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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*Editor*

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

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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 = $\log_2$ ($Pa^2$ 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 = $\log_2$ ($Pa^2$ 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