Newsletter of the NIDCD National Temporal Bone, Hearing and Balance Pathology Resource Registry

Summer 2012 Volume 20, Number 1

the Registry

Contents

Mission Statement

The NIDCD National Temporal Bone, Hearing and Balance Pathology Resource Registry was established in 1992 by the National Institute on Deafness and Other Communication Disorders (NIDCD) of the National Institutes of Health to continue and expand upon the former National Temporal Bone Banks (NTBB) Program. The Registry promotes research on hearing and balance disorders and serves as a resource for the public and the scientific community about research on the pathology of the human auditory and vestibular systems.

Auditory Nerve Degeneration after TTS-Producing Noise

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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

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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

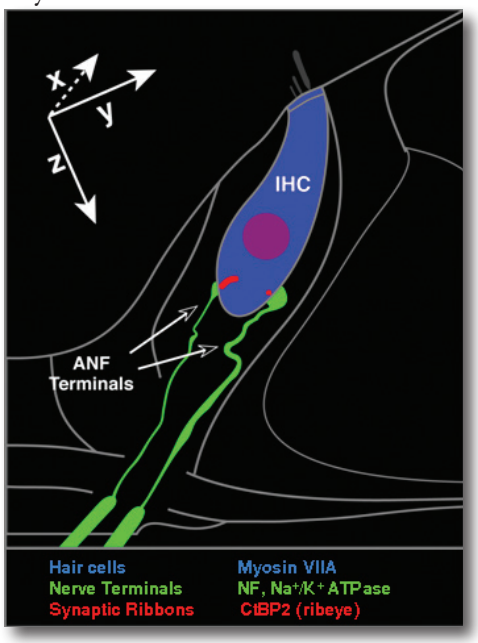
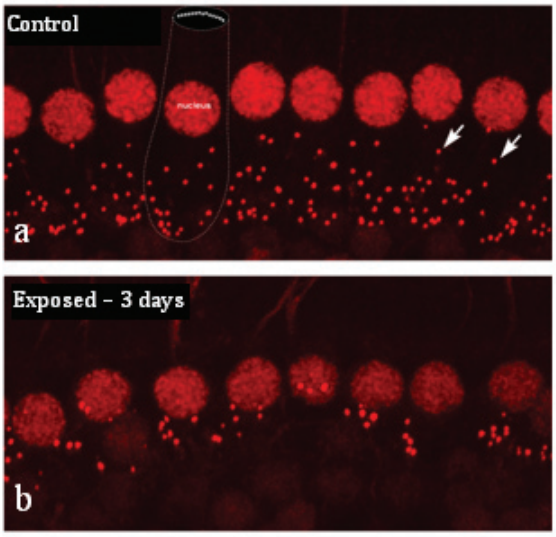


Fig 1 (above). In a normal ear, synaptic ribbons, immunostained with anti-CtBP2 (red) are found on the IHC's basolateral membrane in the subnuclear region. Double- staining for a neuronal marker (Na+/K+ ATPase, green) shows that every ribbon is opposite a terminal and vice versa.

Synapses



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),

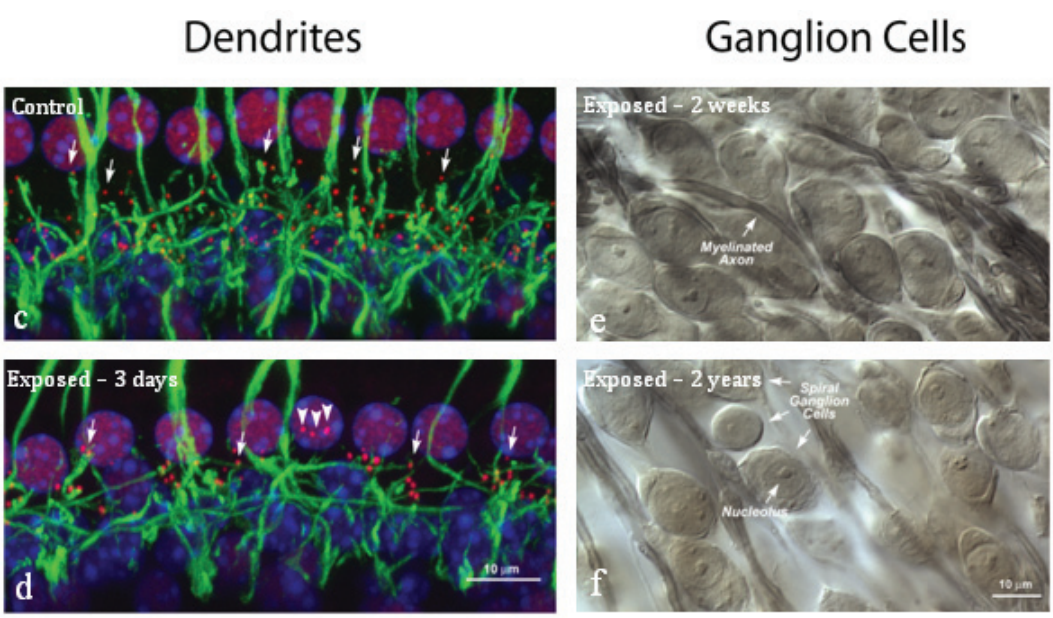


Fig 2 (left). Despite reversibility of threshold shift and intact sensory cells, noise-exposed ears show rapid loss of cochlear synaptic terminals and delayed loss of cochlear ganglion cells. Immunostaining reveals synaptic ribbons (red, anti-CtBP2) and cochlear nerve dendrites (green, anti-neurofilament) in the IHC area of a control and an exposed ear at 3 d post noise (a-d). Light micrographs of osmium-stained plastic sections from the 32 kHz place in noise-exposed ears, either 2 wks or 2 yrs after exposure (e-f).

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).

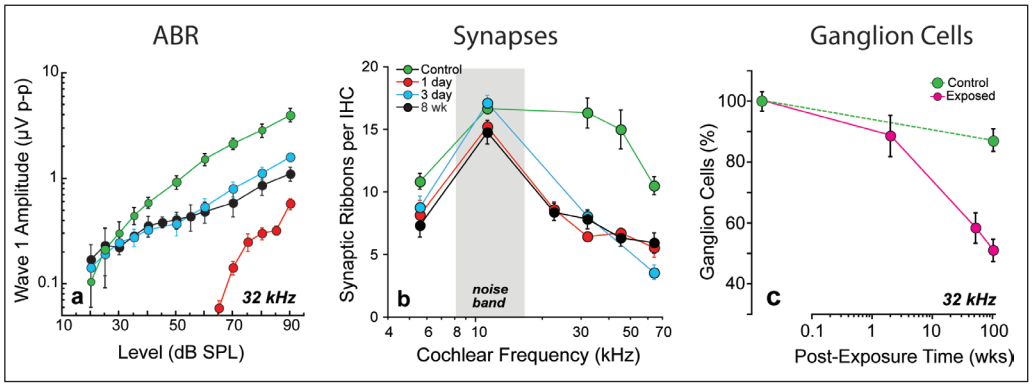


Fig 3 (above). There is a close match between loss of neural amplitudes (a) and synaptic loss (b) in the acute post-exposure timeframe. Ganglion cell loss (c) is significantly delayed, but ultimately proportional to changes seen acutely. (a): Rapid and persistent synaptic loss after noise. Despite threshold recovery, suprathreshold neural responses at high frequencies are permanently attenuated, although recovery of otoacoustic emissions (not shown) suggests cochlear OHCs are normal. At 8 weeks post exposure, suprathreshold amplitudes of ABR wave 1, the far-field response of the cochlear nerve, are less than half their pre-exposure values. Group means (± SE) are shown: n=7–21 ears per group. Key in panel B also applies to panel A. (b): Rapid and persistent synaptic loss after noise. Synaptic ribbon counts in six cochlear regions of control and noise-exposed ears show synaptic loss throughout the basal half of the cochlea. Means (± SE) of synaptic ribbons per IHC were computed from confocal z-stacks such as those in Figure 2 from control ears (n=11) and exposed ears at 6 cochlear locations and 3 post exposure times: 1 d, 3 d, and 8 wk (n = 5-6 ears per group). (c): Slow degeneration of spiral ganglion cells after noise. These counts of spiral ganglion cells from osmium-stained plastic sections are from the 32 kHz place in control (16 wk, n=7; 104 wk, n=12) vs noise-exposed ears held for 3 post-exposure times: 2 weeks (n = 6), 52– 64 wks (n = 7) and 104 weeks (n = 6).

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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Thin-Sheet Laser Imaging Microscopy (TSLIM) of the Human Temporal Bone

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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. sTLIM 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.

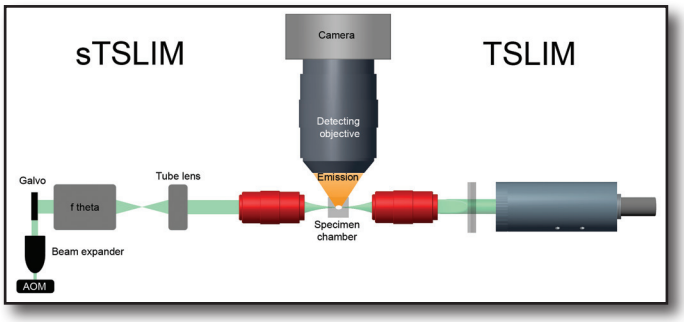

Fig. 1 sTSLIM for nondestructive imaging of the human temporal bone using a thin light-sheet.

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. ■

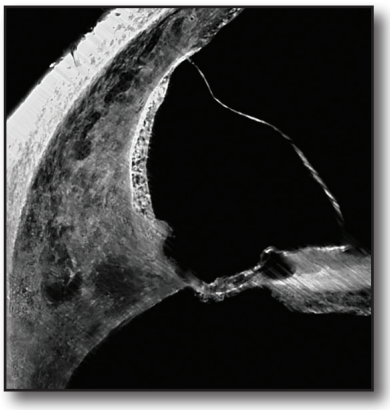

Fig. 2 TSLIM optical section of the scala media from a human cochlea showing the spiral ligament, stria vascularis, and Reissner's membrane.

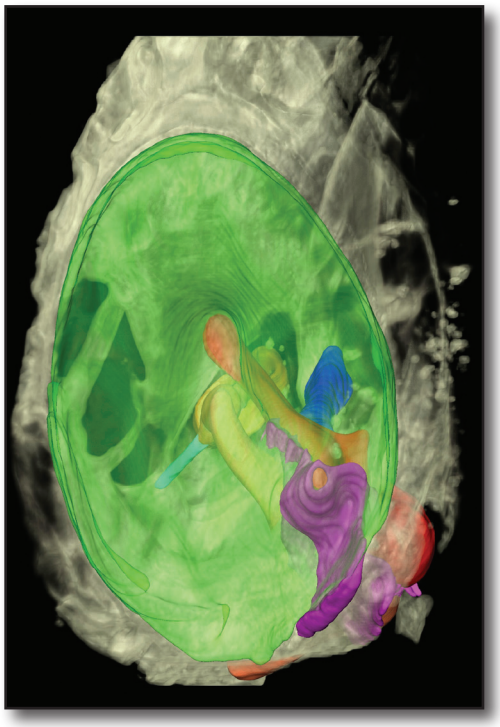

Fig.3. 3D volume reconstruction of normal middle ear structures from sTSLIM image stacks.

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Schröter TJ, Johnson SB, John K, Santi PA (2012). Scanning thin-sheet laser imaging microscopy (sTSLIM) with structured illumination and HiLo background rejection biomedical Opt Express 1: 170-177.

Otopathology Mini-Travel Fellowship Program

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.

These fellowships are available to:
1. U.S. hospital departments who aspire to start a new temporal bone laboratory.
2. Inactive U.S. temporal bone laboratories that wish to reactivate their collections, or
3. Active U.S. temporal bone laboratories that wish to learn new research techniques.

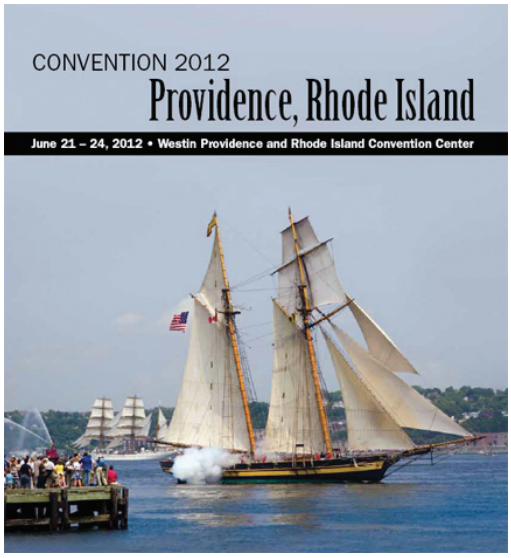
Up to two fellowship awards will be made each year ($1,000 per fellowship). The funds may be used to defray travel and lodging expenses. Applications will be decided on merit. Interested applicants should submit the following:

1. An outline of the educational or training aspect of the proposed fellowship (1-2 pages).
2. Applicant's curriculum vitae.
3. Letter of support from temporal bone laboratory director or department chairman.
4. Letter from the host temporal bone laboratory, indicating willingness to receive the traveling fellow.

Applications should be submited to:
Michael J. McKenna, M.D.
NIDCD Temporal Bone Registry
Massachusetts Eye and Ear Infirmary
243 Charles Street
Boston, MA 02114
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.



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***Acknowledgments*:**
Supported by grants from the National Institute on Deafness and Other Communication Disorders. Figure 1 modified from Lin et al (2011). JARO 12: 605-61. Figures 2,3 modified from Kujawa SG, Liberman MC (2009). J Neurosci. 29(45): 14077-85.

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

The Gift of Hearing and Balance: Learning about Temporal Bone Donation is a 16-page full-color booklet which describes in more detail the benefits of temporal bone research. It also answers commonly asked questions regarding the temporal bone donation process. (Dimensions: 7”x10”)

If you would like to display either or both of these brochures, please complete the form below and return it to the Registry by mail or fax. The brochures will be sent to you **free of charge**. Please circle the amount requested for each brochure or write in amount not listed.

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