, hearing loss research has largely focused on cochlear and cochlear nuclei functioning in animal models of deafness, while alterations in the primary auditory cortex (A1) remain largely unexplored. The long-term objective of this proposal is to investigate mechanisms of plasticity in auditory cortex neurons following bilateral cochlear ablation. The evaluation of auditory cortex neuronal functioning in an animal model of deafness and the progressive identification of neurotransmitter receptor systems that may modulate their activity after hearing loss, may lead to the development of pharmacologic tools to facilitate restorative hearing.

James M. Coticchia, MD, Wayne State University School of Medicine

Nasopharyngeal Biofilms in the Pathogenesis of Recurrent Acute Otitis Media

Ear infections are a significant problem in infants and children. Research has shown bacteria that cause ear infections are resistant to antibiotic therapy and frequently require ear tube placement. By understanding which bacteria form these chronic infections and by evaluating new treatments, we hope to reduce the number of children who require ear tubes. This will allow researchers to understand which bacteria form biofilms; when biofilms develop; help to better understand the role of biofilms in recurrent ear infections; and develop new treatment options for children with frequent ear infections.

Michael R. Deans, PhD, Harvard Medical School, Department of Neurobiology

Genetic Dissection of Planar Cell Polarity Within the Inner Ear

Within the human population, hereditary deafness affects 1:1000 children, and age related hearing loss hinders 33% of individuals over age 60 (NIDCD 2003). One exciting but unexplored hypothesis is that disrupted planar cell polarity underlies some forms of hereditary hearing loss. More significantly, if hair cell regeneration is to be successfully developed as a treatment for hereditary and age-related deafness, replacement hair cells must be properly oriented to be able to respond to sound.

This proposal seeks to identify the mechanisms regulating hair cell polarity during development using the mouse as a model. It is broadly accepted that hearing and balance requires the correct orientation of hair cells and their stereocilia bundles within the inner ear. This patterning is called planar cell polarity and involves the coordinated organization of adjacent hair cells. This project aims to understand the developmental mechanisms generating planar polarization and to determine the effects of hair cell disorganization upon auditory and vestibular function.

Gregory I. Frolenkov, PhD, University of Kentucky

Mechanoelectrical Transduction Without Myosin XVa

The long-term goal is to define the molecular and biophysical mechanisms shaping mechanosensitivity in cochlear hair cells. A common structural feature of hair cells in all vertebrates is the staircase arrangement of stereocilia, which is thought to be critical for mechanotransduction. This study will determine the distinguishing features of mechanotransduction in auditory hair cells of deaf shaker 2 mice that have abnormally short stereocilia due to a mutation in the motor domain of Myosin XVa.

Yan Li, PhD, New York University, School of Medicine

Mouse Models of Human Syndromic Hearing Loss Linked to Mutant MYH9 Alleles

Mutations within the nonmuscle myosin heavy chain type IIA (MYH9) have been linked to human hearing loss. The study will examine the biological role of MYH9 in hearing and the role of its mutant alleles MYH9R702C in hearing loss with the goal of developing and characterizing transgenic mouse models that express the mutant alleles

MYH9R702C which is linked to syndromic hereditary hearing loss in humans. Characterizing these mice models will lead to elucidation of the role of MYH9 in hearing and help to development of therapeutic stategies for circumventing hearing loss due to MYH9 mutation.

Iain M. Miller, PhD, Ohio University

The Distribution of Glutamate Receptors in the Turtle Utricle: A Confocal and Electron Microscope Study

When stimulated by acceleration and head tilt (gravity), sensory hair cells in the turtle utricle, an organ in the inner ear, transmit information about these stimuli to the brain. The long term goal of this research is to understand what role synaptic structure and composition play in the observed spatially heterogeneous and diverse discharge properties of afferents supplying the vestibular end organs, and in particular, the utricle. This knowledge is central for accurate diagnosis and rational treatment strategies for vestibular dysfunction. Vestibular dysfunction is particularly disabling. As our population ages, it becomes increasingly urgent that we decode the working of this poorly understood system.

Lavanya Rajagopalan, PhD, Baylor College of Medicine

The Structural and Functional Basis of Electromotility in Prestin, the Outer Ear Amplifier Protein

Prestin, a membrane protein in outer hair cells in the cochlea, is involved in cochlear amplification leading to frequency sensitivity. The long-term objectives of this study are to understand the molecular basis of prestin function, to advance the field closer to designing therapeutics in certain types of hearing loss. This will provide insight into the molecular basis of prestin-related hearing loss, and can lead to rational design of therapeutics to treat such conditions.

Sonia M. S. Rocha-Sanchez, PhD, Creighton University

Role of Central Auditory Neurons in Pathogenic Mechanism of Progressive High Frequency Hearing Loss (PHFHL)

The long-term objective of this study is to assess the relative contribution of Central Auditory Neurons (CANs) to high frequency hearing loss. The peripheral auditory system suggests that progressive hearing loss is resultant of SGNs and/or IHCs dysfunction. This study proposes to determine the effects of the mutations using genetically engineered mice with DN-KCNQ4 expression specific to CANs. Achieving these objectives will open doors to the formulation of therapeutic modalities and possible interventions to PHFHL treatment. Several clinical applications can be resulted from our research. The understanding of the mechanism(s) underlying KCNQ4-mediated channelopathy and the impact of mutational load in the CANs will enable the formulation of different therapeutical approaches in the treatment of PHFHL. Likewise the development of the transgenic mouse model we are proposing here will open the doors to test diverse therapeutical strategies to delay the onset or diminish the progression and/or severity of PHFHL. Moreover, the methodologies and approaches we are presenting can be later applied to many other age-related hearing diseases and will bring new insights into the development of many other progressive hearing losses.

Wenxue Tang M.D, Emory University School of Medicine

The molecular diversity of gap junction channel systems in the cochlea

Connexin mutations are the leading cause of hereditary deafness. This work aims to identify molecular mechanisms responsible for hearing losses caused by connexin mutations that are found in cells that envelop the auditory nerve fibers. Connexins in this subtype provide electrical insulation for the auditory nerve fibers, like plastic sheaths provide electrical insulation for wires. Dysfunction of this insulation caused by connexin mutations results in a human disease called auditory neuropathy. Our research is aimed at understanding the molecular mechanisms of these genetic hearing defects, so further clinical studies can develop methods to correct the connexin mutations' effects in patients suffering from auditory neuropathy.

The long-term objective of this study is to understand how molecular mechanisms of different subtypes of connexins (Cxs) contribute to cochlear functions. Connexins (Cxs) are a family of proteins constituting the gap junctions (GJs). GJs allow direct intercellular exchanges of nutrients, inorganic ions, signaling molecules. The importance of Cxs in hearing functions has been revealed by large amount of genetic linkage studies showing that mutations in Cx genes are associated with about half of patients with childhood nonsyndromic hearing losses. Mutations in Cx26 are responsible for most of the cases. However, mutations in a myelinating Cx (Cx32) have also been linked to Charcot-Marie-Tooth syndrome that includes hearing defects in many cases. Despite their importance in hearing, we know very little about molecular mechanisms that GJs play in the cochlea.

Kathleen T. Yee, PhD, Tufts University School of Medicine

The Role of Neuregulin1 Signaling in the Developing Cochlear Nucleus

The long-term objective of this study is to understand the genetics of cochlear nucleus neuronal differentiation and specification to examine how information-transmitting cells in the brain (neurons) obtain their identity and acquire

specific characteristics that endow them to perform very specific functions. We are interested in how information-transmitting cells in the brain (neurons) obtain their identity and acquire specific characteristics that endow 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, much less is known about them during formation of the nucleus. Our preliminary data shows that a receptor molecule, erbB4, along with a protein that binds to this receptor and activates it, neuregulin1, are both expressed in the developing cochlear nucleus. ErbB4 and neuregulin1 are molecules that can be associated with components of neurons that are important for neuron-to-neuron communication and interestingly, data supports that NRG1 is a schizophrenia susceptibility gene. Impaired auditory processing is a feature of the schizophrenic disorder and we propose to examine how the cochlear nucleus is altered in mutant mice with impaired erbB4/neuregulin1 signaling. Our findings will reveal how early gene expression controls development of the cochlear nucleus and how erbB4/neuregulin1 signaling may be critical for organization and wiring of the cochlear nucleus in schizophrenia.