

and quantity of RNA was assessed with an Agilent 2100 BioAnalyzer and processed for gene expression analysis with Affymetrix rat230A GeneChip microarrays. Signal expression of transcripts was analyzed using GeneChip Operating Software GCOS 1.1 and transcripts were considered to be present if at least two present calls and one marginal call in either the four untreated or the four treated sample groups were detected. For each transcript, expression comparison analysis was made between each untreated & DEX-treated sample to generate a signal log ratio (SLR) value; the t-test was performed on SLR to identify regulated gene expression. Fold change of the transcript level was derived from the median SLR. Genes were found to be present and regulated in groups thought to participate in: 1) transepithelial Na absorption [Pondugula et al. (2004) *Am J Physiol*], 2) transepithelial calcium absorption [Yamauchi et al. (2005) *ARO*], 3) water transport and 4) regulation of several signal pathways, including PI3-kinase (Na absorption) and cAMP/PKA (Cl secretion). We conclude that the presence and regulation of ion transport-related genes in SCCD cells can account for the observed transport phenomena and for the clinical observation that glucocorticoid administration leads to reduced episodes of vertigo in Meniere's patients. Supported by NIH-NIDCD grants R01-DC00212, NCRR P20 RR17686 & P20 RR16475.

## **25 Global View of Expression Patterns of Mouse Utricle Development**

**MingQian Huang<sup>1</sup>, Cyrille Sage<sup>1</sup>, S Zhong<sup>2</sup>, Yu GUO<sup>2</sup>, W Wong<sup>2</sup>, David Corey<sup>3</sup>, Cheng Li<sup>2</sup>, Zheng-Yi Chen<sup>1</sup>**

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To comprehensively understand the genes and pathways involved in the development and function of the inner ear, oligonucleotide microarray (GeneChip) technology was employed to profile global gene expression of the developing mouse utricle, from E10.5 through P12, at daily intervals. The study surveyed over 45,200 mouse genes and ESTs, and 26,000 genes/ESTs were detected in at least one of the utricle samples. 10.5% (4755) of the expressed transcripts were identified to be developmentally regulated.

Comparisons of expression patterns identified 1587 genes whose expression levels changed between two adjacent stages throughout development (a total of 21 comparisons). Surprisingly 1116 of these genes (70%) showed expression level changes at only five stages, indicating that they are the major transitional events in utricle development. Comparison between E12.5 and E13.5 revealed the largest number of genes within which the expression levels changed between two adjacent stages, coinciding with the beginning of terminal differentiation of the sensory epithelium. *In situ* hybridization studies of many genes showed that the patterns are indicative of their roles in progenitor cell production, hair cell and supporting cell specification, and demarcation for the inner ear sensory epithelium.

Clustering analysis identified co-regulated genes. We identified transcription factors and growth control genes which showed co-expression with the retinoblastoma gene (Rb), a gene that is critical in the cell cycle exit of sensory epithelium. Members of the Notch signaling pathway showed co-expression with Math1, raising the possibility of interactions between the two pathways.

Our studies identified many novel genes that are temporally regulated and potentially play significant roles during utricle development. This analysis provided a basis for similar studies of other inner ear organs such as the cochlea, and also aid in identifying deafness genes.

## **26 Gene Expression Analysis of Specific Cell Types Isolated from Mouse Inner Ear Following Laser Capture Microdissection**

**Kumar Alagramam<sup>1</sup>, Wen Wang<sup>1</sup>, Rick Davis<sup>2</sup>, Charles Wright<sup>3</sup>**

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Laser Capture Microdissection (LCM) allows procurement of specific cell types from microscopic regions of tissue sections, which can then be used for gene expression analysis. Cryosections, rather than formalin-fixed paraffin embedded (FFPE) sections, are preferred because the recovery of quality RNA from FFPE sections can be quite challenging. However, the morphology of the various cell types within the inner ear is well preserved in FFPE sections (compared to cryosections) making it easier to identify the cell types and their boundaries. We explored the possibility of using FFPE for gene expression following LCM. Relatively pure populations of cells from the organ of Corti, spiral ganglion, and the maculae were isolated from eight-micron FFPE sections by LCM (Pixcell II, Arcturus, CA). RNA was extracted from captured cells, amplified and assessed for quality by gel electrophoresis. Expression of a select number of genes were tested by reverse transcription PCR and real-time PCR. We were able to detect the expression of several genes reproducibly. This included housekeeping gene Hprt, deafness genes (ex. Protocadherin 15) and other uncharacterized genes. While the house keeping genes were detected in all cell types, some of the other genes showed a restricted expression pattern. The method described here has potential use in many areas of hearing research. The sensitivity and accuracy of molecular profiling can be increased substantially by focusing the analysis on specific cell types within target tissues. For example, following noise exposure in mice, it would be highly desirable to perform gene expression analysis using RNA isolated from hair cells or spiral ganglion cells, instead of whole inner ear tissue or mixed populations of cells from surface preparations. Further, the method we describe here for mouse FFPE sections could be used for retrospective analysis of human archival ear tissue for investigation in search of disease mechanisms.

ABSTRACTS OF THE TWENTY-EIGHTH ANNUAL  
MIDWINTER RESEARCH MEETING

ASSOCIATION FOR RESEARCH  
IN OTOLARYNGOLOGY



February 19-24, 2005

The Fairmont New Orleans  
New Orleans, Louisiana

**ABSTRACTS OF THE TWENTY-EIGHTH ANNUAL  
MIDWINTER RESEARCH MEETING  
OF THE**

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**A**sso**c**iation for  
**R**esearch in  
**O**tolaryngology

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**February 19 – 24, 2005**

**New Orleans, Louisiana, USA**

**Peter A. Santi, Ph.D.**  
*Editor*

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

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2. Understand current controversies in research methods and findings that bear on this understanding.
3. Understand what are considered to be the key research questions and promising areas of research in otolaryngology.

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## President's Message

Welcome to New Orleans and our 28<sup>th</sup> Annual Midwinter Research Meeting. It is the first meeting of ARO in a major convention city and it promises, as in the past, to be scientifically important and stimulating. New Orleans and the Fairmont Hotel offer opportunities that should enhance the meeting experience.



The Program Committee and its Chair, Bob Shannon (who rotates off the committee this year), have once again done an efficient and thoughtful job of organizing the 1065 contributed and invited abstracts into poster and podium sessions. The scientific program begins Saturday evening with a workshop on Gene Microarray Technology. There are two other workshops on Sunday and Monday evening. The Presidential Symposium on Sunday morning is devoted to the structure and function of biological membranes. Five outstanding scientists address lipid-protein interactions that are at the heart of many sensory, developmental, neuronal and pharmacological events. Membrane-based cell signaling contributes to Planar Cell Polarity and Stereocilia Bundle Development which is the topic of one of the Sunday afternoon symposia. Other symposia at the meeting cover auditory pathways, cochlear implants, gap junctions, music and the brain, neuroethology, brain slices, hereditary hearing loss, and middle ear biomechanics.

The Award of Merit Committee and the ARO Council have selected Edwin W. Rubel for the 2005 Award of Merit. Ed will present the Presidential Lecture late Tuesday afternoon. His title "Gone Fishing; Can We Hook the Modulators of Hair Cell Survival in Zebrafish Lateral Line?" gives an indication as to why he is receiving the award. The Awards & Honors Ceremony that follows his lecture will elaborate on his career and contributions.

The association's journal JARO continues its ascent. The ARO research community together with JARO Editor-in-Chief Eric Young, associate editors and reviewers continues to improve our publication's citation index. Eric and his editorial office deserves a special thanks for initiating and maintaining the online prepublication of papers immediately on acceptance. The publication committee under the leadership of Drs. Popper and Popelka (co-chairs) continues to facilitate communication between the publisher, council and the editorial office helping to assure the fiscal viability of JARO and its important role in the ARO research enterprise.

Research trainees have benefited over the years from the generosity of the NIH/NIDCD, Deafness Research Foundation, American Academy of Otolaryngology - Head & Neck Surgery Foundation, Medtronic Xomed, and American Academy of Audiology/American Academy of Audiology Foundation (AAAF). These organizations sponsor the Travel Award Luncheon and Program which honors recipients of the Midwinter Meeting Travel Awards and their mentors. The Deafness Research Foundation deserves special thanks this year for significantly increasing its support. ARO Secretary/Treasurer, Betsy Keithley, as part of her role as in providing active oversight of ARO finances and documentation of council activities, made sure that applications for travel award and meeting funds were submitted. Betsy and Council Member Steve Rauch end their terms at this meeting. Please thank Betsy and Steve for their tireless efforts on behalf of ARO.

Their hard work has enhanced the organization and in many cases extended beyond the normal call of duty.

Returning and new exhibitors also contribute to the success of the Midwinter meeting with displays that keep us up to date on products, publications, grants and in some cases by sponsoring special receptions. Please be sure to thank them as their participation helps to keep our meeting costs down.

Our meeting in New Orleans would not have come about were it not for the support and hard work of many people. The wisdom and leadership of Council guided the decision to hold the 2005 Midwinter meeting at the Fairmont Hotel in New Orleans. We were able to move at practically the last possible moment because of the able assistance of our management firm (notably our Meetings Manager, Lisa Astorga, and our Executive Director, Darla Dobson). An *ad hoc* site selection committee consisting of Bob Shannon, Charlie Liberman, myself and Lisa Astorga visited potential meeting venues and Council made the final decision. Bob Shannon, Lisa Astorga and the program committee had more than the usual stress of organizing the meeting at a new location. Please thank Bob, Darla, Lisa and all our Council members for their service during a stressful transition period.

The pressures that forced us to move to New Orleans also require we identify and commit to a meeting location for next year (2006) and beyond. A two year lead time is desirable in order to optimize meeting organization and membership attendance. Specifics about our exodus from the Adams Mark hotel and future meeting locations will be discussed during the business meeting on Monday evening. Please attend and make your wishes known. You will have a second opportunity to voice your opinions by completing the post-meeting questionnaire.

Now, let us celebrate science!

**Bill Brownell**



**Edwin W. Rubel, Ph.D.  
2005 Award of Merit Recipient**

**Edwin W Rubel, Ph.D.  
2005 Award of Merit Recipient**

Edwin W Rubel trained in Psychology at Michigan State University and received a doctorate in 1969 for the first comprehensive study of sensory coding in the developing central nervous system. His independent research program of the past three decades has retained a focus on behavior, particularly during auditory development. At the same time, Dr. Rubel's work has provided important insights into biological questions that tend to be viewed in purely cellular and molecular terms: hair cell regeneration, neuron cell death, and dendrite formation. In fact, each of these research areas is a successful offshoot from a laboratory that studies how the chicken auditory system develops and how that development is influenced by the environment.

After a postdoctoral fellowship at UC Irvine studying the physiology of polysensory association cortex, Dr. Rubel began his independent research career in 1971 as an Assistant Professor of Psychology at Yale University. There he began a series of experiments to determine how frequency is represented in the developing chick central auditory pathway. Behavioral experiments in normal and acoustically-deprived hatchling chicks used generalization of distress-call habituation to tones to show that there is perceptual sharpening during the first few days after hatching and that this maturation depends on normal acoustic experience. In parallel with these behavioral studies, Dr. Rubel began a series of experiments that have produced compelling examples of structure-function relationships in the auditory central nervous system. These cellular studies explored the organization and development of the second- and third-order neurons in the chick's auditory pathway, nucleus magnocellularis (NM) and nucleus laminaris (NL). Mapping studies revealed that there is a precise gradient of dendritic structure along the tonotopic axis of NL, which is thought to optimize interaural time difference detection at each frequency. Dr. Rubel (with Steven Young) then discovered that the projection from NM to NL serves as an anatomical delay line, consistent with the Jeffress coincidence-detector model of sound localization. Dr. Rubel then answered one of his initial research questions by showing (with Zaid Smith and Jeffrey Deitch) that the distinctive dorsal and ventral dendritic tufts of these NL neurons are independently maintained by afferent synaptic input from NM. Together, these studies formed one of the most successful fusions of behavioral and cellular neuroscience, and recommended the chick as an exceptional model for hearing research.

Although anatomical findings were consistent with a place code of frequency in the developing chick brainstem auditory nuclei, there was a paradox that had yet to be resolved. Whereas the cochlea itself begins to mature morphologically in the basal region (which responds to higher frequencies in adults), developing animals first respond behaviorally to relatively low frequencies. Dr. Rubel hypothesized that the basal cochlea was at first responsive to lower frequencies and only gradually shifted its responsiveness to higher frequencies. In one line of research begun after his move to the University of Virginia School of Medicine in 1977, Dr. Rubel (with Brenda Ryals) showed that the cochlear damage resulting from high intensity pure tones was found at successively more basal locations during development. In a second line of research,

taking advantage of his meticulous work on the normal development of NM topography, Dr. Rubel (with William Lippe) showed that the frequency map in NM did indeed shift during development. Similar observations have been made in many species over the past two decades, and the development of a shifting place code is now a broadly accepted principle.

One of Dr. Rubel's motivations for launching a systematic investigation of development in the bird auditory system was the mounting evidence that avian auditory neurons and hearing-related behaviors were quite sensitive to manipulation of sound-evoked activity. Experiments in his lab, in which the ear of chick embryos or hatchlings was surgically destroyed, revealed that there is a discrete period of life during which one-third of the neurons in NM die in the absence of cochlear nerve input. After moving to the University of Washington School of Medicine in 1985, Dr. Rubel devoted increasing efforts to understand the mechanisms of the rapid death of some auditory neurons and the survival of others after damage to the ear. The earliest studies (with Oswald Steward, Dianne Durham and Gwen Garden) showed that various cytological markers associated with oxidative metabolism and protein synthesis identified, within hours of deafening, those NM neurons destined to die. Dr. Rubel (with Richard Hyson) then demonstrated that afferent synaptic stimulation is necessary for survival of the NM neurons that otherwise die after deafferentation. Later work (with Lance Zirpel) showed that afferent stimulation acts via metabotropic glutamate receptors to maintain the normal intracellular calcium levels required for neuronal survival in NM. More recent experiments (with Sam Moustafapour) have shown that the normally brief postnatal critical period for susceptibility to deafening-induced cell death in the mouse cochlear nucleus can be extended by blocking expression of the *bcl2* gene or prevented by its over-expression. The chick NM is well-established as the leading model system for understanding the role of synaptic activity on neuron survival.

Dr. Rubel's work on hair cell regeneration in the bird cochlea is an interesting illustration of how basic research often begets clinically-relevant findings. His experiments on cochlear damage with ototoxic agents were initially designed to study the effects of auditory deprivation. Acoustic deprivation with earplugs had not produced significant changes in central auditory anatomy, and Dr. Rubel (with William Lippe) subsequently discovered that spontaneous activity was quite high prior to the onset of hearing. Thus, as he set about to identify a better way to deprive the brain of sound, one of the first studies (with Paul Lambert and Raul Cruz) looked at when cochlear damage first became apparent after exposure to noise or ototoxic drugs. The surprising finding was that damage decreased with survival time, suggesting a repair mechanism. Later, Dr. Rubel (with Brenda Ryals) used tritiated thymidine autoradiography to demonstrate that new cells were indeed generated within the damaged chick cochlea. We should emphasize that the experimental psychologist is never far from the lab bench. Most recently, Dr. Rubel (with Sarah Woolley) has shown that adult songbirds that are deprived of auditory input by hair-cell destruction recall their song when the hair cells regenerate, indicating that a stable template does not require persistent activation.

Dr. Rubel has clearly made key discoveries in diverse areas of developmental neurobiology, and his impact extends well beyond the hearing sciences. However, we in the auditory community have benefited the most from his imagination, energy, and intellect. About 100 students, scientists, and clinicians have been mentored in his laboratory, which has produced over 200 publications. Dr. Rubel founded the Virginia Merrill Bloedel Hearing Research Center, a thriving multidisciplinary enterprise that now embraces 12 departments and over 60 faculty at the University of Washington. He has also served our community as president of ARO and has been a forceful advocate for NIDCD from its inception. It is a welcome tribute that the 2005 Award of Merit should be presented to Edwin W Rubel for his scientific achievements, his scholarship, and his enduring commitment to understand the entire system, from hair cell to behavior.

Thomas N. Parks  
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