Auditory Nerve Degeneration after TTS-Producing Noise

Sharon G. Kujawa1,2,3 and M. Charles Liberman1,2
1Dept. of Otology and Laryngology, Harvard Medical School, Boston, MA, USA
2Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary
3Dept. of Audiology, Massachusetts Eye and Ear Infirmary

Introduction
Overexposure to loud sound can cause hearing loss, the severity of which is shaped by characteristics of the exposure (level, duration, frequency content) and characteristics of the individual (genetics, age). After overexposure, thresholds are immediately elevated, but can recover for several weeks. If the audiogram returns to normal, the noise-induced hearing loss (NIHL) is deemed “temporary”; if recovery is incomplete after a few weeks, the NIHL is considered “permanent”.

Temporary NIHL can arise from largely sub-light microscopic changes to hair cell and neuronal machinery that reversibly compromise threshold sensitivity. In the case of ‘excitotoxic’ synaptic swelling, histologic evidence of noise injury may be captured, but only in the acute post-exposure timeframe. Post-exposure recovery of threshold sensitivity has been assumed to indicate reversal of damage to delicate mechano-sensory and neural structures of the inner ear and no persistent or delayed consequences for auditory function.

Recent work in our laboratory (Kujawa and Liberman 2006; 2009; Lin et al 2011) has shown that significant degeneration of the cochlear nerve occurs after noise exposure, even when there is no hair cell loss, and even if thresholds have returned to normal. Such findings have important implications for standard clinical assessments of noise injury and regulation of noise exposure in humans.

Methods
Animals (CBA/CaJ mice, 16 wk, male) are noise exposed and held with untreated age-, strain- and gender-matched animals for varying post-exposure times. Noise-induced changes in auditory function (thresholds, response growth functions) are quantified using the outer hair cell (OHC)-based DPOAEs and
the first ABR (Wave I) or CAP wave representing summed activity of the cochlear nerve. Together, these techniques allow us to assess OHC vs IHC/neural functional status over a broad range of acoustic frequencies (cochlear places) from the low-frequency apical turn to the high-frequency basal tip.

Histopathology of the cochlear hair cells, nerve terminals and the synapses that connect them is studied by confocal imaging of immunostained cochlear whole mounts. As schematized in Figure 1, synapses are rendered visible by immunostaining for a component of the presynaptic “ribbon” (CtBP2, red), a structure involved in vesicle delivery to the active zone (Khimich et al., 2005). To assess cochlear nerve terminals, we use anti-neurofilament or anti-Na+/K+ATPase (green), to reveal all the unmyelinated nerve fibers in the sensory epithelium, or anti-parvalbumin (a calcium buffer), which stains only the terminal swellings of cochlear nerve fibers under the IHCs. Myosin VIIA aids visualization of hair cells. We also count spiral ganglion cells in osmium-stained, plastic-embedded sections.

Results
Immediately after noise exposure, mice exposed to an 8-16 kHz octave band noise at 100 dB for 2 hrs show a moderate NIHL of 30 – 40 dB, by both ABRs and DPOAEs. Two weeks later, thresholds have returned to normal, as have amplitudes of the OHC-based DPOAEs. However, neural response amplitudes recover to only about 50% of pre-exposure values (Figure 3a), suggesting degeneration of ~ 50% of the cochlear nerve.

For this exposure, there is no loss of hair cells to extended post exposure times. Despite the normal hair cell populations, dramatic degeneration of both presynaptic and postsynaptic elements in the IHC area can be observed throughout the basal cochlea (Figure 2). Roughly 50% of the synaptic connections between hair cells and cochlear neurons disappear acutely after exposure (Figures 2a,b, 3b). Fiber density in the IHC area also is reduced (Figures 2c,d), at all post-exposure times, in proportion to the loss of ribbons. Although functionally disconnected from the hair cell, the cell bodies of these neurons are visible for many months. However, by two years post-exposure (Figures 2e,f, 3c),
ganglion cell loss, too, matches the degree of loss seen acutely in the ABR amplitudes and at the level of the synapse.

**Discussion**

Once an ear has been compromised by noise, the question of whether this insult influences future changes in auditory function is of significant clinical and public health importance given the high prevalence of noise exposure in, and the aging of, our society. Our work demonstrates that noise exposure has significant neurodegenerative consequences, even in ears with recovered threshold sensitivity. It also suggests that current damage risk criteria for human noise exposure may be inadequate, because they are based on the assumption that exposures producing reversible NIHL are benign. Such findings are intriguing in light of human temporal bone studies showing steady loss of cochlear neuronal populations with age, (Otte et al., 1978), even in ears with a full complement of hair cells (Makary et al 2011).

Is it paradoxical that thresholds return to normal despite loss of > 50% of the nerve fibers connecting hair cells to the brain? No – it appears that the fibers that degenerate are selectively those that, pre-exposure, had the highest thresholds. What, then, are the functional consequences of this primary neural degeneration? We believe that the selective loss of these high-threshold neurons will affect hearing in a noisy environment and may explain why this ability decreases so dramatically in the aging ear.

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**References**


New Techniques
from the Otopathology Laboratory at the University of Minnesota

Thin-Sheet Laser Imaging Microscopy (TSLIM) of the Human Temporal Bone
Peter A. Santi, Ph.D., Department of Otolaryngology, University of Minnesota

Thin-Sheet Laser Imaging Microscopy (TSLIM) of the human temporal bone is a new attempt to produce high-resolution, serial optical sections of portions of the human temporal bone from whole specimens. Human temporal bones are fixed, decalcified and chemically cleared to transparency. Movement of the specimen through the light-sheet produces serial optical sections that are well suited for 3D reconstruction of tissue structures and morphometry. Recently, TSLIM (Santi et al., 2009) has been upgraded with a scanning, thin beam configuration (sTSLIM) (Schröter et al., 2012) that allows for imaging 15mm wide specimens with improved image quality (Fig. 1). Since sTSLIM is a fluorescent method and does not require any embedding, structures can be optimally labeled using indirect immunohistochemistry on whole cochleas or other portions of the human temporal bone. sTSLIM minimizes photobleaching of fluorophore labeled structures as the tissue is only exposed to a very thin light-sheet. After TSLIM imaging, specimens can be processed for traditional celloidin sectioning.

We have imaged five human temporal bones and are using the image stacks to produce 3D reconstructions of middle and inner ear structures in normal humans. Fig. 2 is a TSLIM optical section of the scala media of the cochlea that shows good resolution of the soft tissues (spiral ligament, stria vascularis, Reissner’s membrane). Although the resolution is not quite as good as obtained from celloidin sections, images in a TSLIM stack are perfectly aligned and a whole cochlea can be imaged in only 2 hours. Due to greater air spaces and less dense bone, the middle ear can be imaged with even better resolution by sTSLIM. 3D volume reconstructed structures shown in Fig. 3 like the tympanic membrane (green), ossicles (orange and red) and ligaments (blue and purple) can be analyzed morphometrically to estimate volume, shape, and other dimensions. Thus, average models of ear structures can be constructed and compared to the same structures in humans with hearing loss to better understand the histopathological basis of inner ear disorders.


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The NIDCD National Temporal Bone Registry is pleased to announce the availability of mini-travel fellowships. The fellowships provide travel funds for research technicians and young investigators to visit a temporal bone laboratory for a brief educational visit, lasting approximately one week. The emphasis is on the training of research assistants, technicians and junior faculty.

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Massachusetts Eye and Ear Infirmary
243 Charles Street
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michael_mckenna@meei.harvard.edu

Meetings

The Registry plans to exhibit at the 2012 Hearing Loss Association of America Convention to be held in Providence, Rhode Island June 21-24th, and at the 2012 Donor Dash on July 15th in Denver, Colorado.


Acknowledgments:

Correspondence to:
Sharon G. Kujawa, Ph.D
Massachusetts Eye & Ear Infirmary
243 Charles Street, Boston, MA 02114, USA.
Tel: 1-617-573-3745
sharon_kujawa@meei.harvard.edu

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Free Brochures for your Office or Clinic about Temporal Bone Research and Donation

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