Hidden Injury in the Noise-Exposed and Aging Ear

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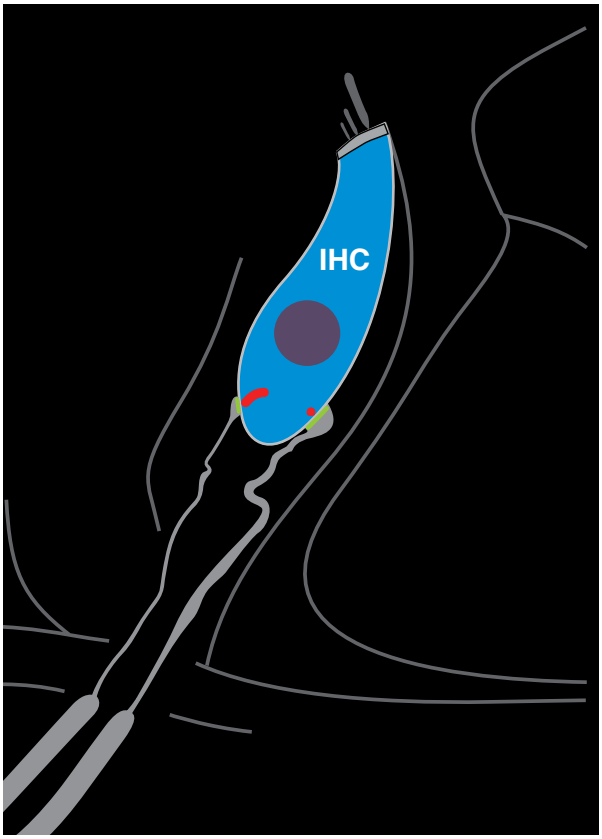
SUMMARY

Noise exposure and aging are two common causes of hearing loss in humans. Historically, the focus has been on hair cell damage and the threshold elevations that this causes. However, we now know that, for both noise and aging, inner hair cell synapses with cochlear afferent neurons are primary targets.Well before threshold elevations and hair cell damage compromise function by reducing the audibility of sound signals, synapse loss compromises function by permanently interrupting sensory-to-neural communication for affected neurons. In unexposed ears, loss of synapses is gradual and modest until advanced age. After noise, it is sudden; up to ~50 percent of synapses can be lost acutely with exposure, including many producing only temporary threshold shifts. In ears receiving even a single "synaptopathic" exposure, subsequent losses with age are accelerated and exaggerated. This loss is not revealed as changes in threshold sensitivity and is not detected by routine light microscopy; thus, it remained hidden from our view. It is nevertheless widespread, and the permanent interruption of information flow from hair cells to auditory nerve fibers that results must have important consequences for hearing function, whether thresholds are elevated or not. Here, the aim is to provide a brief review of this hidden injury in noise and aging and to place it into context relative to the threshold-elevating injuries that have been the focus of regulatory, diagnostic and treatment efforts to date.

Permanent Cochlear Injury After TTS-Producing Noise



Noise-induced hearing loss results when exposure to damaging levels of sound injures delicate inner ear structures and compromises vulnerable inner ear functions. The noise-induced threshold sensitivity loss captured by the audiogram can be temporary or permanent, and the underlying injury subtle or dramatic (Kujawa 2009). Permanent threshold losses after noise are associated with permanent cochlear injury, often hair cell loss or damage. In contrast, complete post-exposure recovery of thresholds has been assumed to indicate a recovered ear and a "safe" exposure,

with no delayed consequences for hearing function as noise-exposed individuals age (Institute of Medicine, 2006; ACOEM, 2012).These assumptions form the basis for noise exposure regulations, they shape our assessments of noise-induced injury and they guide approaches to treatment and prevention. Our recent work in several mammalian models of noise and aging, however, provides strong evidence that they require significant modification.

Thresholds recover, but neural response amplitudes do not. To examine these assumptions, we undertook a series of experiments in which we studied acute and long-term consequences of noise exposure on the ear and hearing. Below, for example (Kujawa & Liberman 2009), we adjusted the level and duration of a single noise exposure to produce a temporary threshold shift (TTS) on the border of reversibility. We used two complementary techniques, outer hair cell-based distortion product otoacoustic emissions (DPOAEs) and neural-based auditory brainstem responses (ABRs), to assess function, much as we do in the clinic. In the same ears, we quantified hair cells, cochlear neurons and the synapses that allow them to communicate with each other. As shown in Figure 1, a two-hour exposure with an octave-band of noise centered in a region of good hearing sensitivity in our mouse model resulted in a ~30–40dB maximum threshold shift by both DPOAEs (Figure 1a) and ABR wave 1 (Figuren 1b) when measured 24 hours after exposure. Threshold sensitivity recovered in the days following exposure and remained stable two and eight weeks later.

Although response thresholds returned to pre-exposure baselines, suprathreshold amplitudes of the DPOAE and ABR wave 1 responses showed a different pattern of recovery with post-exposure time. At 32 kHz, where the TTS was large (Figures 1a, b), the amplitudes of the neural responses displayed a permanent decline relative to the pre-exposure baseline (Figure 1d). In contrast, the amplitude-versus-level functions for the DPOAEs (Figure 1c), which require only presynaptic processes for their generation, recovered to pre-exposure levels. This persistent reduction in the suprathreshold neural response, in combination with full recovery of response thresholds and DPOAE amplitudes, suggested that the OHCs had recovered function but that there was permanent injury involving IHCs, cochlear

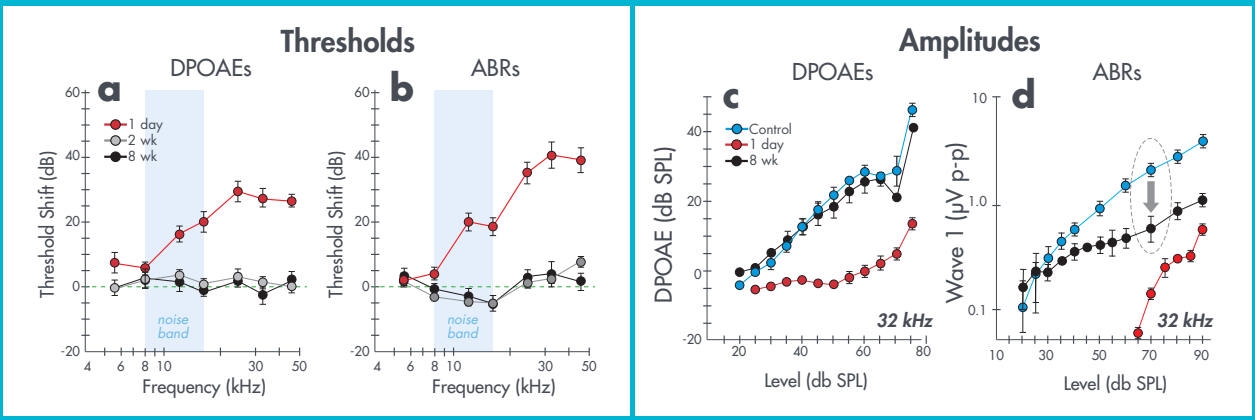


Figure 1. Post-noise threshold shifts vs. suprathreshold amplitude declines. A two-hour noise exposure (8–16 kHz, 100dB SPL) to 16 week-old CBA/CaJ mice produced large, but temporary threshold shifts in DPOAEs (a) and wave 1 of the ABR (b). Thresholds are expressed relative to age-, gender- and strain-matched, unexposed controls. Despite threshold recovery, suprathreshold amplitude of ABR wave 1, the far-field response of the cochlear nerve, recovered incompletely, to less than 50 percent of its pre-exposure value (d) in cochlear frequency regions with maximal TTS. In contrast, DPOAE amplitudes (c) returned to normal, suggesting functional recovery of the OHCs. Means ± SE shown; n=7–21/group. Gray bar in a, b denotes the noise exposure band. Modified from (Kujawa and Liberman, 2009).

afferent neurons or their synaptic connections in basal (high-frequency) cochlear regions. To determine the underlying cause(s), we examined tissues from the same noise-exposed vs. unexposed ears, as is reviewed next.

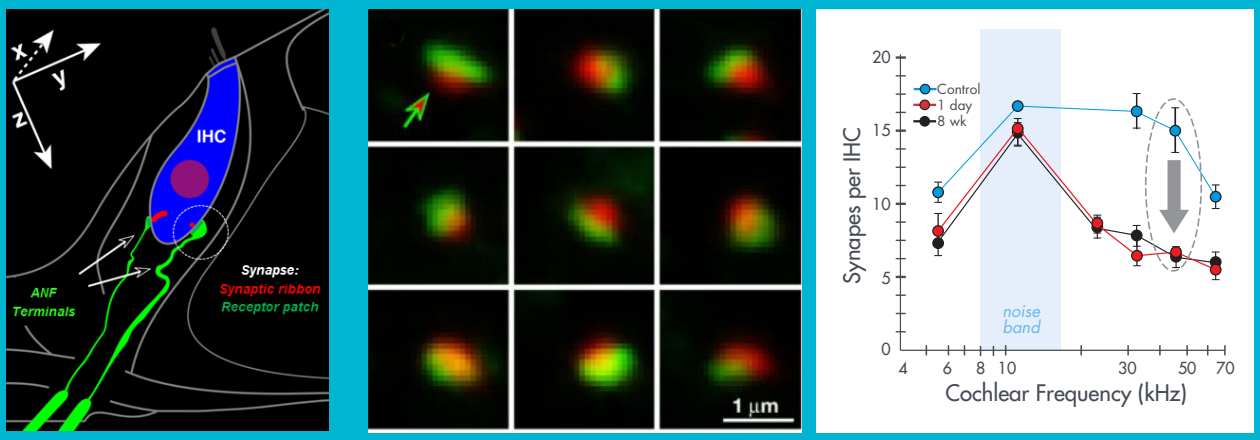
Hair cells remain, but about half lose connections with cochlear neurons. When assessed in the initial hours, days and weeks following this noise exposure, there was no hair cell loss (OHC, IHC) and no loss of spiral ganglion cells relative to counts in unexposed age-, gender- and strain-matched ears. However, direct evidence for lingering injury after noise was evident in the structures that form the synaptic communications between the IHCs and afferent neurons. To briefly review, the IHC-type I afferent fiber synapse is a specialized conduit through which information about the acoustic environment is transmitted to the neuron and ultimately to the brain. In the normal ear, roughly 95 percent of all cochlear afferent nerve fibers make synaptic connections only with IHCs (Spoendlin,1972). Each of these fibers has a cell body in the spiral ganglion, a peripheral axon that passes through the osseous spiral lamina and, in the organ of Corti, an unmyelinated terminal dendrite that forms a single synapse with a single IHC. The synapse is comprised of a synaptic ribbon with its surrounding halo of neurotransmitter-containing vesicles on the hair cell side of the synapse (Nouvian et al., 2006) and a post-synaptic glutamate receptor patch on the cochlear nerve terminal containing AMPA-type receptors for the released neurotransmitter (Matsubara et al., 1996; Ruel et al., 2007). The capabilities of these synapses push biological limits in their ability to rapidly and precisely convey graded temporal information about the stimulus and in maintaining this temporal coding fidelity over a large dynamic range (Moser et al., 2006).

To determine the effects of noise at the level of the synapse, we use immunostaining techniques to make these synaptic structures of interest visible and thus quantifiable. The presynaptic ribbon can be immunostained with antibodies to a protein called CtBP2, which is a prominent component of the ribbon (Khimich et al., 2006). Post-synaptic elements can be immunostained with antibodies to the AMPA-type glutamate receptors (GluA2) found at this synapse (Matsubara et al.,1996) or with antibodies to neurofilament proteins or to a Na+-K+ATPase found in the membranes of the nerve fiber terminals (McLean et al., 2009). Staining with myosin VIIa allows us to better visualize the hair cells. Figure 2a shows a schematic of an IHC with two of its auditory nerve fibers colorized to identify primary structures of interest; Figure 2b shows the paired ribbons and receptor patches as displayed for quantification.

In young, normal ears of CBA/CaJ mice, synapses are most numerous in the mid-frequency region, with roughly 17–18 synapses/IHC (Figure 2c). Noise exposure causes an immediate and dramatic reduction in their number; losses are greatest in regions where the acute TTS is maximum, and in those regions, can reach ~50 percent (Kujawa & Liberman, 2009). Because one auditory nerve fiber makes contact with only one IHC via one synaptic connection, these synapse counts also provide an estimate of the maximum number of auditory nerve fibers that could be carrying information from the cochlea to the brain. ABR wave 1 amplitudes yield permanent and proportional reflections of this loss (Figure 1d; see also Figure 3d) and provide information about the functional integrity of those neurons that remain.

Are all noise exposures synaptopathic?
Noise-induced synaptopathy has now been demonstrated for a wide range of both TTS- and PTS-producing exposures (Kujawa & Liberman, 2009; Kujawa & Liberman, 2006; Kujawa et al., 2011). Not all exposures are acutely synaptopathic, however. By reducing exposure level (sequentially halving the energy by reducing level in 3dB steps) while keeping the frequency content and duration of the noise constant, we identified a TTS-producing exposure (8-16 kHz, 2 h, 91dB SPL) that yielded no persistent ABR wave 1 amplitude

* Spectral iQ is a Starkey brand name.



a b c

Figure 2. Quantifying synapse loss. (a): Colorized schematic shows a single IHC and 2 of the ~20 Type I auditory nerve fibers (ANF) per IHC as they appear in tissue immunolabeled for pre-synaptic ribbons (CtBP2-red), post-synaptic glutamate receptor patches (GluA2-green), post-synaptic ANF terminals (neurofilament or Na-K-ATPase-green) and IHCs (Myosin VIIa, blue): the viewing angle for the x-y projections is noted. (b): To facilitate quantification of synapses, the synaptic region (white box in a) is analyzed using custom software that displays the paired synaptic ribbons and glutamate receptor patches or ANF terminals as x-y projections of the space within 1 µm of each ribbon. (c): Corresponding counts of synapses (means ± SE) in the IHC area from six cochlear locations are given for mice exposed at 16 weeks and assessed 24 hours or 8 weeks after exposure, compared to counts in age-matched, unexposed controls. Results show permanent synaptic loss throughout the basal half of the cochlea. Modified from (Kujawa and Liberman, 2009) and (Fernandez et al 2015).

decline after threshold recovery (Fernandez et al., 2015). Histologic study of these ears also revealed no acute loss of synapses. We then asked whether there are differences in the long-term consequences of exposures that produce acute synapse loss versus those that do not, on structure and function in aging ears.

Aging vs. Aging after Noise

Delayed effects as ears age after noise. The question of whether ears and hearing age differently after noise has received significant experimental attention. In humans, where investigations have concentrated on changes in pure-tone thresholds, consensus opinions concerning possible delayed effects of noise have not yet emerged (Gates et al., 2000; Rosenhall, 2003; Lee et al., 2005; Cruickshanks et al., 2010). The challenges to such study in the human are significant, where a lifetime's worth of noise-exposure events must be determined retrospectively, where confounding influences cannot be readily controlled and may not be known, and where large, genetic heterogeneity influences susceptibility to noise, to aging, and their interactions exists, all with the potential to introduce interindividual variability. Thus, although there is general agreement that threshold losses recorded at advanced age may include a contribution arising from prior noise (Gates & Mills, 2005), the question of whether noise can exert delayed effects on "hearing" remains an open one. Hearing function, of course, depends on more than threshold sensitivity/audibility. Recent laboratory experiments done in genetically-identical individuals receiving identical exposures and then aging in identical environments reveal dramatic and progressive synaptic and neural losses to which threshold measures are largely silent. These studies show that prior noise does, indeed, change the ways ears and hearing age, long after the noise has stopped.

In the experimental series shown for example here (Fernandez et al., 2015), mice were exposed at 16 weeks (when noise vulnerability has stabilized at adult levels) to an 8–16 kHz band of noise that was delivered at either 100dB (synaptopathic) or 91dB (non-synaptopathic) for 2 hr, as discussed

above. They were then held, without additional exposure, for post-exposure times from 1 hour to roughly 2 years of age, along with controls held identically, except for the single noise exposure. Structure and function were assessed at various ages/post-exposure holding times, with results for age-only versus exposed, then aged animals discussed below.

Synapses are most vulnerable in aging ears. Threshold elevations and hair cell losses in unexposed CBA/CaJ mice are minimal until about 2 years of age (Fig. 3a). Thereafter, accelerating threshold deterioration is mirrored by accelerating loss of OHCs; very few IHCs are lost as a function of age in this strain (Sergeyenko et al., 2013).

As with noise injury, however, sensory-to-neural communication shows early compromise in aging ears. Synaptic losses reach roughly 25 to 30 percent in middle age (Fig. 3b), when loss of hair cells is no more than 5 percent and threshold losses are not greater than about 5dB (Fig. 3a). Losses ultimately reach ~40 to 50 percent in the oldest ears. ABR amplitude declines (here, focused on wave 1; Fig. 3c) also begin early and progress steadily, in proportion to the declining synapse counts in the same ears (Fig. 3d). Age-related loss of IHC synapses also is paralleled, with a delay by proportional spiral ganglion cell losses in the same cochlear regions (Fig. 3b). When we compare the ganglion cell losses recorded in these aging mice to those we obtained in an age-graded series of human temporal bones (0–100 years) with full complements of hair cells (Makary et al., 2011), we see that the rate of loss is remarkably similar. Together, these findings suggest that age-related loss of IHC-cochlear nerve synapses may be an early contributor to the performance declines of aging listeners. Are such difficulties exaggerated in aging ears with a history of noise exposure?

Synaptopathic exposure accelerates cochlear aging. Both exposures we used for these experiments produce significant TTS (~30-45dB at 24 hour) as reflected in the DPOAEs and ABR wave 1 responses; frequencies of involvement extended basally, and overall magnitude at 24 hour was greater, for the 100dB noise. For both exposures,

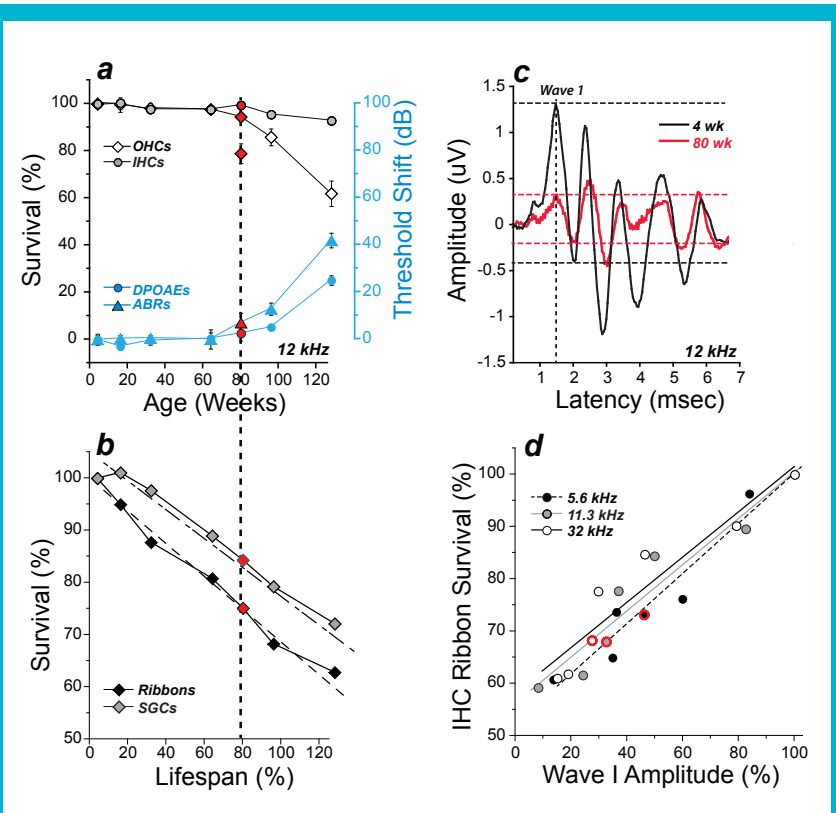


Figure 3. Synaptopathy in the aging ear. (a): Survival of OHCs and IHCs vs. age; shown are values for 12 kHz, means (± SE) relative to survival at 4 weeks. Values at 80 weeks are highlighted by red symbols and vertical dotted line for visual reference. (b): IHC ribbon counts were averaged across cochlear frequency, normalized to life span, and compared to frequency-averaged spiral ganglion cell (SGC) counts from the same animals; comparison of 80 week values (vertical line in a,b) shows that loss of ribbons and SGNs precedes loss of hair cells and threshold sensitivity. (c): Representative ABR waveforms (12 kHz, 80 dB SPL) at 4 weeks (black) versus 80 weeks (red): dotted lines mark Wave 1 peak and trough (d) IHC ribbon survivals are plotted versus mean Wave 1 amplitudes (at 80 dB SPL) from the same animals. Red-circled points are those from the 80 week group. Best-fit lines are shown for each stimulus/cochlear frequency. Modified from Sergeyenko et al., 2013.

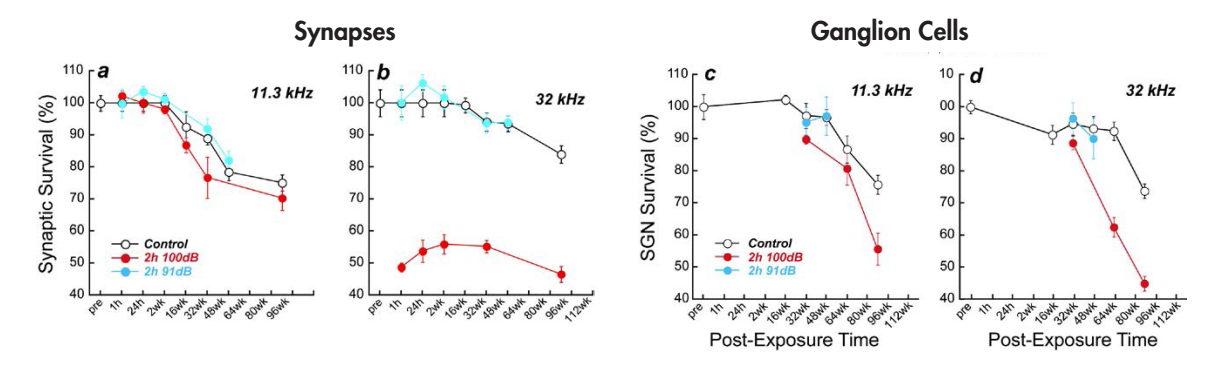


Figure 4. Noise exaggerates synaptic and neural losses in aging ears. Synapse counts (a, b) decline gradually with age (open symbols) and suddenly after noise (100dB at 2 hours); up to 50 percent loss is acutely evident in basal cochlear regions of maximum TTS (b, red symbols) without acute loss in regions of minimal to no TTS (a, open vs. red symbols). With post-exposure time, losses in more apical regions begin to exceed those in age-matched controls. There is no acute synapse loss for the 91dB, 2 hour exposure (a, b, teal) in either cochlear region and no obvious interaction between noise and aging. Ganglion cell counts (c, d) from mice exposed and held identically show similarly exaggerated losses for 100dB but not for 91dB exposure. Means (± SE) are normalized to 16 wk, unexposed values. Modified from (Fernandez et al 2015.)

thresholds returned to baseline, and as expected, there was no hair cell loss (Fernandez et al., 2015).

Despite reversibility of threshold shift and intact sensory cells in these ears, noise-induced degeneration progressed from the IHC-nerve fiber synapse to the nerve cell body with post-exposure time, exaggerating changes otherwise seen with aging alone. Panels of Figures 4a and 4b contrast the gradual and generally mild, age-related loss of synapses with immediate (one hour) and delayed (to 20 months) loss after noise. In the region of maximum TTS (32 kHz; Fig. 4b), synapse loss of ~50 percent is immediately apparent for 100dB but not for 91dB exposure. As ears age after noise exposure, cochlear regions that originally appeared uninvolved in the noise injury (e.g., 11.3 kHz; Fig. 4a) begin to demonstrate synaptic losses in excess of those seen in never exposed animals. Acutely non-synaptopathic (91dB) exposure did not produce this exaggeration of age-related losses, at least to one year. Subsequent loss of the cell bodies of these neurons within the spiral ganglion is slow (Figure 4c and 4d), progressing over one to two years; for the synaptopathic, 100dB exposure, it reaches dramatically exaggerated levels regarding age-alone controls. In terms of function, however, the communication failure occurred long before, with the loss of the synapse. Such diffuse neurodegeneration, which is not detectable by threshold metrics, raises challenges for evaluation and risk assessment, as discussed below.

Threshold-Based Assessments and Implications for Diagnosis and Management

Our standard, threshold-based tests are silent to the synaptopathy. The pure-tone threshold audiogram serves as a primary tool for quantifying the effects of noise and aging on hearing in clinical and occupational settings. Protocols for the measurement of threshold sensitivity are well established, standardized and validated. Threshold data captured from large-scale studies form the basis for population sensitivity norms to which individuals can be compared. Additionally, models of noise-induced hearing loss risk, which form the basis of every existing noise exposure standard, utilize audiometric threshold data.

Audiometric thresholds provide important functional information relative to signal levels required for detection and how this is impaired in aging and after noise. However, pure-tone audiograms provide imperfect reflections of

underlying pathology (Schuknecht & Woellner,1953; Halpin et al.,1994; Moore, 2004; Lobarinas et. al., 2013) and do not capture performance declines that depend on functions in addition to detection. Although audiometric thresholds for pure tones are the current "gold standard" for quantifying noise-induced hearing loss in humans and OAEs can provide additional sensitivity to underlying OHC compromise, these metrics, along with neural response thresholds, can be quite insensitive to even dramatic synaptic and neural loss, as shown here.

High-threshold, low-SR neurons appear most vulnerable. How is it that thresholds can return to normal despite loss of ~50 percent of the nerve fibers connecting hair cells to the brain? It appears that the fibers most vulnerable to noise exposure (Furman et al., 2013) and to aging (Schmiedt et al., 1996) are those that originally had the highest thresholds. Auditory nerve fibers contacting IHCs display a range of sensitivities and, in the normal cochlea, thresholds for fibers with similar best frequencies of response can differ by as much as 60dB (Liberman, 1978). These fibers also differ systematically in their spontaneous rates (SR) of firing, and their sound-driven firing rates vary over different ranges to support a large dynamic range of neural response; high-SR fibers have low (good) thresholds, but their driven rates saturate at levels where high-threshold, low-SR fibers continue to code level with increased firing rate. These features of low-SR neurons make them more resistant to continuous noise masking; as noise level increases, low-threshold, high-SR fibers are the first to be masked, while high-threshold, low-SR fibers are masked last (Costalupes et al., 1984). Thus, although noise-induced and age-related loss of these high-threshold fibers has little impact on overall threshold sensitivity, it is tempting to speculate that such losses, which are well underway by middle age, may contribute to the fairly universal finding that speech-in-noise performance becomes progressively more difficult with age, even when thresholds remain well preserved (Grose et al., 2006; Snell & Frisina, 2000; Ruggles et al., 2012) .

Summary

Noise exposure produces "hearing loss" (threshold elevations) and cochlear injury with effects that are largest at acute post-exposure times (Miller et al., 1963). Commonly, a period of rapid post-exposure recovery is followed by one of relative stability, which can be quite long lasting. This threshold recovery and subsequent stability, however, can mask persistent and progressive cochlear synaptic and neural loss. Accumulating evidence suggests that such loss is widespread, occurring as a primary event in at least two common causes of human hearing loss, noise and aging. Noise-induced synaptopathy has now been demonstrated in several mammalian models. There is no reason to suspect that the human will provide an exception to this general finding.

This work has sobering implications for public and occupational health, as opportunities for TTS-producing noise exposures are common in work and recreational activities and as federal guidelines for allowable noise exposure rely on PTS-based assessments of noise injury. It is now quite clear that these assessments fail to detect what is likely the more common consequence of noise exposure; thus, noise is much more dangerous than we previously thought.

Acknowledgments

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References

ACOEM Task Force on Occupational Hearing Loss. Kirchner DB, Evenson E, Dobie RA, Rabinowitz P, Crawford J, Kopke R, Hudson TW (2012) Occupational noise-induced hearing loss. J Occup Environ Med 54(1):106-108.

Costalupes, J.A., Young, E.D., & Gibson, D.J. (1984). Effects of Continuous Backgrounds on Rate Response of Auditory Nerve Fibers in Cat. *Journal of Neurophysiology, 51*(6), 1326-1344. Retrieved from: http://jn.physiology.org/content/51/6/1326.long

Cruickshanks, K.J., Nondahl, D.M., Tweed, T.S., Wiley, T.L., Klein, B.E., Klein, R., ..., Nash, S.D. (2010). Education, Occupation, Noise Exposure History and the 10-Year Cumulative Incidence of Hearing Impairment in Older Adults. *Hearing Research, 264*(1-2), 3-9. Doi:10.1016/j.heares.2009.10.008

Fernandez, K.A., Jeffers, P.W., Lall, K., Liberman, M.C., & Kujawa, S.G. (2015). Aging after Noise Exposure: Acceleration of Cochlear Synaptopathy in "Recovered" Ears. *The Journal of Neuroscience, 35*(19), 7509-7520. doi:10.1523/JNEUROSCI.5138-14.2015

Furman, A.C., Kujawa, S.G., & Liberman, C.M. (2013). Noise-Induced Cochlear Neuropathy is Selective for Fibers with Low Spontaneous Rates. *Journal of Neurophysiology, 110*(3), 577-586. doi:10.1152/jn.00164.2013

Gates, G.A., & Mills, J.H. (2005). Presbycusis. *Lancet, 366*(9491), 1111-1120. Doi:http://dx.doi.org/10.1016/S0140-6736(05)67423-5

Gates, G.A., Schmid, P., Kujawa, S.G., Nam, B., & D'Agostino, R. (2000). Longitudinal Threshold Changes in Older Men with Audiometric Notches. *Hearing Research, 141*(1-2), 220-228. doi:10.1016/S0378-5955(99)00223-3

Grose, J.H., Hall, J.W. 3rd., & Buss, E. (2006). Temporal Processing Deficits in the Pre-Senescent Auditory System. *The Journal of the Acoustical Society of America, 119*(4), 2305-2315. doi:10.1121/1.2172169

Halpin, C., Thornton, A., & Hasso, M. (1994). Low-Frequency Sensorineural Loss: Clinical Evaluation and Implications for Hearing Aid Fitting. *Ear Hear, 15*(1), 71-81. Doi:10.1097/00003446-199402000-00008

Institute of Medicine. (2006). *Noise and Military Service: Implications for Hearing Loss and Tinnitus.* Humes, L.E., Joellenbeck, L.M., & Durch, J.S. Washington, DC: National Academies Press.

Khimich D, Nouvian R, Pujol R, Tom Dieck S, Egner A, Gundelfinger ED, Moser T (2005) Hair cell synaptic ribbons are essential for synchronous auditory signalling Nature 434:889-894.

Kujawa, S.G. (2009). Noise-Induced Hearing Loss. In J.B. Snow & P.A. Wackym (17th Edition), Ballenger's *Otorhinolaryngology: Head and Neck Surgery* (265-272). Shelton, CT: People's Medical Publishing House – USA.

Kujawa, S.G., & Liberman, M.C. (2006). Acceleration of Age-Related Hearing Loss by Early Noise Exposure: Evidence of a Misspent Youth. *The Journal of Neuroscience, 26*(7), 2115-2123. doi:10.1073/pnas.1222285110

Kujawa, S.G., & Liberman, M.C. (2009). Adding Insult to Injury: Cochlear Nerve Degeneration after "Temporary" Noise-Induced Hearing Loss. *The Journal of Neuroscience, 29*(45), 14077-14085. doi:10.1523/JNEUROSCI.2845-09.2009

Kujawa, S.G., Micucci, S., & Liberman, M.C. (2011). Noise-Induced Primary Neural Degeneration: Effects of Spectrum, Duration, Intensity and Survival. *Midwinter Meeting of the Association for Research in Otolaryngology, 34*, 56.

Lee, F.S., Matthews, L.J., Dubno, J.R., & Mills, J.H. (2005). Longitudinal Study of Pure-Tone Thresholds in Older Persons. *Ear Hear, 26*(1), 1-11. doi:10.1097/00003446-200502000-00001

Liberman, M.C. (1978). Auditory-Nerve Response from Cats Raised in a Low-Noise Chamber. *The Journal of the Acoustical Society of America, 63*(2), 442-455. doi:10.1121/1.381736

Lobarinas, E., Salvi, R., & Ding, D. (2013). Insensitivity of the Audiogram to Carboplatin Induced Inner Hair Cell Loss in Chinchillas. *Hearing Research, 302*, 113-120. doi:10.1016/j.heares.2013.03.012

Matsubara, A., Laake, J.H., Davanger, S., Usami, S., & Ottersen, O.P. (1996). Organization of AMPA Receptor Subunits at a Glutamate Synapse: a Quantitative Immunogold Analysis of Hair Cell Synapses in the Rat Organ of Corti. *The Journal of Neuroscience, 16*(14), 4457-4467. doi:10.1111/j.1460-9568.1997.tb01640.x

Makary CA, Shin J, Kujawa SG, Liberman MC, Merchant SN. (2011) Age-related primary cochlear neuronal degeneration in human temporal bones. *J Assoc Res Otolaryngol 12*(6):711-717

McLean, W.J., Smith, K.A., Glowatzki, E., & Pyott, S.J. (2009). Distribution of the Na, K-ATPase Alpha Subunit in the Rat Spiral Ganglion and Organ of Corti. *Journal of the Association for Research in Otolaryngology, 10*(1), 37-49. doi:10.1007/s10162-008-0152-9

Miller, J.D., Watson, C.S., & Covell, W.P. (1963) Deafening Effects of Noise on the Cat. In *Acta Otolaryngologica: Supplement*, 1-91. Stockholm: Acta oto-laryngologica.

Moore, B.C.J. (2004). Dead Regions in the Cochlea: Conceptual Foundations, Diagnosis, and Clinical Applications. *Ear Hear, 25*(2), 98-116. doi:10.1097/01.AUD.0000120359.49711.D7

Moser, T., Neef, A., Khimich, D. (2006). Mechanisms Underlying the Temporal Precision of Sound Coding at the Inner Hair Cell Ribbon Synapse. *The Journal of Physiology, 576*(1), 55-62. doi:10.1113/jphysiol.2006.114835

Nouvian, R., Beutner, D., Parsons, T.D., & Moser, T. (2006). Structure and Function of the Hair Cell Ribbon Synapse. *The Journal of Membrane Biology, 209*(2-3), 153-165. doi:10.1007/s00232-005-0854-4

Rosenhall, U. (2003). The Influence of Ageing on Noise-Induced Hearing Loss. *Noise Health, 5*(20), 47-53. Retrieved from: http://www.noiseandhealth.org/text.asp?2003/5/20/47/31689

Ruel J, Wang J, Rebillard G, Eybalin M, Lloyd R, Pujol R, Puel JL. (2007) Physiology, pharmacology and plasticity at the inner hair cell synaptic complex. Hear Res. 227:19-27.

Ruggles, D., Bharadwaj, H., & Shinn-Cunningham, B.G. (2012). Why Middle-Aged Listeners Have Trouble Hearing in Everyday Settings. *Current Biology, 22*(15), 1417-1422. doi:10.1016/j.cub.2012.05.025

Schmiedt, R.A., Mills, J.H., & Boettcher, F.A. (1996). Age-Related Loss of Activity of Auditory-Nerve Fibers. *Journal of Neurophysiology, 76*(4), 2799-2803. doi:10.1523/JNEUROSCI.1783-13.2013

Schuknecht, M.D., & Woellner, B.A. (1953). Hearing Losses Following Partial Section of the Cochlear Nerve. *The Laryngoscope, 63*(6), 441-465. Doi:10.1288/00005537-195306000-00001

Sergeyenko, Y., Lall, K., Liberman, M.C., & Kujawa, S.G. (2013). Age-Related Cochlear Synaptopathy: An Early-Onset Contributor to Auditory Functional Decline. *The Journal of Neuroscience, 33*(34), 13686-13694. doi:10.1523/JNEUROSCI.1783-13.2013

Snell, K.B., & Frisina, D.R. (2000). Relationships Among Age-Related Differences in Gap Detection and Word Recognition. *The Journal of the Acoustical Society of America, 107*(3), 1615-1626. doi:10.1121/1.428446

Spoendlin, H. (1972). Innervation Densities of the Cochlea. *Acta oto-laryngologica, 73*(2), 235-248. doi:10.3109/00016487209138937

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