PHARMACEUTICAL INTERVENTIONS FOR HEARING LOSS (PIHL)

Newsletter – Fall 2014/Edition 3

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EDITORIAL

The increasing volume of research activities, from basic research through clinical trials, currently underway to understand the mechanisms behind hearing loss as well as potential pharmaceutical interventions that may reduce this health issue is both incredibly exciting and often difficult to keep up with. You will find that this third edition of the Newsletter is greatly expanded from the first two editions which were focused on concepts from the Animal Committee of the Pharmaceutical Interventions for Hearing Loss Working Group (PIHL WG). This edition grows the Newsletter to encompass the entire PIHL community scope, including a newly broadened spectrum of research highlights and clinical trials than before, in an attempt to create a manageable platform for dissemination and understanding of the current state of the PIHL sciences.

New to this edition are guidelines posed for consideration while performing PIHL research. These guidelines are the result of consensus across a large and diverse community of experts. The HCE's Execute Director, Col Mark Packer, will provide further introduction to that novel section later in this Newsletter.

As I mentioned, a great deal of research activities are currently underway addressing various aspects of noise-induced hearing loss, some of which have already yielded recent publications, while others have begun clinical trials. We would like to briefly touch on two of these published studies which are also incorporated within this Newsletter. The first of these is the article entitled "Efficacy and safety of AM-101 in the treatment of acute inner ear tinnitus—a double-blind, randomized placebo-controlled phase II study". This study evaluated the safety and efficacy of intratympanic AM-101 in 248 patients, aged 16 to 65 years, with acute inner ear tinnitus. Although the study yielded a negative finding for its primary end point of changes in minimum masking level, it did reveal that patients given AM-101 reported greater improvement in tinnitus loudness, annoyance, and sleep difficulties. It also demonstrated the safety of AM-101 as the study drug and that the intratympanic injections were well tolerated. This study also serves to remind researchers that they should give careful consideration of primary endpoints, be they objective or self-reported outcomes, as they define their studies.

In a second article, "Association between GPX-1 single nucleotide polymorphisms and susceptibility to noise-induced hearing loss among Chinese Han population", the authors found a statistically significant association between GPX-1 SNP rs1987628 and the susceptibility to noise-induced hearing loss (NIHL). In fact, in their case-control study, they found an odds ratio of 2.5 when comparing those with the C allele to those of the T allele. Additionally, the CC genotype had an odds ratio of 3.5 compared to the TT genotype. The ability to genetically identify individuals at increased risk for noise-induced hearing loss has both practical and research applications. From the practical sense, screening may allow for people to make better informed decisions as to the type of job and/or level of personal protection that they might choose, whereas researchers will be able to better control for these genetic effects when evaluating other potential

predictors of NIHL, and, potentially, future target populations for pharmaceutical interventions.

While expanding this newsletter's scope, the editors selected new and expanded search terms for both the literature review and the clinicaltrials.gov search. Each of those sections will provide the expanded terms and some insight into the editors' review process. Attempting to retain a scope broad enough to be adequately inclusive yet narrow enough to be appropriately targeted was the goal. This was made difficult at times by interesting observations of, for example, increasing numbers of articles exploring inter-morbidity correlations with hearing loss, such as in "Investigations into Audiovestibular Manifestations in Patients with Psoriatic Arthritis," which could potentially expand the reach of and demand for pharmaceutical interventions beyond our typical noise-exposed and ototoxic population concerns.

Also included in this edition is a list of the on-going clinical trials studying pharmaceutical interventions for hearing loss or tinnitus. It was very exciting to find 29 separate trials listed, ranging from safety trials to determine the local tolerance of repeated treatment cycles of AM-101 to Phase 3 trials such as the one being used to determine the efficacy of D-Methionine in preventing NIHL or reducing tinnitus secondary to live fire military training. The scope of agents and the wide range of study populations will assist future researchers in developing their own protocols. For researchers looking to fund future work, from basic science to clinical trial, be sure to check out the Funding Opportunities section at the end of the Newsletter.

Martin D. Slade, MPH –Yale University School of Medicine

Tanisha L. Hammill, MPH – DOD Hearing Center of Excellence

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RESEARCH GUIDANCES

Director's Foreword

The Pharmaceutical Interventions for Hearing Loss (PIHL) working group was chartered in 2012 with the purpose of reviewing and maintaining state of the science knowledge that supports translational therapies for the prevention and rescue of noise induced hearing loss. This foundational knowledge was to be used to spotlight minimal functional performance requirements of potential agents, and importantly, to identify the evidence-based laboratory, animal, exposure, and clinical assessment methodologies that underscore best practices and could be used to promote comparability across trials investigating new drug development.

The guidance beginning in this newsletter edition is the culmination of two years of working group discussion, literature review, and open dialogue during two states of the science symposia by the experts working with front-running candidate drugs and actually performing these investigations. All discussion focused on analyzing the issues most relevant to participation in Investigational New Drug (IND) development and translation of the science for the prevention and/or rescue of hearing loss. So, while players are certainly free to attack the field and advance the ball according to their preferred style and philosophy of play, we believe that PIHL advisors have "striped the field" to define and meter forward motion. And, while these views are not necessarily based on rigorous systematic review, the process of subject matter review, debate and experience, coupled with PIHL advisory consensus to bridge literature gaps in this developing field of research, allows us to confidently recommend appropriate standards and technologies to guide future PIHL studies.

Both Military and Civilian noise threats continue to claim casualties. Degradation of quality of life and limitations in communication, opportunities and performance can be expected to continue to escalate, marginalizing significant portions of the nation. Advancements in the PIHL arena are critical to ebbing this tide. The HCE will continue to facilitate extramural collaborations with Military study populations and by establishing requirements for technology transition to the DoD. Finally, the HCE is happy to continue to "officiate" play by recommending and administratively facilitating research methodology for DoD stakeholder implementation.

It has been my privilege to work with a passionate PIHL working group on developing these guidelines and I am honored to endorse the recommendations proposed herein. I believe in the competitive process, but feel that competition is enhanced by collegial and collaborative rules of engagement. I feel confident that this competing field of experts has defined a functional way ahead that will boost the progress of everyone interested in participating in the advancement of Pharmaceutical Interventions for Hearing loss.

~ Col Mark Packer, USAF, MC, FS

Executive Director, DOD Hearing Center of Excellence

Temporary and Permanent Noise-Induced Threshold Shifts

Allen F. Ryan PhD, Sharon G. Kujawa PhD, Tanisha L. Hammill MPH, Colleen Le Prell PhD, Jonathan Kil MD

Definition of Temporary and Permanent Threshold Shifts

Hearing loss due to noise has been recognized in humans for centuries. However, it was only in the 20th century that the phenomenon of noise-induced hearing loss (NIHL) was rigorously investigated in animals, allowing a more accurate determination and definition of the disease. Such studies have demonstrated that exposure to excessive sound produces hearing loss (threshold sensitivity loss), with the magnitude of the initial shift and the degree of recovery depending on characteristics of the exposure in the level, time and frequency domains, and on characteristics of the individual, as noted below. Threshold shifts that recover to baseline levels in the hours, days or weeks following exposure are termed temporary threshold shifts (TTS). More injurious exposures can produce threshold sensitivity losses containing both temporary and permanent components, in which the majority of the TTS resolves but a measurable permanent threshold shift (PTS) has evolved (e.g. Eldredge et al., 1973; Ryan and Bone, 1976). Threshold shifts of up to ~50 dB immediately after a single noise exposure can recover completely, while more extensive immediate hearing losses are likely to result in permanent losses of hearing sensitivity (Ryan and Bone, 1976). Continuous or repeated exposures to noise that only induce a TTS, may evolve to a PTS if repeated (Lonsbury-Martin, 1987), as occurs in occupational noise exposure. Therefore, PTS can be defined as noise-induced threshold shift that persists after a period of recovery subsequent to the exposure. In animal models, recovery has been reported for periods extending up to 3 weeks, therefore it may be premature to define a threshold shift as temporary until at least 3 weeks post-exposure, when a permanent threshold shift arises.

While the smallest level of TTS or PTS that can be reliably measured in an individual has not been well defined given test-to-test variability in individuals, several standards have been set for what is considered a significant hearing loss or "standard threshold shift" (STS). The Occupational Safety and Health Administration (OSHA) states that an STS is a 10 decibel (dB) increase in hearing threshold averaged across 2000, 3000 and 4000 Hz in the same ear from an individual's baseline or recent annual audiogram (29 CFR 1910.95). An STS is a reportable work related injury once it has been reconfirmed with a retest within 30 days of the initial test and results in a hearing threshold of at least 25 dB in the affected ear. Therefore, most occupational hearing loss or PTS is under reported since OSHA only requires an STS to be reported.

The Department of Defense policy for the military's Hearing Conservation Program (HCP) and the American Speech-Language-Hearing Association (ASHA) similarly define STS by a 10 dB shift average using the same frequencies, "in either ear without age

corrections" (DoDI 6055.12, 2010 [currently under revision]). In contrast, the National Institute of Occupational Safety and Health (NIOSH) recommended definition of an STS is "an increase of 15 dB in hearing threshold level (HTL) at 500, 1000, 2000, 3000, 4000, or 6000 Hz in either ear, as determined by two consecutive audiometric tests," with the second test required to reduce false-positive findings (NIOSH 1998). A significant negative STS (improved hearing) is further defined by the DoD as a decrease of 10 dB or greater change (improvement in hearing) for the average of 2, 3 and 4 kHz in either ear. An early warning shift STS (decrease in hearing) is defined as a 10 dB or greater change at 1, 2, 3 or 4 kHz in either ear. Therefore, a consistent measure between TTS and PTS involves a 10 dB shift from baseline hearing involving one or more frequencies in the same ear.

Characteristics of PTS

PTS is sensorineural and varies across frequencies, depending on characteristics of the exposure, the transmission characteristics of the external and middle ears, and the innate sensitivity of different regions of the cochlea to damage.

Noise damage is typically most extensive at frequencies above those of the exposure (Cody and Johnstone, 1981), a phenomenon well explained by nonlinearities in the cochlear mechanical response to sound (Robles and Ruggero, 2001). This is most apparent for TTS and for low levels of PTS. However, noises to which human ears are exposed often are broadband in frequency composition. These signals are shaped (some frequencies amplified, others reduced by filtering) by passage through the external and middle ears (Rosowski, 1991). Resonance in the ear canal produces amplification of acoustic frequencies whose wavelengths are approximately 4 times the length of the canal, which for humans results in enhancement of frequencies around 4 kHz. This contributes to an enhanced "notch" of PTS at 4-6 kHz for exposure to broad-band stimuli. Finally, as with many other forms of damage, the basal cochlea appears to be most vulnerable to noise. While the reason for this is not entirely clear, it may be related to higher levels of antioxidants in apical hair cells as well as higher rates of metabolic activity in basal hair cells (Sha et al., 2001). This basal sensitivity results in a tendency for TTS and PTS to be more extensive at high frequencies.

Characteristics of TTS

TTS is a change in hearing threshold that recovers to pre-exposure levels (baseline) over time. The amount of time to recover to baseline may be relatively fast (minutes to hours) or slow (days to weeks). The severity of the initial insult, as well as the time course of the recovery, are dependent on a number of factors including: the type of insult or trauma, the intensity and duration of the insult (single vs repeated, short vs long exposures), and the stimulus type (impulse/impact sound or continuous noise including wide or narrow-band noise). Individual susceptibility is dependent on the use of hearing protective devices, the quiet time or rest between exposures, and the level of hearing loss prior to exposure. Individual susceptibility to TTS may also be influenced by age, sex, prior history or noise exposure, diabetes, genotype and other personal or environmental factors

such as smoking and diet. While these factors are at play for PTS as well, unlike PTS, TTS is a change in hearing sensitivity which recovers to baseline or within test/retest criteria in minutes, hours, days or weeks with the upper limit being 30 days post exposure. TTS and PTS outcomes will vary as a function of the insult and individual factors.

Historically, TTS was largely thought to be a mechanical process that involved structures within the outer and middle ear including the ear drum, ossicular chain and middle ear muscles through the acoustic reflex. Extremely intense noise exposure is also known to mechanically damage the cochlea, disrupting the connections between the tectorial membrane and outer hair cell stereocilia, damaging the stereocilia themselves, breaching the integrity of the reticular lamina or even disrupting the basilar membrane.

However, recent work in several preclinical studies has demonstrated a significant involvement of several sensorineural inner ear structures including hair cells and their stereocilia, supporting cells within the organ of Corti, endothelial cells and fibrocytes within the stria vascularis and spiral ligament, and dendritic processes of the auditory nerve (Mulroy et al., 1990; Kujawa and Liberman, 2009). Molecular and biochemical changes have been identified that include pro-inflammatory and pro-apoptotic processes (Henderson et al., 2006). These changes have been shown to alter the normal function of several critical processes within the cochlea including the endolymphatic potential that drives hair cell depolarization (Yan et., al., 2013), cellular membranes and mitochondria responsible for hair cell and supporting cell activity, and neural innervation of the inner hair cell that conduct impulses to the auditory brainstem. In addition, changes in the activity or metabolism of neurons in the cochlear nucleus, superior-olivary complex and inferior colliculus have been observed (Ryan et al., 1992). In support of this noise-induced change in inner ear biology and pharmacology and its relevance in establishing the TTS, several preclinical studies have demonstrated a significant reduction in TTS when the animals were administered otoprotective compounds or drugs immediately prior to noise exposure (Siedman et al., 1993; Attias et al., 2004; Yamasoba et al., 2005; Lynch and Kil, 2005; Kil et al., 2007).

Mechanisms of PTS

While intense sounds such as blast can damage the conductive apparatus of the outer and middle ears, producing permanent hearing loss through tympanic membrane rupture or ossicular dislocation, PTS is generally considered to be a sensorineural phenomenon restricted to the cells of the cochlea. The most recognized cause of PTS is damage to and loss of cochlear hair cells. The mechanisms by which this damage can occur are not known with certainty. However, there is extensive evidence implicating the generation of reactive oxygen species (ROS) within hair cells during and after overexposure (Henderson et al., 2006). This leads to the activation of stress signaling pathways such as the JNK MAP kinase cascade (Pirvola et al., 2000), which can in turn lead to cell damage, apoptosis and/or necrosis (Bohne et al., 2007). The biochemical pathways leading to hair cell damage/death are undoubtedly complex, and also appear to include competing survival pathways that attempt to rescue hair cells and

restore their function. It is the balance of these competing pathways that determine the fate of the cell. The outer hair cells, responsible for the exquisite sensitivity and frequency and selectivity of the cochlea, are the most sensitive to damage (Eldredge et al., 1973; Ryan and Bone, 1976).

Noise also can target hair cell synapses and neurons directly, even when the hair cells themselves remain and recover normal function. The insult is seen acutely as a glutamate-like 'excitotoxicity' that includes swelling and retraction of afferent terminals from beneath inner hair cells (Robertson 1983). Recent work in animal models shows that noise-induced loss of synapses and afferent terminals is rapid and permanent (Kujawa and Liberman 2009; Lin et al 2011). Loss of spiral ganglion neurons is comparatively slow, and can be 'primary,' that is, occurring without noise-induced hair cell loss (Kujawa and Liberman 2006; 2009; Lin et al 2011) or 'secondary' to the loss of their inner hair-cell targets (Bohne, 1997; Puel, 1998). Such synaptic and neural loss can exacerbate the functional consequences of noise exposure by reducing the ability of the VIIIth nerve to encode auditory signals with fidelity, with or without loss of threshold sensitivity (Bharadwaj et al 2014). Thus lack of PTS does not imply that auditory function is normal.

It should be noted that our understanding of the mechanisms of NIHL remain incomplete. For example, many of the mechanisms that have been proposed to mediate hearing loss would take considerable time to develop. However impulse exposures, even those that do not result in PTS, produce hearing loss essentially instantaneously, without immediate loss of cells. Presumably this represents a disruption of cochlear cells at the microstructural and protein levels. In another example, it has recently been suggested that the initial 10-15 dB of TTS may serve as a mechanism to extend the dynamic range of hearing, rather than representing a damage mechanism (Housley et al., 2013). Further studies of NIHL mechanisms are clearly warranted.

Consequences of PTS

ASHA uses the following threshold-based definitions of hearing loss: none (normal hearing) (-10 to 15 dB), slight (16-24 dB), mild (25-40 dB), moderate (41-55 dB), moderately severe (56-70 dB) severe (71-90 dB) or profound (>91 dB). Thus 10 dB of PTS would have different consequences depending upon the initial level of hearing, for example leaving one individual with normal hearing (by definition) while increasing hearing loss from mild to moderate in another. One reasonable strategy may be to calculate the resulting hearing handicap as per the AAO-1979 criteria, or using the ASHA criteria, both of which incorporate a low fence of 25-dB HL. Key differences include the frequencies included in the calculation (AAO-1979: 0.5, 1, 2, and 3 kHz; ASHA: 1, 2, 3, and 4 kHz) and the growth rate for impairment for PTA thresholds above the low fence value (AAO-1979: 1.5% per dB; ASHA: 2% per dB). However, if PTA thresholds were below 25 dB HL after the exposure, the PTS would be "missed" using this scheme, as there is deemed to be no handicap below the low fence value. Moreover, functional losses that have no threshold change correlate would not be recognized using these strategies.

The consequences of threshold sensitivity loss have been well documented in animal studies of auditory physiology and psychophysical studies of human auditory function. Loss of 40 dB of hearing sensitivity is associated with a loss of outer hair cells, which as noted above are responsible for the lower ranges of hearing sensitivity, and for the sharply tuned responses of the cochlea to individual frequencies. The loss of these cells leads to a degraded ability to discriminate sounds, especially in noisy environments. More severe hearing loss is associated with the loss of inner hair cells, which transmit sensory information from the cochlea to the central auditory system. Loss of all inner hair cells from a cochlear region eliminates auditory responses, and loss from the entire cochlea results in total deafness.

Of course, the consequences of PTS are dependent upon the degree and frequency range of the loss, and total loss of hearing from noise exposure is rare. However, with PTS leading to moderate and especially severe hearing loss, many facets of life become extremely challenging (Arlinger, 2003). Communication is significantly impacted. This can lead to difficulty in performing military duties or in obtaining/retaining civilian employment. Social interactions are also heavily impacted, with the result that individuals with hearing loss can become withdrawn and isolated. This can in turn lead to depression and possibly cognitive decline (Arlinger, 2003). In the case of blast injury, hearing loss can exacerbate the effects of traumatic brain injury (TBI), even when TBI is mild (Lew et al., 2009). Another consequence of noise exposure is an increase in sensitivity to other forms of hearing loss, including ototoxicity (Bone and Ryan, 1978) and aging (Campo et al., 2011).

There is also a strong, positive correlation between the presence of noise-induced permanent hearing loss and tinnitus (Mazurek et al., 2010). While tinnitus can be a benign condition, a large fraction of individuals with tinnitus experience distress that can be extreme (e.g. Gomaa et al., 2013). A lesser correlation is observed for hyperacusis (e.g. Jansen et al., 2009), another negative sequela of PTS.

References

Arlinger S. Negative consequences of uncorrected hearing loss--a review. Int J. Audiol. 2003 Jul;42 Suppl 2:2S17-20.

ASHA. http://www.asha.org/public/hearing/Degree-of-Hearing-Loss/

Attias J, Sapir S, Bresloff I, Reshef-Haran I, Ising H. Reduction in noise-induced temporary threshold shift in humans following oral magnesium intake. Clin Otolaryngol 2004; 29:635-41.

Bharadwaj HM, Verhulst S, Shaheen L, Liberman MC, Shinn-Cunningham BG. Cochlear neuropathy and the coding of supra-threshold sound. Front Syst Neurosci. 2014 Feb 21;8:26. Doi: 10.3389/fnsys.2014.00026. eCollection 2014.

Bohne BA, Harding GW, Lee SC. Death pathways in noise-damaged outer hair cells. Hear Res. 2007 Jan;223(1-2):61-70.

Bohne BA. Time course of nerve-fiber regeneration in the noise-damaged mammalian cochlea. Int J Dev Neurosci. 1997 Jul;15(4-5):601-17.

Bone RC, Ryan AF. Audiometric and histologic correlates of the interaction between kanamycin and subtraumatic levels of noise in the chinchilla. Otolaryngology. 1978 May-Jun;86(3 Pt 1):ORL400-4.

Campo P, Venet T, Rumeau C, Thomas A, Rieger B, Cour C, Cosnier F, Parietti-Winkler C. Impact of noise or styrene exposure on the kinetics of presbycusis. Hear Res. 2011 Oct;280(1-2):122-32.

Clark WW, Bohne BA. Effects of noise on hearing. JAMA. 1999 May 5;281(17):1658-9.

Cody AR, Johnstone BM. Acoustic trauma: single neuron basis for the "half-octave shift". J Acoust Soc Am. 1981 70:707–711.

Eldredge DH, Mills JH, Bohne BA. Anatomical, behavioral, and electrophysiological observations on chinchillas after long exposures to noise. Adv Otorhinolaryngol. 1973;20:64-81.

Gomaa MA, Elmagd MH, Elbadry MM, Kader RM. Depression, Anxiety and Stress Scale in patients with tinnitus and hearing loss. Eur Arch Otorhinolaryngol. 2013 Sep 27. [Epub ahead of print].

Henderson D, Bielefeld EC, Harris KC, Hu BH. The role of oxidative stress in noise-induced hearing loss. Ear Hear 2006; 27:1-19

Housley GD, Morton-Jones R, Vlajkovic SM, Telang RS, Paramananthasivam V, Tedros SF, Wong AC, Foud KE, Cederholm JM, Sivakuraran Y, Snguanwongchai P, Khakh BS, Cockayne DA, Thorne PR, Ryan AF. ATP-gated ion channels mediate adaptation to elevated sound levels. Proc Natl Acad Sci U S A. 2013; 110:7494-7499.

Jansen EJ, Helleman HW, Dreschler WA, de Laat JA. Noise induced hearing loss and other hearing complaints among musicians of symphony orchestras. Int Arch Occup Environ Health. 2009 Jan;82(2):153-64.

Kil J, Pierce C, Tran H, Gu R, Lynch ED. Ebselen treatment reduces noise induced hearing loss via the mimicry and induction of glutathione peroxidase. Hear Res. 2007 Apr; 226(1-2):44-51.

Kirchner DB, Evenson E, Dobie RA, Rabinowitz P, Crawford J, Kopke R, Hudson WT. Occupational Noise-Induced Hearing Loss. JOEM 2012; 54(1):106-108.

Kujawa SG, Liberman MC. Acceleration of age-related hearing loss by early noise exposure: evidence of a misspent youth. J Neurosci. 2006 Feb 15;26(7):2115-23.

Kujawa SG, Liberman MC. Adding insult to injury: cochlear nerve degeneration after "temporary" noise-induced hearing loss. J Neurosci. 2009 Nov 11;29(45):14077-85.

Lew HL, Garvert DW, Pogoda TK, Hsu PT, Devine JM, White DK, Myers PJ, Goodrich GL. Auditory and visual impairments in patients with blast-related traumatic brain injury: Effect of dual sensory impairment on Functional Independence Measure. J Rehabil Res Dev. 2009;46(6):819-26.

Lin HW, Furman AC, Kujawa SG, Liberman MC. Primary neural degeneration in the Guinea pig cochlea after reversible noise-induced threshold shift. J Assoc Res Otolaryngol. 2011 Oct; 12(5):605-16.

Lonsbury-Martin BL, Martin GK, Bohne BA. Repeated TTS exposures in monkeys: alterations in hearing, cochlear structure, and single-unit thresholds. J Acoust Soc Am. 1987 May;81(5):1507-18.

Lynch ED, Kil J. Compounds for the prevention and treatment of noise-induced hearing loss. Drug Discovery Today 2005; 10:1291-8.

Mazurek B, Olze H, Haupt H, Szczepek AJ. The more the worse: the grade of noise-induced hearing loss associates with the severity of tinnitus. Int J Environ Res Public Health. 2010 Aug;7(8):3071-9.

Mulroy MJ, Fromm RF, Curtis S. Changes in the synaptic region of auditory hair cells during noise-induced temporary threshold shift. Hear Res 1990; 49(1-3):79-87.

Pirvola U, Xing-Qun L, Virkkala J, Saarma M, Murakata C, Camoratto AM, Walton KM, Ylikoski J. Rescue of hearing, auditory hair cells, and neurons by CEP-1347/KT7515, an inhibitor of c-Jun N-terminal kinase activation. J Neurosci. 2000 Jan 1;20(1):43-50.

Puel JL, Ruel J, Gervais d'Aldin C, Pujol R. Excitotoxicity and repair of cochlear synapses after noise-trauma induced hearing loss. Neuroreport. 1998 Jun 22;9(9):2109-14.

Robertson D. Functional significance of dendritic swelling after loud sounds in the guinea pig cochlea. Hear Res. 1983 9:263–278.

Robles L, Ruggero MA. Mechanics of the mammalian cochlea. Physiol Rev. 2001 81:1305–1352.

Rosowski JJ. The effects of external- and middle-ear filtering on auditory threshold and noise-induced hearing loss. J Acoust Soc Am. 1991; 90(1):124-135.

Ryan A, Bone RC. Noise-induced threshold shift and cochlear pathology in the Mongolian gerbil. J Acoust Soc Am. 1978 Apr; 63(4):1145-51.

Ryan AF, Axelsson GA, Woolf NK. Central auditory metabolic activity induced by intense noise exposure. Hear Res 1992; 61(1-2):24-30.

Sha S-H, Taylor R, Forge A, Schacht J. Differential vulnerability of basal and apical hair cells is based on intrinsic susceptibility to free radicals. Hear Res. 2001; 155:1-8.

Seidman MD, Shivapuja BG, Quirk WS. The protective effects of allopurinol and superoxide dismutase on noise-induced cochlear damage. Otolaryngol Head Neck Surg 1993; 109(6):1052-6.

Thurston FE. The worker's ear: a history of noise-induced hearing loss. Am J Ind Med. 2013 Mar;56(3):367-77.

Yamasoba T, Pourbakht A, Sakamoto T, Suzuki M. Ebselen prevents noise-induced excitotoxicity and temporary threshold shift. Neurosci Lett 2005; 380:234-8.

Yan D, Zhu Y, Walsh T, et al. Mutation of the ATP-gated P2X2 receptor leads to progressive hearing loss and increased susceptibility to noise. Proc Natl Acad Sci U S A. Feb 5, 2013; 110(6): 2228–2233.

Guidelines for Adult Auditory Threshold Measurement for Significant Noise Induced Threshold Shift

Kathleen Campbell PhD, Michael Hoffer MD, Tanisha Hammill MPH, Jonathan Kil MD, Colleen LePrell PhD

Introduction

At minimum, all clinical trials should meet all International Conference on Harmonization (ICH) guidelines for clinical trials (http://www.ich.org/). Currently no US Food and Drug Administration (FDA) guidance exists specifically for pharmacological intervention studies for hearing loss or tinnitus. However, reproducible, validated methodologies of the highest rigor must be employed. Test equipment, test environment, procedures, and personnel must meet all relevant American Speech-Language Hearing association (ASHA), American Academy of Audiology (AAA), American National Standards Institute (ANSI) standards and guidelines and military standards where applicable.

General Test Environment

Audiologic testing ought to be conducted in a testing environment meeting current ANSI standards, including annual inspection. Note that ANSI standards have different specifications for single and double walled sound booths for permissible external ambient noise levels. Levels both outside and within booths should be measured to verify compliance with ANSI guidelines.

Personnel

All study testing for hearing should optimally be performed by audiologists having at least a master's degree in audiology from a graduate program accredited regionally and by ASHA. In addition, each audiologist must hold a current audiology license in those states where licensure applies. Certain study design elements, such as subject screening for further audiological follow-up testing or longitudinal study follow up, may warrant testing performed by technicians who are certified by the Council for Accreditation in Occupational Hearing Conservation (CAOHC) and under the ultimate supervision of an audiologist. The audiologists or technicians will be responsible for all testing and for maintaining all audiologic data files for all patients enrolled in the study. For other countries, testing should be conducted by the equivalent personnel in the host country recognizing that in other countries, trained audiometric technicians may be the most skilled and appropriate personnel delivering the audiologic standard of care in that country. Further, it is incumbent on the study team to assure that all staff responsible for audiological testing in foreign-speaking nations both understand the protocol and ensure that language barriers do not introduce study confounds.

Equipment Standards

Test equipment, test environment, and procedures must meet all relevant ASHA, AAA, ANSI and JHACO standards and guidelines in the United States or equivalent in other countries.

Calibration

To ensure accurate and reliable auditory threshold determination, at minimum, audiologic test equipment should include calibrated audiometers for the conventional frequency range of 0.25-8 kHz, otoscope, and immittance audiometry, test environment (minimum single walled sound booth), procedures, and personnel meeting all relevant ASHA, AAA, ANSI, and Joint Hospital Accreditation Commission (JHACO) standards and guidelines in the United States or equivalent standards appropriate to the test site country.

All audiologic testing must be conducted using an audiometer calibrated at least annually in a test environment meeting ANSI specifications. If immittance audiometry is included, then the immitance bridge must also be calibrated at least once annually (ASHA 1987).

Test Procedures for Pure Tone Threshold Testing

Regardless of study design, subject audiological exams ought to include a detailed history including any preceding noise exposure and the use of hearing protective devices (HPDs), and whether additional complaints of aural pain, fullness, pressure, or tinnitus are present. A physical examination including otoscopy needs to be conducted with the appearance of the ear drum and any defects or abnormalities of the external ear canal noted. Evidence of middle ear disease including tympanic perforation requires further medical evaluation by an otolaryngologist. An audiometric examination should include tympanometry, a non-invasive test of middle ear function, and conventional pure tone audiometry (air 0.5, 1, 2, 3, 4, 6, 8 kHz.; bone 0.5, 1, 2, 4 kHz); a behavioral test of conductive and sensorineural hearing loss should be completed.

Pure-tone air conduction threshold testing both at baseline and post-test two to three weeks after noise exposure, will be conducted utilizing the modified Hughson-Westlake procedure. Pure-tone air-conduction testing will be conducted at 0.5, 1, 2, 3, 4, 6, 8 kHz. Bone conduction testing will be conducted at .5, 1, 2, 4 kHz if the pure tone air-conduction threshold at that frequency is greater than or equal to 15 dB HL.

Pure-tone threshold testing should be conducted using the modified Hughson Westlake procedure (Carhart and Jerger 1959, ASHA 1987) as follows: Initial descent towards threshold is accomplished in 10-dB steps. Beginning with the first non-response, level is increased by 5-dB for each non-response, and decreased by 10-dB after each correct detection response. Threshold is defined as the lowest level at which two responses are obtained out of three presentations on an ascending run.

At the baseline visit, pure-tone air-conduction testing will be immediately repeated at 1 and 2 kHz to determine that the subject provides reliable responses. Responses will be

considered reliable if retest thresholds at both frequencies do not exceed ± 5 dB of the previously obtained threshold response. This method of verifying threshold reliability for ototoxicity monitoring in clinical populations is based on Fausti, et al., 1999 and Campbell et al., 2003. The timing of the baseline and follow-up tests may vary by study. Clinically, for acute acoustic trauma inclusion studies, an individual presenting clinically with a complaint of hearing loss or tinnitus ought to be evaluated as soon as possible to determine the extent of the injury and provide a diagnosis and prognosis

Determination of PTS, TTS and Otoprotection

If a diagnosis of STS is made (see STS Definition paper), the subject should be counseled to reduce their noise exposure and be retested after 30 days from last noise exposure. Subjects with an STS should also undergo a final tympanogram screen to rule out middle ear pathology as a cause of the hearing loss. If they pass the tympanogram screen in both ears, they should be referred for a diagnostic audiologic follow-up exam. If they do not pass the tympanogram screen they will be referred for otologic check and then diagnostic audiological assessment.

If it is determined that a permanent threshold shift (PTS) has occurred, then the PTS must be recorded. Determination of work-related injury or hearing loss should be documented with relevant (i.e., OSHA, DOD) criteria and reported as required. The affected individual needs to be counseled on the importance of HPDs, on limiting their future noise exposures, and the potential for progressive hearing loss that could impair speech discrimination and result in permanent disability.

Study Design and Data Capture

Ideally, a computerized study database should be configured so that when the post-noise audiogram is entered in the database, criteria for hearing change will automatically be calculated and flagged, so that the examiner can recheck the frequencies in question.

Experimental design consists of within-subjects serial testing in which baseline standard frequency audiograms (as outlined above) are initially acquired by comparing similar measures obtained at the end of the study period to the relevant pre-exposure measure, reliable noise-induced changes in pure-tone hearing threshold can be identified. Thus, subjects will serve as their own control for identifying hearing change.

References

Campbell, Kathleen CM, Kelly, Ellen, Targovnik, Nan, Hughes, Larry F., Van Saders, Claudia, Gottleib, Alice B., Dorr, Mary Beth, Leighton, Anton. Audiologic Monitoring for Potential Ototoxicity in a Phase I Clinical Trial of a New Glycopeptide Antibiotic. Journal of the American Academy of Audiology: Special Edition on Ototoxicity 14(3):157-169. 2003.

Carhart, R., and Jerger J.F. (1959) Preferred Method for Clinical Determination of Pure-Tone Thresholds. Journal of Speech and Hearing Disorders, 24:330-45.

Biomarkers of Oxidative Damage and Inflammation: Experiences in Hearing and Balance Disorders

Gerald M. Haase MD, Kedar N. Prasad PhD

Oxidative stress from excessive production of free radicals is recognized as a causative factor in noise-induced hearing disorders (Henderson, Bielefeld, Harris, & Hu 2006; Neri et al., 2006; Ohlemiller, Wright, & Dugan, 1999). Pro-inflammatory cytokines from acute and chronic inflammation are also implicated in auditory injury (Chen et al., 2007; Derebery, 1996). Therefore, it is not surprising that several groups of investigators have reported that antioxidants (that also produce anti-inflammatory effects and quench free radicals at appropriate doses) demonstrate benefits in animal models and human trials (Ewert et al.,2012; Haase, Prasad, Cole, Baggett-Strehlau, & Wyatt, 2011; Hatano, Uramoto, Okabe, Furukawa, & Ito, 2008; Kopke et al., 2005; Le Prell, Hughes, & Miller, 2007).

A logical derivative from these experiences would suggest that biomarkers of oxidative damage and inflammation have value as appropriate secondary outcomes parameters in clinical studies regarding interventions for hearing disorders. While the list of potential biomarkers is extensive, it seems reasonable to highlight some of the most clinically relevant and well-studied in each category, including those with which we have had direct positive experience.

Oxidative stress involves direct injury to lipids, DNA and proteins, each of which is related to different biomarkers of damage. Markers of inflammation and antioxidant levels also reflect the degree of injury. The following measures are recommended as an initial relevant panel:

Markers of Oxidative Stress

In order to obtain a reliable indication of oxidative damage, at least one representative from each category of lipid peroxidation, adducts of DNA and oxidation of proteins, should be determined. Published studies that have only measured one or even two biomarkers of the same molecule may provide inadequate information.

Malondialdehyde (MDA) is an intracellular aldehyde formed during the metabolism of polyunsaturated fatty acids and a product of prostaglandin biosynthesis. It is one of the most abundant biomarkers of lipid peroxidation, a process resulting from increased cellular oxidative stress (Feng, Hu, Marnett, & Tang, 2006). One specific assay is based on the reaction of a chromogenic agent with MDA and the spectral absorbance is read at 586nm. The analysis can be performed on both plasma and urine in prospective human trials (Coppes et al., 2006).

F2-isoprostane, resulting from the non-enzymatic free radical-catalyzed peroxidation of arachidonic acid, is considered by some investigators to be a more specific product of

lipid peroxidation and a more sensitive indicator of oxidative damage in vivo (Montuschi , Barnes, & Roberts, 2004). Measurements based on gas chromatography, mass spectrometry and immunoassays are available for biological fluids and tissues. Plasma levels can be determined, but because of ease of collection and stability, measures of urinary levels are frequently utilized, such as in randomized human trials involving military subjects (Hodgdon et al., 2008).

Endogenous damage to nuclear or mitochondrial DNA can be caused by oxidative stress. One of the predominant oxidative lesions and most widely detected DNA adducts is 8-hydroxy deoxyguanosine (Marnett ,1999,2002). Detection in urine is commonly available employing mass spectrometry and immunochemical techniques and has been a beneficial marker in clinical trials evaluating oxidative stress occurring in harsh military environments (Hodgdon et al., 2008.).

Nitrosylative stress causes post-translational protein modifications and occurs with increased production of nitric oxide which is oxidized to form peroxynitrite. Peroxynitrite ca uses nitration of tyrosine and forms 3-nitrotyrosine (Ceriello, 2002; Xiao, Nel, & Loo, 2005). Nitrotyrosine in plasma can be detected by mass spectrometry, immune-blotting and gel electrophoresis. Similarly, oxidation of amino acid residues on proteins is a sensitive indicator of cellular damage. The modifications result in the formation of protein carbonyls (Chevion, Berenshtein, & Stadtman , 2000). These substances react with dinitrophenylhydrazine and the subsequent bound product can be measured in plasma by enzyme-linked immunosorbent assay technology.

Biomarkers of Inflammation

Pro- and anti-inflammatory cytokines are released by immune cells during the response to injury. During the acute phase, anti-inflammatory cytokines dominate to assist in repair, whereas pro-inflammatory cytokines are predominant in the chronic phase. An Increase in the level of two such substances, tumor necrosis factor-alpha and interleukin-6, participates in the pathologic processes of many chronic diseases, including hearing disorders (Clark, 2007; Heinrich et al., 2003). In addition, C-reactive protein is a well-established biomarker of inflammation, infection and tissue damage with relevant predictive clinical value (Pepys, & Hirschfield, 2003). Specific analysis of these substances in plasma can be performed by solid-phase enzyme-linked immunosorbent assay.

Nitric oxide (NO) synthases are enzymes that catalyze production of NO from L-arginine. One form, inducible nitric oxide synthase (iNOS), is of particular value because it is only expressed after cellular activation and produces the most sustained periods of NO presence. iNOS is expressed in a parallel manner in inflammatory processes in humans and rodents making it a valuable experimental biomarker of damage in animal models (Chavko et al., 2008). It is usually measured in plasma samples.

Levels of Glutamate

The excitatory amino acid, glutamate, plays a significant role in the progression of traumatic brain injury and related conditions such as hearing and balance dysfunction

(Gopinath, Valadka , Goodman, & Robertson, 2004). Since increased levels of glutamate are detected with increased severity of injury, these measurements in plasma should be determined. The most accurate technical methods employ high performance liquid chromatography.

Antioxidant levels

The degree of oxidative damage can also be determined by antioxidant levels in plasma and the level of alpha-tocopherol (lipid soluble) has proved to be a credible marker. These determinations have been valuable in demonstrating restoration of critical antioxidant levels as well as documenting subject compliance in consuming the study medication (Hodgdon et al., 2008). Glutathione (water soluble) is the most abundant intracellular antioxidant and is present in millimolar rather than micromolar amounts. However, some investigators have demonstrated that under certain circumstances, oxidized glutathione can also promote oxidative processes (Pompella, Visvikis, Paolicchi, & Casini, 2003). Therefore, it is generally thought that the ratio of reduced to oxidized glutathione is the most predictive measurement of the severity of injury (Pastore et al., 2001). A reliable assay employs a thiol-scavenging agent that reduces total glutathione without interfering with the action of glutathione reductase. The change in absorbance of the detectable product is determined by high performance liquid chromatography.

References

Ceriello, A. (2002). Nitrotyrosine: new findings as a marker of postprandial oxidative stress. *Int J Clin Pract Suppl, 129*, 51-58.

Chavko M, McCarron R, Prasad KN, et al. (2008). Prevention of post-blast wave inflammation by antioxidant micronutrients. Naval Medical Research Center, Silver Spring, Maryland.

Chen, Y., Huang, W. G., Zha, D. J., Qiu, J. H., Wang, J. L., Sha, S. H., & Schacht, J. (2007). Aspirin attenuates gentamicin ototoxicity: from the laboratory to the clinic. *Hear Res, 226*(1-2), 178-182.

Chevion, M., Berenshtein, E., & Stadtman, E. R. (2000). Human studies related to protein oxidation: protein carbonyl content as a marker of damage. *Free Radic Res, 33*(108), S99-108.

Clark, I. A. (2007). How TNF was recognized as a key mechanism of disease. *Cytokine Growth Factor Rev, 18*(3-4), 335-343.

Coppes Z, DeVecchi S, Coppes P, et al. (2006). A multiple micronutrient supplement increases immune competency and decreases oxidative stress in human volunteers: a pilot study. University of Uruguay, Montevideo.

Derebery, M. J. (1996). Allergic and immunologic aspects of Meniere's disease. *Otolaryngol Head Neck Surg, 114*(3), 360-365.

Ewert, D. L., Lu, J., Li, W., Du, X., Floyd, R., & Kopke, R. (2012). Antioxidant treatment reduces blast-induced cochlear damage and hearing loss. *Hear Res, 285*(1-2), 29-39.

Feng, Z., Hu, W., Marnett, L. J., & Tang, M. S. (2006). Malondialdehyde, a major endogenous lipid peroxidation product, sensitizes human cells to UV- and BPDE-induced killing and mutagenesis through inhibition of nucleotide excision repair. *Mutat Res, 601*(1-2), 125-136.

Gopinath, S. P., Valadka, A. B., Goodman, J. C., & Robertson, C. S. (2000). Extracellular glutamate and aspartate in head injured patients. *Acta Neurochir Suppl, 76*, 437-438.

Haase, G. M., Prasad, K. N., Cole, W. C., Baggett-Strehlau, J. M., & Wyatt, S. E. (2011). Antioxidant micronutrient impact on hearing disorders: concept, rationale, and evidence. *Am J Otolaryngol, 32*(1), 55-61.

Hatano, M., Uramoto, N., Okabe, Y., Furukawa, M., & Ito, M. (2008). Vitamin E and vitamin C in the treatment of idiopathic sudden sensorineural hearing loss. *Acta Otolaryngol, 128*(2), 116-121.

Heinrich, P. C., Behrmann, I., Haan, S., Hermanns, H. M., Muller-Newen, G., & Schaper, F. (2003). Principles of interleukin (IL)-6-type cytokine signalling and its regulation. *Biochem J, 374*(Pt 1), 1-20.

Henderson, D., Bielefeld, E. C., Harris, K. C., & Hu, B. H. (2006). The role of oxidative stress in noise-induced hearing loss. *Ear Hear, 27*(1), 1-19.

Hodgdon J, Prasad K, Cole WC, et al. (2008). Beneficial effects of micronutrients on oxidative damage during intensive military training. Naval Health Research Center, San Diego, California.

Kopke, R., Bielefeld, E., Liu, J., Zheng, J., Jackson, R., Henderson, D., & Coleman, J. K. (2005). Prevention of impulse noise-induced hearing loss with antioxidants. *Acta Otolaryngol, 125*(3), 235-243.

Le Prell, C. G., Hughes, L. F., & Miller, J. M. (2007). Free radical scavengers vitamins A, C, and E plus magnesium reduce noise trauma. *Free Radic Biol Med, 42*(9), 1454-1463.

Marnett, L. J. (1999). Lipid peroxidation-DNA damage by malondialdehyde. *Mutat Res, 424*(1-2), 83-95.

Marnett, L. J. (2002). Oxy radicals, lipid peroxidation and DNA damage. *Toxicology, 182*, 219-222.

Montuschi, P., Barnes, P. J., & Roberts, L. J., 2nd. (2004). Isoprostanes: markers and mediators of oxidative stress. *Faseb J, 18*(15), 1791-1800.

Neri, S., Signorelli, S., Pulvirenti, D., Mauceri, B., Cilio, D., Bordonaro, F., . . . Tsami, A. (2006). Oxidative stress, nitric oxide, endothelial dysfunction and tinnitus. *Free Radic Res, 40*(6), 615-618.

Ohlemiller, K. K., Wright, J. S., & Dugan, L. L. (1999). Early elevation of cochlear reactive oxygen species following noise exposure. *Audiol Neurootol, 4*(5), 229-236.

Pastore, A., Piemonte, F., Locatelli, M., Lo Russo, A., Gaeta, L. M., Tozzi, G., & Federici, G. (2001). Determination of blood total, reduced, and oxidized glutathione in pediatric subjects. *Clin Chem, 47*(8), 1467-1469.

Pepys, M. B., & Hirschfield, G. M. (2003). C-reactive protein: a critical update. *J Clin Invest, 111*(12), 1805-1812.

Pompella, A., Visvikis, A., Paolicchi, A., De Tata, V., & Casini, A. F. (2003). The changing faces of glutathione, a cellular protagonist. *Biochem Pharmacol, 66*(8), 1499-1503.

Xiao, G. G., Nel, A. E., & Loo, J. A. (2005). Nitrotyrosine-modified proteins and oxidative stress induced by diesel exhaust particles. *Electrophoresis, 26*(1), 280-292.

Measurement of Tinnitus

James Henry, PhD

Tinnitus researchers and clinical audiologists routinely obtain measures of tinnitus perception (Cope, Baguley,& Moore, 2011; Davis, Paki, & Hanley, 2007; Henry, & Meikle, 2000; Henry, 2004; Hiller, & Goebel, 2007; Jastreboff, Hazell, & Graham, 1994; Johnson, Brummett, & Schleuning, 1993). Over 30 years ago formal efforts were undertaken by the CIBA Foundation in London to promote international cooperation in tinnitus research (Evered, & Lawrenson, 1981; McFadden,1982). A central concept in the CIBA Symposium was that standardization of tinnitus measures would advance international understanding and facilitate work on tinnitus. As a result of these efforts a clinical assessment battery was recommended to include pitch match, loudness match, maskability, and residual inhibition. Vernon and Meikle (1981) published procedural details for these tests (requiring special equipment that was available at the time). Currently, most audiologists who perform tinnitus evaluations use their clinical audiometer in some manner to obtain the measures. Although the clinical value of these measures is questioned, they currently are used most commonly to enhance counseling.

To date there is no known method for reducing the perception of tinnitus, which would normally be experienced as a reduction in tinnitus loudness. The problem with using psychoacoustic measures to assess outcomes of treatment for tinnitus is thus twofold: (1) the measures have not been shown to correlate with changes in functional effects of tinnitus; and (2) methods do not exist to suppress or eliminate (i.e., cure) tinnitus. For these reasons, outcomes assessment in tinnitus research relies mainly on participants' subjective ratings of functional effects of tinnitus. Numerous questionnaires have been developed for this purpose, all of which were statistically validated for intake assessment. None, however, was specifically designed and tested to maximize responsiveness to intervention-related change. Further, no single questionnaire covered all dimensions of tinnitus functional impact, and all differed with respect to format, scaling, and wording of items. Consequently, it was difficult to compare intervention effects obtained in different clinics and in clinical trials. This has resulted in a lack of available systematic reviews, which are important for determining the clinical effectiveness of the various treatment options (Kamalski, Hoekstra et al. 2010).

A new self-report questionnaire, the Tinnitus Functional Index (TFI) has become available (Meikle et al., 2012). The TFI has documented validity both for scaling the negative impact of tinnitus for use in intake assessment and for measuring intervention-related changes ("responsiveness") in the functional effects of tinnitus. Because of its responsiveness to treatment-related change, as well as its other psychometric properties and comprehensive coverage of the domains of tinnitus impact, the TFI can be used as a standard instrument for both clinical and research settings. For evaluating tinnitus impact at intake, TFI mean scores can be stratified into five levels:

1. Not a problem: M = 14 (range: 0-17)
2. Small problem: M = 21 (range: 18-31)
3. Moderate problem: M = 42 (range: 32-53)
4. Big problem: M = 65 (range: 54-72)
5. Very big problem: M = 78 (range: 73-100)

As another way to interpret TFI scores, preliminary data support the following:

- <25 = relatively mild tinnitus (little or no need for intervention)
- 25-50 = significant problems with tinnitus (possible need for intervention)
- >50 = tinnitus severe enough to qualify for more aggressive intervention

The topic of minimum clinically important change in questionnaire index scores has generated substantial debate among measurement experts. A major issue is the considerable individual differences between patients in regard to what they consider a "meaningful change." Also, statistical demonstrations of differences between treatment groups are not necessarily indicative of changes that patients consider important or meaningful. What change in the TFI index score might our subjects consider meaningful? Using the criterion groups approach (described above), mean change scores exhibit an orderly progression from Much or Moderately improved through Unchanged to Moderately or Much worse. We interpret these data as suggesting a reduction in TFI scores of ~13 points should be meaningful to patients (there are considerable individual differences between patients in regard to what they consider a "meaningful change").

In addition to the TFI, a Visual Numeric (loudness rating) Scale (VNS) should be administered to research participants/patients at each visit (Folmer, et al., 2001). Participants should complete the scale at each appointment prior to any audiometric or psychoacoustic testing to ensure that the rating of tinnitus loudness is not affected by auditory stimulation. Careful instructions are given to participants to ensure that only a vertical line is drawn on the scale (as compared to a circle or shaded area). They are instructed: "On the scale below, please draw a vertical line to indicate the loudness of your tinnitus at this moment."

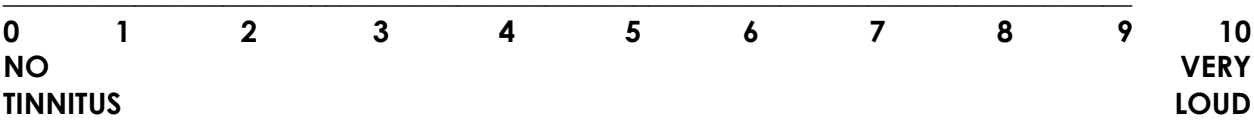

Figure 1. Visual Numeric (loudness rating) Scale (VNS) for self-rated tinnitus loudness. Participants will be instructed: "On the scale below, please draw a vertical line to indicate the loudness of your tinnitus at this moment." The VNS should be completed in a quiet exam room (not a sound booth) prior to any testing with auditory stimuli.

The Tinnitus Ototoxicity Monitoring Interview (TOMI) was developed as a clinical tool to detect tinnitus onset or changes in the tinnitus percept during treatment with potentially

ototoxic drugs. Portions of the TOMI were adapted from the TRT Initial Interview (Henry et al., 2003). The TOMI is a one-page instrument that can be completed normally within about 5 minutes. Ideally, the TOMI should be administered by an audiologist or ENT physician. Because it is fully scripted, the TOMI can also be administered by a nurse or other health care professional who may not be familiar with clinical tinnitus issues, in which case the patient's responses should be reviewed by an audiologist or ENT physician.

References

Cope, T. E., Baguley, D. M., & Moore, B. C. (2011). Tinnitus loudness in quiet and noise after resection of vestibular schwannoma. *Otol Neurotol, 32*(3), 488-496.

Davis, P. B., Paki, B., & Hanley, P. J. (2007). Neuromonics Tinnitus Treatment: third clinical trial. *Ear Hear, 28*(2), 242-259.

Evered, D., & Lawrenson, G. (Eds.). (1981). Tinnitus. Ciba Foundation Symposium 85. London: Pitman Books, Ltd.

Henry, J. A., & Meikle, M. B. (2000). Psychoacoustic measures of tinnitus. *J Am Acad Audiol, 11*(3), 138-155.

Henry, J. A. (2004). Audiologic assessment of the tinnitus patient. In J. B. Snow (Ed.), Tinnitus: Theory and Management (pp. 220-236). Lewiston, NY: BC Decker, Inc.

Hiller, W., & Goebel, G. (2007). When tinnitus loudness and annoyance are discrepant: audiological characteristics and psychological profile. *Audiol Neurootol, 12*(6), 391-400.

Jastreboff, P. J., Hazell, J. W., & Graham, R. L. (1994). Neurophysiological model of tinnitus: dependence of the minimal masking level on treatment outcome. *Hear Res, 80*(2), 216-232.

Johnson, R. M., Brummett, R., & Schleuning, A. (1993). Use of alprazolam for relief of tinnitus. A double-blind study. *Arch Otolaryngol Head Neck Surg, 119*(8), 842-845.

Meikle, M. B., Henry, J. A., Griest, S. E., Stewart, B. J., Abrams, H. B., McArdle, R., . . . Vernon, J. A. (2012). The tinnitus functional index: development of a new clinical measure for chronic, intrusive tinnitus. *Ear Hear, 33*(2), 153-176.

McFadden, D. (1982). Tinnitus-Facts, Theories and Treatments. Washington, D.C.: National Academy Press.

Vernon, J. A., & Meikle, M. B. (1981). Tinnitus masking:unresolved problems. *Ciba Found Symp, 85*, 239-262.

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Non-Cochlear Effects of Noise

Howard Greene, PhD

The consequences of population growth, urbanization, and technological developments include the continued growth of "noise pollution". Although a great deal of recent research has focused on investigation of its health effects, its "toxicity" was recognized thousands of years ago (Berglund and Lindvall, eds., 1995; Lee and Fleming, 2002). Chariots in ancient Rome were banned from the streets at night to prevent the noise of the wheels clattering on paving stones from disrupting sleep and annoying the citizens. Centuries later, cities in medieval Europe either banned horses and horse drawn carriages from the streets at night or covered the stone streets with straw to reduce noise and ensure residents' peaceful sleep. In eighteenth century Philadelphia, the framers of the constitution had nearby cobblestone streets covered with earth to prevent noise-induced interruptions (Goines and Hagler, 2007).

Our knowledge of the cochlear effects of noise has increased dramatically over the last few decades, and is now understood at the molecular level. In contrast, the non-cochlear effects are less clear, although many are intuitively obvious. The World Health Organization (WHO) estimated that in high-income western European countries (population approximately 340 million), at least 1 million healthy life-years (disability-adjusted life-years, DALYs) are lost every year because of environmental noise (Fritschi, 2011). The most investigated endpoints are sleep disturbance, cardiovascular health, cognitive impairment (mainly in children), and perceived annoyance (Basner, et al., 2013).

The Link between Chronic Noise Exposure and Adverse Health Effects

Beyond communication, hearing informs us about the environment. We are constantly analyzing what we hear. This is a complex process and requires that pathways distribute information across various areas of the brain and central nervous system. Noise effects depend upon the integrated meaning assigned to a host of characteristics (source, onset, duration, frequencies, intensity, whether exposure is voluntary or involuntary, whether it is regarded as useful or necessary, pleasant or unpleasant, etc.), which ultimately depends upon the instantaneous state of the person who hears it. Saunders (1956) referred to the myriad of interacting, transient, internal factors as "moderating variables". These properties of the listener determine how the body will respond.

In other words, there is only limited theoretical understanding of non-auditory noise effects, and knowledge of possible mechanisms and modifiers is little more than suggestive (Babisch, 2002; Guski, 1999). Health effects attributed to noise exposure are mediated by physiological and/or psychological responses, which often overlap, and may not be separable, especially when physiological effects are the underlying cause of the psychological stress and vice versa.

Physiological Models

These models hypothesize a link between noise and health that is mediated by either the:

1. Sympathetic nervous system and the secretion of catecholamines, or
2. The pituitary-adrenocortical axis, based on a process called the general adaptation syndrome (Selye, 1956), more recently described terms of allostasis (Sterling and Eyer, 1988) or allostatic load (McEwen and Stellar, 1993).

Psychological Models

From the psychological perspective, four major constructs have been proposed to account for the non-cochlear effects of noise:

1. Information overload
2. Arousal
3. Coping strategies
4. Loss of control

Health Effects

Sleep Disturbance

Undisturbed sleep of sufficient length is necessary for daytime alertness and performance, quality of life, and health (Muzet, 2007; Fritschi et al., 2011). Therefore, sleep disturbance is regarded as the most deleterious non-auditory effect of environmental noise. Humans perceive, evaluate, and react to environmental sounds, even while asleep (Dang-Vu et al., 2010). Sound pressure levels as low as L_{Amax} 33dB can induce physiologic reaction during sleep, including autonomic, motor, and cortical arousals (e.g., tachycardia, body movements, and awakenings) (Muzet, 2007; Basner et al., 2006). Reaction to noise while sleeping depends not only on the number of noise events and their acoustical properties, but also on situational moderators (e.g., sleep stage; Basner, et al, 2010) and individual noise susceptibility (Dang-Vu et al., 2010). The elderly, children, shift-workers, and people with a pre-existing sleep disorder are the at risk groups for noise induced sleep disturbance. Repeated arousals interfere with sleep structure, including delayed sleep onset and early awakenings, reduced deep (slow wave) and REM sleep, and an increase in time spent awake and in superficial sleep stages. Short-term effects of disturbed sleep include impaired mood, daytime sleepiness, and impaired cognitive performance (Basner, 2008; Elmenhorst et al., 2010).

Cardiovascular Disease

Noise exposure causes a number of short-term physiological activation responses mediated through the autonomic nervous and endocrine systems (including increased heart rate and blood pressure, peripheral vasoconstriction), and causes the release of stress hormones (including catecholamines and glucocorticoids).

Long-term studies have provided biological mechanisms and plausibility for the hypothesis that long-term exposure to environmental noise affects the cardiovascular system in humans and animals, and causes manifest disease (including hypertension,

ischemic heart disease, and stroke) in animals (Babisch, 2011). However, effects in humans and animals cannot be directly compared. The effect mechanism is thought to be the general stress model, which comprises the two pathways discussed earlier: The direct (physiological) pathway (non-conscious stress from interactions between the central auditory system and other regions of the CNS), and the indirect (psychological) pathway (emotional stress due to the cognitive reaction to noise). The latter is certainly different in humans (WHO, 2011).

The association of noise exposure and cardiovascular disease is supported by several epidemiology studies of occupationally-(van Kempen, et al., 2002; and Tomei, et al., 2010; Davies and van Kamp, 2012) and environmentally (Huss, et al., 2010; Sorensen, et al., 2011; and Gan et al., 2012) exposed populations. However, the studies are not completely convincing. The risk estimates for occupational noise at ear-damaging intensities tend to be higher than are those for environmental noise, but still relatively small (RR<2) – within the range where they could be explained by incomplete control for confounding and/or various biases, e.g., reporting bias, and selection bias.

Babisch (2011) points out an additional obstacle to Interpretation. Non-auditory noise effects do not follow the toxicological principle of dosage. This means that it is not simply the accumulated sound energy that causes the adverse effects (dealing with decibels is not like summing up micrograms as we do for chemical exposures). Instead, the individual situation and disturbed activity need to be taken into account (time activity patterns). It may be very well that 80 decibels has less effect than 65 decibels when carrying out mental tasks at home or 50 decibels when trying to sleep. In this respect, the evening hours, when people come home from work for relaxation and the nighttime, when the body physically recovers from daytime load and brain restoration takes place, may be particularly important with respect to noise-induced health effects. Sleep is also an important modulator of cardiovascular function. Noise-disturbed sleep, in this respect, must be considered as a particular potential pathway for the development of cardiovascular disorders.

Cognitive Effects

More than 20 studies have shown environmental noise exposure has a negative effect on children's learning outcomes and cognitive performance (Evans and Hygge, 2007), and that children with chronic aircraft, road traffic, or rail noise exposure at school have poorer reading ability, memory, and performance on national standardized tests than do children who are not exposed to noise at school (Hygge et al., 2002; Bronzaft, 1981; Lercher et al., 2003). The RANCH study of 2844 children aged 9 – 10 years attending 89 schools around Heathrow (London, UK), Schiphol (Amsterdam, the Netherlands), and Madrid-Barajas (Spain) airports showed a linear exposure/effect relationship between aircraft noise exposure at school and a child's reading comprehension and recognition memory after adjusting for a range of socioeconomic factors (Stansfeld et al., 2005; Clark et al., 2006). This linear association between exposure and effect suggests that there is no effect threshold, and any reduction in noise level at school should improve a child's cognition.

Annoyance

Annoyance is the most prevalent community response in a population exposed to environmental noise. Noise-related annoyance can result from interference with daily activities, feelings, thoughts, sleep, or rest, and may be accompanied by anger, displeasure, exhaustion, and by stress-related symptoms. In severe forms, it could be thought to affect wellbeing and health, and because of the large number of people affected, annoyance substantially contributes to the burden of disease from environmental noise.

Conclusions

The evidence for non-cochlear effect of noise on health is strongest for annoyance, sleep, and cognitive performance in adults and children. Occupational noise exposure shows some association with increased blood pressure. Dose-response relationships can be demonstrated for annoyance and, less consistently for blood pressure. The effects of noise are strongest for those outcomes that, like annoyance, can be classified under "quality of life" rather than illness. Nevertheless, what these effects lack in severity, they make up for in number of people affected.

Adaptation to long-term noise needs further study. Most people exposed to chronic environmental noise, for example from major airports, tend to tolerate it (they do not move). Yet questionnaire studies suggest that high levels of annoyance do not decline over time. One possible explanation is that adaptation to noise is achieved with a cost to health. These points ought to be taken into consideration when designing a research study involving noise exposures for heightened protection of human subjects as well as for additional data collection considerations to pursue.

References

Babisch W. The noise/stress concept, risk assessment and research needs. *Noise and Health*, 2002, 4(16):1–11.

Babisch W. Cardiovascular effects of noise. In: Nriagu JO, ed. Encyclopedia of Environmental Health. Burlington: Elsevier, 2011: 532-42.

Basner M, Samel A, Isermann U. Aircraft noise effects on sleep: application of the results of a large polysomnographic field study. *J Acoust Soc Am* 2006; 119: 2772–84.

Basner M, Müller U, Griefahn B. Practical guidance for risk assessment of traffic noise effects on sleep. *Appl Acoust* 2010; 71: 518–22.

Basner M. Nocturnal aircraft noise increases objectively assessed daytime sleepiness. *Somnologie* 2008; 12: 110-117.

Basner M, Muller U, Elmenhorst EM. Single and combined effects of air, road, and rail traffic noise on sleep and recuperation. *Sleep* 2011; 34: 11-23.

Basner M, Babisch W, Davis A, Brink M, Clark C, Janssen S, and Stansfeld S. Auditory and non-auditory effects of noise on health. www.thelancet.com, October 30, 2013.

Berglund B, Lindvall T. (eds.) Community Noise. *Archives of the Center for Sensory Research.*1995; 2:1-195.

Bronzaft AL. The effect of a noise abatement program on reading ability. *J Environ Psychol* 1981; 1: 215-222.

Clark C, Martin R, van Kempen E, et al. Exposure-effect relations between aircraft and road traffic noise exposure at school and reading comprehension: the RANCH project. *Am J Epidemiol* 2006; 163: 27-37.

Dang-Vu TT, McKinney SM, Buxton OM, Solet JM, Ellenbogen JM. Spontaneous brain rhythms predict sleep stability in the face of noise. *Curr Biol* 2010; 20: R626-27.

Davies H and van Kamp IV. Noise and cardiovascular disease: a review of the literature 2008-2011. *Noise Health* 2012; 14: 287-291.

Elmenhorst EM, Elmenhorst D, Wenzel J, et al. Effects of nocturnal aircraft noise on cognitive performance in the following morning: dose-response relationships in laboratory and field. *Int Arch Occup Environ Health* 2010; 83: 743–51.

Evans G, Hygge S. Noise and performance in adults and children. In: Luxor L, Prasher D, eds. Noise and its effects. London: Whurr Publishers, 2007.

Fields JM. Effects of Personal and Situational Variables on Noise Annoyance with Special Reference to Implications for En Route Noise. Report No.: FAA-AEE-92-03. Washington, DC: Federal Aviation Administration and NASA, 1992

Flindall, IH and Stallen, PJM. Non-acoustical factors in environmental noise, *Noise and Health* 1999; Vol. 3, pp. 11–16.

Fritschi L, Brown AL, Kimi R, Schwela DH, Kephalopoulos S, eds. Burden of disease from environmental noise. Bonn: World Health Organization, 2011.

Gan WQ, Davies HW, Koehoorn M, Brauer M. Association of long-term exposure to community noise and traffic-related air pollution with coronary heart disease mortality. *Am J Epidemiol* 2012; 175: 898–906.

Guski R. Personal and social variables as co-determinants of noise annoyance. *Noise and Health* 1999;1(3):45-56.

Goines L and Hagler L. Noise Pollution: A Modern Plague. *South Med J*. 2007; 100(3):287-294.

Hatfield, J, Job, RFS, Carter, NL, Peploe, P, Taylor, R and Morrell, S. The influence of psychological factors on self-reported physiological effects of noise. *Noise and Health* 2001, Vol. 3, pp. 1–13.

Hudspeth AJ and Konishi M. Auditory neuroscience: Development, transduction and integration', *Proceedings of the National Academy of Sciences 2000* Vol. 97, No. 22, pp 11690–691.

Huss A, Spoerri A, Egger M, Röösli M, for the Swiss National Cohort Study Group. Aircraft noise, air pollution, and mortality from myocardial infarction. *Epidemiology* 2010; 21: 829-836.

Hygge S, Evans GW, Bullinger M. A prospective study of some effects of aircraft noise on cognitive performance in schoolchildren. *Psyhchol Sci* 2002; 13: 469-474.

Job RFS. Noise sensitivity as a factor influencing human reaction. Noise and Health 1999, Vol. 3, pp. 57–68.Lee CSY, Fleming GG. General Health Effects of Transportation Noise. U.S. Department of Transportation. dts-34-RR297-LR2. Washington, DC, 2002

Lercher P, Evans GW, Meis M. Ambient noise and cognitive processes among primary schoolchildren. Environ Behav 2003; 35:725-735.

Manabe Y, Kurokawa T, Saito T, Saito H. Vestibular dysfunction in noise induced hearing loss .*Acta Otolaryngol Suppl*. 1995;519:262-4.

McEwen BS and Stellar E. Stress and the individual. Mechanisms leading to disease. Arch Intern Med 1993 Sep 27; 153(18):2093-2101.

Muzet A. Environmental noise, sleep and health. *Sleep Med Rev* 2007; 11: 135-142.

Oosterveld WJ, Polman AR, Schoonheyt J. Vestibular implications of noise-induced hearing loss.Br J Audiol. 1982 Nov;16(4):227-32

Saunders, DR 1956. Moderator variables in prediction. *Journal of Educational and Psychological Measurement* 1956; Vol. 16, pp. 209–22.Selye H. Stress and Disease. Science. 1955; 122:625-631.

Shupak A, Bar-El E, Podoshin L, Spitzer O, Gordon CR, Ben-David. Vestibular findings associated with chronic noise induced hearing impairment. *Acta Otolaryngol*. 1994 Nov;114(6):579-85.

Sørensen M, Hvidberg M, Andersen ZJ, et al. Road traffic noise and stroke: a prospective cohort study. *Eur Heart J* 2011; 32: 737-744.

Sørensen M, Andersen ZJ, Nordsborg RB, et al. Road traffic noise and incident myocardial infarction: a prospective cohort study. *PLoS One* 2012; 7: e32983

Stansfeld SA, Berglund B, Clark C, et al. for the RANCH study team. Aircraft and road traffic noise and children's cognition and health: a cross-national study. *Lancet* 2005; 365: 1942-1949.

Suter, H. Noise and its Effects. Administrative Conference of the United States, 1991 (http://www.nonoise.org/library/suter/suter.htm).

Tomei G, Fioravanti M, Cerratti D, et al. Occupational exposure to noise and the cardiovascular system: a meta-analysis. *Sci Total Envion* 2010; 408: 681-689.

Vallet, M. Sleep Disturbance by Noise: Recent Orientations. Noise Effects 1998 Congress, pp. 421–427.

Sydney.van Kempen EEMM, Kruize H, Boshuizen HC, Ameling CB, Staatsen BAM, de Hollander AEM. The association between noise exposure and blood pressure and ischemic heart disease: a meta-analysis. *Environ Health Perspect* 2002; 110: 307-317.

World Health Organization. Burden of disease from environmental noise: Quantification of healthy life year lost in Europe. Theakston F (ed), The WHO European Centre for Environment and Health, Bonn Office. 2011.

The Genetic and Epigenetic Basis of Noise-Induced Hearing Loss

Royce E Clifford MD, Michael E Hoffer MD, Rick A Rogers PhD

Noise-induced hearing loss can be examined using the model of the junction of environmental effects on the genome and its subsequent expression in phenotype. Individual human susceptibility to noise depends on the type of noise – continuous versus impulse versus explosive, as well as intensity and frequencies, acting in concert with the cellular reaction determined by an individual's DNA (Taggart et al., 2001). Putative genes and their single nucleotide polymorphisms (SNPs) have been studied in humans and in the laboratory; however, the application of animal studies to humans is challenging, due to the varying extent of noise exposure in both industrial and military populations, different SNPs between human and animals, and the need for population studies large enough to demonstrate statistical significance (Annelies Konings, Van Laer, & Van Camp, 2009). Over the last decade, human and laboratory studies have concentrated on candidate genes based on oxidative stress and apoptosis pathways, potassium channel recycling in the inner ear, tip-link genes, and Hsp70 genes (Sliwinska-Kowalska & Pawelczyk, 2013).

The cochlea maintains constant metabolic activity; mechanosensory outer hair cells (OHCs) respond to sound stimuli by adjusting length and breadth, and nerve cells fire a constant baseline stream of signals to the brain. The synthesis of reactive oxidative species (ROS) is a key factor in the maintenance of cellular equilibrium and it is the cell's ability to neutralize ROC species via glutathione, methionine sulfide reductase and other pathways to support cellular metabolism in response to noise exposure. However, when stimulated by excess noise, cochlear cells produce increasing levels of ROS, overwhelming this protective mechanism. ROS that linger in the cell without removal can cause damage to membranes, DNA, and proteins. Any defect in one of the proteins involved either in neutralizing or in reconstituting the normal glutathione level may decrease the cochlea's ability to respond to this stress, resulting in apoptosis or cell death.

Simultaneously with reaction to noise, the cochlea must maintain its electric potential across two fluid compartments, the scala media and scala vestibulae. Other studies suggest a SNP dysregulation of potassium channels across this fluid barrier may lead to degradation of the potential (Sliwinska-Kowalska & Pawelczyk, 2013).

Epidemiologic genetic studies have implicated SNPs occurring in oxidative pathway genes as well as potassium recycling genes, including glutathione S-transferase (GSTM) isoforms, GSTP, glutathione peroxidase (GPX1), glutathione S-reductase (GSR), superoxide dismutase (SOD), paraoxonase (PON2), catalase (CAT), KCNE1, and others (Carlsson et al., 2005; Fortunato et al., 2004; A Konings et al., 2007; Laer et al., 2006; Lin & Wu, 2009; Rabinowitz et al., 2002). The results may hold promise in directing therapeutic

intervention. Lin & Wu (2009) reported that those members of the population who had glutathione S-transferase (GST) T1 and M1 SNPs responded to N-acetylcysteine more consistently than workers exposed to industrial noise with either GST-T1 or GST-M1 SNPs in prevention of temporary threshold shifts. Different regional populations may have diverse SNPs because of global genetic drift, mitigating against drawing general conclusions from a specific population. Thus what may be an important SNP in one part of the world may not be relevant on another continent. In addition, these SNPs have focused on those occurring in exons; whereas SNPs in introns have yet to be addressed. Increasing evidence shows that introns can be involved in control of up regulation, down regulation, enhancement, and directing RNA-replication via epigenetic means.

Research in various animal species has identified genes, proteins, and chromosome areas associated with acoustic susceptibility. This includes oxidative pathways as well as apoptosis genes, genes involved in the initial immune and inflammatory response, and others. Genes crucial to OHC and IHC survival after acoustic trauma include caspase-3, caspase-9, bcl-2, c-Jun N-terminal kinase (JNK), p38 mitogen-activated protein kinases (Cheng, Cunningham, & Rubel, 2005; Minal Patel et al., 2013; Ruan et al., 2007; Yamashita, Minami, Kanzaki, Ogawa, & Miller, 2008), as well as proteins involved in inflammation, i.e., ICAM-1, NF kappaβ, 5 (MCP-5), monocyte chemoattractant protein 1 (MCP-1), MIP-1beta, P-selectin, PECAM-1, IL-6 (Adams, Seed, Lu, Landry, & Xavier, 2009; Seidman et al., 2009; Shi & Nuttall, 2007; Tornabene, Sato, Pham, Billings, & Keithley, 2006; Wakabayashi et al., 2010). Whether these correlate with human SNPs will require population studies.

Another approach to clarification of relevant genes has included quantitative trait locus (QTL) maps for mouse strains resistant to acoustic trauma (Ohlemiller, 2008; Street et al., 2014). QTL mapping identifies stretches of the genome that are involved with protection from noise trauma on different chromosomes in mouse models, and will be helpful in identifying both relevant proteins as well as non-transcribed areas of the mouse genome. Matching these stretches of chromosome with relevant human areas is possible with bioinformatics methods that are publically accessible.

Clearly SNPs are not be the entire picture, and it is evident that there are numerous epigenetic factors in play as well, including methylation patterns of DNA, histone markers, and RNA regulation both pre-and post-transcription and translation. Changes in expression of enzymes can be controlled by other proteins or methods, i.e., RNA inhibition (RNAi), and thus may not be evident in studies of SNPs. Differential gene expression measurement following acoustic trauma has helped to identify up-regulation and down-regulation of cell systems occurring in response to noise associated with the immune system and inflammation, transcription factors, cytokines, protein synthesis, metabolism, cytoskeletal proteins, calcium balance, oxidative, apoptotic systems, and heat-shock proteins (Cho, Gong, Kanicki, Altschuler, & Lomax, 2004; Gratton et al., 2011; Han et al., 2012; Kirkegaard et al., 2006; Ohlemiller, 2008). These elucidated pathways will be important for studies of therapeutic intervention for prevention of hearing loss as

well as treatment. Identification of individuals who have deficits in these pathways can help drive preventive treatment modalities.

In the past decade, it has become clear that protein pathways are not the entire picture. Only 20% of the genome is translated into proteins; on the other hand, greater than 80% of DNA is transcribed into RNA. Untranslated DNA is no longer mistakenly considered "junk DNA," but is transcribed into long non-coding RNA (lncRNA) greater than 200 nucleotides in length, microRNA (miRNA) and small nucleolar RNA (snoRNA) of approximately 20-22 nucleotides in length. These RNAs are involved in expression of genes pre- and post-transcription.

MicroRNAs, non-coding RNAs of 20-22 nucleotides, modulate mRNA level by degradation through the RISC pathway (Patel, 2013). Thus, these provide post-transcriptional/pre-translation control of RNAs, and have been characterized pre- and post-acoustic trauma in the lab (M. Patel & Hu, 2012; Minal Patel et al., 2013). Both up-regulated and down-regulated miRNAs have been described, predominantly associated with cell death and apoptosis.

It is now estimated that there are greater than 60,000 lncRNAs. Those that have been identified sustain histone methylation identical to active promoters of DNA, are expressed at low levels, and are less conserved than protein-coding transcripts. They usually have the genetic structure of 1-2 exons. The study of lnRNAs is in its infancy, but some appear to bring together trans- elements of DNA sequences and recruit epigenetic modifiers, i.e., methylation of histones. Polycomb repressor proteins are complexes that contain 20% expressed lncRNA, and may write and erase chromatin marks, regulate enhancers, and thus regulate the 3D structure of chromosomes with looping and activation of distant promoters (Kornfeld & Brüning, 2014). In the cochlea, Meg3/Gtl2, a maternally-expressed RNA has been characterized important for development (Manji et al., 2006), and others have been associated with tissue homeostasis (lnc-RAP and HI-LNC) and inflammation (Cui et al., 2014).

In conclusion, genes and SNPs involved in NIHL remain a fruitful topic for investigation, the study of epigenetics, i.e., lncRNA, miRNA, is a promising field yet in its infancy. Appropriate tools are being developed and refined to explore the interface of environment and cellular mechanisms. New avenues of research in the areas of characterization of the cochlea's response to stress, and in the field of therapeutic intervention will yield important insight into the mechanism of NIHL.

References

Adams, J. C., Seed, B., Lu, N., Landry, A., & Xavier, R. J. (2009). Selective activation of nuclear factor kappa B in the cochlea by sensory and inflammatory stress. *Neuroscience*, *160*(2), 530–539.

Carlsson, P. I., Van Laer, L., Borg, E., Bondeson, M. L., Thys, M., Fransen, E., & Van Camp, G. (2005). The influence of genetic variation in oxidative stress genes on human noise susceptibility. *Hear Res*, *202*(1-2), 87–96.

Cheng, A. G., Cunningham, L. L., & Rubel, E. W. (2005). Mechanisms of hair cell death and protection. *Current Opinion in Otolaryngology & Head and Neck Surgery*, *13*(6), 343–8.

Cho, Y., Gong, T. W., Kanicki, A., Altschuler, R. A., & Lomax, M. I. (2004). Noise overstimulation induces immediate early genes in the rat cochlea. *Brain Res Mol Brain Res*, *130*(1-2), 134–148.

Cui, H., Xie, N., Tan, Z., Banerjee, S., Thannickal, V. J., Abraham, E., & Liu, G. (2014). The human long noncoding RNA lnc-IL7R regulates the inflammatory response. *European Journal of Immunology*, *44*(7), 2085–95.

Fortunato, G., Marciano, E., Zarrilli, F., Mazzaccara, C., Intrieri, M., Calcagno, G., … Sacchetti, L. (2004). Paraoxonase and superoxide dismutase gene polymorphisms and noise-induced hearing loss. *Clinical Chemistry*, *50*(11), 2012–2018.

Gratton, M. A., Eleftheriadou, A., Garcia, J., Verduzco, E., Martin, G. K., Lonsburyemartin, B. L., & Vázquez, A. E. (2011). Noise-induced changes in gene expression in the cochleae of mice differing in their susceptibility to noise damage. *Hearing Research*, *277*(1-2), 211–26.

Han, Y., Hong, L., Zhong, C., Chen, Y., Wang, Y., Mao, X., … Qiu, J. (2012). Identification of new altered genes in rat cochleae with noise-induced hearing loss. *Gene*, *499*(2), 318–22.

Kirkegaard, M., Murai, N., Risling, M., Suneson, A., Jarlebark, L., & Ulfendahl, M. (2006). Differential gene expression in the rat cochlea after exposure to impulse noise. *Neuroscience*, *142*(2), 425–435.

Konings, A., Van Laer, L., Pawelczyk, M., Carlsson, P. I., Bondeson, M. L., Rajkowska, E., Van Camp, G. (2007). Association between variations in CAT and noise-induced hearing loss in two independent noise-exposed populations. *Hum Mol Genet*, *16*(15), 1872–1883.

Konings, A., Van Laer, L., & Van Camp, G. (2009). Genetic studies on noise-induced hearing loss: a review. *Ear and Hearing*, *30*(2), 151–9.

Kornfeld, J.-W., & Brüning, J. C. (2014). Regulation of metabolism by long, non-coding RNAs. *Frontiers in Genetics*, *5*(March), 57.

Laer, L. Van, Carlsson, Ã. P., Ottschytsch, N., Bondeson, M., Konings, A., Vandevelde, A., Van Camp, G. (2006). The Contribution of Genes Involved in Potassium- Recycling in the Inner Ear to Noise-Induced Hearing Loss. *Human Mutation*, *27*(8), 786–795.

Lin, C. Y., & Wu, J. L. (2009). Glutathione S-transferase M1, T1, and P1 polymorphisms as susceptibility factors for noise-induced temporary threshold shift. *Hear Res*, *257*(1-2), 8–15.

Manji, S. S. M., Sørensen, B. S., Klockars, T., Lam, T., Hutchison, W., & Dahl, H.-H. M. (2006). Molecular characterization and expression of maternally expressed gene 3 (Meg3/Gtl2) RNA in the mouse inner ear. *Journal of Neuroscience Research*, *83*(2), 181–90.

Ohlemiller, K. K. (2008). Recent findings and emerging questions in cochlear noise injury. *Hearing Research*, *245*(1-2), 5–17.

Patel, M., Cai, Q., Ding, D., Salvi, R., Hu, Z., & Hu, B. H. (2013). The miR-183/Taok1 target pair is implicated in cochlear responses to acoustic trauma. *PloS One*, *8*(3),

Patel, M., & Hu, B. (2012). MicroRNAs in Inner Ear Biology and Pathogenesis. *Hear Res*, *287*(1-2), 6–14.

Rabinowitz, P. M., Wise, J. P., Mobo, B. H., Antonucci, P. G., Powell, C., & Slade, M. (2002). Antioxidant status and hearing function in noise-exposed workers. *Hearing Research*, *173*(1-2), 164–171.

Ruan, Q., Wang, D., Gao, H., Liu, A., Da, C., Yin, S., & Chi, F. (2007). The effects of different auditory activity on the expression of phosphorylated c-Jun in the auditory system. *Acta Otolaryngol*, *127*(6), 594–604.

Seidman, M. D., Tang, W., Shirwany, N., Bai, U., Rubin, C. J., Henig, J. P., & Quirk, W. S. (2009). Anti-intercellular adhesion molecule-1 antibody's effect on noise damage. *Laryngoscope*, *119*(4), 707–712.

Shi, X., & Nuttall, A. L. (2007). Expression of adhesion molecular proteins in the cochlear lateral wall of normal and PARP-1 mutant mice. *Hear Res*, *224*, 1–14.

Sliwinska-Kowalska, M., & Pawelczyk, M. (2013). Contribution of genetic factors to noise-induced hearing loss: a human studies review. *Mutation Research*, *752*(1), 61–5.

Street, V, Kujawa, S. G., Manichaikul, A., Broman, K. W., Kallman, J. C., Shilling, D. J., Tempel, B. L. (2014). Resistance to Noise-Induced Hearing Loss in 129S6 and MOLF Mice: Identification of Independent, Overlapping, and Interacting Chromosomal Regions. *JARO*, Jun 21 [Epub ahead of print]

Taggart, R. T., McFadden, S. L., Ding, D. L., Henderson, D., Jin, X., Sun, W., & Salvi, R. (2001). Gene Expression Changes in Chinchilla Cochlea from Noise-Induced Temporary Threshold Shift. *Noise Health*, *3*(11), 1–18.

Tornabene, S. V, Sato, K., Pham, L., Billings, P., & Keithley, E. M. (2006). Immune cell recruitment following acoustic trauma. *Hearing Research*, *222*(1-2), 115–124.

Wakabayashi, K., Fujioka, M., Kanzaki, S., Okano, H. J., Shibata, S., Yamashita, D., Okano, H. (2010). Blockade of interleukin-6 signaling suppressed cochlear inflammatory response and improved hearing impairment in noise-damaged mice cochlea. *Neuroscience Research*, *66*(4), 345–352.

Yamashita, D., Minami, S. B., Kanzaki, S., Ogawa, K., & Miller, J. M. (2008). Bcl-2 genes regulate noise-induced hearing loss. *J Neurosci Res*, *86*(4), 920–928.

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Statistical Considerations

Martin D. Slade, MPH

There are several statistical issues to consider when planning a study to measure hearing loss. These range from the learning curve associated with audiometric testing to the fact that multiple measurements of hearing level are recorded on a subject each time that they undergo a hearing evaluation. The following sections describe statistical issues that need to be considered, often recommending methodologies to utilize.

Audiometric Learning Curve

Literature suggests that individuals new to audiometric testing experience a learning curve during which time their measured hearing improves. This usually occurs over the first 4 tests (Royster & Royster, 1986). Due to this effect, an individual's first 4 audiometric tests should be discounted when calculating changes in threshold hearing levels. One potential solution to this issue is to have individual's that have had less than 4 audiometric tests to undergo several rounds of audiometric testing prior to recording a baseline threshold level.

Definition of Hearing Loss / Multiple Comparisons

Perhaps the greatest issue with regard to studies involving hearing loss as an outcome is the fact that threshold hearing levels are measured at many different frequencies. For audiometric testing, these typically include 0.5, 1, 2, 3, 4, 6, and 8k Hz. Therefore, seven separate hearing threshold levels are recorded. Analyses conducted to determine if there is an effect of an intervention at any of these frequencies result in seven statistical tests being performed. Thus, to be able to show an effect with 95% confidence ($\alpha=0.05$), utilizing the Bonferroni correction for multiple comparisons, the observed probability (p-value) would need to be below $0.05/7 = 0.0071$. Given that there were seven frequencies included in this analysis meant that we were taking an average of both ears. If, instead, we were looking at right and left ears separately, then there would be 14 different statistical tests performed and the required p-value (to demonstrate an effect with 95% confidence) would need to be below $0.05/14 = 0.0036$. Any additional definitions of hearing loss such as averages of certain frequencies would only add to the number of statistical tests performed thereby making the required p-value even harder to demonstrate.

There are two different approaches to reducing the multiple comparisons problem. The first of these is to define one specific definition of hearing loss to be used as the primary objective. Thus, a definition such as the average of frequencies 2, 3, and 4k Hz across both ears would eliminate the entire multiple comparison issue. Other definitions of hearing loss can surely be evaluated, but they should be explicitly denoted as secondary analyses. These should be considered as exploratory analyses and would, therefore, be reported without correcting for multiple comparisons. Note, however,

that any significant predictors will need to be replicated in a follow-up trial whereby this definition of hearing loss becomes the primary outcome.

A second approach to reducing the multiple comparisons problem would be to substantially increase the sample size such that the collected data could be randomly split. The first group would be used in an exploratory analysis whereby various definitions of hearing loss could be evaluated. Once that is completed and the desired definition of hearing loss determined, the second group would be used to confirm the results. This second analysis would also be the one that is reported (Muller, Otto & Benignus, 1983).

Some researchers have suggested that a repeated measures ANOVA be utilized as a way to remove the problem of multiple comparisons. Unfortunately, without interactions, the model assumes that the intervention affects not only both ears, but also all of the frequencies, in a like manner. If, however, only certain frequencies are actually affected, this method will lose substantial power. This limitation can be overcome by including interactions, but this will substantially decrease the power of the study.

Along with continuous definitions of hearing loss, binary definitions of hearing shifts should also be considered. In choosing this definition the amount of hearing loss expected to occur during the trial needs to be taken into account such that there are enough expected cases (shifts). This should be determined from power calculations to determine sample size requirements.

Independence/Dependence of Left and Right Ear Test Results

There has been previous discussion as to whether an analysis that utilizes a subject's right ear results separately from their left ear results should be considered independent. In other words, should they count as two independent tests? The simple answer is that these results are NOT independent. They are both occurring within a single individual, so if there are genetic effects that related to hearing loss, then that person's genetics are affecting both these measurements. The modeling of this type of data should incorporate a random effect of individual to control for the covariance of the measurements.

References

Royster, J. D., & Royster, L. H. (1986). Using audiometric data base analysis. *Journal of Occupational and Environmental Medicine*, *28*(10), 1055-1068.

Muller, K. E., Otto, D. A., & Benignus, V. A. (1983). Design and analysis issues and strategies in psychophysiological research. *Psychophysiology*, *20*(2), 212-218.

Use of Otoacoustic Emissions to Assess the Efficacy of a Pharmaceutical Otoprotective Agent

Dawn Konrad-Martin PhD, Garnett P. McMillan PhD, Lynne Marshall PhD, Judi A. Lapsley Miller PhD, Gayla L. Poling PhD

Benefits and Limitations to the Use of OAEs

Otoacoustic emissions (OAEs) are byproducts of the outer hair cell (OHC)-based nonlinear cochlear processing that takes place in healthy, mammalian ears. They provide an indirect, noninvasive measure of the OHC electro-mechanical response that enables the auditory system to encode a large range of stimulus levels, discriminate small differences in sound frequencies and detect low-level sounds. Environmental exposures that damage OHCs make cochlear processing more linear, and are associated with abnormal loudness sensation, impaired frequency selectivity, increased pure-tone thresholds and reduced or absent OAEs. These relationships motivate the use of OAEs to identify damage from environmental exposures (e.g., noise) and assess the efficacy with which otoprotectants mitigate the damage.

OAEs are generally considered more sensitive to changes in cochlear function than hearing threshold measures. Evidence shows that distortion-product otoacoustic emissions (DPOAEs) and transient-evoked otoacoustic emissions (TEOAEs) change following noise exposure even when pure-tone thresholds measured contemporaneously remain stable (e.g., Engdahl et al., 1996; Marshall et al., 2009; Seixas et al., 2005). This is consistent with histological results obtained in chinchillas that indicate up to 30% of the outer hair cells that would respond at a given cochlear location can be damaged before producing an ABR threshold shift (Bohne and Clark, 1982). When shifts in OAE level and hearing thresholds from noise do co-occur, the OAE changes can take place at (Marshall et al., 2001) or below (Helleman and Dreschler, 2012) the frequencies that show a hearing loss. Corresponding changes across the two measures are more often found for temporary (hearing) threshold shift (TTS) than for permanent (hearing) threshold shift (PTS) (Marshall et al., 2001). Additionally, for TTS, the time course of OAE and audiometric changes appears to be similar (humans: Sutton et al., 1994; Marshall et al., 2001; animals: Kujawa and Liberman, 2009). Within a clinical trial, OAE measures are therefore expected to be a sensitive indicator of cochlear damage involving the OHC system and protection achieved from a drug therapy. Finally, OAE testing causes no discomfort to- and requires minimal compliance from- the test subject, and involves minimal time and cost to perform.

There are limitations and caveats of their use. Limitations include that OAEs are influenced by factors unrelated to cochlear function (e.g., round-trip middle ear transmission, strength of efferent feedback to the middle ear and cochlea, individual- and frequency specific- variations in cochlear reflectance), so protocols must be

designed carefully. For example, the fact that OAEs are sensitive to changes in middle ear function means that a test to identify any conductive involvement (e.g., tympanometry) must be included in the protocol so that any OAE changes can be interpreted. Certain patient populations (e.g., children, patients with certain infectious diseases, patients receiving chemotherapy, etc.) are prone to fluctuating middle-ear pressure and conductive loss from otitis media, which can make OAE testing (and hearing monitoring in general) problematic. To this end, documentation of extensive history of middle ear problems is warranted in a clinical trial in which OAE outcomes are incorporated.

OAE results are subject to variability due to limitations of current calibration methods, poor probe fitting techniques, analysis techniques, and any exposure to damaging agents not explicitly being monitored (e.g., use of power tools, personal music players, etc.) during the time between tests. All evoked OAEs arise from a mixture of distortion and reflection emission sources, which can render them challenging to interpret and difficult to relate to underlying basilar membrane processing, particularly when current clinical protocols are used. For example, so called, "mixed"-source DPOAEs may decrease in level following damage, but are also frequently found to become larger (Helleman and Dreshler, 2012). Evidence suggests that basal components may "fill in" regions of damage unless low stimulus levels are used (Martin et al., 2011). Finally, there is the lack of consensus about which OAE measurement protocols are best, and how to define clinically meaningful changes in the measures.

Pros and Cons of Specific OAE Protocols

Ultimately, an OAE protocol in a clinical trial should depend on the clinical or research question being investigated and the population being tested. Additionally, a protocol should be theoretically sound, based on known patterns of damage, involve minimal time, generate valid results in the majority of individuals tested, and be accurate and repeatable (Konrad-Martin et al., 2012). In the meantime, the choice of OAE protocols is limited for those who use clinical OAE systems, which provide a narrow range of well-researched test protocols for DPOAEs or TEOAEs, and sometimes both.

DPOAEs and TEOAEs are the OAE types most commonly used clinically in part because they were historically easier to measure than stimulus-frequency OAEs (SFOAEs). SFOAEs are considered the most frequency-specific and the simplest in terms of source generation, particularly when elicited at low stimulus levels; however, recent evidence suggests that evoked OAEs of all types are less frequency-specific and more complex in their generation than formally believed (Shera and Guinan, 1999; Martin et al., 2011). When obtained at low stimulus levels rather than the usual high-level clinical system default settings, an individual TEOAE frequency component appears to arise as a single source reflection emission comparable to an SFOAE (Kalluri and Shera, 2007). One drawback is that many clinical patients will not have TEOAEs at low levels, potentially limiting their utility for tracking functional changes. The broadband TEOAE stimulus makes the measure attractive for rapid testing of a wide range of frequencies, at least

until swept tone algorithms for DPOAEs and SFOAEs become clinically available. However, DPOAEs remain favored for making high-frequency measures (above 4 kHz) because current clinical systems extract TEOAEs in a way that removes high-frequency response components.

In a comparison of DPOAE levels, response growth functions, and group delays in a population of adults exposed to an ototoxin, thresholds and group delays identified changes more often than did DPOAE level obtained with moderate-level stimuli (Katbamna et al., 1999). In noise-exposed populations, lower overall primary levels with greater L1-L2 separation done in fine stimulus frequency step-sizes increased the sensitivity of DPOAEs to detect post-exposure changes (Delb et al., 1999; Engdahl and Kemp, 1996; Sutton et al., 1994).

Based on current models of OAE generation, certain OAE protocols may provide a more direct measure of certain aspects of the cochlear mechanical response then others. Phase gradient delays of low-level reflection emissions can be used to estimate frequency tuning (Shera et al., 2002) and their thresholds and levels may provide an indication of the gain of the cochlear amplifier; distortion emission thresholds, response growth and maximum amplitudes provide an indication of the strength and form of the basilar membrane nonlinearity (Shera and Guinan, 1999). The use of canned protocols and analysis programs available on clinical equipment has effectively limited these potentially powerful measures to the domain of research.

Active areas of research include comparing the sensitivity (to cochlear insult) and retest-reliability of emerging OAE protocols and analyses, and substantive improvements in OAE measurement system capabilities. The protocols, analyses and calibration techniques available with most clinical systems, though well-researched, generally have not caught up with the state of the science. Because of this and due to physical limitations of current systems, clinical OAE measurements are reliable at measurement frequencies up to only 6 to 8 kHz. Still, even basic, moderate-level DPOAEs and TEOAEs are able to separate normal from impaired ears quite well (Gorga et al., 1997; Hussain et al., 1999), and can indicate early changes in cochlear function from noise and ototoxins. Further, most clinical systems allow some control over stimulus levels and frequency step sizes.

Specific Recommendations

Due to the lack of consensus about how to measure and interpret changes in OAEs clinically, it could be argued that they are not well suited as a primary outcome measure in a clinical trial to determine the efficacy of an otoprotective agent. However, having data related to cochlear function that were obtained using a sensitive measure free from confounding changes in cognitive processes of attention and memory would clearly add value to results of a clinical trial. Their use should be seriously considered if time and measurement conditions permit.

Statistical Considerations. The standard clinical trial design for evaluating drug efficacy is suitable for use with OAE outcome data. Briefly, baseline OAE measurements are

taken, subjects are randomized to one or more active treatment groups and placebo, and are followed through and immediately after exposure. Statistical tests of treatment efficacy are based on contrasts of the follow-up measurements. Adjustment for baseline OAE response is possible using one of several approaches (Fitzmaurice et al., 2004). In contrast to standard clinical trial design, it is standard clinical practice to compare changes in OAEs to shift reference standards established in a healthy, homeostatic population. We do not recommend this approach in clinical trials, since the goal of such trials is to evaluate the efficacy of a new therapy on changes in cochlear function as opposed to identifying alarming changes with reference to a standard population. In addition to standard concerns with longitudinal studies, OAE outcomes introduce some further challenges. In particular, multiple stimulus frequencies and test levels are likely to be used, generating complex multivariate outcomes. Careful consideration of the statistical model is necessary, with guidance from an experienced statistician recommended, to enhance the accuracy of the statistical tests. Finally, it is not unusual for OAEs to fall below noise after exposure. Such measurements will appear as missing, which can seriously bias outcomes if the probability of falling below the noise floor is associated with any treatment effects. In fact, it is reasonable to expect that this is a common occurrence. One approach is to use censored data models (e.g. 'survival analysis') for these trials; another is to set such responses to the noise floor or system distortion level. Such approaches should be considered carefully on a case-by-case basis.

Because the tester's retest reliability is critical to successful serial OAE measurements, it is also recommended that each tester or group of testers assess (using a statistically sound method) and document their test-retest reliability for the specific protocol to be used in the clinical trial.

Testing Procedures. The twin goals are first to determine valid baseline measures with further characterization of test-retest repeatability (considering noise and distortion generated by the equipment, middle ear function, patient and test environment, probe placement, etc.). The typical moderate- or high-level clinical protocols available with most standard OAE measurement equipment can be used to obtain a gross assessment of cochlear function over a broad range of frequencies. Minimal additional test considerations include the use of a lower-level frequency sweep (e.g., 45 dB SPL), perhaps with fine frequency step measurements, as well as multiple levels measured at up to a few vulnerable frequencies. For this more detailed coverage of the frequency and/or level space, a narrower test frequency region should be targeted based on the mechanism and pattern of damage expected from the exposure. The approach of using a targeted region of testing near the highest frequency that elicits a 6-10 dB SNR DPOAE response appears to be useful for monitoring cisplatin ototoxicity among cancer patients in whom cochlear damage begins at the high frequency coding base and proceeds apically (Ress et al., 1999; Reavis et al., 2008; Dille et al., 2010). In an industrial noise setting, more detailed OAE measures may be fruitful in the 2 to 6 kHz range.

References

Delb, W., Hoppe U., Liebel J., and Iro H. (1999). "Determination of acute noise effects using distortion product otoacoustic emissions," *Scan. Audiol.* 28(2): 67-776.

Dille, M.F., Konrad-Martin, D., Gallun, F., Helt, W.J., Gordon, J.S., et al. (2010). "Tinnitus onset rates form chemotherapeutic agents and ototoxic antibiotics: Results of a large prospective study," *J. Am. Acad. Audiol*. 21: 409 – 417.

Engdahl, B., and Kemp, D.T. (1996). "The effect of noise exposure on the details of distortion product otoacoustic emissions in humans," *J. Acoust. Soc. Am.*, 99(3): 1573 – 1587.

Gorga, M., Neely, S., Ohlrich, B., Redner, J., and Peters, J. (1997). "From laboratory to clinic: A large scale study of distortion product otoacoustic emissions in ears with normal hearing and ears with hearing loss," *Ear. Hear.* 18: 440–455.

Harding, G., and Bohne, B. (2004). "Temporary DPOAE level shifts, ABR threshold shifts and histopathological damage following below-critical level noise exposure," *Hear. Res.* 196: 94–108.

Helleman, H.W., and Dreshler, W.A. (2012). "Overall versus individual changes for otoacoustic emissions and audiometry in a noise-exposed cohort," *Int. J. Audiology* 51: 362 – 372.

Hussain, D.M., Gorga, M.P., Neely S.T., Keefe D.H., Peters J. (1999). Transient evoked otoacoustic emissions in patients with normal hearing and in patients with hearing loss. *Ear. Hear*. 19(6): 434-49.

Kalluri, R., and Shera, C.A. (2007). "Near equivalence of human click-evoked and stimulus-frequency otoacoustic emissions," *J. Acoust. Soc. Am*. 121(4): 2097-110.

Katbamna, B., Homnick, D.N., and Marks, J.H. (1999). "Effects of chronic tobramycin treatment on distortion product otoacoustic emissions," *Ear. Hear*. 20(5): 393-402.

Konrad-Martin, D., Reavis, K.M., McMillan, G.P., and Dille, M.F. (2012). "Multivariate DPOAE metrics for identifying changes in hearing: Perspectives from ototoxicity monitoring," *International J Audiol*, 51(S1): S51-S62.

Kujawa, S.G., and Liberman, M.C. (2009). "Adding insult to injury: Cochlear nerve degeneration after ' temporary ' noise-induced hearing loss," *J Neurosci* 29(45): 14077 – 14085.

Marshall, L., Lapsley-Miller, J.A., and Heller, L.M. (2001). "Distortion-product otoacoustic emissions as a screening tool for noise-induced hearing loss," *Noise Health* 3: 43 – 60.

Marshall, L., Lapsley-Miller, J.A., and Heller, L.M. (2009). "Detecting incipient inner-ear damage from impulse noise with otoacoustic emissions," *J. Acoust. Soc. Am.* 125: 995 – 1013.

Martin, G.K., Stagner, B.B., Chung, Y.S., and Lonsbury-Martin, B.L. (2011). "Characterizing distortion-product otoacoustic emission components across four species," *J. Acoust. Soc. Am.* 129: 3090 – 3103.

Reavis, K.M., McMillan G., Austin D., Gallun F., Fausti S. et al. (2011). "Distortion-product otoacoustic emission test performance for ototoxicity monitoring," *Ear. Hear.* 32(1): 61 – 74.

Seixas N.S., Goldman B., Sheppard L., Neitzel R., Norton S. et al. (2005). "Prospective noise induced changes to hearing among construction industry apprentices," *Occup Environ Med* 62: 309 – 317.

Shera C.A., Guinan J.J., and Oxenham A.J. (2002). "Revised estimates of human cochlear tuning from otoacoustic and behavioral measurements," *Proc. Natl. Acad. Sci. USA* 99(5): 3318-23.

Shera C.A., and Guinan J.J. (1999). "Evoked otoacoustic emissions arise by two fundamentally different mechanisms: a taxonomy for mammalian OAEs," *J. Acoust. Soc. Am.* 105(2 Pt1): 782-98.

Sutton, L.A., Lonsbury-Martin, B.L., Martin, G.K., and Whitehead, M.L. (1994). "Sensitivity of distortion-product otoacoustic emissions in humans to tonal over-exposure: Time course of recovery and effects of lowering L2," *Hear. Res.* 75(1 – 2): 161 – 174.

Supra-threshold Testing Using Speech-in-Noise and Auditory Evoked Potentials

Colleen G. Le Prell PhD, Douglas S. Brungart PhD

In this white paper, we discuss the use of supra-threshold tests, including supra-threshold auditory brainstem response (ABR) and speech-in-noise testing, for early detection of noise-induced hearing loss (NIHL) and for assessing the effectiveness of interventions designed to prevent NIHL. In humans, the accepted clinical standard for detecting NIHL is the behavioral audiogram, which is based on the absolute detection threshold of narrow-band noises or tones (for detailed review, see Le Prell & Lobarinas, 2015). There is little question that the audiogram is a reliable and sensitive measure of NIHL in human listeners. However, recent results from noise exposed animals suggest that NIHL can cause substantial neurodegeneration in the peripheral auditory system without degrading the pure tone audiometric threshold, as measured by the threshold ABR (Le Prell & Bao, 2012; Kujawa &Liberman, 2009). This suggests that clinical measures of auditory performance that are conducted with stimuli presented above the detection threshold, such as speech-in-noise testing or the supra-threshold ABR, may be more sensitive than the behavioral audiogram in detecting early stage NIHL in listeners with audiometric thresholds within normal limits. Also, from a more practical standpoint, it is often difficult to find locations with low enough ambient noise levels for the collection of valid behavioral audiometric threshold data in the austere working environments where occupational noise exposures are most likely to occur. This can cause logistical difficulties in the design of studies aimed at the detection of small changes in the hearing abilities of noise exposed listeners. These environmental concerns can provide additional motivation to use supra-threshold speech-in-noise testing or supra-threshold ABR responses as a supplement to the behavioral audiogram in the measurement of small changes in hearing loss in noise-exposed listeners.

Speech-Based Functional Tests

Individuals with high-frequency hearing loss are impaired in terms of their ability to understand speech in noise because some parts of the speech signal are inaudible due to their elevated thresholds (Quist-Hanssen et al., 1979; Badri et al., 2011). However, most hearing scientists believe that, in addition to this *audibility* component, that there is also a *distortion* component of hearing loss that causes hearing impaired listeners to perform poorly on tests of speech-in-noise perception even when all components of the speech signal are theoretically audible (Plomp, 1986). Consequently, the American Academy of Otolaryngology (AAO) recently recommended word recognition test scores be collected in *all* clinical trials that assess auditory function (Gurgel et al., 2012). Although the absolute nature of this guidance has been questioned by the American Academy of Audiology, which believes that speech-in-noise testing, specifically, is not optimal for *all* clinical trial populations (Carlson, 2013), there are many reasons to

believe that tests using speech- in-noise backgrounds may play an important role in providing a "stress test" for auditory function (Wilson, 2011) that may be more sensitive to small changes in hearing ability than a behavioral audiogram. Moreover, interest and enthusiasm for speech-in-noise tasks as a proxy for auditory function should be high given suggestions that loss of neural connections from inner hair cells, resulting in decreased ABR amplitude *in the absence of overt threshold shift,* may underlie speech-in-noise discrimination deficits (Kujawa &Liberman, 2009; Lin et al., 2011; Makary et al., 2011).

The impact that interfering noise has on speech perception has long been a subject of interest to hearing scientists, and over the past 70 years a substantial number of speech-in-noise tests have emerged in the literature. Most of these tests were initially designed for use in hearing research (e.g., Carhart & Tillman, 1970), although many of them have also been adapted for use in the clinic (Carhart, 1951, for review, see Wilson, 2011). Early example of tests that were initially designed primarily for research have included the Speech Perception in Noise (SPIN) Test (Kalikow et al., 1977), the Revised Spin Test (R-SPIN) (Bilger et al., 1984), the Connected Speech Test (CST), (Cox et al., 1987; Cox et al., 1988), the Speech Intelligibility Rating (SIR) test (McDaniel & Cox, 1992; Beck & Speaks, 1993), and the Revised Speech Intelligibility Rating (RSIR) test (Speaks et al., 1994).

In recent years, a few speech-in-noise tests have begun to be used more widely in the clinic. Two examples are the Hearing in Noise Test (HINT) as modified for American English (Nilsson et al., 1994) and the Speech Recognition in Noise Test (SPRINT) (AR 40-501; Standards of Medical Fitness, http://www.apd.army.mil/pdffiles/r40_501.pdf), both of which are used primarily for evaluating the fitness for duty of hearing impaired individuals in high-risk occupations such as police and fire departments and in the military. Three other widely-used speech-in-noise tests include the Quick Speech-In-Noise (QuickSIN) test (Killion et al., 2004), the Words-in-Noise (WIN) test (for review, see Wilson, 2011), and the Oldenburg Matrix Sentence Tests (OLSA) (Wagener, Brand, & Kollmeier, 1999), which is used primarily in Europe. These tests are used to assess general speech-in-noise performance and, in some cases, to assess hearing aid or cochlear implant candidacy. It is difficult to provide good (evidence-based) guidance on the selection of speech-in-noise tasks for clinical trials, as there have not been many studies that provide empirical data directly comparing performance of participants across speech-in-noise tests, although there are some notable examples of studies that do offer back-to-back comparisons, such as Wilson et al. (2007) and Grant and Walden (2013). One finding of these back-to-back comparisons is that the WIN tends to be one of the more challenging tests for young listeners with normal hearing, which may be helpful in ensuring that no floor effects occur in studies designed to detect small changes in hearing in this population.

In general, the correlation between speech-in-noise scores and the behavioral audiogram is not very high, suggesting that factors other than pure audibility have a strong influence on speech perception performance in hearing impaired listeners

(Smorenberg, 1992). However, recent research has shown certain speech-in-noise tests, and in particular those that adaptively determine the speech reception threshold of three-digit sequences presented in noise, can be reasonably effective in identifying individuals with abnormal hearing (i.e., Pure Tone Averages at 0.5, 1.0, and 2.0 kHz >20 dB), even when administered over a relatively crude telephone line (Watson, Kidd, Miller, Smits, & Humes, 2012).

In terms of their utility for assessing small changes in hearing in a clinical evaluation of oto-protectants, there are potential advantages to the use of speech-in-noise tests as a supplement to the pure tone audiogram. Speech-in-noise stimuli can and should be presented well above threshold levels, meaning that they could potentially be conducted under noise-attenuating headphones in office or clinic spaces with background noise levels that would be too high to obtain a behavioral audiogram. They also provide an objective measure of performance that allows listeners to be provided with correct answer feedback and may be more stable over time than the subjective judgment of the presence or absence of a low-level tone. Finally, they provide a stimulus with sufficient ecological validity to independently sustain a claim of efficacy in a clinical trial focused on the elimination of NIHL: few would question the utility of an intervention that preserved speech-in-noise performance even if it had no impact on the pure tone audiogram. However, it must also be recognized that studies focused on the effectiveness of NIHL interventions are implicitly based on sequential changes in metrics that occur within a single subject pre- and post-noise exposure, and that there is very little data currently available to suggest how effective speech-in-noise tests might be when used in this fashion.

One fundamental issue that must be addressed is the possibility of learning effects across repeated administrations of the same speech-in-noise test. Most tests with open-set speech materials have at least a few different lists of words that are purported to be "equivalently difficult", but when using one of these tests the experimenter always has to make a trade-off between the number of times the test is administered and the number of lists to use per test (to improve reliability). Closed-set tests, such as the OLSA or the Triple-Digit test may be more suitable for studies evaluating longitudinal changes in hearing loss.

Also, although it is well known that speech-in-noise performance degrades with increasing hearing loss, the temporal relationship between speech-in-noise performance and hearing loss is not known. It may be the case that small changes in the speech-in-noise score will be seen before changes in the behavioral audiogram are detectable, or it may be that changes in the audiogram will always occur before a change in speech in noise performance. Thus, at this point, it must be admitted that there is some risk that speech-in-noise testing will not be as sensitive as the audiogram for detecting changes in hearing sensitivity. Nevertheless, the inclusion of speech-in-noise testing where possible is recommended, as the potential benefits of finding small changes in hearing that are not detectable on the behavioral audiogram are likely to

outweigh the relatively modest costs of including a short speech-in-noise test battery as part of the pre- and post- noise exposure evaluation.

Auditory Brainstem Response (ABR)

ABR supra-threshold input-output functions are considered to be a "gold standard" metric for assessing lasting effects of noise on the neural population in animal testing. Interest in ABR tests in humans has been increasing given reported loss of neural connections from inner hair cells, with corresponding decrease in Wave I ABR amplitude, after noise exposures that produce robust threshold shift (i.e., approximately 40-dB temporary threshold shift, or "TTS" (see "Temporary and Permanent Noise-Induced Threshold Shifts" guidance document for further definition), measured 24-hours post-noise) in rodents (Kujawa & Liberman, 2009; Lin et al., 2011; Wang & Ren, 2012). Limited physiological data from humans have not shown corresponding deficits however. There were no deficits in ABR amplitude in either Veterans with known noise exposure (Konrad-Martin et al., 2012) or professional pop/rock musicians (Samelli et al., 2012) compared to control subjects. Additional controlled studies are needed. If human ABR amplitude varies with noise exposure history, in the absence of threshold deficits, we may ultimately gain insight into the "critical boundary" at which noise becomes hazardous to synapses in human ears, a boundary that is not currently known for either humans or laboratory animals (for discussion of critical boundary, see Le Prell et al., 2012; Spankovich et al., 2014). One problem in human testing of the ABR is that the absolute level of the ABR can vary according to the placement and sensitivity of the electrodes used to make the measurement. If an attempt to measure Wave I ABR recordings is attempted as part of a clinical trial, every effort must be made to increase the sensitivity of the measure. Possible strategies include using electrodes that utilize the ear canal as a recording site to improve sensitivity (Gaddam & Ferraro, 2008), and normalizing the electrode sensitivity by using the of Wave I/ Wave V amplitude ratio rather than an absolute measure of Wave I output (Musiek et al., 1984). Amplitude of Wave I may not be the only evoked potential of interest. Recently, Ruggles et al. (2011; 2012) identified variability in the auditory brainstem frequency-following response in normal-hearing young adults as a key factor for difficulty discriminating sounds in noise. Other groups are independently arriving at similar conclusions; Hopkins and Moore (2011) also reported reduced sensitivity to temporal fine structure cues may underlie speech perception difficulties.

Challenges to the field

Oto-protection studies have focused on the ability of agents to reduce or prevent threshold shifts. However, an emerging body of evidence suggests that changes in supra-threshold hearing may also be important. Provocative data from animal studies suggest that robust TTS can be accompanied by damage to auditory nerve fiber synapses. Deficits on speech in noise tasks are common, but there is little agreement which tests to use to document deficits. Noise-induced ABR amplitude changes are a "hot-topic" in animal models, but such changes have not been established in humans.

This is clearly relevant to long-term trajectory of noise-induced changes in hearing. Slow-to-develop loss of the spiral ganglion fibers and increased changes in hearing associated with aging were observed over the course of the mouse life span after robust TTS (Kujawa & Liberman, 2006). Those data contradict a long-standing assumption that, as long as thresholds recover, the ear has completely recovered. These reports have raised important questions, such as whether a more moderate TTS could also induce long-term deficits through similar neural-based mechanisms. The "critical boundary" at which long-term synaptic deficits are first induced is not known in rodents or humans (for discussion, see Le Prell et al., 2012; Spankovich et al., 2014), and is subject to some debate. Some evidence suggests the phenomena is likely in humans (Plack et al., 2014). In contrast, the Institute of Medicine (2005) report on Noise and Military Service specifically concluded, "The committee's understanding of the mechanisms and processes involved in the recovery from noise exposure suggests, however, that a prolonged delay in the onset of noise-induced hearing loss is unlikely."

Given all the evidence suggesting that some functional changes in hearing can occur prior to the point where a significant threshold shift is seen in the audiogram, we believe that strong arguments can be made for the inclusion of some supra-threshold measure of performance as part of any large-scale study designed to detect small changes in hearing in noise-exposed populations, including those focused on evaluating the efficacy of interventions for noise-induced hearing loss. Unfortunately, because few if any studies to date have compared the sensitivity of different speech-in-noise tests to longitudinal, within-subject changes in hearing ability, it is difficult to define a single best practice for conducting such tests. If speech in noise testing is confined to just a small number of measurement sessions per subject, any of a number of clinical tests could be used for the pre- and post- testing, including the widely-available WIN and QuickSIN. However, if a larger number of periodic test sessions are required, it may be desirable to switch to a closed set test such as the OLSA or the Triple Digit test, which can be scored automatically and can be administered as many times as necessary without significant learning effects. If time permits and it is possible to include a speech-in-noise test more sensitive than the speech-in-noise tests typically included in a standard clinical battery, then it might be reasonable to include a more challenging speech perception condition where the target talker is presented over headphones at a simulated location in front of the listener and two flanking maskers speech maskers are located to the left and the right of the target talker. Examples of such tests include the modified version of the QuickSIN, where the target talker is time-compressed and presented in the presence of simulated room reverberation proposed by Brungart, Sheffield, and Kubli (2014); the modified version of the Coordinate Response Measure proposed by Gallun, Diedesch, and Campbell (2013); or the Listening in Spatialized Noise test (LISN-S), proposed by the National Acoustics Laboratory in Australia (Cameron, Glyde & Dillon, 2011). These tests have all been shown to be more sensitive than standard speech-in-noise tests to auditory deficits in listeners who complain about speech in noise problems but have relatively normal audiometric thresholds. Because there is so little information on longitudinal changes in speech-in-noise perception in noise exposed populations,

we contend that the most important consideration right now is to make every effort to ensure that *some* speech-in-noise testing is included in the design of large-scale studies examining the effects of noise exposure on hearing ability. We believe the costs of adding an additional speech-in-noise measure would, in most cases, be relatively minor compared to the large-scale logistical costs of identifying and sequentially testing a large population of listeners pre- and post- noise exposure, and that the results of these studies will provide invaluable information that will help shape the design of future studies examining interventions for NIHL and answer basic questions about human hearing that have applications far beyond the results of any single study conducted in this area.

References

Badri R, Siegel JH, Wright BA (2011) Auditory filter shapes and high-frequency hearing in adults who have impaired speech in noise performance despite clinically normal audiogramsa. J Acoust Soc Am 129:852-863.

Beck WG, Speaks C (1993) Intelligibility of selected passages from the Speech Intelligibility Rating (SIR) test. J Speech Hear Res 36:1075-1082.

Bilger RC, Nuetzel JM, Rabinowitz WM, Rzeczkowski C (1984) Standardization of a test of speech perception in noise. J Speech Hear Res 27:32-48.

Brungart, D. S., Sheffield, B. M., & Kubli, L. R. (2014). Development of a test battery for evaluating speech perception in complex listening environments. The Journal of the Acoustical Society of America, 136(2), 777-790.

Cameron, S., Glyde, H., & Dillon, H. (2011). Listening in Spatialized Noise—Sentences Test (LiSN-S): Normative and Retest Reliability Data for Adolescents and Adults up to 60 Years of Age. Journal of the American Academy of Audiology, 22(10), 697-709.

Carhart R (1951) Basic principles of speech audiometry. Acta Otolaryngol 40:62-71.

Carhart R, Tillman TW (1970) Interaction of competing speech signals with hearing losses. Arch Otolaryngol 91:273-279.

Carlson DL (2013) American Academy of Audiology response to Gurgel et al. Otolaryngol Head Neck Surg 149:349-350.

Cox RM, Alexander GC, Gilmore C (1987) Development of the Connected Speech Test (CST). Ear Hear 8:119S-126S.

Cox RM, Alexander GC, Gilmore C, Pusakulich KM (1988) Use of the Connected Speech Test (CST) with hearing-impaired listeners. Ear Hear 9:198-207.

Gaddam A, Ferraro JA (2008) ABR recordings in newborns using an ear canal electrode. Int J Audiol 47:499-504.

Gallun, F. J., Diedesch, A. C., Kampel, S. D., & Jakien, K. M. (2013). Independent impacts of age and hearing loss on spatial release in a complex auditory environment. Frontiers in neuroscience, 7.

Grant KW, Walden TC (2013) Understanding excessive SNR loss in hearing-impaired listeners. J Am Acad Audiol 24:258-273; quiz 337-258.

Gurgel RK, Jackler RK, Dobie RA, Popelka GR (2012) A new standardized format for reporting hearing outcome in clinical trials. Otolaryngol Head Neck Surg 147:803-807.

Hopkins K, Moore BC (2011) The effects of age and cochlear hearing loss on temporal fine structure sensitivity, frequency selectivity, and speech reception in noise. J Acoust Soc Am 130:334-349.

Institute of Medicine (2005) Noise and Military Service: Implications for Hearing Loss and Tinnitus. Washington D. C.: The National Academies Press.

Kalikow DN, Stevens KN, Elliott LL (1977) Development of a test of speech intelligibility in noise using sentence materials with controlled word predictability. J Acoust Soc Am 61:1337-1351.

Killion MC, Niquette PA, Gudmundsen GI, Revit LJ, Banerjee S (2004) Development of a quick speech-in-noise test for measuring signal-to-noise ratio loss in normal-hearing and hearing-impaired listeners. J Acoust Soc Am 116:2395-2405.

Konrad-Martin D, Dille MF, McMillan G, Griest S, McDermott D, Fausti SA, Austin DF (2012) Age-related changes in the auditory brainstem response. J Am Acad Audiol 23:18-35; quiz 74-15.

Kujawa SG, Liberman MC (2006) Acceleration of age-related hearing loss by early noise exposure: evidence of a misspent youth. J Neurosci 26:2115-2123.

Kujawa SG, Liberman MC (2009) Adding insult to injury: cochlear nerve degeneration after "temporary" noise-induced hearing loss. J Neurosci 29:14077-14085.

Le Prell CG, Bao J (2012) Prevention of noise-induced hearing loss: potential therapeutic agents. In: Noise-Induced Hearing Loss: Scientific Advances, Springer Handbook of Auditory Research(Le Prell, C. G. et al., eds), pp 285-338 New York: Springer Science+Business Media, LLC.

Le Prell CG, Dell S, Hensley BN, Hall JWI, Campbell KCM, Antonelli PA, Green GE, Miller JM, Guire K (2012) Digital music exposure reliably induces temporary threshold shift (TTS) in normal hearing human subjects. Ear Hear 33:e44-58.

Le Prell CG, Lobarinas E (2015) Strategies for assessing antioxidant efficacy in clinical trials. In: Oxidative Stress in Applied Basic Research and Clinical Practice: Free Radicals in ENT Pathology(Miller, J. M. et al., eds) New York: Springer.

Lin HW, Furman AC, Kujawa SG, Liberman MC (2011) Primary neural degeneration in the guinea pig cochlea after reversible noise-induced threshold shift. J Assoc Res Otolaryngol 12:605-616.

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 12:711-717.

McDaniel DM, Cox RM (1992) Evaluation of the speech intelligibility rating (SIR) test for hearing aid comparisons. J Speech Hear Res 35:686-693.

Musiek, F. E., Kibbe, K., Rackliffe, L., & Weider, D. J. (1984). The auditory brain stem response IV amplitude ratio in normal, cochlear, and retrocochlear ears.*Ear and hearing*, *5*(1), 52-55.

Nilsson M, Soli SD, Sullivan JA (1994) Development of the Hearing in Noise Test for the measurement of speech reception thresholds in quiet and in noise. J Acoust Soc Am 95:1085-1099.

Plack CJ, Barker D, Prendergast G (2014) Perceptual consequences of "hidden" hearing loss Trends in Hearing 18.

Plomp, R. (1986). A signal-to-noise ratio model for the speech-reception threshold of the hearing impaired. *Journal of Speech and Hearing Research, 29*(2), 146-154.

Quist-Hanssen S, Thorud E, Aasand G (1979) Noise-induced hearing loss and the comprehension of speech in noise. Acta oto-laryngologica Supplementum 360:90-95.

Ruggles D, Bharadwaj H, Shinn-Cunningham BG (2011) Normal hearing is not enough to guarantee robust encoding of suprathreshold features important in everyday communication. Proc Natl Acad Sci U S A 108:15516-15521.

Ruggles D, Bharadwaj H, Shinn-Cunningham BG (2012) Why middle-aged listeners have trouble hearing in everyday settings. Current Biology 22:1417-1422.

Samelli AG, Matas CG, Carvallo RM, Gomes RF, de Beija CS, Magliaro FC, Rabelo CM (2012) Audiological and electrophysiological assessment of professional pop/rock musicians. Noise Health 14:6-12.

Smoorenburg, G. F. (1992). Speech reception in quiet and in noisy conditions by individuals with noise-induced hearing loss in relation to their tone audiogram. The journal of the acoustical society of America, 91(1), 421-437.

Spankovich C, Griffiths SK, Lobarinas E, Morgenstein KE, de la Calle S, Ledon V, Guercio D, Le Prell CG (2014) Temporary threshold shift after impulse-noise during video game play: Laboratory data. Int J Audiol 53 Suppl 2:S53-65.

Speaks C, Trine TD, Crain TR, Niccum N (1994) A Revised Speech Intelligibility Rating (RSIR) test: listeners with normal hearing. Otolaryngol Head Neck Surg 110:75-83.

Wang Y, Ren C (2012) Effects of repeated "benign" noise exposures in young CBA mice: shedding light on age-related hearing loss. J Assoc Res Otolaryngol 13:505-515.

Watson, C. S., Kidd, G. R., Miller, J. D., Smits, C., & Humes, L. E. (2012). Telephone screening tests for functionally impaired hearing: Current use in seven countries and development of a US version. *Journal of the American Academy of Audiology*, *23*(10), *757-767*.

Wilson RH (2011) Clinical experience with the words-in-noise test on 3430 veterans: comparisons with pure-tone thresholds and word recognition in quiet. J Am Acad Audiol 22:405-423.

Wilson RH, McArdle RA, Smith SL (2007) An Evaluation of the BKB-SIN, HINT, QuickSIN, and WIN Materials on Listeners With Normal Hearing and Listeners With Hearing Loss. J Speech Lang Hear Res 50:844-856.

AUTHOR BIOGRAPHIES

Douglas S. Brungart, PhD, is currently the Chief Scientist at the Audiology and Speech Center at Walter Reed National Military Medical Center, which serves as the flagship entity for clinical care in audiology and speech pathology within the DOD medical system and has a long-standing history of conducting world-class clinical and applied research in speech and audiology with direct relevance to military medicine. The Center is currently working with the U.S. Army Public Health Command in a major effort to conduct clinical and field studies to support the objectives of the U.S. Army Hearing Program, including an initiative to develop new "Auditory Fitness for Duty" standards for military personnel. Dr. Brungart obtained his doctorate in Computer Science and Electrical Engineering from the Massachusetts Institute of Technology in 1998. From 1993 to 2009, he worked at the Battlespace Acoustics Branch at the Air Force Research Laboratory at Wright-Patterson AFB, OH. Since 2009, he has been the Chief Scientist at the Audiology and Speech Center at Walter Reed National Military Medical Center in Bethesda, MD. Dr. Brungart has published many peer-reviewed journal articles on auditory perception, and holds more than 10 U.S. patents related to auditory perception and speech communication. In 2009, he was designated as a "Fellow" by the Acoustical Society of America. Since 2011, Dr. Brungart has served as the Interim Director of Research for the HCE, where his scientific research and subject matter expertise is being utilized to support the HCE mission.

Kathleen Campbell, PhD, distinguished Scholar and Professor at Southern Illinois University (SIU) School of Medicine, ran audiology clinics in the US and Canada for over 25 years and audiology research for over 30 years. She served on the American Academy of Audiology (AAA) Board of Directors, received an AAA Presidential Citation, 2 Medical Innovators Award and was made a Fellow by the American Speech Language Hearing Association. She received the Mensa Copper Black Award for her patents, and the Board of Trustees Award for her contributions in chairing the Mensa national research awards committee. She was the 2012 Inventor of the Year for the Southern Illinois University System, and was the 2014 SIU Scholar of Excellence, SIUs highest distinction. She authored _Essential Audiology for Physicians_ and edited/authored _Pharmacology and Ototoxicity for Audiologists_. She has received a number of grants from NIH and other agencies for her research in otoprotective agents and is the inventor of the protective agent D-methionine patents. Her grants and patent income to SIU exceeds 8.2 million dollars. Her patents are owned by her employer, SIU School of Medicine. She is currently running FDA approved, and DOD funded Phase 3 clinical trials of D-methionine prevention and treatment of noise induced hearing loss and tinnitus, in US Army troops.

Royce Ellen Clifford, MD, received a MPH degree at Harvard in 2006, followed by the Harvard Public Health Innovator Award in 2013 for continuing publications in the fields of acoustic trauma and hearing loss. As a board-certified Ear, Nose, and Throat Surgeon and Aerospace/Preventive Medicine Physician, she has spent the last decade in basic

science and operational research focusing on noise and resultant hearing loss in the military. While on deployment in the Middle East, including Iraq, she obtained first-hand knowledge of the operational effects of explosive devices on military personnel. During her tenure as Officer-In-Charge of the Aviation Clinic at Marine Corps Air Base Camp Pendleton, she has worked with Naval Medical Center San Diego and Harvard towards the goal of finding effective modes of intervention for the treatment and prevention of noise-induced hearing loss. She flew 2 years with a San Diego-based F/A-18 squadron and has served in the aviation community for 14 years, including two years as Senior Medical Officer on the aircraft carrier USS GEORGE WASHINGTON (CVN-73). She is currently a Visiting Scientist at Harvard School of Public Health and operational advisor to Code 42 at Office of Naval Research on projects involving noise-induced hearing loss.

Howard Greene, PhD, MS, MBA, is a research epidemiologist with the Geneva Foundation and located supporting the DOD Hearing Center of Excellence onsite at the Navy Medical Center San Diego. Dr. Green's current research interests include the effects of impulse noise on hearing. He received his PhD in Environmental Medicine/ Epidemiology and MS in Inhalation Toxicology from New York University, and his MBA from the University of Southern California. He has a broad background in occupational and environmental epidemiology, inhalation toxicology, risk assessment, exposure modeling, and statistics and is the co-author of a patent for the "Performance Assurance Computerized Test", a test of fitness for duty.

Gerald M. Haase, MD, is Clinical Professor of Surgery, University of Colorado, School of Medicine and Children's Hospital Colorado. He is also Chief Medical Officer of Premier Micronutrient Corporation and has conducted medical research and clinical trials for three decades including surgical innovations in oncology and development of novel strategies for antioxidant micronutrient therapy. He was educated at the Johns Hopkins University and Tufts University School of Medicine and received graduate honors in research. His postgraduate training was from the University of Colorado, Health Sciences Center, Children's Hospital Medical Center, Boston, and Nationwide Children's Hospital, Columbus, Ohio. He is licensed to practice in Colorado, Massachusetts and Ohio, and held certification from the American Board of Surgery in General Surgery, Pediatric Surgery and Critical Care. Dr. Haase was Chairman of the Department of Surgery, Children's Hospital Colorado, a consultant surgeon, Department of the Army, Fitzsimmons Medical Center and a Director on the National Board of the American Cancer Society. He has published 180 scientific papers, holds eight U.S. patents for antioxidant micronutrient therapy and been the recipient of clinical research grants and contracts funded at a several million dollar cumulative level. He was an editorial reviewer for several medical journals and an editorial board member of The Annals of Surgical Oncology. He is a member of more than 25 professional societies including the American Association for Cancer Research, International College of Surgeons, New York Academy of Sciences and American College of Physician Executives.

Tanisha L. Hammill holds Master's degrees in Ethics/Religious Studies and Public Health Administration. She has ten years of experience as an administrator for hearing programs in the US military. Her duties include reports and inputs to policy changes, regulatory planning and adherence, enterprise-wide research programmatic design, coordination and execution. She has specific program oversight responsibilities in Fitness for Duty and Return to Duty Standards, Pharmaceutical Interventions for Hearing Loss (PIHL), Allied NeuroSensory Warrior-Related Research (ANSW2R) and the Collaborative Auditory & Vestibular Research Network (CAVRN).

James A. Henry, PhD, is employed as a Research Career Scientist at the VA National Center for Rehabilitative Auditory Research located at the Portland, Oregon VA Medical Center. He is also Research Professor in the Dept. of Otolaryngology-Head & Neck Surgery at Oregon Health & Science University (OHSU) in Portland. He has conducted tinnitus clinical research since 1995. He recently served on the AAO-HNS committee that developed the clinical guidelines for tinnitus.

Michael Hoffer, MD, FACS, is currently serving as a Professor of Otolaryngology at the University of Miami and Academic Liaison for the DoD Hearing Center of Excellence. Dr. Hoffer assumed these roles after an over twenty year military career. During his time in the military and at the University, Dr. Hoffer performs both basic and clinical research and serves as a practicing clinical neurotologist. Dr. Hoffer's lab focuses on traumatic damage to the inner ear and brain. He has authored or co-authored over sixty papers and has a particular expertise in dizziness and balance disorders as well as neurosensory consequences after mild traumatic brain injury (mTBI) Dr. Hoffer and his collaborators have done pioneering work on pharmaceutical countermeasures for mTBI as well as optimized diagnosis and management of neurosensory disorders seen after mTBI.

Jonathan Kil, MD, is the co-founder and Chief Medical Officer of Sound Pharmaceuticals, a private biotechnology company in Seattle that has developed three proprietary product pipelines aimed at improving and restoring auditory function in humans. Dr. Kil has expertise in basic, preclinical and clinical studies involving noise-induced and chemotherapy-induced hearing loss. He has been the principal investigator of several federal grants and R&D awards for his work in hearing research that has examined the mechanisms of inner ear hair cell loss, repair and protection, and supporting cell and hair cell regeneration via p27Kip1 inhibition totaling $5.8M. Dr. Kil has lead the FDA interactions on two INDs with the FDA and has now completed an interventional Ph-II clinical trial with SPI-1005 in NIHL. He is the inventor on several issued US patents and serves as a Board Trustee to the University of Washington's Harborview Medical Center and is Board President of the Academy for Precision Learning, an independent autism inclusion school in Seattle.

Dawn Konrad-Martin, PhD, CCC-A, is a Research Investigator at the VA RR&D Center of Excellence, the National Center for Rehabilitative Auditory Research (NCRAR) located in Portland, Oregon, and is Associate Professor in the Department of Otolaryngology at Oregon Health and Sciences University. Her research contributions include early efforts to examine distortion-product otoacoustic emission (DPOAE) source contributions, one

of the first reports of stimulus-frequency otoacoustic emissions measured in impaired ears, and the use of clinical decision theory techniques to examine DPOAE test performance for identifying changes in hearing from ototoxic drugs. Her current research involves developing OAE-based techniques for ototoxicity monitoring, and determining the auditory system deficits responsible for impaired speech understanding among individuals who are older and/or have diabetes. Prior to joining the NCRAR, Dawn completed a PhD in Audiology at the University of Washington, a Post-Doctoral Fellowship at Boys Town National Research Hospital, and was an Assistant Professor in the Dept. of Communication Disorders and Sciences at Rush University. She currently serves on the Department of Defense Pharmaceutical Interventions for Hearing Loss Advisory Board, and was recently nominated to serve on ASHA's Research and Scientific Affairs Committee (RSAC).

Sharon G. Kujawa, PhD, is an Associate Professor of Otology and Laryngology, Harvard Medical School. She is Director of the Department of Audiology and a Senior Scientist in the Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary, Boston. She serves on the faculty of the Harvard Program in Speech and Hearing Biosciences and Technology. Work in the Kujawa Lab focuses on understanding how vulnerability to noise-induced hearing loss is shaped by genetic background, how noise exposure alters the way ears and hearing age, and how these consequences of exposure can be manipulated pharmacologically to reveal underlying mechanisms or for treatment or prevention.

Colleen LePrell, PhD, is an Associate Professor and Interim Chair of the Department of Speech, Language, and Hearing Sciences at the University of Florida, where she also directs the Center for Hearing Research. She has received research funding from the National Institutes of Health, Department of Defense, several foundations, and industry, for basic scientific research and translational studies assessing protection against temporary hearing loss in humans. Current research programs in her laboratory at the University of Florida include ongoing efforts to identify and prevent the progression of biochemical processes that lead to cell death in the inner ear, as well as industry-sponsored clinical research.

Lynne Marshall, PhD, is a Senior Research Audiologist at the Naval Submarine Medical Research Laboratory in Groton, Connecticut. She also is a Jayhawker from the University of Kansas, where she obtained master's degrees in Speech Pathology and in Audiology, and a Ph.D. in Speech and Hearing Science. Following a clinical fellowship year in audiology at the Upstate Medical Center in Syracuse, New York, she spent several years in Omaha, Nebraska, where she was Clinical Coordinator of Audiology at the University of Nebraska Medical Center, a faculty member at the University of Nebraska, and also did postdoctoral work at Boys Town National Research Hospital. At the Naval Submarine Medical Research Laboratory she first did auditory sonar research, and now is focused on noise-induced hearing loss including the potential role of otoacoustic emissions in Hearing Conservation Programs, educational programs using

hearing-loss simulation for Hearing Conservation applications, and decreasing noise-induced hearing loss in the Navy and Marine Corps.

Garnett P. McMillan, PhD, is biostatistician with the National Center for Rehabilitative Auditory Research at the Portland VA Medical Center. Dr. McMillan is an expert in computational statistics, statistical approaches to patient screening, diagnostic test development, linear and non-linear models, and Bayesian inference. He currently provides statistical support to over 30 projects funded by VA-RR&D, NIH, and industry sponsors spanning diverse topics such as tinnitus treatment, chemotherapy and cystic fibrosis management, sound localization, and the auditory epidemiology of multiple sclerosis, diabetes and Parkinson's disease.

Judi Lapsley Miller, PhD, is a hearing-research consultant, with a background in psychophysics, otoacoustic emissions, middle-ear power analysis, and hearing conservation. Her forte is experimental design and data analysis, and she is typically responsible for the bit in the middle of research papers - the method and results. Judi is based in Wellington, New Zealand.

Col Mark Packer, MD, is the Executive Director for the Hearing Center of Excellence (HCE). He graduated from the Uniformed Services University of the Health Sciences in 1995 as a member of the Alpha Omega Alpha medical honor society. He completed a general surgery internship at Wright State University in Dayton, Ohio, in 1996, and was board certified in Otolaryngology Head and Neck surgery upon finishing his residency training in the San Antonio Uniformed Services Health Education Consortium in 2002. Most recently, he graduated from fellowship training in neurotology and cranial base surgery at The Ohio State University in 2008, and practices as a board certified Neurotologist within the San Antonio Military Health System.

Gayla L. Poling, PhD, CCC-A, is a Research Audiologist in the Clinical Care, Rehabilitation and Restoration Directorate, Defense Hearing Center of Excellence, Contractor with The Geneva Foundation. She earned her M.A. in Speech and Hearing Science from The Ohio State University and completed her Clinical Fellowship at Henry Ford Hospital in Detroit, MI. She practiced as a clinical audiologist at the Mayo Clinic (Rochester, MN) prior to returning to Ohio State where she earned a Ph.D. in Hearing Science. She completed postdoctoral training focused on translational research at the Medical University of South Carolina and Northwestern University. Dr. Poling's primary research interest is in the early detection and prevention of hearing loss due to noise and other environmental factors (ototoxicity).

Kedar N. Prasad, PhD, obtained a Master's degree in Zoology from the University of Bihar in India, and a Ph.D. degree in Radiation Biology from the University of Iowa, Iowa City. He went to Brookhaven National Laboratory for Post-doctoral training. Dr. Prasad Joined the Department of Radiology at the University of Colorado medical School where he became Professor and the Director for the Center for Vitamins and Cancer Research. He published over 250 papers in peer-reviewed journals including Nature, Science, and Proceedings of the National Academy of Sciences (PNAS) supported by

the NIH. He also contributed several book chapters and abstracts as well as authored or edited 25 books on radiobiology, neurodegenerative disease, and nutrition and cancer. He was a member of several professional organizations, and served as an ad-hoc member of various Study Sections of the NIH. He was a frequently invited speaker at National and International meetings on nutrition and cancer and neurological diseases. In 1982, he was invited by the Nobel Prize Committee to nominate a candidate for the Nobel Prize in Medicine. He delivered the 1999 Harold Harper Lecture at the meeting of the American College of Advancement of Medicine. He is a former President of the International Society for Nutrition and Cancer. Since 2005, he is Chief Scientific Officer of Premier Micronutrient Corporation.

Allen Ryan, PhD, obtained a joint doctoral degree in neurophysiology and psychology from the University of Washington, studying the neural interface between hearing and behavior. He completed a postdoctoral fellowship in auditory neurobiology at Northwestern University, focused on the function of the outer hair cell. He then joined the Department of Otolaryngology at the University of California, San Diego and the San Diego VA Medical Center, where he directs programs in auditory research. A major focus is the cellular substrates of hearing loss, including cell death and survival signaling, molecular genetics and biology, the regulation of genes in hair cells and cochlear proteomics. A second area of research is the interface between cochlear neurons and hair cells, including neural contributions to hearing loss and the role of Type II spiral ganglion neurons. He has published over 340 journal articles and book chapters on these and other topics.

Martin Slade, MPH, is a Lecturer in Medicine and in Public Health at Yale University School of Medicine. He currently serves as the Director of Research for Yale Occupational & Environmental Medicine. He serves as a member of the Department of Defense Hearing Center of Excellence Pharmaceutical Interventions for Hearing Loss Working Group. Over the past 15 years, Mr. Slade has focused on the development of analytical models to evaluate the effects of physical, social, genetic and environmental factors on the patterns of disease and injury within the workplace setting, including non-traditional settings such as the military and merchant mariners. In recent years, he has been the lead statistician on four clinical trials conducted by the Department of Defense to determine the effect of pharmacological agents on prevention of hearing loss as well as mild traumatic brain injuries. More generally, he oversees the analyses required to deterministically model risk factors of occupational disease and injury.

RECENTLY PUBLISHED LITERATURE

Articles determined to be of particular interest will be listed with full abstract in "Research Highlights" below, followed by the remainder of the "Relevant Literature," all published between April 1, 2014 (the end of the last Newsletter search term) and September 4, 2014.

RESEARCH HIGHLIGHTS

Editors evaluated over 320 article abstracts and full text articles as needed for inclusion in this edition's listing of recently published PIHL-related literature. While the final retention of articles was a subjective decision by the editors, care was taken to ensure that articles met at least a basic criterion of relevance or interest to the PIHL community. Articles which examined ototoxic drugs were generally not retained while those examining drugs to prevent ototoxicity were included, for example. Likewise, studies about efficacy of hearing conservation programs or industrial hygiene were not included, nor properly searched for, but select studies with relevance to PIHL-related population selection or study design implications have been included. Searching only PubMed, the following searches were conducted:

"Hearing Injury": 51 articles reviewed by abstract; 30 retained

"Hearing Loss" AND "Prevention": 51 articles reviewed by abstract; 32 retained

"Hearing Loss" AND "Treatment": 196 articles reviewed by abstract; 74 retained

"Hearing Loss" AND "Prophylaxis": 0 articles reviewed (duplicates of other categories); 0 retained

"Hearing Loss" AND "Therapy": 3articles reviewed by abstract; 0 retained

"Hearing Loss" AND "Ototoxic": 11 articles reviewed by abstract; 8 retained

"Hearing Loss" AND "Ototoxicity": 8 articles reviewed by abstract; 3 retained

How to Bury the Dead: Elimination of Apoptotic Hair Cells from the Hearing Organ of the Mouse
J Assoc Res Otolaryngol, 30, 30. (2014)

Anttonen, T., Belevich, I., Kirjavainen, A., Laos, M., Brakebusch, C., Jokitalo, E., & Pirvola, U.

Hair cell death is a major cause of hearing impairment. Preservation of surface barrier upon hair cell loss is critical to prevent leakage of potassium-rich endolymph into the

organ of Corti and to prevent expansion of cellular damage. Understanding of wound healing in this cytoarchitecturally complex organ requires ultrastructural 3D visualization. Powered by the serial block-face scanning electron microscopy, we penetrate into the cell biological mechanisms in the acute response of outer hair cells and glial-like Deiters' cells to ototoxic trauma in vivo. We show that Deiters' cells function as phagocytes. Upon trauma, their phalangeal processes swell and the resulting close cellular contacts allow engulfment of apoptotic cell debris. Apical domains of dying hair cells are eliminated from the inner ear sensory epithelia, an event thought to depend on supporting cells' actomyosin contractile activity. We show that in the case of apoptotic outer hair cells of the organ of Corti, elimination of their apices is preceded by strong cell body shrinkage, emphasizing the role of the dying cell itself in the cleavage. Our data reveal that the resealing of epithelial surface by junctional extensions of Deiters' cells is dynamically reinforced by newly polymerized F-actin belts. By analyzing Cdc42-inactivated Deiters' cells with defects in actin dynamics and surface closure, we show that compromised barrier integrity shifts hair cell death from apoptosis to necrosis and leads to expanded hair cell and nerve fiber damage. Our results have implications concerning therapeutic protective and regenerative interventions, because both interventions should maintain barrier integrity.

Hair cell regeneration after ATOH1 gene therapy in the cochlea of profoundly deaf adult guinea pigs
PLoS One, 9(7). (2014)

Atkinson, P. J., Wise, A. K., Flynn, B. O., Nayagam, B. A., & Richardson, R. T.

The degeneration of hair cells in the mammalian cochlea results in permanent sensorineural hearing loss. This study aimed to promote the regeneration of sensory hair cells in the mature cochlea and their reconnection with auditory neurons through the introduction of ATOH1, a transcription factor known to be necessary for hair cell development, and the introduction of neurotrophic factors. Adenoviral vectors containing ATOH1 alone, or with neurotrophin-3 and brain derived neurotrophic factor were injected into the lower basal scala media of guinea pig cochleae four days post ototoxic deafening. Guinea pigs treated with ATOH1 gene therapy, alone, had a significantly greater number of cells expressing hair cell markers compared to the contralateral non-treated cochlea when examined 3 weeks post-treatment. This increase, however, did not result in a commensurate improvement in hearing thresholds, nor was there an increase in synaptic ribbons, as measured by CtBP2 puncta after ATOH1 treatment alone, or when combined with neurotrophins. However, hair cell formation and synaptogenesis after co-treatment with ATOH1 and neurotrophic factors remain inconclusive as viral transduction was reduced due to the halving of viral titres when the samples were combined. Collectively, these data suggest that, whilst ATOH1 alone can drive non-sensory cells towards an immature sensory hair cell phenotype in

the mature cochlea, this does not result in functional improvements after aminoglycoside-induced deafness.

Auditory and non-auditory effects of noise on health
Lancet, 383(9925), 1325-1332. (2014)

Basner, M., Babisch, W., Davis, A., Brink, M., Clark, C., Janssen, S., & Stansfeld, S

Noise is pervasive in everyday life and can cause both auditory and non-auditory health effects. Noise-induced hearing loss remains highly prevalent in occupational settings, and is increasingly caused by social noise exposure (eg, through personal music players). Our understanding of molecular mechanisms involved in noise-induced hair-cell and nerve damage has substantially increased, and preventive and therapeutic drugs will probably become available within 10 years. Evidence of the non-auditory effects of environmental noise exposure on public health is growing. Observational and experimental studies have shown that noise exposure leads to annoyance, disturbs sleep and causes daytime sleepiness, affects patient outcomes and staff performance in hospitals, increases the occurrence of hypertension and cardiovascular disease, and impairs cognitive performance in schoolchildren. In this Review, we stress the importance of adequate noise prevention and mitigation strategies for public health.

Association between ambient noise exposure, hearing acuity, and risk of acute occupational injury
Scand J Work Environ Health, 19(10). (2014)

Cantley, L. F., Galusha, D., Cullen, M. R., Dixon-Ernst, C., Rabinowitz, P. M., & Neitzel, R. L.

OBJECTIVE: This study aimed to examine the associations between acute workplace injury risk, ambient noise exposure, and hearing acuity, adjusting for reported hearing protection use. **METHODS:** In a cohort of 9220 aluminum manufacturing workers studied over six years (33 300 person-years, 13 323 person-jobs), multivariate mixed effects models were used to estimate relative risk (RR) of all injuries as well as serious injuries by noise exposure category and hearing threshold level (HTL) adjusting for recognized and potential confounders. **RESULTS:** Compared to noise <82 dBA, higher exposure was associated with elevated risk in a monotonic and statistically significant exposure-response pattern for all injuries and serious injuries with higher risk estimates observed for serious injuries [82-84.99 dBA: RR 1.26, 95% confidence interval (95% CI) 0.96-1.64; 85-87.99 dBA: RR 1.39, 95% CI 1.05-1.85; ≥88 dBA: RR 2.29, 95% CI 1.52-3.47]. Hearing loss was associated with increased risk for all injuries, but was not a significant predictor of risk for the subset of more serious injuries. Compared to those without hearing loss, workers with

HTL ≥25 dB had 21% increased all injury risk (RR 1.21, 95% CI 1.09-1.33) while those with HTL 10-24.99 dB had 6% increased risk (RR 1.06, 95% CI 1.00-1.13). Reported hearing protection type did not predict injury risk. **CONCLUSION:** Noise exposure levels as low as 85 dBA may increase workplace injury risk. HTL was associated with increased risk for all, but not the subset of serious, injuries. Additional study is needed both to confirm the observed associations and to explore causal pathways.

Prevention and restoration of hearing loss associated with the use of cisplatin
Biomed Res Int, 925485(10), 22. (2014)

Chirtes, F., & Albu, S.

BACKGROUND: Cisplatin is a well-known platinum-based chemotherapeutic agent used for the treatment of various malignant tumours. A frequent side effect of cisplatin therapy is ototoxicity. Unfortunately, currently there are no available treatments. **MATERIALS AND METHODS:** Experimental, clinical studies and reviews published between 2004 and 2014 in the English medical literature concerning ototoxicity were selected using Medline, PubMed, and Google Scholar databases. Inclusion criteria were cisplatin-induced ototoxicity and therapy aimed at preventing or curing this disorder. Molecular mechanisms and clinical, audiological, and histological markers of cisplatin-induced ototoxicity are described. Moreover, experimental and clinical strategies for prevention or treatment of hearing loss were also reviewed. **RESULTS AND DISCUSSION:** Experimental studies demonstrate a wide range of otoprotective molecules and strategies efficient against cisplatin-induced hearing loss. However, only dexamethasone proved a slight otoprotective effect in a clinical study. **CONCLUSION**: Further research must be completed to bring future therapeutic options into clinical setting.

Noise-induced hearing loss in Korean workers: co-exposure to organic solvents and heavy metals in nationwide industries
PLoS One, 9(5). (2014)

Choi, Y. H., & Kim, K.

Noise exposure is a well-known contributor to work-related hearing loss. Recent biological evidence suggests that exposure to ototoxic chemicals such as organic solvents and heavy metals may be additional contributors to hearing loss. However, in industrial settings, it is difficult to determine the risks of hearing loss due to these chemicals in workplaces accompanied by excessive noise exposure. A few studies suggest that the effect of noise may be enhanced by ototoxic chemicals. Therefore,

this study investigated whether co-exposure to organic solvents and/or heavy metals in the workplace modifies the risk of noise exposure on hearing loss in a background of excessive noise. METHODS: We examined 30,072 workers nationwide in a wide range of industries from the Korea National Occupational Health Surveillance 2009. Data on industry-based exposure (e.g., occupational noise, heavy metals, and organic solvents) and subject-specific health outcomes (e.g., audiometric examination) were collected. Noise was measured as the daily 8-h time-weighted average level. Air conduction hearing thresholds were measured from 0.5 to 6 kHz, and pure-tone averages (PTA) (i.e., means of 2, 3, and 4 kHz) were computed. RESULTS: In the multivariate linear model, PTA increment with occupational noise were 1.64-fold and 2.15-fold higher in individuals exposed to heavy metals and organic solvents than in unexposed individuals, respectively. CONCLUSION: This study provides nationwide evidence that co-exposure to heavy metals and/or organic solvents may exacerbate the effect of noise exposure on hearing loss in workplaces. These findings suggest that workers in industries dealing with heavy metals or organic solvents are susceptible to such risks.

Personal noise dosimeters: accuracy and reliability in varied settings
Noise Health, 16(70), 143-148. (2014)

Cook-Cunningham, S. L.

This study investigated the accuracy, reliability, and characteristics of three brands of personal noise dosimeters (N = 7 units) in both pink noise (PN) environments and natural environments (NEs) through the acquisition of decibel readings, Leq readings and noise doses. Acquisition periods included repeated PN conditions, choir room rehearsals and participant (N = 3) Leq and noise dosages procured during a day in the life of a music student. Among primary results: (a) All dosimeters exhibited very strong positive correlations for PN measurements across all instruments; (b) all dosimeters were within the recommended American National Standard Institute (ANSI) SI.25-1991 standard of ±2 dB (A) of a reference measurement; and (c) all dosimeters were within the recommended ANSI SI.25-1991 standard of ±2 dB (A) when compared with each other. Results were discussed in terms of using personal noise dosimeters within hearing conservation and research contexts and recommendations for future research. Personal noise dosimeters were studied within the contexts of PN environments and NEs (choral classroom and the day in the life of collegiate music students). This quantitative study was a non-experimental correlation design. Three brands of personal noise dosimeters (Cirrus doseBadge, Quest Edge Eg5 and Etymotic ER200D) were tested in two environments, a PN setting and a natural setting. There were two conditions within each environment. In the PN environment condition one, each dosimeter was tested individually in comparison with two reference measuring devices (Ivie and Easera) while PN was generated by a Whites Instrument PN Tube. In condition two, the PN procedures

were replicated for longer periods while all dosimeters measured the sound levels simultaneously. In the NE condition one, all dosimeters were placed side by side on a music stand and recorded sound levels of choir rehearsals over a 7-h rehearsal period. In NE, condition two noise levels were measured during a day in the life of college music students. Three participants each wore two types of dosimeters for an 8-h period during a normal school day. Descriptive statistical analyzes including means, standard deviation and Pearson product-moment correlation. The primary finding is that the dosimeters in this study recorded results within ±2 dB of either a reference measurement or within dosimeters in all four conditions examined. All dosimeters studied measured steady noise source accurately and consistently, with strong positive correlations across all instruments. Measurements acquired during choral rehearsals indicated a maximum of 1.5 dB difference across dosimeters. The Etymotic research personal noise dosimeters (ER200D) could provide individuals and schools of music with a relatively inexpensive tool to monitor sound doses. Findings from this study suggest that the three brands of dosimeters tested will provide reliable Leq levels and hearing dosages in both PN and natural settings

Steroids for treatment of sudden sensorineural hearing loss: A meta-analysis of randomized controlled trials
Laryngoscope, 21(10), 24834. (2014)

Crane, R. A., Camilon, M., Nguyen, S., & Meyer, T. A.

OBJECTIVE: To systematically review the available evidence regarding steroid treatment for sudden sensorineural hearing loss (SSHL) and to update prior analyses when possible. **DATA SOURCES:** OVID Medline. **REVIEW METHODS:** An electronic database search (OVID Medline) was performed with the objective of identifying all randomized controlled trials examining the use of steroids for the treatment of SSHL. The search was limited to English-language publications between January 1980 and June 2013. Reference lists were examined for additional articles. **RESULTS:** A total of 15 articles including 1,166 subjects were included in three separate analyses. Three articles (181 subjects) were included in the steroid versus placebo analysis, which resulted in an odds ratio of 1.52 (95% confidence interval [CI]: 0.83-2.77). Six articles (702 subjects) were included in the systemic versus intratympanic steroids analysis, which resulted in an odds ratio of 1.14 (95% CI: 0.82-1.59). Six articles (283 subjects) were included in the salvage treatment analysis, which resulted in an odds ratio of 6.04 (95% CI: 3.26-11.2). **CONCLUSIONS:** A meta-analysis of randomized controlled trials does not support the use of steroids over placebo in this condition, a finding consistent with previous analyses. Although systemic or intratympanic steroid administration does not have a significant treatment effect, steroids for salvage treatment of patients failing traditional therapy appear to have an effect. However, this result should be interpreted with caution given the poor quality of component trials. Implications for clinical practice and future trial design are discussed.

Comparison of the effects of N-acetyl-cysteine and ginseng in prevention of noise induced hearing loss in male textile workers
Noise Health, 16(71), 223-227. (2014)

Doosti, A., Lotfi, Y., Moossavi, A., Bakhshi, E., Talasaz, A. H., & Hoorzad, A.

Previous studies revealed the role of antioxidant agents in prevention of noise induced hearing loss (NIHL). The aim of this study was to compare the protective effect of N-acetyl-cysteine (NAC) and ginseng on protection of NIHL in textile workers exposed to continuous noise in daily working. In this study, 48 participants were randomly allocated to three groups; Group I received NAC 1200 mg/day, Group II received ginseng 200 mg/day, and Group III (control group) received no supplement. Pure tone audiometry and high frequency audiometry were performed preshift before and after 14 days (on day 15). Linear regression analysis results showed reduced noise-induced temporary threshold shift (TTS) for NAC and ginseng groups at 4, 6 and 16 kHz ($P < 0.001$) in both ears. Furthermore, the protective effects were more prominent in NAC than ginseng. Our results show that NAC and ginseng can reduce noise induced TTS in workers exposed to occupational noise. Further studies are needed to prove antioxidants benefits in hearing conservation programs.

Do hearing protectors protect hearing?
Am J Ind Med, 57(9), 1001-1010. (2014)

Groenewold, M. R., Masterson, E. A., Themann, C. L., & Davis, R. R.

BACKGROUND: We examined the association between self-reported hearing protection use at work and incidence of hearing shifts over a 5-year period. **METHODS:** Audiometric data from 19,911 workers were analyzed. Two hearing shift measures-OSHA standard threshold shift (OSTS) and high-frequency threshold shift (HFTS)-were used to identify incident shifts in hearing between workers' 2005 and 2009 audiograms. Adjusted odds ratios were generated using multivariable logistic regression with multi-level modeling. **RESULTS:** The odds ratio for hearing shift for workers who reported never versus always wearing hearing protection was nonsignificant for OSTS (OR 1.23, 95% CI 0.92-1.64) and marginally significant for HFTS (OR 1.26, 95% CI 1.00-1.59). A significant linear trend towards increased risk of HFTS with decreased use of hearing protection was observed ($P = 0.02$). **CONCLUSION:** The study raises concern about the effectiveness of hearing protection as a substitute for noise control to prevent noise-induced hearing loss in the workplace.

Systemic lipopolysaccharide induces cochlear inflammation and exacerbates the synergistic ototoxicity of kanamycin and furosemide
J Assoc Res Otolaryngol, 15(4), 555-570. (2014)

Hirose, K., Li, S. Z., Ohlemiller, K. K., & Ransohoff, R. M.

Aminoglycoside antibiotics are highly effective agents against gram-negative bacterial infections, but they cause adverse effects on hearing and balance dysfunction as a result of toxicity to hair cells of the cochlea and vestibular organs. While ototoxicity has been comprehensively studied, the contributions of the immune system, which controls the host response to infection, have not been studied in antibiotic ototoxicity. Recently, it has been shown that an inflammatory response is induced by hair cell injury. In this study, we found that lipopolysaccharide (LPS), an important component of bacterial endotoxin, when given in combination with kanamycin and furosemide, augmented the inflammatory response to hair cell injury and exacerbated hearing loss and hair cell injury. LPS injected into the peritoneum of experimental mice induced a brisk cochlear inflammatory response with recruitment of mononuclear phagocytes into the spiral ligament, even in the absence of ototoxic agents. While LPS alone did not affect hearing, animals that received LPS prior to ototoxic agents had worse hearing loss compared to those that did not receive LPS pretreatment. The poorer hearing outcome in LPS-treated mice did not correlate to changes in endocochlear potential. However, LPS-treated mice demonstrated an increased number of CCR2(+) inflammatory monocytes in the inner ear when compared with mice treated with ototoxic agents alone. We conclude that LPS and its associated inflammatory response are harmful to the inner ear when coupled with ototoxic medications and that the immune system may contribute to the final hearing outcome in subjects treated with ototoxic agents.

Effects of substance P during the recovery of hearing function after noise-induced hearing loss
Brain Res, 25, 187-196. (2014

Kanagawa, E., Sugahara, K., Hirose, Y., Mikuriya, T., Shimogori, H., & Yamashita, H.

Substance P (SP) is a widely distributed neurotransmitter in living tissues and is involved in various repair processes. We investigated the possibility that SP may ameliorate cochlear hair cell damage produced by noise exposure. The present study examined the effect of SP in protecting the cochlea from noise damage in guinea pigs exposed to noise after an infusion of SP into the inner ear. Changes in the hearing threshold (auditory brain response, ABR), number of synaptic ribbons, and the appearance of the outer hair cells after noise exposure were analyzed at 2 severity levels of noise-induced hearing loss. The moderate noise-induced hearing loss (110dB, 3h) group showed

recovery in the ABR threshold over time, finally reaching a level slightly above pre-exposure levels, with only slight injury to the synaptic ribbons and minimal changes in the appearance of the outer hair cells. Our results indicated that in moderate hearing loss, SP exhibited a protective effect on the inner ear, both functionally and structurally. While the final magnitude of ABR threshold elevation was greater in severe noise-induced hearing loss, the synaptic ribbons and outer hair cells showed signs of severe damage.

Efficacy of intratympanic steroid treatment for idiopathic sudden sensorineural hearing loss after failure of intravenous steroid treatment
Nihon Jibiinkoka Gakkai Kaiho, 117(6), 802-808. (2014)

Kawano, T., Matsuura, M., Ishitoya, J., & Oridate, N.

This study investigated the efficacy of intratympanic steroid (ITS) therapy as a salvage treatment for idiopathic sudden sensorineural hearing loss after failure of intravenous steroid (IVS) therapy. Systemic steroid therapy is the only standard drug therapy. However, ethically, we could not simply compare ITS with IVS. Conventionally, we have treated idiopahic sudden sensorineural hearing loss patients after failure of systemic steroid therapy with the double combined therapy IVS and hyperbaric oxygen (HBO), as the salvage modality. We examined the effect of ITS by adding it to the double combined therapy with IVS and HBO. Retrospectively, we clinically examined the effect of double combined therapy with IVS and HBO (A group) for 31 patients (12 men and 19 women) (median age: 54 years) with sudden hearing loss after failure of systemic steroid therapy between June, 2003 and July, 2010. Prospectively, we also examined clinically the effect of triple combined therapy with IVS and HBO, ITS (B group) for 29 patients (17 men and 12 women) (median age: 51 years) with sudden hearing loss after failure of systemic steroid therapy between August, 2010 and April, 2012. In the examination of patients treated within 30 days from the onset, one patient (3.2%) demonstrated remarkable recovery, 6 patients (19.4%) demonstrated mild recovery, while no change was noted in 24 patients (77.4%) in the A group. In the B group, 5 patients (17.2%) demonstrated complete recovery, 3 patients (10.3%) demonstrated remarkable recovery, mild recovery was seen in 14 patients (48.3%), and the remaining 7 patients (24.1%) showed no change. There was a significant difference ($p < 0.05$) between the A group and the B group. Furthermore, the hearing improvement in group B in five pure tone average was significantly better than in the group A ($p < 0.05$). We concluded that the B group demonstrated better hearing improvement than the A group. Therefore, ITS could be effective for idiopathic sudden sensorineural hearing loss patients after failure of systemic steroid therapy.

Assessment of nutrient supplement to reduce gentamicin-induced ototoxicity.
J Assoc Res Otolaryngol, 15(3), 375-393. (2014)

Le Prell, C. G., Ojano-Dirain, C., Rudnick, E. W., Nelson, M. A., DeRemer, S. J., Prieskorn, D. M., & Miller, J. M.

Gentamicin is an aminoglycoside antibiotic used to treat gram-negative bacterial infections. Treatment with this antibiotic carries the potential for adverse side effects, including ototoxicity and nephrotoxicity. Ototoxic effects are at least in part a consequence of oxidative stress, and various antioxidants have been used to attenuate gentamicin-induced hair cell death and hearing loss. Here, a combination of nutrients previously shown to reduce oxidative stress in the hair cells and attenuate hearing loss after other insults was evaluated for potential protection against gentamicin-induced ototoxicity. Guinea pigs were maintained on a nutritionally complete standard laboratory animal diet or a diet supplemented with β-carotene, vitamins C and E, and magnesium. Three diets with iterative increases in nutrient levels were screened; the final diet selected for study use was one that produced statistically reliable increases in plasma levels of vitamins C and E and magnesium. In two separate studies, significant decreases in gentamicin-induced hearing loss at frequencies including 12 kHz and below were observed, with less benefit at the higher frequencies. Consistent with the functional protection, robust protection of both the inner and outer hair cell populations was observed, with protection largely in the upper half of the cochlea. Protection was independently assessed in two different laboratories, using two different strains of guinea pigs. Additional in vitro tests did not reveal any decrease in antimicrobial activity with nutrient additives. Currently, there are no FDA-approved treatments for the prevention of gentamicin-induced ototoxicity. The current data provide a rationale for continued investigations regarding translation to human patients.

Growth factors have a protective effect on neomycin-induced hair cell loss.
Cell Biol Int, 23(10), 10347. (2014)

Lou, X., Yuan, H., Xie, J., Wang, X., Yang, L., & Zhang, Y.

We have demonstrated that selected growth factors are involved in regulating survival and proliferation of progenitor cells derived from the neonatal rat organ of Corti (OC). The protective and regenerative effects of these defined growth factors on the injured organ of Corti were therefore investigated. The organ of Corti dissected from the Wistar rat pups (P3-P5) was split into apical, middle, and basal parts, explanted and cultured with or without neomycin and growth factors. Insulin-like growth factor-1 (IGF-1), fibroblast growth factor-2 (FGF-2), and epidermal growth factor (EGF) protected the inner hair cells (IHCs) and outer hair cells (OHCs) from neomycin ototoxicity. Using EGF, IGF-1, and FGF-2 alone induced no protective effect on the survival of auditory hair

cells. Combining 2 growth factors (EGF + IGF-1, EGF + FGF-2, or IGF-1 + FGF-2) gave statistically protective effects. Similarly, combining all three growth factors effectively protected auditory hair cells from the ototoxic insult. None of the growth factors induced regeneration of hair cells in the explants injured with neomycin. Thus various combinations of the three defined factors (IGF-1, FGF-2, and EGF) can protect the auditory hair cells from the neomycin-induced ototoxic damage, but no regeneration was seen. This offers a possible novel approach to the treatment of hearing loss.

Blast-induced tinnitus and spontaneous activity changes in the rat inferior colliculus
Neurosci Lett, 580, 47-51. (2014)

Luo, H., Pace, E., Zhang, X., & Zhang, J.

High-pressure blast shockwaves are known to cause tinnitus. Imaging studies have shown that blast-induced tinnitus may result from damage to the inner ear structures and/or direct brain impact that trigger a cascade of neuroplastic changes in both auditory and non-auditory centers. Nevertheless, information is still lacking on the neurophysiological mechanisms underlying blast-induced tinnitus. In this study, we used a rat model and investigated the effect of blast-induced tinnitus on spontaneous activity in the inferior colliculus (IC) at one day, one month, and three months following blast. Our results showed that rats with behavioral evidence of tinnitus exhibited hyperactivity in all frequency regions at one day post-blast. Although the induced hyperactivity persisted throughout a three-month recording period, it was more robust in middle frequency loci at one month after blast exposure and in middle-to-high-frequency loci at three months after blast. Our results also showed increased bursting rate in the low and middle frequency regions at one day after blast, in the middle frequency region at one month after blast, and in all frequency regions at three months after blast. The findings suggest that neuroplasticity as reflected by shifted tonotopic representations of hyperactivity and bursting activity subserves blast-induced tinnitus and hearing impairment

Blast-Induced tinnitus and spontaneous firing changes in the rat dorsal cochlear nucleus
J Neurosci Res, 92(11), 1466-1477. (2014)

Luo, H., Pace, E., Zhang, X., & Zhang, J.

Exposure to high-pressure blast shock waves is known to cause tinnitus. Although the underlying mechanisms may involve damage to structures in the ear and/or direct brain impact, which triggers a cascade of neuroplastic changes in both auditory and nonauditory centers, it remains unclear how the induced neuroplasticity manifests neurophysiologically. This study investigates the influence of blast exposure on

spontaneous firing rates (SFRs) in the dorsal cochlear nucleus (DCN) and its time course in rats with blast-induced tinnitus. Each rat was exposed to a single blast at 22 psi. Behavioral evidence of tinnitus was measured by using a gap-detection acoustic startle-reflex paradigm. SFRs were measured 1 day, 1 month, and 3 months after blast exposure. The results showed that nine rats with blast-induced tinnitus and hearing loss developed hyperactivity immediately and that the induced hyperactivity persisted in six rats with tinnitus at 1 month after blast exposure. At 3 months after blast exposure, however, the induced hyperactivity of four rats with tinnitus transitioned to hypoactivity. In addition, the 20-30-kHz, and >30-kHz regions in the DCN of rats with and without blast-induced tinnitus were more affected than other frequency regions at different recovery time points after blast exposure. These results demonstrate that the neural mechanisms underlying blast-induced tinnitus are substantially different from those underlying noise-induced tinnitus.

Therapeutic effect of sildenafil on blast-induced tinnitus and auditory impairment
Neuroscience, 269, 367-382. (2014)

Mahmood, G., Mei, Z., Hojjat, H., Pace, E., Kallakuri, S., & Zhang, J. S.

Blast-induced tinnitus, along with associated auditory impairment and traumatic brain injury, is a primary concern facing military service members. To search for treatment, we investigated the therapeutic effects of sildenafil, a phosphodiesterase-5 inhibitor, given its vasodilatory effects and evidence suggesting its beneficial effects on noise-induced hearing loss. Rats were subjected to three consecutive blast exposures at 22 psi and were monitored for tinnitus using a gap-detection acoustic startle reflex paradigm. Hearing thresholds and detection were tested using auditory brainstem responses and prepulse inhibition, respectively. Blasted rats were either treated with sildenafil or tap water following blast exposure, while age-matched sham control rats were treated with sildenafil and no blast exposure. Our results showed that sildenafil did not effectively prevent acute tinnitus onset and hearing impairment. Instead, sildenafil significantly suppressed high-frequency tinnitus from 3 to 6 weeks after blast exposure and reduced hearing impairment during the first week after blast exposure. Complex results were observed in the startle force data, where sildenafil-treated rats displayed significantly reduced startle force compared to the untreated blasted group, suggesting possible mitigation of traumatic brain injury and suppression of hyperacusis-like percepts. Taken together, sildenafil showed a therapeutic effect on blast-induced tinnitus and audiological impairment in a time-dependent manner. Other regimens such as higher dosage prior to blast exposure and combination with other treatments deserve further investigation to optimize the therapeutic effects.

Prevalence of workers with shifts in hearing by industry: a comparison of OSHA and NIOSH Hearing Shift Criteria

J Occup Environ Med, 56(4), 446-455. (2014)

Masterson, E. A., Sweeney, M. H., Deddens, J. A., Themann, C. L., & Wall, D. K.

OBJECTIVE: The purpose of this study was to compare the prevalence of workers with National Institute for Occupational Safety and Health significant threshold shifts (NSTS), Occupational Safety and Health Administration standard threshold shifts (OSTS), and with OSTS with age correction (OSTS-A), by industry using North American Industry Classification System codes. **METHODS:** From 2001 to 2010, worker audiograms were examined. Prevalence and adjusted prevalence ratios for NSTS were estimated by industry. NSTS, OSTS, and OSTS-A prevalences were compared by industry. **RESULTS:** Twenty percent of workers had an NSTS, 14% had an OSTS, and 6% had an OSTS-A. For most industries, the OSTS and OSTS-A criteria identified 28% to 36% and 66% to 74% fewer workers than the NSTS criteria, respectively. **CONCLUSIONS:** Use of NSTS criteria allowing for earlier detection of shifts in hearing is recommended for improved prevention of occupational hearing loss.

Effects of chronic furosemide on central neural hyperactivity and cochlear thresholds after cochlear trauma in Guinea pig

Front Neurol, 5(146). (2014)

Mulders, W. H., McMahen, C., & Robertson, D.

Increased neuronal spontaneous firing rates have been observed throughout the central auditory system after trauma to the cochlea and this hyperactivity is believed to be associated with the phantom perception of tinnitus. Previously, we have shown in an animal model of hearing loss, that an acute injection with furosemide can significantly decrease hyperactivity after cochlear trauma and eliminate behavioral evidence of tinnitus of early onset. However, furosemide also has the potential to affect cochlear thresholds. In this paper, we measured the effects of a chronic (daily injections for 7 days) furosemide treatment on the spontaneous firing rate of inferior colliculus neurons and on cochlear thresholds in order to establish whether a beneficial effect on hyperactivity can be obtained without causing additional hearing loss. Guinea pigs were exposed to a 10-kHz, 124 dB, 2 h acoustic trauma, and after 5 days of recovery, were given daily i.p. injections of 80 mg/kg furosemide or an equivalent amount of saline. The activity of single IC neurons was recorded 24 h following the last injection. The furosemide treatment had no effect on cochlear thresholds compared to saline injections but did result in significant reductions in spontaneous firing rates recorded in inferior colliculus. These results that suggest a long-term beneficial effect of furosemide on hyperactivity after cochlear trauma may be achievable without detrimental effects on hearing, which is important when considering therapeutic potential.

Attenuation of noise-induced hearing loss using methylene blue
Cell Death Dis, 24(5), 170. (2014)

Park, J. S., Jou, I., & Park, S. M.

The overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) has been known to contribute to the pathogenesis of noise-induced hearing loss. In this study, we discovered that in BALB/c mice pretreatment with methylene blue (MB) for 4 consecutive days significantly protected against cochlear injury by intense broad-band noise for 3 h. It decreased both compound threshold shift and permanent threshold shift and, further, reduced outer hair cell death in the cochlea. MB also reduced ROS and RNS formation after noise exposure. Furthermore, it protected against rotenone- and antimycin A-induced cell death and also reversed ATP generation in the in vitro UB-OC1 cell system. Likewise, MB effectively attenuated the noise-induced impairment of complex IV activity in the cochlea. In addition, it increased the neurotrophin-3 (NT-3) level, which could affect the synaptic connections between hair cells and spiral ganglion neurons in the noise-exposed cochlea, and also promoted the conservation of both efferent and afferent nerve terminals on the outer and inner hair cells. These findings suggest that the amelioration of impaired mitochondrial electron transport and the potentiation of NT-3 expression by treatment with MB have a significant therapeutic value in preventing ROS-mediated sensorineural hearing loss.

Role of cysteinyl leukotriene signaling in a mouse model of noise-induced cochlear injury
Proc Natl Acad Sci U S A, 111(27), 9911-9916 (2014)

Park, J. S., Kang, S. J., Seo, M. K., Jou, I., Woo, H. G., & Park, S. M.

Noise-induced hearing loss is one of the most common types of sensorineural hearing loss. In this study, we examined the expression and localization of leukotriene receptors and their respective changes in the cochlea after hazardous noise exposure. We found that the expression of cysteinyl leukotriene type 1 receptor (CysLTR1) was increased until 3 d after noise exposure and enhanced CysLTR1 expression was mainly observed in the spiral ligament and the organ of Corti. Expression of 5-lipoxygenase was increased similar to that of CysLTR1, and there was an accompanying elevation of CysLT concentration. Posttreatment with leukotriene receptor antagonist (LTRA), montelukast, for 4 consecutive days after noise exposure significantly decreased the permanent threshold shift and also reduced the hair cell death in the cochlea. Using RNA-sequencing, we found that the expression of matrix metalloproteinase-3 (MMP-3) was up-regulated after noise exposure, and it was significantly inhibited by montelukast.

Posttreatment with a MMP-3 inhibitor also protected the hair cells and reduced the permanent threshold shift. These findings suggest that acoustic injury up-regulated CysLT signaling in the cochlea and cochlear injury could be attenuated by LTRA through regulation of MMP-3 expression. This study provides mechanistic insights into the role of CysLTs signaling in noise-induced hearing loss and the therapeutic benefit of LTRA.

Protective effect of rasagiline in aminoglycoside ototoxicity
Neuroscience, 265, 263-273. (2014)

Polony, G., Humli, V., Ando, R., Aller, M., Horvath, T., Harnos, A., Zelles, T.

Sensorineural hearing losses (SNHLs; e.g., ototoxicant- and noise-induced hearing loss or presbycusis) are among the most frequent sensory deficits, but they lack effective drug therapies. The majority of recent therapeutic approaches focused on the trials of antioxidants and reactive oxygen species (ROS) scavengers in SNHLs. The rationale for these studies was the prominent role of disturbed redox homeostasis and the consequent ROS elevation. Although the antioxidant therapies in several animal studies seemed to be promising, clinical trials have failed to fulfill expectations. We investigated the potential of rasagiline, an FDA-approved monomanine oxidase type B inhibitor (MAO-B) inhibitor type anti-parkinsonian drug, as an otoprotectant. We showed a dose-dependent alleviation of the kanamycin-induced threshold shifts measured by auditory brainstem response (ABR) in an ototoxicant aminoglycoside antibiotic-based hearing loss model in mice. This effect proved to be statistically significant at a 6-mg/kg (s.c.) dose. The most prominent effect appeared at 16kHz, which is the hearing sensitivity optimum for mice. The neuroprotective, antiapoptotic and antioxidant effects of rasagiline in animal models, all targeting a specific mechanism of aminoglycoside injury, may explain this otoprotection. The dopaminergic neurotransmission enhancer effect of rasagiline might also contribute to the protection. Dopamine (DA), released from lateral olivocochlear (LOC) fibers, was shown to exert a protective action against excitotoxicity, a pathological factor in the aminoglycoside-induced SNHL. We have shown that rasagiline enhanced the electric stimulation-evoked release of DA from an acute mouse cochlea preparation in a dose-dependent manner. Using inhibitors of voltage-gated Na(+)-, Ca(2+) channels and DA transporters, we revealed that rasagiline potentiated the action potential-evoked release of DA by inhibiting the reuptake. The complex, multifactorial pathomechanism of SNHLs most likely requires drugs acting on multiple targets for effective therapy. Rasagiline, with its multi-target action and favorable adverse effects profile, might be a good candidate for a clinical trial testing the otoprotective indication.

Development of the hearing protection assessment (HPA-2) questionnaire
Occup Med, 64(3), 198-205. (2014)

Reddy, R., Welch, D., Ameratunga, S., & Thorne, P.

BACKGROUND: Noise-induced hearing loss (NIHL) remains an important occupational health issue as the second most commonly self-reported occupational injury or illness. The incorrect and inconsistent use of hearing protection devices (HPDs) compromises their effectiveness in preventing NIHL.**AIMS:** To describe the development of an easily administered yet robust questionnaire to investigate factors that influence HPD use. **METHODS:** A hearing protection assessment (HPA-2) questionnaire was developed using items based on themes identified in our previous research. These fell into two classes: supports and barriers to wearing HPD, which formed two scales within the questionnaire. The questionnaire, which also included demographic items, was administered to workers from 34 manufacturing companies. The internal consistency of the scales was tested, and factor analysis was conducted to investigate the underlying structure of the scales. **RESULTS:** Of the 1053 questionnaires distributed, 555 completed questionnaires were received giving a response rate of 53%. The Cronbach's alpha for the barriers scale (a = 0.740) and supports scale (a = 0.771) indicated strong internal reliability of the questionnaire. The supports and barriers were further described as five key factors (risk justification, HPD constraints, hazard recognition, behaviour motivation and safety culture) that influence hearing protection behaviour. Workers who reported always using HPDs had more supports across these factors, while those who did not always wear HPDs reported more barriers. **CONCLUSIONS:** The HPA-2 questionnaire may be useful in both research and interventions to understand and motivate hearing protection behaviour by identifying and targeting supports and barriers to HPD use at different levels of the ecological model.

Development and evaluation of a questionnaire to assess knowledge, attitudes, and behaviors towards hearing loss prevention
Int J Audiol, 53(4), 209-218. (2014)

Saunders, G. H., Dann, S. M., Griest, S. E., & Frederick, M. T.

OBJECTIVE: To develop and evaluate a questionnaire assessing knowledge, attitudes, and behaviors (KAB) as they pertain to hearing conservation, using the constructs of the health belief model (HBM).**DESIGN:** The KAB was completed by 235 participants. Relationships between knowledge and attitudes about hearing and hearing conservation, participation in noisy activities, and use of hearing protection were examined. **STUDY SAMPLE:** 117 males and 118 females aged between 18 and 80 years (mean = 42.3, SD = 4.1) recruited from the Portland VA Medical Center, local universities, and a community college. **RESULTS:** Knowledge scores ranged from 15.6% to 93.8%. Factor analyses revealed six attitude factors, interpreted as measuring perceived susceptibility, perceived severity, perceived benefits, perceived barriers, perceived self-efficacy, and cues to action. Over 95% of participants routinely participated in at least

one noisy activity but few used hearing protection while doing so. The attitude scores of individuals who used hearing protection differed significantly from the scores of those who did not. **CONCLUSIONS:** Significant relationships between use of hearing protection and scores on the KAB provide validation that the HBM is a valuable framework for understanding hearing health behaviors, and evidence that the KAB is a valid tool for assessing these attitudes and behaviors.

Is there a relationship between brain-derived neurotrophic factor for driving neuronal auditory circuits with onset of auditory function and the changes following cochlear injury or during aging?

Neuroscience. 2014 Jul 24. pii: S0306-4522(14)00588-0. doi: 10.1016/j.neuroscience.2014.07.025.

Schimmang, T., Duran Alonso, B., Zimmermann, U., & Knipper, M.

Brain-derived neurotrophic factor, BDNF, is one of the most important neurotrophic factors acting in the peripheral and central nervous system. In the auditory system its function was initially defined by using constitutive knockout mouse mutants and shown to be essential for survival of neurons and afferent innervation of hair cells in the peripheral auditory system. Further examination of BDNF null mutants also revealed a more complex requirement during re-innervation processes involving the efferent system of the cochlea. Using adult mouse mutants defective in BDNF signaling, it could be shown that a tonotopical gradient of BDNF expression within cochlear neurons is required for maintenance of a specific spatial innervation pattern of outer hair cells and inner hair cells. Additionally, BDNF is required for maintenance of voltage-gated potassium channels (K_V) in cochlear neurons, which may form part of a maturation step within the ascending auditory pathway with onset of hearing and might be essential for cortical acuity of sound-processing and experience-dependent plasticity. A presumptive harmful role of BDNF during acoustic trauma and consequences of a loss of cochlear BDNF during aging are discussed in the context of a partial reversion of this maturation step. We compare the potentially beneficial and harmful roles of BDNF for the mature auditory system with those BDNF functions known in other sensory circuits, such as the vestibular, visual, olfactory, or somatosensory system.

Heptanol application to the mouse round window: a model for studying cochlear lateral wall regeneration

Otolaryngol Head Neck Surg, 150(4), 659-665. (2014)

Stevens, S. M., Xing, Y., Hensley, C. T., Zhu, J., Dubno, J. R., & Lang, H.

OBJECTIVE: Identify cells supporting cochlear lateral wall regeneration. **STUDY DESIGN:** Prospective controlled trial. **SETTING:** Laboratory. Human presbyacusis occurs, in part,

secondary to age-related degeneration of cochlear lateral wall structures such as the stria vascularis and spiral ligament fibrocytes. This degeneration is likely linked to the diminished regenerative capacity of lateral wall cells with age. While lateral wall regeneration is known to occur after an acute insult, this process remains poorly understood and the cells capable of self-replication unidentified. We hypothesized that spiral ligament fibrocytes constitute these proliferative cells. **SUBJECTS AND METHODS:** To test the hypothesis, an acute ototoxic insult was created in 65 normal-hearing, young adult mice via cochlear exposure to heptanol. Sacrifice occurred at 1 to 60 days posttreatment. Auditory brainstem responses, 5-ethynyl-2'-deoxyuridine assay, and immunostaining were used to assess regeneration. **RESULTS:** Posttreatment hearing thresholds were elevated in nearly all treated mice. Selective fibrocyte apoptosis and strial injury were observed at the time of peak hearing loss around 1 to 7 days posttreatment. Cellular proliferation was detected in the region of type II fibrocytes during this time. Hearing thresholds plateaued at 7 days posttreatment followed by a significant recovery of both hearing and morphologic appearance. Permanent outer hair cell degeneration was observed. **CONCLUSIONS:** Heptanol application to the round window of young adult mice is a rapid, selective, and reliable technique for investigating proliferation in the cochlear lateral wall. The data indirectly showed that spiral ligament fibrocytes may be the proliferative cells of the cochlear lateral wall. Further studies of this process are needed.

Efficacy and Safety of AM-111 in the Treatment of Acute Sensorineural Hearing Loss: A Double-Blind, Randomized, Placebo-Controlled Phase II Study
Otol Neurotol, 35(8), 1317-1326. (2014)

Suckfuell, M., Lisowska, G., Domka, W., Kabacinska, A., Morawski, K., Bodlaj, R., Meyer, T.

OBJECTIVE: To evaluate the efficacy and safety of AM-111, a c-Jun N-terminal Kinase (JNK) ligand, in patients with acute sensorineural hearing loss (ASNHL).

STUDY DESIGN: Prospective, double-blind, randomized, placebo-controlled study with follow-up visits on Days 3, 7, 30, and 90. **SETTING:** Twenty-five European sites (academic tertiary referral centers, private ENT practices). **PATIENTS:** Approximately 210 patients aged 18 to 61 years presenting within 48 hours after acute acoustic trauma or idiopathic sudden sensorineural hearing loss with mean hearing loss of 30 dB or greater at the 3 most affected contiguous test frequencies. **INTERVENTIONS:** Single-dose intratympanic injection of AM-111 (0.4 or 2.0 mg/ml) or placebo; optionally, oral prednisolone if hearing improvement was less than 10 dB at Day 7.**MAIN OUTCOME MEASURES:** Efficacy was assessed by absolute hearing improvement (primary end point, Day 7), percentage hearing improvement, complete hearing recovery, speech discrimination improvement, and complete tinnitus remission. Safety was evaluated by the frequency of clinically relevant hearing deterioration and adverse events. **RESULTS:** The study failed to demonstrate a treatment benefit for the entire study population

because mild-to-moderate ASNHL cases showed unexpectedly strong spontaneous recovery. In severe-to-profound ASNHL patients (threshold ≥60 dB), AM-111 0.4 mg/ml showed statistically significant, clinically relevant, and persistent improvements in hearing and speech discrimination and higher tinnitus remission compared with placebo. The study drug and the intratympanic injections were well tolerated. **CONCLUSION:** The study established proof of concept for AM-111 in the treatment of severe-to-profound ASNHL. Control for spontaneous hearing recovery is essential for ASNHL studies.

Experimental study of local inner ear gene therapy for controlling autoimmune sensorineural hearing loss
Biomed Res Int, 134658(10), 7. (2014)

Tan, C. Q., Gao, X., Cai, W. J., Qian, X. Y., Lu, L., & Huang, H.

This study aimed to investigate the efficacy of gene therapy for treating autoimmune sensorineural hearing loss (ASHL) via local administration of a recombinant adenovirus vector containing the Fas ligand or interleukin IL-10 gene. Guinea pigs were divided into four groups, with different microinjections in the scala tympani. Group A were injected with FasL-EGFP, B with IL-10-EGFP, C with EGFP, and D with artificial perilymph. Seven days later, auditory brain-stem response (ABR) was tested, and the temporal bone was stained and observed by light microscopy. The spiral ligament and basement membrane were observed using transmission electron microscopy. FasL and IL-10 expression were examined using immunofluorescence histochemistry. Immunohistochemical analysis showed that the recombinant adenovirus vector in Groups A, B, and C can transfect the stria vascularis, the spiral ligament, the organ of Corti, the spiral ganglion, the region surrounding the small blood vessel in the modiolus, and the cochlear bone wall. Compared with those in Groups C and D, the ABR wave III mean thresholds were significantly lower and the inner ear immunoinflammatory responses in Groups A and B were significantly alleviated. Inhibition of immunoinflammatory response alleviated immunoinflammatory injury and auditory dysfunction. This technique shows potential as a novel therapy for ASHL.

A new grading system for ototoxicity in adults
Ann Otol Rhinol Laryngol, 123(10), 711-718. (2014)

Theunissen, E. A., Dreschler, W. A., Latenstein, M. N., Rasch, C. R., van der Baan, S., de Boer, J. P., Zuur, C. L.

OBJECTIVE: This study aimed to propose an ototoxicity grading system sensitive to the effect of ototoxicity on specific daily life situations like speech intelligibility and the

perception of ultra-high sounds and to test its feasibility compared to current criteria. **METHODS:** Pure tone averages (PTAs) for speech perception (1-2-4 kHz) and ultra-high frequencies (8-10-12.5 kHz) were incorporated. Threshold shift and hearing level posttreatment were taken into account. Criteria were tested on head and neck cancer patients treated with (chemo-) radiotherapy ([C]RT) and compared with the Common Terminology Criteria for Adverse Events version 4 (CTCAEv4) and the American Speech-Language-Hearing Association criteria (ASHA). **RESULTS:** Grades 1 and 2 were based on threshold shifts from baseline (in dB) and subjective complaints. Grades 3 and 4 were defined as treatment-induced hearing loss of ≥ 35 dB at PTA 1-2-4 kHz and ≥ 70 dB at PTA 1-2-4 kHz, respectively. In high-dose cisplatin CRT incidences by the new criteria, CTCAEv4 and ASHA were comparable (78%-88%). In RT and low-dose cisplatin CRT, incidences were 36% to 39% in the new criteria versus 22% to 53% in CTCAEv4 and ASHA. **CONCLUSION:** The new criteria show an increased sensitivity to ototoxicity compared to CTCAEv4 and ASHA and provide insight into the effect of hearing loss on certain daily life situations. The new grading system seems feasible for clinic and research purposes.

Therapeutic potential of a gamma-secretase inhibitor for hearing restoration in a guinea pig model with noise-induced hearing loss
BMC Neurosci, 15(66), 1471-2202. (2014)

Tona, Y., Hamaguchi, K., Ishikawa, M., Miyoshi, T., Yamamoto, N., Yamahara, K., Nakagawa, T.

BACKGROUND: Notch signaling plays a crucial role in the fate determination of cochlear progenitor cells, hair cells, and supporting cells in the developing cochlea. Recent studies have demonstrated the temporal activation of Notch signaling in damaged mature cochleae, and have demonstrated the induction of new hair cells by pharmacologically inhibiting Notch signaling. The present study aimed to illustrate the feasibility of pharmacologically inhibiting Notch signaling by using a gamma-secretase inhibitor for treating sensorineural hearing loss. **RESULTS:** The effect of the sustained local delivery of MDL28170, a gamma-secretase inhibitor, on hearing and hair cell induction was tested in a guinea pig model with noise-induced hearing loss. MDL28170 was directly delivered into the cochlear fluids via a micro-osmotic pump. Drug application was initiated 7 days after noise exposure. Measurements of auditory brainstem responses revealed better hearing in the MDL28170-treated animals than in the vehicle controls. Histological analysis demonstrated a higher number of outer hair cells in the MDL28170-treated cochleae than the vehicle-treated cochleae. **CONCLUSION:** These findings strongly suggest that local sustained delivery of a gamma-secretase inhibitor into the cochlea could be a novel strategy for treating acute hearing loss that is refractory to conventional treatment.

Protective effects of Ginkgo biloba extract EGb 761 against noise trauma-induced hearing loss and tinnitus development
Neural Plast, 427298(10), 17. (2014)

Tziridis, K., Korn, S., Ahlf, S., & Schulze, H.

Noise-induced hearing loss (NIHL) and resulting comorbidities like subjective tinnitus are common diseases in modern societies. A substance shown to be effective against NIHL in an animal model is the Ginkgo biloba extract EGb 761. Further effects of the extract on the cellular and systemic levels of the nervous system make it a promising candidate not only for protection against NIHL but also for its secondary comorbidities like tinnitus. Following an earlier study we here tested the potential effectiveness of prophylactic EGb 761 treatment against NIHL and tinnitus development in the Mongolian gerbil. We monitored the effects of EGb 761 and noise trauma-induced changes on signal processing within the auditory system by means of behavioral and electrophysiological approaches. We found significantly reduced NIHL and tinnitus development upon EGb 761 application, compared to vehicle treated animals. These protective effects of EGb 761 were correlated with changes in auditory processing, both at peripheral and central levels. We propose a model with two main effects of EGb 761 on auditory processing, first, an increase of auditory brainstem activity leading to an increased thalamic input to the primary auditory cortex (AI) and second, an asymmetric effect on lateral inhibition in AI

Efficacy and safety of AM-101 in the treatment of acute inner ear tinnitus--a double-blind, randomized, placebo-controlled phase II study
Otol Neurotol, 35(4), 589-597. (2014)

van de Heyning, P., Muehlmeier, G., Cox, T., Lisowska, G., Maier, H., Morawski, K., & Meyer, T.

OBJECTIVE: To evaluate the efficacy and safety of intratympanic AM-101 in patients with persistent acute inner ear tinnitus after acute acoustic trauma, idiopathic sudden sensorineural hearing loss (ISSNHL), or acute otitis media. **STUDY DESIGN:** Prospective, double-blind, randomized, placebo-controlled study with follow-up visits on Days 7, 30, and 90.**SETTING:** Twenty-eight European sites (academic tertiary referral centers and private ENT practices).**PATIENTS:** 248 patients aged 16 to 65 years. **INTERVENTIONS:** Three intratympanic injections of AM-101 (0.27 or 0.81 mg/ml) or placebo over 3 consecutive days. **MAIN OUTCOME MEASURES:** Efficacy was assessed by changes in minimum masking level (MML; primary end point), loudness match, tinnitus loudness, tinnitus annoyance, and sleep difficulties on a 0 to 100 numerical rating scale, THI-12 questionnaire, and patient global impression of change. Safety was evaluated using the frequency of clinically relevant hearing deterioration and adverse events. **RESULTS:** The study overall failed to demonstrate a treatment benefit based on the change in

MML. However, AM-101 0.81 mg/ml showed statistically significantly better improvement for tinnitus loudness, annoyance, sleep difficulties, and tinnitus impact in patients with tinnitus after noise trauma or otitis media. The subgroup of ISSNHL-related tinnitus patients did not show conclusive results. The study drug and I.T. injections were well tolerated. **CONCLUSION:** The study established proof of concept for AM-101 in the treatment of tinnitus arising from cochlear glutamate excitotoxicity. Patient-reported outcomes seem to be more relevant and reliable efficacy measures for assessing treatment-related changes in tinnitus than psychoacoustic tests.

Inflammation is associated with a worsening of presbycusis: evidence from the MRC national study of hearing
Int J Audiol, 53(7), 469-475. (2014)

Verschuur, C., Agyemang-Prempeh, A., & Newman, T. A.

OBJECTIVE: Inflammaging, a state of chronic inflammation in the elderly, is now thought to be a key element of the ageing process and contributor to age-related disease. In a previously published study, we identified a significant association between inflammation levels and severity of presbycusis among individuals aged 63 to 73 ('younger old") within an available audiometric range 0.5 to 4 kHz. Our aim was to see if this association would be identified among participants in the MRC national study of hearing, and whether the strength of the association would increase with greater age, or for very low or very high audiometric frequencies.**DESIGN:** Cross-sectional analysis of cohort data.**STUDY SAMPLE:** Three hundred and sixty community-dwelling adults age 60 years and over, representing all those with white blood cell count and audiometric data available.**RESULTS:** A significant independent association between (higher) WBC and (worse) hearing level was identified. This effect increased with age. The strongest association was among those over 75, for whom average hearing threshold levels among those with lower WBC was 17 dB better than those with higher WBC.**CONCLUSIONS:** The current findings support an association between inflammaging (a condition potentially amenable to pharmacological treatment or lifestyle management) and presbycusis.

Protective effect of dexmedetomidine on noise-induced hearing loss
Laryngoscope, 124(5), 7. (2014)

Wen, J., Xiao, Y., Bai, Y. X., & Xu, M.

OBJECTIVES/HYPOTHESIS: Noise generated by instruments, such as mastoid or craniotomy drills, may cause hearing damage by reducing the cochlear blood flow

(CoBF). This study investigated whether dexmedetomidine can lessen noise-induced hearing loss (NIHL) in a guinea pig model. **STUDY DESIGN**: Animal study using noise stimulation and measurement of hearing and CoBF in guinea pigs. **METHODS**: Guinea pigs (n = 8 animals/group) were treated by saline vehicle (control group), dexmedetomidine (1, 3, and 10 μg/kg dex groups), saline and noise (noise group), or 3 μg/kg dexmedetomidine and noise (dex+noise group). For noise exposure, octave band noise at 124 dB sound pressure level was administered to animals for 2 hours. Blood pressure (BP) and CoBF were monitored continuously. Auditory function was measured by the auditory brain-stem response (ABR) before and 1 hour, 3 hours, 8 hours, and 10 days after noise exposure. Plasma norepinephrine (NE) was measured at baseline and 30, 60, 90, and 120 minutes after noise exposure by high-performance liquid chromatography (HPLC). **RESULTS**: Noise exposure caused temporary and permanent hearing damage. Dexmedetomidine concentrations of 1 μg/kg and 3 μg/kg dose dependently improved CoBF. Administration of 10 μg/kg dexmedetomidine drastically reduced BP and CoBF. Pretreatment with 3 μg/kg dexmedetomidine alleviated the noise-induced reduction in CoBF and improved hearing function by decreasing the permanent and temporary threshold shifts. **CONCLUSION**: Dexmedetomidine displayed protective effects against NIHL in this animal model, suppressing activation of the sympathetic nervous system and improving CoBF. These findings could have clinical relevance and deserve further investigation.

Association between GPX-1 single nucleotide polymorphisms and susceptibility to noise-induced hearing loss among Chinese Han population
Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi, 32(8), 568-572. (2014)

Wen, X., Qiu, C., Li, X., Lin, H., & Huang, Y.

OBJECTIVE: To investigate the association between single nucleotide polymorphisms (SNPs) in glutathione peroxidase 1 (GPX-1) gene, rs3448, rs1050450, rs1800668, and rs1987628, and the susceptibility to noise-induced hearing loss (NIHL) among Chinese Han population.**METHODS:** A case-control study was conducted to investigate the threshold shift of the left ear at 3000 Hz among the workers of Chinese Han population whowere exposed to the samelevel of soundpressure. Two hundred and one (10%) of the subjects with the highest level of threshold shift were recruited in susceptible group, while 202 of (10%) of the subjects with the lowest level of threshold shift were recruited in tolerant group. Targeted occupational health survey and questionnaire survey were performed among these people. For each individual, genome DNA was extracted from 5 ml of fasting peripheral venous blood. Four SNPs (GPX-1 rs3448, rs1050450, rs1800668, and rs1987628) were genotyped by the TaqMan SNP genotyping kit. The main effects of SNPs and the association between NIHL susceptibility and SNPs were analyzed by logistic regression.**RESULTS:** The C allele of rs1987628 was a risk factor for NIHL, with an odds ratio (OR) of 2.531 (95%CI: 1.878-3.411) as compared with the T allele. The CC genotype of rs1987628 was more associated with NIHL than the TT genotype (OR =

3.500, 95%CI: 1.984-6.174; adjusted OR = 3.544, 95% CI: 1.974 ~ 6.364).**CONCLUSION:** Among Chinese Han population, GPX-1 SNP rs1987628 may be associated with the susceptibility to NIHL.

Protective role of L-ascorbic acid, N-acetylcysteine and apocynin on neomycin-induced hair cell loss in Zebrafish
J Appl Toxicol, 4(10). (2014)

Wu, C. Y., Lee, H. J., Liu, C. F., Korivi, M., Chen, H. H., & Chan, M. H.

Hair cells are highly sensitive to environmental insults and other therapeutic drugs. The adverse effects of drugs such as aminoglycosides can cause hair cell death and lead to hearing loss and imbalance. The objective of the present study was to evaluate the protective activity of L-ascorbic acid, N-acetylcysteine (NAC) and apocynin on neomycin-induced hair cell damage in zebrafish (Danio rerio) larvae at 5 days post fertilization (dpf). Results showed that the loss of hair cells within the neuromasts of the lateral lines after neomycin exposure was evidenced by a significantly lower number of neuromasts labeled with fluorescent dye FM1-43FX observed under a microscope. Co-administration with L-ascorbic acid, NAC and apocynin protected neomycin-induced hair cell loss within the neuromasts. Moreover, these three compounds reduced the production of reactive oxygen species (ROS) in neuromasts exposed to neomycin, indicating that their antioxidant action is involved. In contrast, the neuromasts were labeled with specific fluorescent dye Texas-red conjugated with neomycin to detect neomycin uptake. Interestingly, the uptake of neomycin into hair cells was not influenced by these three antioxidant compounds. These data imply that prevention of hair cell damage against neomycin by L-ascorbic acid, NAC and apocynin might be associated with inhibition of excessive ROS production, but not related to modulating neomycin uptake. Our findings conclude that L-ascorbic acid, NAC and apocynin could be used as therapeutic drugs to protect aminoglycoside-induced listening impairment after further confirmatory studies.

Development of a cell-based treatment for long-term neurotrophin expression and spiral ganglion neuron survival
Neuroscience, 277, 690-699. (2014)

Zanin, M. P., Hellstrom, M., Shepherd, R. K., Harvey, A. R., & Gillespie, L. N.

Spiral ganglion neurons (SGNs), the target cells of the cochlear implant, undergo gradual degeneration following loss of the sensory epithelium in deafness. The preservation of a viable population of SGNs in deafness can be achieved in animal models with exogenous application of neurotrophins such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3. For translation into clinical application,

a suitable delivery strategy that provides ongoing neurotrophic support and promotes long-term SGN survival is required. Cell-based neurotrophin treatment has the potential to meet the specific requirements for clinical application, and we have previously reported that Schwann cells genetically modified to express BDNF can support SGN survival in deafness for 4weeks. This study aimed to investigate various parameters important for the development of a long-term cell-based neurotrophin treatment to support SGN survival. Specifically, we investigated different (i) cell types, (ii) gene transfer methods and (iii) neurotrophins, in order to determine which variables may provide long-term neurotrophin expression and which, therefore, may be the most effective for supporting long-term SGN survival in vivo. We found that fibroblasts that were nucleofected to express BDNF provided the most sustained neurotrophin expression, with ongoing BDNF expression for at least 30weeks. In addition, the secreted neurotrophin was biologically active and elicited survival effects on SGNs in vitro. Nucleofected fibroblasts may therefore represent a method for safe, long-term delivery of neurotrophins to the deafened cochlea to support SGN survival in deafness.

Inner ear stem cells derived feeder layer promote directional differentiation of amniotic fluid stem cells into functional neurons
Hear Res, 11, 57-64. (2014)

Zong, L., Chen, K., Zhou, W., Jiang, D., Sun, L., Zhang, X., & Jiang, H.

Intact spiral ganglion neurons are required for cochlear implantation or conventional hearing amplification as an intervention for sensorineural hearing loss. Treatment strategies to replace the loss of spiral ganglion neurons are needed. Recent reports have suggested that amniotic fluid-derived stem cells are capable of differentiating into neuron-like cells in response to cytokines and are not tumorigenic. Amniotic fluid stem cells represent a potential resource for cellular therapy of neural deafness due to spiral ganglion pathology. However, the directional differentiation of amniotic fluid stem cells is undetermined in the absence of cytokines and the consequence of inner ear supporting cells from the mouse cochlea organ of Corti on the differentiation of amniotic fluid stem cells remains to be defined. In an effort to circumvent these limitations, we investigated the effect of inner ear stem cells derived feeder layer on amniotic fluid stem cells differentiation in vitro. An inner ear stem cells derived feeder layer direct contact system was established to induce differentiation of amniotic fluid stem cells. Our results showed that inner ear stem cells derived feeder layer successfully promoted directional differentiation of amniotic fluid stem cells into neurons with characteristics of functionality. Furthermore, we showed that Wnt signaling may play an essential role in triggering neurogenesis. These findings indicate the potential use of inner ear stem cells derived feeder layer as a nerve-regenerative scaffold. A reliable and effective amniotic fluid stem cell differentiation support structure provided by inner ear stem cells derived feeder layer should contribute to efforts to translate cell-based strategies to the clinic.

RELEVANT LITERATURE

Aksoy, F., Dogan, R., Ozturan, O., Eren, S. B., Veyseller, B., Pektas, A., & Huseyinbas, O. (2014). **Protective effect of trimetazidine on amikacin-induced ototoxicity in rats**. *Int J Pediatr Otorhinolaryngol, 78*(4), 663-669.

Alzahrani, M., Ratelle, J., Cavel, O., Laberge-Malo, M., & Saliba, I. (2014). **Hearing loss in the shaken baby syndrome**. *Int J Pediatr Otorhinolaryngol, 78*(5), 804-806.

Amor-Dorado, J. C., Barreira-Fernandez, M. P., Pina, T., Vazquez-Rodriguez, T. R., Llorca, J., & Gonzalez-Gay, M. A. (2014). **Investigations into Audiovestibular Manifestations in Patients with Psoriatic Arthritis**. *J Rheumatol, 1*, 140559.

Atkinson, P. J., Wise, A. K., Flynn, B. O., Nayagam, B. A., & Richardson, R. T. (2014). **Viability of long-term gene therapy in the cochlea**. *Sci Rep, 4*(4733).

Auchter, M., & Le Prell, C. G. (2014). **Hearing loss prevention education using adopt-a-band: changes in self-reported earplug use in two high school marching bands**. *Am J Audiol, 23*(2), 211-226.

Bass, J. K., Huang, J., Onar-Thomas, A., Chang, K. W., Bhagat, S. P., Chintagumpala, M., . . . Gurney, J. G. (2014). **Concordance between the chang and the International Society of Pediatric Oncology (SIOP) ototoxicity grading scales in patients treated with cisplatin for medulloblastoma**. *Pediatr Blood Cancer, 61*(4), 601-605.

Battaglia, A., Lualhati, A., Lin, H., Burchette, R., & Cueva, R. (2014). **A prospective, multi-centered study of the treatment of idiopathic sudden sensorineural hearing loss with combination therapy versus high-dose prednisone alone: a 139 patient follow-up**. *Otol Neurotol, 35*(6), 1091-1098.

Belhadj, Z., Kandouci, C., & Abdelkrim Kandouci, B. (2014). 0098 **Occupational deafness due to co-exposure to noise and ototoxic agents**. *Occup Environ Med, 71*(1), 2014-102362.

Berger, A. C., Kelly, J. J., Lajoie, P., Shao, Q., & Laird, D. W. (2014). **Mutations in Cx30 that are linked to skin disease and non-syndromic hearing loss exhibit several distinct cellular pathologies**. *J Cell Sci, 127*(Pt 8), 1751-1764.

Blakley, B. W., Alsaleh, S., Dewji, Z., Qureshy, K., Berard, S., & Xie, L. (2014). **Steroids in aminoglycoside-containing ear drops: do they reduce cochlear toxicity?** *Laryngoscope, 124*(5), 1209-1213.

Bogaz, E. A., Suzuki, F. A., Rossini, B. A., Inoue, D. P., & Penido Nde, O. (2014). **Glucocorticoid influence on prognosis of idiopathic sudden sensorineural hearing loss**. *Braz J Otorhinolaryngol, 80*(3), 213-219.

Bonyadi, M. J., Fotouhi, N., & Esmaeili, M. (2014). **Spectrum and frequency of GJB2 mutations causing deafness in the northwest of Iran**. *Int J Pediatr Otorhinolaryngol, 78*(4), 637-640.

Brito, R., Sheth, S., Mukherjea, D., Rybak, L. P., & Ramkumar, V. (2014). **TRPV1: A Potential Drug Target for Treating Various Diseases**. *Cells, 3*(2), 517-545.

Budimcic, M., Seke, K., Krsmanovic, S., & Zivic, L. (2014). **Auditory risk behaviours and hearing problems among college students in Serbia**. *Med Glas, 11*(2), 361-366.

Carleton, B. C., Ross, C. J., Pussegoda, K., Bhavsar, A. P., Visscher, H., Lee, J. W., . . . Hayden, M. R. (2014). **Genetic markers of Cisplatin-induced hearing loss in children**. *Clin Pharmacol Ther, 96*(3), 296-298.

Carter, L., Williams, W., Black, D., & Bundy, A. (2014). **The leisure-noise dilemma: hearing loss or hearsay? What does the literature tell us?** *Ear Hear, 35*(5), 491-505.

Chen, L., Yu, N., Lu, Y., Wu, L., Chen, D., Guo, W., . . . Zhai, S. (2014). **Hydrogen-saturated saline protects intensive narrow band noise-induced hearing loss in guinea pigs through an antioxidant effect**. *PLoS One, 9*(6).

Chien, W. W., Monzack, E. L., McDougald, D. S., & Cunningham, L. L. (2014). **Gene Therapy for Sensorineural Hearing Loss**. *Ear Hear, 27*, 27.

Choi, J., Chang, J., Jun, H. J., Im, G. J., Chae, S. W., Lee, S. H., . . . Park, H. C. (2014). **Protective role of edaravone against neomycin-induced ototoxicity in zebrafish**. *J Appl Toxicol, 34*(5), 554-561.

Cohen, B. E., Durstenfeld, A., & Roehm, P. C. (2014). **Viral causes of hearing loss: a review for hearing health professionals**. *Trends Hear, 29*(18), 2331216514541361.

Colevas, A. D., Lira, R. R., Colevas, E. A., Lavori, P. W., Chan, C., Shultz, D. B., & Chang, K. W. (2014). **Hearing evaluation of patients with head and neck cancer: Comparison of Common Terminology Criteria for Adverse Events, Brock and Chang adverse event criteria in patients receiving cisplatin**. *Head Neck, 15*(10), 23714.

Dallan, I., Fortunato, S., Casani, A. P., Bernardini, E., Sellari-Franceschini, S., Berrettini, S., & Nacci, A. (2014). **Long-term follow up of sudden sensorineural hearing loss patients treated with intratympanic steroids: audiological and quality of life evaluation**. *J Laryngol Otol, 128*(8), 669-673.

Dammeyer, P., Hellberg, V., Wallin, I., Laurell, G., Shoshan, M., Ehrsson, H., . . . Kirkegaard, M. (2014). **Cisplatin and oxaliplatin are toxic to cochlear outer hair cells and both target thioredoxin reductase in organ of Corti cultures**. *Acta Otolaryngol, 134*(5), 448-454.

Dinh, M. L., Koppel, S. J., Korn, M. J., & Cramer, K. S. (2014). **Distribution of glial cells in the auditory brainstem: Normal development and effects of unilateral lesion**. *Neuroscience, 278*, 237-252.

Dong, Y., Ding, D., Jiang, H., Shi, J. R., Salvi, R., & Roth, J. A. (2014). **Ototoxicity of paclitaxel in rat cochlear organotypic cultures**. *Toxicol Appl Pharmacol, 30*(14), 00314-00317.

Erdur, O., Kayhan, F. T., & Cirik, A. A. (2014). **Effectiveness of intratympanic dexamethasone for refractory sudden sensorineural hearing loss**. *Eur Arch Otorhinolaryngol, 271*(6), 1431-1436.

Fang, B., & Xiao, H. (2014). **Rapamycin alleviates cisplatin-induced ototoxicity in vivo**. *Biochem Biophys Res Commun, 448*(4), 443-447.

Fetoni, A. R., Eramo, S. L., Paciello, F., Rolesi, R., Podda, M. V., Troiani, D., & Paludetti, G. (2014). **Curcuma longa (curcumin) decreases in vivo cisplatin-induced ototoxicity through heme oxygenase-1 induction**. *Otol Neurotol, 35*(5), 0000000000000302.

Filipo, R., Attanasio, G., Russo, F. Y., Cartocci, G., Musacchio, A., De Carlo, A., . . . Covelli, E. (2014). **Oral versus Short-Term Intratympanic Prednisolone Therapy for Idiopathic Sudden Hearing Loss**. *Audiol Neurootol, 19*(4), 225-233.

Gatto, M. P., Fioretti, M., Fabrizi, G., Gherardi, M., Strafella, E., & Santarelli, L. (2014). **Effects of potential neurotoxic pesticides on hearing loss: a review**. *Neurotoxicology, 42*, 24-32.

Gopel, W., Berkowski, S., Preuss, M., Ziegler, A., Kuster, H., Felderhoff-Muser, U., . . . Herting, E. (2014). **Mitochondrial mutation m.1555A>G as a risk factor for failed newborn hearing screening in a large cohort of preterm infants**. *BMC Pediatr, 14*(1), 210.

Gu, X., Guo, L., Ji, H., Sun, S., Chai, R., Wang, L., & Li, H. (2014). **Genetic testing for sporadic hearing loss using targeted massively parallel sequencing identifies 10 novel mutations**. *Clin Genet, 22*(10), 12431.

Huth, M. E., Popelka, G. R., & Blevins, N. H. (2014). **Comprehensive Measures of Sound Exposures in Cinemas Using Smart Phones**. *Ear Hear, 29*, 29.

Hutten, M., Dhanasingh, A., Hessler, R., Stover, T., Esser, K. H., Moller, M., . . . Scheper, V. (2014). **In vitro and in vivo evaluation of a hydrogel reservoir as a continuous drug delivery system for inner ear treatment**. *PLoS One, 9*(8).

Ivory, R., Kane, R., & Diaz, R. C. (2014). **Noise-induced hearing loss: a recreational noise perspective**. *Curr Opin Otolaryngol Head Neck Surg, 22*(5), 394-398.

Kalinec, G. M., Thein, P., Parsa, A., Yorgason, J., Luxford, W., Urrutia, R., & Kalinec, F. (2014). **Acetaminophen and NAPQI are toxic to auditory cells via**

oxidative and endoplasmic reticulum stress-dependent pathways. *Hear Res, 313*, 26-37.

Kanzaki, S., Sakagami, M., Hosoi, H., Murakami, S., & Ogawa, K. (2014). **High fibrinogen in peripheral blood correlates with poorer hearing recovery in idiopathic sudden sensorineural hearing loss**. *PLoS One, 9*(8).

Kennedy, T. A., Avey, G. D., & Gentry, L. R. (2014). **Imaging of temporal bone trauma**. *Neuroimaging Clin N Am, 24*(3), 467-486.

Kikkawa, Y. S., Nakagawa, T., Taniguchi, M., & Ito, J. (2014). **Hydrogen protects auditory hair cells from cisplatin-induced free radicals**. *Neurosci Lett, 579*, 125-129.

Kim, D. K., Park, Y., Back, S. A., Kim, H. L., Park, H. E., Park, K. H., . . . Park, S. N. (2014). **Protective effect of unilateral and bilateral ear plugs on noise-induced hearing loss: functional and morphological evaluation in animal model**. *Noise Health, 16*(70), 149-156.

Kim, H. J., Oh, G. S., Choe, S. K., Kwak, T. H., Park, R., & So, H. S. (2014). **NAD(+) Metabolism in Age-Related Hearing Loss**. *Aging Dis, 5*(2), 150-159.

Kim, J., Cho, H. J., Sagong, B., Kim, S. J., Lee, J. T., So, H. S., . . . Choo, Y. S. (2014). **Alpha-lipoic acid protects against cisplatin-induced ototoxicity via the regulation of MAPKs and proinflammatory cytokines**. *Biochem Biophys Res Commun, 449*(2), 183-189.

Kitcher, E. D., Ocansey, G., Abaidoo, B., & Atule, A. (2014). **Occupational hearing loss of market mill workers in the city of Accra, Ghana**. *Noise Health, 16*(70), 183-188.

Kokong, D. D., Bakari, A., & Ahmad, B. M. (2014). **Ototoxicity in Nigeria: why it persists**. *Ear Nose Throat J, 93*(7), 256-264.

Liebau, A., & Plontke, S. K. (2014). [**Local drug therapy for inner ear hearing loss**.]. *Hno, 11*, 11.

Lin, H. Y., Shih, S. C., Chuang, C. K., Lee, K. S., Chen, M. R., Lin, H. C., . . . Lin, S. P. (2014). **Assessment of hearing loss by pure-tone audiometry in patients with mucopolysaccharidoses**. *Mol Genet Metab, 111*(4), 533-538.

Liu, K., Shi, C., Sun, Y., Xu, Y., Shi, L., Wang, X., . . . Yang, S. (2014). **Dynamic distribution of ototoxic gentamicin entry into inner hair cells of mice**. *Acta Otolaryngol, 134*(4), 345-351.

Luzzi, R., Belcaro, G., Hu, S., Dugall, M., Hosoi, M., Cacchio, M., . . . Corsi, M. (2014). **Improvement in symptoms and cochlear flow with pycnogenol in patients with Meniere's disease and tinnitus**. *Minerva Med, 105*(3), 245-254.

Memari, F., & Hassannia, F. (2014). **Effect of Intratympanic Dexamethasone on Controlling Tinnitus and Hearing loss in Menier's Disease**. *Iran J Otorhinolaryngol, 26*(76), 129-133.

Modayil, P. C., Lloyd, G. W., Mallik, A., & Bowdler, D. A. (2014). **Inner ear damage following electric current and lightning injury: a literature review**. *Eur Arch Otorhinolaryngol, 271*(5), 855-861.

Mohan, S., Smyth, B. J., Namin, A., Phillips, G., & Gratton, M. A. (2014). **Targeted Amelioration of Cisplatin-Induced Ototoxicity in Guinea Pigs**. *Otolaryngol Head Neck Surg, 30*, 0194599814544877.

Mujica-Mota, M. A., Salehi, P., Devic, S., & Daniel, S. J. (2014). **Safety and otoprotection of metformin in radiation-induced sensorineural hearing loss in the guinea pig**. *Otolaryngol Head Neck Surg, 150*(5), 859-865.

Na, Y., Joo, H. S., Yang, H., Kang, S., Hong, S. H., & Woo, J. (2014). **Smartphone-based hearing screening in noisy environments**. *Sensors, 14*(6), 10346-10360.

Nakajima, Y. (2014). **Signaling regulating inner ear development: cell fate determination, patterning, morphogenesis, and defects**. *Congenit Anom, 8*(10), 12072.

Novoa, E., Gartner, M., & Henzen, C. (2014). **Systemic effects of intratympanic dexamethasone therapy**. *Endocr Connect, 3*(3), 127-131.

O'Brien, I., Ackermann, B. J., & Driscoll, T. (2014). **Hearing and hearing conservation practices among Australia's professional orchestral musicians**. *Noise Health, 16*(70), 189-195.

Oue, S., Jervis-Bardy, J., Stepan, L., Chong, S., & Shaw, C. K. (2014). **Efficacy of low-dose intratympanic dexamethasone as a salvage treatment for idiopathic sudden sensorineural hearing loss: the Modbury Hospital experience**. *J Laryngol Otol, 128*(2), 24.

Ozkul, Y., Songu, M., Basoglu, M. S., Ozturkcan, S., & Katilmis, H. (2014). **Evaluation of the protective effect of alpha-lipoic acid on cisplatin ototoxicity using distortion-product otoacoustic emission measurements: an experimental animal study**. *J Craniofac Surg, 25*(4), 1515-1518.

Palmer, K., D'Angelo, S., Clare Harris, E., Linaker, C., & Coggon, D. (2014). 0066 **Problems of vision, hearing and balance and risks of workplace injury**. *Occup Environ Med, 71*(1), 2014-102362.

Park, S. H., & Moon, I. S. (2014). **Round window membrane vibration may increase the effect of intratympanic dexamethasone injection**. *Laryngoscope, 124*(6), 1444-1451.

Park, S. Y., Seo, J. H., Kim, J. K., & Park, K. H. (2014). **Effects of korean red ginseng on hearing and blood glucose levels in steroid therapy for sudden sensorineural hearing loss**. *Clin Exp Otorhinolaryngol, 7*(3), 170-174.

Paz, Y. M. C., Beaty, D., Lopez-Cortes, A., & Proano, I. (2014). **Frequency of GJB2 and del(GJB6-D13S1830) mutations among an Ecuadorian mestizo population**. *Int J Pediatr Otorhinolaryngol, 78*(10), 1648-1654.

Peleva, E., Emami, N., Alzahrani, M., Bezdjian, A., Gurberg, J., Carret, A. S., & Daniel, S. J. (2014). **Incidence of platinum-induced ototoxicity in pediatric patients in Quebec**. *Pediatr Blood Cancer, 61*(11), 2012-2017.

Pinyon, J. L., Tadros, S. F., Froud, K. E., AC, Y. W., Tompson, I. T., Crawford, E. N., . . . Housley, G. D. (2014). **Close-field electroporation gene delivery using the cochlear implant electrode array enhances the bionic ear**. *Sci Transl Med, 6*(233), 3008177.

Rachakonda, T., Shimony, J. S., Coalson, R. S., & Lieu, J. E. (2014). **Diffusion tensor imaging in children with unilateral hearing loss: a pilot study**. *Front Syst Neurosci, 8*(87).

Ralli, M., Troiani, D., Podda, M. V., Paciello, F., Eramo, S. L., de Corso, E., . . . Fetoni, A. R. (2014). **The effect of the NMDA channel blocker memantine on salicylate-induced tinnitus in rats**. *Acta Otorhinolaryngol Ital, 34*(3), 198-204.

Ramsebner, R., Koenighofer, M., Parzefall, T., Lucas, T., Schoefer, C., & Frei, K. (2014). **Despite a lack of otoacoustic emission, word recognition is not seriously influenced in a TECTA DFNA8/12 family**. *Int J Pediatr Otorhinolaryngol, 78*(5), 837-842.

Rance, G., Chisari, D., O'Hare, F., Roberts, L., Shaw, J., Jandeleit-Dahm, K., & Szmulewicz, D. (2014). **Auditory neuropathy in individuals with Type 1 diabetes**. *J Neurol, 261*(8), 1531-1536.

Ruan, Q., Zeng, S., Liu, A., Chen, Z., Yu, Z., Zhang, R., . . . Wang, J. (2014). **Overexpression of X-Linked Inhibitor of Apoptotic Protein (XIAP) reduces age-related neuronal degeneration in the mouse cochlea**. *Gene Ther, 21*(10), 77.

Rudnicki, A., Shivatzki, S., Beyer, L. A., Takada, Y., Raphael, Y., & Avraham, K. B. (2014). **microRNA-224 regulates Pentraxin 3, a component of the humoral arm of innate immunity, in inner ear inflammation**. *Hum Mol Genet, 23*(12), 3138-3146.

Salehi, P., Akinpelu, O. V., Waissbluth, S., Peleva, E., Meehan, B., Rak, J., & Daniel, S. J. (2014). **Attenuation of cisplatin ototoxicity by otoprotective effects of nanoencapsulated curcumin and dexamethasone in a guinea pig model**. *Otol Neurotol, 35*(7), 1131-1139.

Schink, T., Kreutz, G., Busch, V., Pigeot, I., & Ahrens, W. (2014). **Incidence and relative risk of hearing disorders in professional musicians**. *Occup Environ Med, 71*(7), 472-476.

Seo, Y. J., Jeong, J. H., Choi, J. Y., & Moon, I. S. (2014). **Neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio: novel markers for diagnosis and prognosis in patients with idiopathic sudden sensorineural hearing loss**. *Dis Markers, 702807*(10), 7.

Sheppard, A., Hayes, S. H., Chen, G. D., Ralli, M., & Salvi, R. (2014). **Review of salicylate-induced hearing loss, neurotoxicity, tinnitus and neuropathophysiology**. *Acta Otorhinolaryngol Ital, 34*(2), 79-93.

Slattery, E. L., Oshima, K., Heller, S., & Warchol, M. E. (2014). **Cisplatin exposure damages resident stem cells of the mammalian inner Ear**. *Dev Dyn, 29*(10), 24150.

Son, H. J., Karkas, A., Buchanan, P., Giurintano, J. P., Theodosopoulos, P., Pensak, M. L., & Samy, R. N. (2014). **Spontaneous cerebrospinal fluid effusion of the temporal bone: repair, audiological outcomes, and obesity**. *Laryngoscope, 124*(5), 1204-1208.

Sun, S., Yu, H., Honglin, M., Ni, W., Zhang, Y., Guo, L., . . . Li, H. (2014). **Inhibition of the Activation and Recruitment of Microglia-Like Cells Protects Against Neomycin-Induced Ototoxicity**. *Mol Neurobiol, 1*, 1.

Sun, W., Fu, Q., Zhang, C., Manohar, S., Kumaraguru, A., & Li, J. (2014). **Loudness perception affected by early age hearing loss**. *Hear Res, 313*, 18-25.

Suzuki, H., Tabata, T., Koizumi, H., Hohchi, N., Takeuchi, S., Kitamura, T., . . . Ohbuchi, T. (2014). **Prediction of Hearing Outcomes by Multiple Regression Analysis in Patients With Idiopathic Sudden Sensorineural Hearing Loss**. *Ann Otol Rhinol Laryngol, 18*, 000348941453860 6.

Takagi, T., Yoshida, T., Okada, M., Hata, R., Hato, N., Gyo, K., & Hakuba, N. (2014). **Intravenous administration of bone marrow mononuclear cells alleviates hearing loss after transient cochlear ischemia through paracrine effects**. *Neuroreport, 16*, 16.

Takanashi, Y., Misu, T., Oda, K., Miyazaki, H., Yahata, I., Hidaka, H., . . . Katori, Y. (2014). **Audiological evidence of therapeutic effect of steroid treatment in neuromyelitis optica with hearing loss**. *J Clin Neurosci, 29*(14), 00310-00315.

Tan, C. Q., Gao, X., Guo, L., & Huang, H. (2014). **Exogenous IL-4-expressing bone marrow mesenchymal stem cells for the treatment of autoimmune sensorineural hearing loss in a guinea pig model**. *Biomed Res Int, 856019*(10), 22.

Terashima, M., Fujita, Y., Togashi, Y., Sakai, K., De Velasco, M. A., Tomida, S., & Nishio, K. (2014). **KIAA1199 interacts with glycogen phosphorylase kinase beta-subunit (PHKB) to promote glycogen breakdown and cancer cell survival**. *Oncotarget, 5*(16), 7040-7050.

Thirumala, P. D., Krishnaiah, B., Crammond, D. J., Habeych, M. E., & Balzer, J. R. (2014). **Analysis of wave III of brain stem auditory evoked potential waveforms during microvascular decompression of cranial nerve VII for hemifacial spasm**. *J Clin Neurophysiol, 31*(2), 127-132.

Tian, C., Kim, Y. J., Lim, H. J., Kim, Y. S., Park, H. Y., & Choung, Y. H. (2014). **Red ginseng delays age-related hearing and vestibular dysfunction in C57BL/6 mice**. *Exp Gerontol, 57*, 224-232.

Tian, G., Sawashita, J., Kubo, H., Nishio, S. Y., Hashimoto, S., Suzuki, N., . . . Higuchi, K. (2014). **Ubiquinol-10 supplementation activates mitochondria functions to decelerate senescence in senescence-accelerated mice**. *Antioxid Redox Signal, 20*(16), 2606-2620.

Tropitzsch, A., Arnold, H., Bassiouni, M., Muller, A., Eckhard, A., Muller, M., & Lowenheim, H. (2014). **Assessing cisplatin-induced ototoxicity and otoprotection in whole organ culture of the mouse inner ear in simulated microgravity**. *Toxicol Lett, 227*(3), 203-212.

Vambutas, A., Lesser, M., Mullooly, V., Pathak, S., Zahtz, G., Rosen, L., & Goldofsky, E. (2014). **Early efficacy trial of anakinra in corticosteroid-resistant autoimmune inner ear disease**. *J Clin Invest, 124*(9), 4115-4122.

van As, J. W., van den Berg, H., & van Dalen, E. C. (2014). **Medical interventions for the prevention of platinum-induced hearing loss in children with cancer**. *Cochrane Database Syst Rev, 1*(7).

Verver, E. J., Freriks, K., Sas, T. C., Huygen, P. L., Pennings, R. J., Smeets, D. F., . . . Kunst, H. P. (2014). **Karyotype-specific ear and hearing problems in young adults with turner syndrome and the effect of oxandrolone treatment**. *Otol Neurotol, 35*(9), 1577-1584.

Vicente-Herrero, M. T., Lladosa Marco, S., Ramirez-Iniguez de La Torre, M. V., Terradillos-Garcia, M. J., & Lopez-Gonzalez, A. A. (2014). [**Evaluation of hearing loss parameters in workers and its relationship with fasting blood glucose levels**]. *Endocrinol Nutr, 61*(5), 255-263.

Vincenti, V., Magnan, J., & Zini, C. (2014). **Cochlear effects of intraoperative use of Mesna in cholesteatoma surgery**. *Acta Biomed, 85*(1), 30-34.

Vitanza, N. A., Shaw, T. M., Gardner, S. L., Allen, J. C., Harter, D. H., & Karajannis, M. A. (2014). **Noncarboplatin-induced Sensorineural Hearing Loss in a Patient With an Intracranial Nongerminomatous Germ Cell Tumor**. *J Pediatr Hematol Oncol, 22*, 22.

Vohr, B. (2014). **Speech and language outcomes of very preterm infants**. *Semin Fetal Neonatal Med, 19*(2), 78-83.

Whitehorn, H., Sibanda, M., Lacerda, M., Spracklen, T., Ramma, L., Dalvie, S., & Ramesar, R. (2014). **High prevalence of cisplatin-induced ototoxicity in Cape Town, South Africa**. *S Afr Med J, 104*(4), 288-291.

Winther Frederiksen, T., Ramlau-Hansen, C. H., Stokholm, Z. A., Vestergaard, J. M., & Kolstad, H. A. (2014). 0069 **Psychosocial work factors, occupational noise exposure, common mental disorders, and the risk of tinnitus**. *Occup Environ Med, 71*(1), 2014-102362.

Wittig, J., Wittekindt, C., Kiehntopf, M., & Guntinas-Lichius, O. (2014). **Prognostic impact of standard laboratory values on outcome in patients with sudden sensorineural hearing loss**. *BMC Ear Nose Throat Disord, 14*(6), 1472-6815.

Womack, A. M., Hayes-Jordan, A., Pratihar, R., Barringer, D. A., Hall, J. H., Jr., Gidley, P. W., & Lewin, J. S. (2014). **Evaluation of Ototoxicity in Patients Treated With Hyperthermic Intraperitoneal Chemotherapy (HIPEC) With Cisplatin and Sodium Thiosulfate**. *Ear Hear, 14*, 14.

Wood, J. W., Bas, E., Gupta, C., Selman, Y., Eshraghi, A., Telischi, F. F., & Van De Water, T. R. (2014). **Otoprotective properties of mannitol against gentamicin induced hair cell loss**. *Otol Neurotol, 35*(5), 0000000000000342.

Xiao, Y., Wen, J., Bai, Y., Duan, N., & Jing, G. X. (2014). **Different effects of propofol and isoflurane on cochlear blood flow and hearing function in Guinea pigs**. *PLoS One, 9*(5).

Yamaguchi, T., Nagashima, R., Yoneyama, M., Shiba, T., & Ogita, K. (2014). **Disruption of ion-trafficking system in the cochlear spiral ligament prior to permanent hearing loss induced by exposure to intense noise: possible involvement of 4-hydroxy-2-nonenal as a mediator of oxidative stress**. *PLoS One, 9*(7).

Yang, C., Zhang, W., Liu, X. L., Liang, Y., Yuan, Y. W., Ren, C., & Peng, J. H. (2014). **Localization of prestin and expression in the early period after radiation in mice**. *Eur Arch Otorhinolaryngol, 17*, 17.

Yasui, N., Adachi, N., Kato, M., Koh, K., Asanuma, S., Sakata, H., & Hanada, R. (2014). **Cisplatin-induced hearing loss: the need for a long-term evaluating system**. *J Pediatr Hematol Oncol, 36*(4), 0000000000000028.

Zagolski, O., & Strek, P. (2014). **Tinnitus pitch and minimum masking levels in different etiologies**. *Int J Audiol, 53*(7), 482-489.

Zhan, Y., Wu, X., Hu, Y., Huang, X., Duan, J., Chen, H., . . . Kong, W. (2014). [**DNA microarray screening analysis in children with profound hearing impairment in Hubei province**]. *Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi, 28*(10), 680-684.

Zhang, G., Tang, Z., Yao, Y., & Wang, H. (2014). [**Investigation of noise hazards and hearing status of workers in outdoor quarries**]. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi, 32*(8), 597-599.

Zhang, Y., Jia, S., & Jiang, W. G. (2014). **KIAA1199 and its biological role in human cancer and cancer cells (review)**. *Oncol Rep, 31*(4), 1503-1508.

CLINICAL TRIALS

ClinicalTrials.gov was searched using the following search terms: "noise induced hearing loss," "hearing loss" AND "pharmaceutical" and "tinnitus" AND "pharmaceutical." "Include only open studies" was selected and the search results, retrieved Sep 2014, derived 4, 64 and 28 results, respectively, for a total of 96 results. 15 duplicates were removed leaving 82 studies for review.

Studies were further eliminated from inclusion based on subjective determination of relevance by the editors for a total of 29 studies included below. It should be noted that relevance was considered broadly as any studies of potential interest, including in secondary outcomes listed, to any one of the PIHL committee focus areas (see editor's introduction for the general listing of these). An exception to the PIHL focus areas used was the category of noise exposure, to include both measurement and preventative assessments, as this opens such a large category of studies, not all of which would necessarily categorize as a clinical trial nor be required to register in clinicaltrials.gov, and thus inclusion herein would produce an indeterminately incomplete set. In studies where primary or secondary outcomes assessed an intervention for hearing or tinnitus outcomes the studies were included, whereas studies which only captured hearing or tinnitus outcomes as adverse events were excluded. This most predominantly occurred in ototoxicity studies. All studies are listed in chronological order (using study start date), newest first.

TITLE: SPI-1005 for Prevention and Treatment of Chemotherapy Induced Hearing Loss

CT.gov ID: NCT01451853

Responsible Party: Sound Pharmaceuticals, Incorporated

Target Condition(s): Lung Cancer; Head and Neck Cancer; Hearing Loss; Ototoxicity; Tinnitus; Neuropathy

Intervention: Drug: SPI-1005 Low Dose; Drug: SPI-1005 Middle Dose; Drug: SPI-1005 High Dose; Drug: Placebo

Phase: 2

Study Start Date: Nov-14

Description Provided: Chemotherapy treatment with platinum based agents is well noted to cause ototoxicity. It is the objective of this study to determine the safety and efficacy of SPI-1005 at three dose levels when delivered orally twice daily for 3 days, surrounding each cycle of platinum chemotherapy in head and neck or non-small cell lung cancer patients to prevent and treat chemotherapy induced hearing loss and tinnitus.

TITLE: NAC to Prevent Cisplatin-induced Hearing Loss

CT.gov ID: NCT02094625

Responsible Party: Etan Orgel, Children's Hospital Los Angeles

Target Condition(s): Neuroectodermal Tumors, Primitive; Liver Neoplasms; Neoplasms, Germ Cell and Embryonal; Osteosarcoma; Other Childhood Cancers Using Cisplatin-based Regimens

Intervention: Drug: N-Acetylcysteine

Phase: 1

Study Start Date: Jul-14

Description Provided: Cisplatin is a key chemotherapy agent for the treatment of multiple childhood cancers but causes permanent hearing loss. This study investigates the drug N-acetylcysteine (NAC) to determine the dose necessary to protect hearing and also how well tolerated NAC is when combined with chemotherapy.

TITLE: Safety, Tolerability and Efficacy for CGF166 in Patients With Bilateral Severe-to-profound Hearing Loss

CT.gov ID: NCT02132130

Responsible Party: Novartis (Novartis Pharmaceuticals)

Target Condition(s): Severe-to-profound Bilateral Hearing Loss with Intact Vestibular Function in the Non-operative Ear.

Intervention: Drug: CGF166

Phase: 1, 2

Study Start Date: Jun-14

Description Provided: The current study will evaluate the safety, tolerability, and potential efficacy of CGF166 and the associated delivery procedures in patients with severe-to-profound bilateral hearing loss. Eligible patients are required to have documented, non-fluctuating hearing loss. Part A will include a safety and tolerability cohort (N=3). Patient dosing will be staggered; dosing the next patient in a cohort will be based on a safety review of all available data through 4 weeks post-dose of the previously dosed patient(s). Part B includes a volumetric escalation design to evaluate infusion volumes of the same CGF166 concentration (5.0 x 10E11vp/mL) in 4 cohorts of patients (n=3/cohort; total of 12 patients). Part C is an expansion cohort of the highest safe and tolerable dose identified in Part B, for further assessment of efficacy.

TITLE: Study of Lamotrigine to Treat Ménière's Disease

CT.gov ID: NCT02158585

Responsible Party: Lixin Zhang, Medical Director of the Dizziness and Balance Center, Dent Neuroscience Research Center

Target Condition(s): Meniere's Disease; Ménière's Vertigo; Vertigo, Intermittent; Vertigo, Aural

Intervention: Drug: Lamotrigine; Drug: Placebo

Phase: 3

Study Start Date: Jun-14

Description Provided: This double-blinded study evaluates the frequency of vertigo attacks and the quality of life of patients diagnosed with Ménière's disease after being randomly assigned to take a placebo or lamotrigine. Primary Outcome Measures:
•Change in Ménière's vertigo attack frequency. Secondary Outcome Measures:
•Responder rate
Percentage of patients with > 50% reduction in vertigo attack frequency from baseline at end of lead-in period to end of study period
•Change in length of vertigo attack-free intervals
•Change in hearing loss
•Change in symptom severity scores on the Ménière's Disease Patients-Oriented Severity Index
•Change in Tinnitus Handicap Inventory (THI) score
•Rating of Meniere's disease severity on the Clinical Global Impression Scale
•Change in Dizziness Handicap Inventory (DHI) score
•Change in rating of symptom impact on daily life on the American Academy of Otolaryngology-Head and Neck Surgery (AAO-HNS) Ménière's disease self-assessment
•Change in number and percentage of vertigo attack-free days per 28 days period
•Change in number and percentage of patients who are vertigo attack-free
•Rating of drug effect on the Clinical Global Impression Scale
•Rating of total improvement on the Clinical Global Impression Scale

TITLE: Prevention of Noise-induced Hearing Loss

CT.gov ID: NCT02049073

Responsible Party: Judith Lieu, Washington University School of Medicine

Target Condition(s): Noise-induced Hearing Loss

Intervention: Drug: Zonisamide; Drug: Methylprednisolone

Phase: 1, 2

Study Start Date: Jun-14

Description Provided: Noise-induced hearing loss affects an estimated 5% of the worldwide population, with 30-40 million Americans exposed to hazardous sound or noise levels regularly. Sources of noise may be occupational, blast noise, or recreational. Trauma to the inner ear can occur through transient hearing loss or permanent hearing loss. Although hearing recovers after temporary transient hearing loss, growing evidence suggests that repeated temporary transient hearing loss may lead to a permanent hearing loss. Currently, there are no treatments and there are no known medications that can be used clinically to prevent noise-induced hearing loss in humans.

The long-term goal of this research is to find medications that can prevent noise-induced hearing loss. The purpose of the present pilot study is to evaluate zonisamide and methylprednisolone as medications to prevent temporary transient hearing loss in humans.

TITLE: AM-101 in the Treatment of Post-Acute Tinnitus 1
CT.gov ID: NCT01934010
Responsible Party: Auris Medical, Inc.
Target Condition(s): Tinnitus
Intervention: Drug: AM-101
Phase: 3
Study Start Date: Jun-14
Description Provided: The purpose of this research study is to test the safety and local tolerance of repeated treatment cycles of AM-101.

TITLE: AM-101 in the Treatment of Post-Acute Tinnitus 2
CT.gov ID: NCT02040207
Responsible Party: Auris Medical, Inc.
Target Condition(s): Tinnitus
Intervention: Drug: AM-101
Phase: 3
Study Start Date: Jun-14
Description Provided: The purpose of this research study is to test the safety and local tolerance of repeated treatment cycles of AM-101.

TITLE: Commercial Lidocaine Patch as a Treatment for Ear-ringing
CT.gov ID: NCT02088866
Responsible Party: Rodney Diaz, MD, University of California, Davis
Target Condition(s): Tinnitus
Intervention: Drug: Transdermal Lidocaine
Phase: 0

Study Start Date: Mar-14
Description Provided: The purpose of this investigation is to evaluate if topically applied lidocaine, in the form of lidocaine patches, reduces the burden of chronic subjective tinnitus in a consistent and measurable way.

TITLE: AM-101 in the Treatment of Acute Tinnitus 2
CT.gov ID: NCT01803646
Responsible Party: Auris Medical, Inc.
Target Condition(s): Tinnitus
Intervention: Drug: AM-101; Drug: Placebo
Phase: 3
Study Start Date: Feb-14
Description Provided: The purpose of this research study is to test the safety and effectiveness of the study drug, AM-101. AM-101 is tested for the treatment of tinnitus that started as the result of an injury to the inner ear or due to middle ear inflammation (otitis media). Subjects with tinnitus can take part in the study, if their tinnitus started within the last 3 months.

TITLE: Sudden Hearing Loss Multi-center Clinical Trial
CT.gov ID: NCT02026479
Responsible Party: Li Sheng Yu, Professor, Peking University People's Hospital
Target Condition(s): Full-frequency Sudden Hearing Loss
Intervention: Drug: Dexamethasone Phosphate; Drug: Ginaton
Phase: International study; none listed
Study Start Date: Jan-14
Description Provided: The incidence of sudden hearing loss is rising obviously resent year, Glucocorticoids have obtained obvious effect in the treatment of sudden deafness. Postauricular hypodermic injection is the latest findings in clinical work and a new noninvasive way of administration which is gradually expanding research. The aim of this experiment is to verify and explore the efficacy and safety of the postauricular injection treatment with different doses of Glucocorticoids.

TITLE: AM-101 in the Treatment of Acute Tinnitus 3
CT.gov ID: NCT02040194
Responsible Party: Auris Medical, Inc.
Target Condition(s): Tinnitus
Intervention: Drug: AM-101; Drug: Placebo
Phase: 3
Study Start Date: Jan-14
Description Provided: The purpose of this research study is to test the safety and effectiveness of the study drug, AM-101. AM-101 is tested for the treatment of tinnitus

that started as the result of an injury to the inner ear or due to middle ear inflammation (otitis media). Subjects with tinnitus can take part in the study, if their tinnitus started within the last 3 months or within the last 4 to 12 months.

TITLE: Effectiveness of Cannabis in the Treatment of Tinnitus Patients
CT.gov ID: NCT01969474
Responsible Party: Oron Yahav, Wolfson Medical Center
Target Condition(s): Tinnitus
Intervention: Drug: Cannabis; Drug: Placebo
Phase: 1
Study Start Date: Dec-13
Description Provided: The hypothesis of the study is that the use of Cannabis will attenuate the tinnitus level as experienced by the patients.

TITLE: Montelukast for Children With Chronic Otitis Media With Effusion (COME): A Double-blind, Placebo-controlled Study
CT.gov ID: NCT01967498
Responsible Party: Tel-Aviv Sourasky Medical Center
Target Condition(s): Chronic Otitis Media With Effusion; Conductive Hearing Loss
Intervention: Drug: Montelukast
Phase: International study; none listed
Study Start Date: Nov-13
Description Provided: The purpose of our double-blind, placebo controlled study is to test the hypothesis that montelukast therapy might be associated with improved hearing in certain sub populations of children suffering from OME.

TITLE: OTO-104 for the Treatment of Meniere's Disease
CT.gov ID: NCT01412177
Responsible Party: Otonomy, Inc.
Target Condition(s): Meniere's Disease; tinnitus secondary outcome measure
Intervention: Drug: OTO-104; Drug: Placebo
Phase: 2
Study Start Date: Nov-13
Description Provided: The purpose of this study is to evaluate the effectiveness of OTO-104 for the treatment of Meniere's disease with secondary outcome measures of tinnitus reports.

TITLE: Latanoprost for the Treatment of Menière's Disease
CT.gov ID: NCT01973114
Responsible Party: Synphora AB

Target Condition(s): Menière's Disease; secondary Speech, HL
Intervention: Drug: Latanoprost; Other: Placebo
Phase: 2, 3
Study Start Date: Oct-13
Description Provided: The purpose of the study is to evaluate the dose regimen, efficacy and safety of latanoprost for the treatment of Menière's disease.

TITLE: Phase 3 Clinical Trial: D-Methionine to Reduce Noise-Induced Hearing Loss (NIHL)
CT.gov ID: NCT01345474
Responsible Party: Southern Illinois University
Target Condition(s): Noise-Induced Hearing Loss
Intervention: Drug: D-methionine, oral liquid suspension; Other: Placebo Comparator
Phase: 3
Study Start Date: Sep-13
Description Provided: This prospective study is a randomized, double-blind, placebo-controlled Phase 3 clinical trial of oral D-met to reduce noise-induced hearing loss (NIHL) and tinnitus. The goal of the study is to develop a safe, oral pharmacological agent to augment physical hearing protectors for noise exposures that exceed the protective capabilities of ear plugs and/or muffs. The study population is a cohort of Drill Sergeant (DS) instructor trainees during and 22 days after their 11 day weapons training. The primary objective of this study is to determine the efficacy of D-Met in preventing NIHL or reducing tinnitus secondary to a minimum of 500 rounds of M-16 weapons training occurring over an 11 day period.

TITLE: Dose-Response Study of MDMA-assisted Psychotherapy in People With PTSD
CT.gov ID: NCT01958593
Responsible Party: Multidisciplinary Association for Psychedelic Studies
Target Condition(s): Posttraumatic Stress Disorder; tinnitus as secondary outcome
Intervention: Drug: Comparator-dose MDMA; Drug: Full-dose MDMA
Phase: 2
Study Start Date: Sep-13
Description Provided: This small ("pilot") study is designed to provide information on whether the combination of psychotherapy with the drug MDMA is safe and helpful for people with post-traumatic stress disorder (PTSD). The researchers will use the results of this study to design more studies of this treatment. The study compares a comparator and a full dose. For each session, there will be an initial dose possibly followed 1.5 to 2.5 hours later by a dose half the size of the initial dose. The study will measure symptoms of PTSD, depression, general psychological well-being, sleep quality, feelings that the self or world is unreal (dissociation), potentially positive effects of surviving traumatic events and cognitive function (thinking, memory and attention). People experiencing pain or tinnitus (ringing in the ears) will record their symptoms throughout the study. Seven people will be randomly (by chance) assigned to receive full-dose MDMA and five will

be randomly assigned to receive a comparator. There will be three preparatory psychotherapy sessions before the first experimental session, and subjects will have supportive or "integrative" sessions after each MDMA-assisted psychotherapy session. Subjects will meet with a male and female psychotherapist for all experimental sessions and for sessions before and after each experimental session. Subjects who received comparator can enroll in Stage 2, where they will have three open-label MDMA-assisted psychotherapy sessions, meaning everyone will know they are receiving an active dose of MDMA. Subjects receiving full dose in Stage 1 will have a third experimental session.. Symptoms of PTSD and other symptoms will be measured again at least 12 months after each subject has started the study.

TITLE: The Effects of Gevokizumab in Corticosteroid-resistant Subjects With Autoimmune Inner Ear Disease
CT.gov ID: NCT01950312
Responsible Party: XOMA (US) LLC
Target Condition(s): Autoimmune Inner Ear Disease
Intervention: Drug: gevokizumab
Phase: 2
Study Start Date: Aug-13
Description Provided: The purpose of this study is to determine if gevokizumab therapy may be an alternate therapy in patients with steroid resistant Autoimmune Inner Ear Disease. Primary Outcome Measures: •Improved hearing threshold, as defined by an improvement in either the PTA (Pure Tone Average) of >=5 dB (Decibel), or 12% in the WRS (Word Recognition Score)

TITLE: Efficacy, Safety, and Tolerability of Ancrod in Patients With Sudden Hearing Loss
CT.gov ID: NCT01621256
Responsible Party: Nordmark Arzneimittel GmbH & Co. KG
Target Condition(s): Hearing Loss; Deafness; Hearing Loss, Sensorineural; Hearing Disorders; Ear Diseases
Intervention: Drug: Ancrod; Drug: Saline solution
Phase: 1, 2
Study Start Date: May-13
Description Provided:
The purpose of this study is to determine whether ancrod is effective and safe in the treatment of sudden sensorineural hearing loss (SSHL).

TITLE: Preventing Nephrotoxicity and Ototoxicity From Osteosarcoma Therapy
CT.gov ID: NCT01848457
Responsible Party: Children's Hospital of Philadelphia
Target Condition(s): Osteosarcoma; Nephrotoxicity; Ototoxicity

Intervention: Drug: Pantoprazole; Drug: High-dose methotrexate infusion duration
Phase: 2
Study Start Date: Apr-13
Description Provided: Osteosarcoma is the most common type of bone cancer in children, adolescents and young adults. Treatment with surgery and a combination of three conventional chemotherapy drugs can cure nearly two-thirds patients with osteosarcoma, but the treatment can also cause irreversible damage to the kidneys and cause permanent hearing loss. The purpose of this study is to evaluate new approaches to prevent these side effects without interfering with the beneficial effects of the chemotherapy drugs on the cancer by using our knowledge of how the drugs damage the kidney and cochlear hair cells in the ear to selectively block these side effects. Preventing these side effects without interfering with the anti-cancer effect of the drugs will improve the outcome in survivors and may also improve the effectiveness of the chemotherapy regimen by preventing treatment delays and dose reductions that are often caused by the side effects. Patients will be carefully monitored to ensure that the new interventions do not adversely affect response to the treatment and do not increase the other side effects of the chemotherapy. Specifically, we will monitor the nutritional status of the patients closely and ask patients to complete a survey describing the side effects after each treatment cycle. We will also collect a small sample of cancer tissue at the time of biopsy and surgery from each patient on this study for testing to determine new classes of anti-cancer drugs currently under development may have a role in treating osteosarcoma. If effective, these new approaches to prevent kidney damage and hearing loss will be applicable in other types of cancers treated with the same chemotherapy drugs.

TITLE: Prevention of Noise-induced Damage by Use of Antioxidants
CT.gov ID: NCT01727492
Responsible Party: Ethisch Comité UZ Antwerpen, University Hospital, Antwerp
Target Condition(s): Noise-induced Tinnitus; Noise-induced Hearing Loss
Intervention: Drug: Antioxidantia
Phase: International study; none listed
Study Start Date: Nov-12
Description Provided: The current study is a dubble-blinde placebo-controlled cross-over study verifying the preventive effect of antioxidants on noise-induced hearing loss (NIHL) and noise-induced tinnitus (NIT). The antioxidants comprise of a mixture of magnesium and n-acetylcystein which should be taken 1h before leisure noise above 100dB for at least 30 minutes.

TITLE: Intratympanic Steroid Treatment For The Prevention Of Inner Ear Toxicity Associated With Systemic Treatment With Cisplatin.
CT.gov ID: NCT01285674
Responsible Party: Ziv Hospital

Target Condition(s): Cisplatin; Ototoxicity; Intratympanic Steroids
Intervention: Drug: Intra-tympanic Cisplatinum
Phase: International study; none listed
Study Start Date: Jan-11
Description Provided: In this study we will aim to determine if cisplatin ototoxicity can be prevented by intratympanic administration of corticosteroids.

TITLE: Fludrocortisone for Sudden Hearing Loss
CT.gov ID: NCT01186185
Responsible Party: Anh Nguyen-Huynh, Oregon Health and Science University
Target Condition(s): Hearing Loss, Sensorineural
Intervention: Drug: Fludrocortisone
Phase: None listed
Study Start Date: Aug-10
Description Provided: The standard of care treatment of sudden hearing loss uses a type of steroid called glucocorticoid. Examples of glucocorticoids are prednisone, methylprednisolone and dexamethasone. Not everybody recovers hearing with glucocorticoid treatment. Fludrocortisone is a different type of steroid called mineralocorticoid. Unlike glucocorticoids, which work by reducing inflammation, mineralocorticoids work by changing salt and fluid balance. In animal studies, fludrocortisone is at least as effective as glucocorticoid in preserving hearing. Fludrocortisone is not approved for the treatment of sudden hearing loss. The purpose of this study is to test whether fludrocortisone can treat sudden hearing loss.

TITLE: Transtympanic Ringer's Lactate for the Prevention of Cisplatin Ototoxicity
CT.gov ID: NCT01108601
Responsible Party: McGill University Health Center
Target Condition(s): Hearing Loss
Intervention: Drug: Ringer's Lactate (0.03% Ciprofloxacin)
Phase: 1, 2
Study Start Date: Apr-08
Description Provided: Cisplatin and carboplatin induce ototoxicity manifested as sensorineural hearing loss, tinnitus, and/or vestibular disturbances. Ototoxicity is induced via damage to inner ear structures by reactive oxygen species. Previous animal studies demonstrated that transtympanic injection of Ringer's Lactate (RL) provided near complete otoprotective effect against cisplatin. The purpose of this study is to determine if transtympanic administration of Ringer's Lactate via a pressure equalising (PE) tube in patients undergoing platinum based chemotherapy treatment will prevent tinnitus, vestibular dysfunction and hearing loss especially at high frequencies. Pre- and post- chemotherapy treatment audiometry will be measured and statistically analysed for significance.

TITLE: Does Aspirin Have a Protective Role Against Chemotherapeutically Induced Ototoxicity?
CT.gov ID: NCT00578760
Responsible Party: University Health Network, Toronto
Target Condition(s): Hearing Loss; Ototoxicity
Intervention: Drug: aspirin; Drug: placebo
Phase: International study; none listed
Study Start Date: Feb-08
Description Provided: Aspirin (ASA) has been shown, in an animal model, to attenuate the ototoxic properties of cisplatin. The researchers plan to investigate this in patients undergoing cisplatin chemotherapy. The researchers hypothesise that low-dose aspirin can prevent cisplatin induced ototoxicity in the clinical setting.

TITLE: Zinc to Treat Tinnitus
CT.gov ID: NCT00683644
Responsible Party: University of Iowa
Target Condition(s): Tinnitus
Intervention: Dietary Supplement: Zinc sulfate
Phase: 2
Study Start Date: Jan-08
Description Provided: There is widespread belief and some evidence to indicate that zinc can successfully treat tinnitus. Zinc deficiency is more likely to occur in the elderly . The primary objective of this study is to establish the effectiveness of zinc for the treatment of tinnitus in individuals 60 years of age and older. Subjects will be randomly assigned to either receive zinc daily or a placebo. After 4 months and a 1-month wash-out, the subjects will be crossed over to the other group.

TITLE: Cisplatin With or Without Sodium Thiosulfate in Treating Young Patients With Stage I, Stage II, or Stage III Childhood Liver Cancer
CT.gov ID: NCT00652132
Responsible Party: National Cancer Institute (NCI)
Target Condition(s): Liver Cancer; Ototoxicity
Intervention: Drug: cisplatin; Drug: sodium thiosulfate; Genetic: gene rearrangement analysis; Genetic: microarray analysis; Genetic: proteomic profiling; Other: immunohistochemistry staining method; Other: laboratory biomarker analysis; Procedure: adjuvant therapy; Procedure: neoadjuvant therapy; Procedure: therapeutic conventional surgery
Phase: 3
Study Start Date: Dec-07
Description Provided: RATIONALE: Drugs used in chemotherapy, such as cisplatin, work in different ways to stop the growth of tumor cells, either by killing the cells or by

stopping them from dividing. Chemoprotective drugs, such as sodium thiosulfate, may protect normal cells from the side effects of chemotherapy. It is not yet known whether giving sodium thiosulfate is effective in reducing hearing damage caused by cisplatin in treating young patients with liver cancer. PURPOSE: This randomized phase III trial is studying how well sodium thiosulfate works to decrease hearing loss caused by cisplatin in treating young patients with stage I, stage II, or stage III childhood liver cancer.

TITLE: Applying Proton Pump Inhibitor to Prevent and Treat Acute Fluctuating Hearing Loss in Patients With SLC26A4 Mutation
CT.gov ID: NCT00789061
Responsible Party: National Taiwan University Hospital
Target Condition(s): Hearing Loss
Intervention: Drug: Proton pump inhibitor
Phase: 2, 3
Study Start Date: Aug-06
Description Provided: Disequilibrium between acid and base in the inner ear was suggested to be an important factor leading to hearing impairment associated with SLC26A4 mutations. For acid-base homeostasis in the inner ear, gastric-type proton pumps might demonstrate antagonistic effects to pendrin, the protein encoded by SLC26A4. To investigate whether proton pump inhibitors might prevent or treat acute fluctuating hearing loss related to SLC26A4 mutations, we launch the current double-blind randomized clinical trial.

TITLE: Bed Rest for Idiopathic Sudden Sensorineural Hearing Loss
CT.gov ID: NCT00416143
Responsible Party: Sheba Medical Center
Target Condition(s): Sudden Loss of Hearing
Intervention: Procedure: bed rest; Drug: prednisone - oral corticosteroid 1mg/kg/D for 1 week
Phase: 2, 3
Study Start Date: Jun-06
Description Provided: sudden sensorineural hearing loss:
- idiopathic in most cases
- 5-20/100,000 new cases annually in the U.S
- no establishes pathogenesis
- treated with oral steroids in most cases
- ~50% improvement in hearing levels
- bed rest - acceptable treatment, not well investigated

FUNDING OPPORTUNITIES

Refer to the HCE website (http://hearing.health.mil/Research/FundingInformation.aspx) for additional hearing-related research funding opportunities.

DoD USARMMC FY15 Broad Agency Announcement for Extramural Medical Research

Award Organization: USARMMC
Announcement #: W81XWH-BAA-15-1

Date Released: October 1, 2014

Date Closed: September 30, 2015

Web site: http://www.grants.gov/view-opportunity.html?oppId=267548

Notes: In accordance with FAR 6.102, projects funded under this BAA must be for basic and applied research and that part of development not related to the development of a specific system or hardware procurement. Projects must be for scientific study and experimentation directed toward advancing the state-of-the-art or increasing knowledge or understanding rather than focusing on a specific system or hardware solution. Research and development funded through this BAA is intended and expected to benefit and inform both military and civilian medical practice and knowledge.

Long Range Broad Agency Announcement for Navy and Marine Corps Science and Technology

Award Organization: ONR
Announcement #: ONRBAA15-001
Date Released: September 30, 2014

Date Closed: September 30, 2015

Web site: http://www.onr.navy.mil/~/media/Files/Funding-Announcements/BAA/2015/15-001.ashx

Notes: This BAA is intended for proposals related to basic research, applied research, or advanced technology development and that part of development not related to the development of a specific system or hardware procurement. Potential Offerors are urged to check the program areas that they are interested in throughout the year for updates to thrust areas and research priorities on the ONR website at http://www.onr.navy.mil.

NIDCD Research on Hearing health Care (R01)

Award Organization: National Institutes of Health/NIDCD
Announcement #: PA-14-091

Date Released: February 5, 2014

Date Closed: May 7, 2017

Web site: http://grants.nih.gov/grants/guide/pa-files/PA-14-091.html

Notes: Funds support research leading to accessible and affordable hearing health care (HHC). The overarching emphasis is on the acquisition of knowledge that can be rapidly translated into new or enhanced approaches for access, assessment or interventions with a goal to delivering better hearing health care outcomes. Applications should seek quality approaches that are effective, affordable and deliverable to those who need them as well as implementable and sustainable in settings beyond the research environment.

NIDCD Research on Hearing Health Care (R21)

Award Organization: National Institutes of Health/NIDCD
Announcement #: PA-14-090

Date Released: February 5, 2014

Date Closed: May 7, 2017

Web site: http://grants.nih.gov/grants/guide/pa-files/PA-14-090.html

Notes: The National Institute on Deafness and Other Communication Disorders (NIDCD) invites applications for Clinical Research Center Grants designed to advance the diagnosis, prevention, treatment, and amelioration of human communication disorders. For this announcement, Clinical Research is defined as research involving individuals with communication disorders or data/tissues from individuals with a communication disorder. Examples of such research include but are not limited to, studies of the prevention, pathogenesis, pathophysiology, diagnosis, treatment, management or epidemiology of a disease or disorder of hearing, balance, smell, taste, voice, speech, or language

NIDCD Research on Hearing Health Care (R21)

Award Organization: National Institutes of Health
Announcement #: PA-14-090

Date Released: May 16, 2014

Date Closed: May 07, 2017

Web site:
http://hearing.health.mil/exitwarning.aspx?link=http://grants.nih.gov/grants/guide/pa-files/PA-14-090.html

Notes: This FOA encourages Research Project Grant (R21) applications from institutions/organizations to support research leading to accessible and affordable hearing health care (HHC). The overarching emphasis is on the acquisition of knowledge that can be rapidly translated into new or enhanced approaches for access, assessment or interventions with a goal to delivering better hearing health care outcomes. Applications should seek quality approaches that are effective, affordable and deliverable to those who need them as well as implementable and sustainable in settings beyond the research environment.

NIDCD Research Grants for Translating Basic Research into Clinical Tools (R01)

Award Organization: National Institutes of Health
Announcement #: PAR-14-009

Date Released: November 22, 2013

Date Closed: February 3, 2017

Web site: http://grants.nih.gov/grants/guide/pa-files/PAR-14-009.html

Notes: The NIDCD is encouraging applications which translate basic research findings into clinical tools for better human health in the NIDCD mission areas of hearing, balance, smell, taste, voice, speech and language. The intent of this Funding Opportunity Announcement (FOA) is to provide a new avenue for basic scientists, clinicians and clinical scientists to jointly initiate and conduct translational research projects. The scope of this FOA includes a range of activities to encourage translation of basic research findings which will impact the diagnosis, treatment and prevention of communication disorders. Multi-institutional, multi-disciplinary, and academic-industrial collaborations studies are encouraged. This FOA is not intended for health services/outcome studies, the extension of ongoing clinical research studies, the optimization of current clinical protocols, or pre-translational studies. Connection to the clinical condition must be clearly established and the outcomes of the grant must have practical clinical impact.

NIDCD Clinical Research Grant (P50)

Award Organization: National Institutes of Health
Announcement #: PAR-13-277

Date Released: July 19, 2013

Date Closed: June 2, 2017

Web site: http://grants.nih.gov/grants/guide/pa-files/PAR-13-277.html

Notes: The National Institute on Deafness and Other Communication Disorders (NIDCD) invites applications for Clinical Research Center Grants designed to advance the diagnosis, prevention, treatment, and amelioration of human communication disorders. For this announcement, Clinical Research is defined as research involving individuals with communication disorders or data/tissues from individuals with a communication disorder. Examples of such research include but are not limited to, studies of the prevention, pathogenesis, pathophysiology, diagnosis, treatment, management or epidemiology of a disease or disorder of hearing, balance, smell, taste, voice, speech, or language.

NIDCD Research on Hearing Health Care (R21/33)

Award Organization: National Institutes of Health
Announcement #: PFA-DC-14-001

Date Released: April 15, 2013

Date Closed: February 24, 2015

Web site: http://grants.nih.gov/grants/guide/rfa-files/RFA-DC-14-001.html

Notes: This funding opportunity announcement (FOA) invites Exploratory/Developmental Phased Innovation (R21/R33) grant applications to support research and/or infrastructure needs leading to more accessible and affordable hearing health care (HHC). The proposed research aims should lead to the delivery of better healthcare access and outcomes and be directed to solutions that are effective, affordable and deliverable to those who need them. Outcomes and health services research are also responsive to this FOA. This FOA provides support for up to two years (R21 phase) for preliminary/development studies, followed by possible transition of up to four years of expanded research and development support (R33 phase). The total project period for an application submitted in response to this FOA may not exceed five years. This FOA requires measurable R21 milestones.

NIDCD Research Career Enhancement Award for Established Investigators (K18)

Award Organization: National Institutes of Health
Announcement #: PAR-13-186

Date Released: April 10, 2013

Date Closed: May 8, 2016

Web site: http://grants.nih.gov/grants/guide/pa-files/PAR-13-186.html

Notes: The purpose of the NIDCD Research Career Enhancement Award for Established Investigators (K18) program is to enable established, proven investigators to augment or

redirect their research programs through the acquisition of new research skills to answer questions relevant to the hearing, balance, smell, taste, voice, speech and language sciences.

Disorders of Human Communication: Effectiveness, Outcomes and Health Services Research (R01)

Award Organization: National Institutes of Health
Announcement #: PAR-13-102

Date Released: February 1, 2013

Date Closed: May 7, 2016

Web site: http://grants.nih.gov/grants/guide/pa-files/PA-13-102.html

Notes: The purpose of this Funding Opportunity Announcement (FOA) is to support effectiveness, outcomes and health services research in the NIDCD mission areas of hearing, balance, smell, taste, voice, speech and language.

Disorders of Human Communication: Effectiveness, Outcomes, and Health Services Research (R21)

Award Organization: National Institutes of Health
Announcement #: PA-13-103

Date Released: February 1, 2013

Date Closed: May 7, 2016

Web site: http://grants.nih.gov/grants/guide/pa-files/PA-13-103.html

Notes: The purpose of this Funding Opportunity Announcement (FOA) is to support effectiveness, outcomes and health services research in the NIDCD mission areas of hearing, balance, smell, taste, voice, speech and language.

NIDCD Small Grant Program (R03)

Award Organization: National Institutes of Health
Announcement #: PAR-13-057

Date Released: December 18, 2012

Date Closed: October 28, 2015

Web site: http://grants.nih.gov/grants/guide/pa-files/PAR-13-057.html

Notes: The NIDCD Small Grant Program (R03) is intended to support basic and clinical research of scientists who are beginning to establish an independent research career. It

cannot be used for thesis or dissertation research. The research must be focused on one or more of the areas within the biomedical and behavioral scientific mission of the NIDCD: hearing, balance, smell, taste, voice, speech, or language. The NIDCD R03 grant mechanism supports different types of projects including secondary analysis of existing data; small, self-contained research projects; development of research methodology; translational research; outcomes research; and development of new research technology. Irrespective of the type of project, the intent of the NIDCD R03 is for the Program Director(s)/Principal Investigator(s) (PD(s)/PI(s) to obtain sufficient preliminary data for a subsequent R01 application.

http://hearing.health.mil/EducationAdvocacy/Newsletters.aspx

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