

**ABSTRACTS OF THE THIRTIETH ANNUAL  
MIDWINTER RESEARCH MEETING**

# **ASSOCIATION FOR RESEARCH IN OTOLARYNGOLOGY**



**February 10-15, 2007**

**The Hyatt Regency Denver  
Denver, Colorado, USA**

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

*Editor*

Association for Research in Otolaryngology  
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using this method to screen for genes specifically expressed in otic- or neural-specific cells of the auditory or vestibular systems. Gal4-based cassettes are inserted into the zebrafish germline by injection of pseudotyped retroviral vectors or Tol2 transposases into early embryos. The injected founder fish are crossed to a reporter line carrying a UAS:GAP43-DsRed-Express fusion transgene whose expression requires Gal4 protein. Only cells expressing a trapped gene will make Gal4 protein and transactivate the reporter whose presence can be detected by fluorescence screening of live F1 embryos. Fish with relatively specific fluorescence in peripheral or central components of mechanosensory systems will be bred and the trapped genes will be cloned. One major advantage of a Gal4 gene-trap design is its potential for targeting bioactive molecules to specific cells in vivo. This can be accomplished by crossing a particular Gal4-trap line (i.e., the activator line) with a transgenic line carrying a target gene placed downstream of a UAS sequence (i.e., the effector line). A drug-inducible form of Gal4 (GeneSwitch, Invitrogen) will permit even more control over the onset of effector protein expression. As proof-of-principle, we plan to create an effector line with UAS upstream of a toxin gene. When crossed to any of the Gal4 activator lines, we expect the toxin will specifically kill only those cells expressing the trapped gene. This should prove especially powerful for selective ablation of subsets of CNS neurons to assess their role in development and/or in behavior. Finally, we hope to test the trapped genes for possible roles in hearing and balance using loss-of-function approaches, such as morpholino-based gene knockdowns or breeding trapped lines to homozygosity. Supported by NOHR and DRF.

### **329** Death Pathways in Noise-Exposed Outer Hair Cells

Barbara A. Bohne<sup>1</sup>, Gary W. Harding<sup>1</sup>, Steve C. Lee<sup>1</sup>

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Using morphological criteria, death pathways in OHCs were determined in organs of Corti (OC) exposed to an octave band of noise (OBN) with a center frequency of either 0.5 or 4 kHz at a moderate sound pressure level (SPL). The specimens were part of our collection of plastic-embedded flat preparations of chinchilla cochleae. Three death pathways were identified: 1) oncosis - OHCs were swollen & pale-staining with a swollen nucleus; 2) apoptosis - OHCs were shrunken & dark-staining with a pyknotic nucleus; & 3) a third death pathway - OHCs had no basolateral plasma membrane, a nucleus deficient in nucleoplasm & cellular debris arranged in the shape of an intact OHC. To minimize the secondary loss of OHCs that occurs post-exposure, the specimens used for quantitative analysis of death pathways had the following characteristics: a) the level to which they were exposed was  $\leq 95$  dB SPL; b) the exposure duration was 6-216 h; c) the cochleae were fixed in-vivo 1-2 h post-exposure; & d) there were no focal OHC lesions in the OC. Fifty-eight noise-exposed cochleae in our collection met these criteria. The specimens had a variable amount of OHC loss, minimal IHC loss, rare pillar loss, & no spiral ganglion

cell loss. The cochleae were grouped by total exposure energy [ $E = \log_2 (\text{Pa}^2 \text{ seconds})$ ] into 7 Groups with energies ranging from 7.77-17.74 for the 0.5-kHz OBN & 6 Groups with energies ranging from 5.11-14.75 for the 4-kHz OBN. In all specimens, degenerating & missing OHCs were classified as to which death pathway the cells were following. Nine non-noise-exposed cochleae were also evaluated for OHC death pathways. The number of OHCs following the third death pathway was significantly greater in the noise-exposed cochleae than the non-noise-exposed cochleae for total exposure energies greater than that produced by a 0.5-kHz OBN at 75 dB SPL for 216 h (i.e., 13.26) or a 4-kHz OBN at 57 dB SPL for 48 h (i.e., 5.11). In cochleae exposed to either octave band, OHCs dying by oncosis or apoptosis were uncommon. Further work must be done on cell-death pathways in the noise-damaged cochlea to determine how the prevalence of the different pathways changes with exposure parameters.

### **330** High-Frequency Noise-Induced TTS Correlates with Outer-Pillar Pathology

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Studies have reported a correlation between noise-induced TTS & structural changes in the organ of Corti such as disarray of stereocilia, swelling of afferent nerve fibers & distortion of supporting-cell bodies. However, no pathological change has been found consistently, & the mechanism of TTS remains unclear. The present study sought to quantify pillar-cell buckling in cochleae fixed when TTS was present & in those fixed after recovery from TTS. Noise-exposed chinchilla cochleae were selected from our permanent collection using the following criteria: a) the exposure was a 4-kHz OBN at 57-86 dB SPL for 24-216 h; b) animals were 1-3-yr-old; c) 34 cochleae were fixed 0-d post-exposure & 12 were fixed after 20-30 d of recovery. Eight control cochleae were also evaluated. All cochleae had been fixed with 1% osmium tetroxide & embedded in plastic. After polymerization, the cochlear ducts were dissected into flat preparations & examined by phase-contrast microscopy. Grade 0 indicated no outer-pillar damage. Grade 1 indicated bowing of the outer-pillar bodies. Grades 2, 3 & 4 indicated that the outer pillars were slightly, moderately, or severely buckled, respectively. For each cochlea, total exposure energy [ $E = \log_2 (\text{Pa}^2 \text{ seconds})$ ] was calculated. E ranged from 5.11 (Group 1; 57 dB SPL, 48 h) to 16.92 (Group 6; 86 dB SPL, 216 h). In the apical half of the cochleae, there was little damage to outer pillars in all Groups. In the basal half of the cochleae, little pillar damage was present in Groups 1-3. Cochleae in Groups 4-6 sustained increasing pillar damage that was concentrated in the base. This damage was significantly different from controls at 78-93% distance from the apex for Group 4; 8-13%, 38%, 53% & 63-88% for Group 5; & 73-83% for Group 6. For the 20-30-d recovery cochleae, the average pillar grades differed significantly from controls for Group 5 at 38%, 53-58% & 78% & for Group 6 at 8% distance. Thus, high-frequency noise exposures that produce TTS also lead to outer-pillar damage, the degree of which increases with E. Recovery

from TTS correlates with repair of outer-pillar damage. The data presented here support the hypothesis that outer-pillar damage is a mechanism for TTS.

### **331 Is Plasma Membrane Leakage a Sign of the Shift of the HC Death Pathway From Apoptosis to Necrosis Following Exposure to Intense Noise?**

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It has been known for years that apoptotic cells preserve their membrane integrity until the late phases of the apoptotic process. Therefore, occurrence of membrane leakage is commonly considered an important sign of cessation of apoptosis or occurrence of necrosis. Here we reported that noise-induced hair cell (HL) apoptosis appear have different pattern of the apoptotic progression. Instead of switching to necrosis, HC apoptosis persist even after the cells lost their membrane integrity. Chinchillas were exposed to an impulse noise (155 dB SPL) or a high level of a continuous noise. Following exposure to the intense noise, nuclear condensation developed rapidly in the center of the cochlear lesion. At the same time, the cells showed the sign of membrane leakage as evidenced by strong uptake of propidium iodide or trypan blue. Although the HCs lost their membrane integrity, the apoptotic phenotypes continue to manifest. SDH activity and mitochondrial membrane potential were preserved in apoptotic HCs until the late phases of apoptosis, suggesting the maintenance of the mitochondrial energetic function. Nuclear condensation continued, leading to generation of small nuclear fragments and, eventually, complete nuclear degradation. More importantly, caspase activity was preserved. Interesting, the HCs with membrane leakage had a relatively intact membrane enclosure as shown by semi-thin sections of the organ of Corti. The plasma membrane of apoptotic HCs confined the cellular organelles within the cells. In contrast, the necrotic cells exhibited the membrane gaps which allow the release of cellular organelles to the extracellular space. Collectively, our observations indicate that loss of membrane integrity did not shift the apoptotic process to necrosis. Apoptotic cells, even with membrane leaks, remain in the apoptotic pathway until complete degradation of the cells.

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### **332 Regulation of Cell Death Pathways Following Exposure to Intense Noise**

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The coexistence of apoptotic and necrotic outer hair cell (OHC) death has been found in the organ of Corti following exposure to intense noise. However, the cellular mechanisms that regulate the propensity of cell death

toward apoptosis or necrosis following exposure to intense noise are unknown. The current study was designed to examine the role of energy supply in controlling the cell death propensity toward apoptosis or necrosis following exposure to intense noise. In the first part of the study, the mitochondrial energetic function was compromised by application of 3-Nitropropionic acid (3-NP, 20 mM or 50 mM), an irreversible inhibitor of succinate dehydrogenase (SDH) before or after exposure to an impulse noise at 155 dB SPL. The results showed that inhibition of SDH activity could reduce the rate of the progression of certain apoptotic events, including F-actin cleavage and nuclear degradation, at the early phase of cochlear pathogenesis. However, SDH inhibition could not prevent the initiation of OHC apoptosis. Although SDH inhibition drove a small portion of OHCs to die through secondary necrosis, there was no major shift of cell death pathways from apoptosis to necrosis following the mitochondrial impairment. In the second part of the study, the blood supply to the cochlea was stopped before exposure to the noise. The results showed that complete cessation of the energy supply to the HCs switch apoptosis to necrosis as evidenced by the presence of a large quantity of HCs with swollen nuclei. Although activation of caspases (3, 8 and 9) in dying cells persisted, the level of caspase activity appeared reduced. Collectively, the results of the study suggest that while the mitochondrial energetic function plays an important role in the apoptotic process, it is not an obligatory component for initiation of OHC apoptosis. Other energy sources, such as cytosolic glycolysis, can participant in the regulation of the apoptotic process in the event of mitochondrial dysfunction.

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### **333 The Dynamics of Mitogen-Activated Protein Kinases in the Cochlea After Acoustic Trauma**

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Mitogen-activated protein kinases, or MAPK (ERK, JNK and p38) are regulators of the intracellular response to different stressors and growth/differentiation factors (Kyriakis and Avruch, 2001). MAPK are expressed in both auditory brainstem neurons (Suneja et al., 2003), as well as in the cochlea (Hess et al., 2002), yet their function is not completely understood. Reductions in hearing thresholds were found after pre-treatment with the inhibitor of JNK (Pirvola et al., 2000) suggesting a protective role in the cochlea. In order to investigate the mechanisms responsible for auditory protection we analyzed the phospho-MAPK expression patterns at different time points after i) a temporary (TTS) or, ii) a permanent (PTS) noise-induced hearing loss. We found the pattern of phospho-MAPK expression after a TTS was different from the expression after PTS. To study the mechanisms underlying the different MAPK expression patterns, we analyzed the total content of several cytokines and BDNF in cochlea tissues using ELISA, and the expression of TNF receptors and TrkB in the cochlea using immunocytochemistry and western blot. These findings will help to understand the mechanisms of hearing loss