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Unraveling the Mystery of Hair Cell Death from Noise

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Ever since a physician named Bernadino Ramazini (1700) first described tinnitus and hearing loss in coppersmiths in *De Morbis Artificum Diatriba* (*Diseases of Workers*),[1] scientists have been trying to understand how noise damages the ear. At that time, Ramazini stated;

"To begin with, the ears are injured by that perpetual din, and in fact the whole head, inevitably, so that workers of this class become hard of hearing and, if they grow old at this work, completely deaf. For that incessant noise beating on the eardrum makes it lose its natural tonus; the air within the ear reverberates against its sides, and this weakens and impairs all the apparatus of hearing."[1]

Over the next 150 years, scientists explored the gross anatomy of human cadavers to begin to explore the link between high levels of noise and hearing loss. Thurston provides an excellent summary of the general history of noise-induced hearing loss (NIHL) for those interested in the subject.[2]

With regard to the anatomical and physiological basis of NIHL, Fosbroke in 1831 appears to be the first to recognize that continuous noise exposure over a period of years results in *gradual onset* hearing loss by the age of 40 or 50 years.[3] In fact, his analogy regarding the cumulative nature of the noise damage from 1831 is just as relevant today as it was then;

"It is cumulative which makes it more difficult to guard against. It is like tobacco. One cigarette will not kill you and one day at work will not make you deaf. Neither will two or three – but where is the line? It is easy to say "one more exposure is not going to make any difference."[3]

Fosbroke also stated that unidentified writers of that era attributed the gradual onset noise-induced hearing loss (NIHL) to paralysis of the auditory nerve, due to permanent over-tension of the tympanic membrane.[3] Toynbee differentiated sudden onset acoustic trauma from gunshots due to ankylosis of the stapes footplate as a different form of damage from exposure to continuous high level noise sources attributed to expansion of the auditory nerve in the labyrinth.[4] In 1882, as microscope technology and cell staining advancements were made, Habermann described atrophy to nerve fibers and the organ of Corti as a consequence of long-term noise exposure in a metal worker that died suddenly after being struck by a locomotive he did not hear approaching.[5] Hair cells, along with their specific locations, were not implicated until 1717 when Fraser and Fraser reported in the "The Morbid Anatomy of War Injuries of the Ear;

"(ii) The neuro-epithelium (hair cells) of Corti's organ are first affected, later the supporting cells are involved. The ganglion cells and nerve fibres are secondarily affected. The condition is one of so-called "degenerative neuritis." (iii) The part of the Corti's organ affected depends on the pitch

of the sound. If the noise be of high pitch the neuro-epithelim at the base of the cochlear is involved. If the noise be of medium pitch, Corti's organ in the middle coil is affected; while if the noise be of low pitch, degeneration is found in a portion of the Corti's organ nearer the apex."

Hallowell Davis' classic experiments in the 1940s further linked the hair cell damage to hearing loss and he also reported cochlear neuron degeneration and capillary vasoconstriction as a consequence of hazardous noise exposure.[6] Continued advancements in electron microscopes and cell staining techniques assist today's scientists in capturing and describing the histopathology of specific sound sources and exposure durations. We've known for almost 300 years that noise damages hair cells, it is the "HOW" of this damage that continues to challenge today's scientists such as Eric Bielefeld. What follows is Eric's personal story, and his attempt to "unravel" the complexities of cochlear cell death in the cochlea.

At the AudiologyNOW conference in 2002 in Philadelphia, I had the opportunity as a graduate student to help develop and present a talk on the mechanisms of hair cell injury underlying NIHL along with my fellow PhD student, Kelly Harris and my mentor, Don Henderson. In 2006, we expanded the presentation into a review paper for *Ear and Hearing* entitled, "Oxidative Stress in Noise-induced Hearing Loss." I believe that I learned more working on that article than I have for any other project in my career. Nearly 13 years after the presentation at AudiologyNOW, I had the opportunity to join Deanna Meinke at the 2015 National Hearing Conservation Association (NHCA) conference for another presentation on the mechanisms of hair cell death in noise-induced hearing loss. It was an opportunity to re-visit the topics from 2002 and 2006, review what has changed, and what still needs exploration and clarification. One thing is certain, the hair cell death process continues to become more complex as we learn more.

The first key topic underlying cochlear cell death from noise is oxidative stress. Oxidative stress results from an imbalance between reactive oxygen species (ROS) and antioxidants. Extensive evidence dating back to the early 1990s and continuing through current experimentation[7–20] has implicated oxidative stress as a key underlying mechanism of NIHL. What remains largely speculative is the origin of the cochlear oxidative stress. Noise induces a burst of ROS formation that causes oxidative stress.[21–24] But the question of what causes the burst of ROS remains unanswered.

Multiple possibilities exist, and it is quite possible that multiple sources of ROS exist within the noise-exposed cochlea. Blood flow changes that result in ischemia (restriction of blood flow to tissue) and reperfusion (blood flow return to tissue after ischemia) are known to induce ROS formation (reviewed in Chan),[25] but the literature on the influence of noise on cochlear blood flow is inconsistent due to the use of different species, measurement times and approaches, and noise parameters.[26–30] Therefore, cochlear blood flow changes may be a factor in ROS formation, but it is unclear how much of a factor.

Enzymatic reactions may also play a role in ROS formulation, specifically, the reaction in which the molecule NADPH donates an electron to molecular oxygen (O_2) to form superoxide (O_2^-). This reaction is catalyzed by NDAPH oxidase. NADPH oxidase has been shown to be activated in the noise-exposed cochlea,[31] and inhibition of NADPH oxidase in the cochlea reduces noise-induced threshold shift.[32]

The severe metabolic demand placed on the cells of the cochlea that is exposed to persistent high-level noise has also been proposed as a source of ROS formation33 but the hypothesis has been

refuted on the grounds that over-driving of the mitochondria would deplete oxygen and ROS rather than producing them.[34] Instead it was proposed that dis-regulation of an enzyme in the Krebs' cycle of mitochondrial respiration, ?-ketoglutarate dehydrogenase, may be a key underlying event in cochlear ROS formation.[34] The cause of the dis-regulation of ?-ketoglutarate dehydrogenase in the noise-exposed cochlear cells remains the subject of hypotheses, and is a promising area for future investigation.

The second key topic to review about NIHL is the pattern of cell death. Cochlear cell death from noise is a primarily a combination of necrosis and apoptosis[9,33] along with an additional third pathway that does not fit either category's criteria.[35] Necrosis is a passive form of cell death in which the cell and nucleus swell and eventually can burst open. Necrosis is not an energy-consuming process and can result in inflammation to tissue. Apoptosis is a controlled disassembly of the cell through a series of enzymatic reactions that initiate and execute the cell death process. Apoptosis is energy consumptive, and does not result in inflammation of the neighboring tissue.

The discovery of apoptosis in the noise-exposed cochlea was a major advancement in the understanding of NIHL, and for developing novel routes for prevention and treatment. What remains an enormous research undertaking is determining the sequence of molecular signals bridging from the noise insult to the complete execution of apoptotic cell death. Review of the literature reveals this cataloging to be an immense task for which considerable work still needs to be done. Since noise causes a combination of mechanical injuries to the structure of the organ of Corti and its individual cells, along with metabolic stress to the cells themselves, determining what stresses are triggering apoptosis is extremely challenging. To confuse the issue further, different noise exposures (for example long-duration continuous noise versus short-duration impulse noise) are likely to affect the organ of Corti in different ways and lead to different sequences of apoptosis.

Rather than beginning with the triggering event for apoptosis, it is clearer to begin with the execution of the cell's death and work backward toward the triggering event that initiates the sequence of events leading to apoptosis. For many routes of apoptosis, the final execution of cell death requires caspases. Caspase are enzymes that can initiate the apoptosis phase, or physically cleave the cell into pieces for export from the body. In particular, caspase-3 is an executor caspase whose action is one of the final events in many cells' apoptosis pathways. Caspase-3 activation has been detected in the noise-exposed organ of Corti.[36] Additionally, caspase-8 and caspase-9 were also detected.[36] This is interesting because caspase-8 is heavily involved in the extrinsic apoptosis pathway, in which the toxic insult to the cell come from outside the cell and reaches the plasma membrane. Caspase-9 is involved in the intrinsic pathway, in which the apoptosis trigger originates from inside the cell at the mitochondria or nucleus. Seeing activation of both caspase-8 and -9 indicates multiple apoptosis sequences taking place concurrently within the same tissue.

Cytochrome c release from the mitochondria[36] and translocation of Endonuclease G from the mitochondria to the nucleus[37] have been detected in the noise-exposed cochlea. Both are key steps in the mitochondrial intrinsic apoptosis pathway, supporting its involvement in NIHL. Further implicating the mitochondrial pathway is the involvement of the Bcl-2 family, a complex family of proteins involved in intrinsic mitochondrial apoptosis, some of which signal for cell survival and some of which signal for cell death. The Bcl-2 cell survival proteins have been detected in the cochlea after noise exposure that induces temporary threshold shift (and thus the proteins promote recovery) while the pro-cell death Bcl-2 proteins have been detected after noise that induces permanent threshold shift.[38] While the mitochondrial intrinsic pathway is clearly involved in noise-

induced apoptosis, it is certainly not the only major pathway, and the list of involved proteins provided here is not close to complete.

The next several years of research hold significant promise for further detailing the complex apoptosis signaling events in the noise-exposed cochlea, as well as identifying the triggering events the initiate the apoptosis process. As we learn more about the mechanisms of cochlear cell death, we will have the opportunity to intervene and prevent NIHL. The future will be exciting for audiology as we potentially become involved in administering antioxidant therapies in advance of noise exposure or preventing the cascade of events that lead to ROS formulation, cochlear cell death and NIHL post exposure.

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